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; Purity: Unlabelled brodifacoum: [redacted]) [14C]-brodifacoum ([redacted]): radiochemical purity [redacted] % (with *cis:trans* ratio of [redacted]) [14C]-brodifacoum ([redacted]): radiochemical purity [redacted] % (with *cis:trans* ratio of [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 [14C]-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.

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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). X

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.

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.

	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	Include revised version. **3.1.2.2 Purity**
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

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

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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	

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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		

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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

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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		

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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		

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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		

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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		

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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

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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

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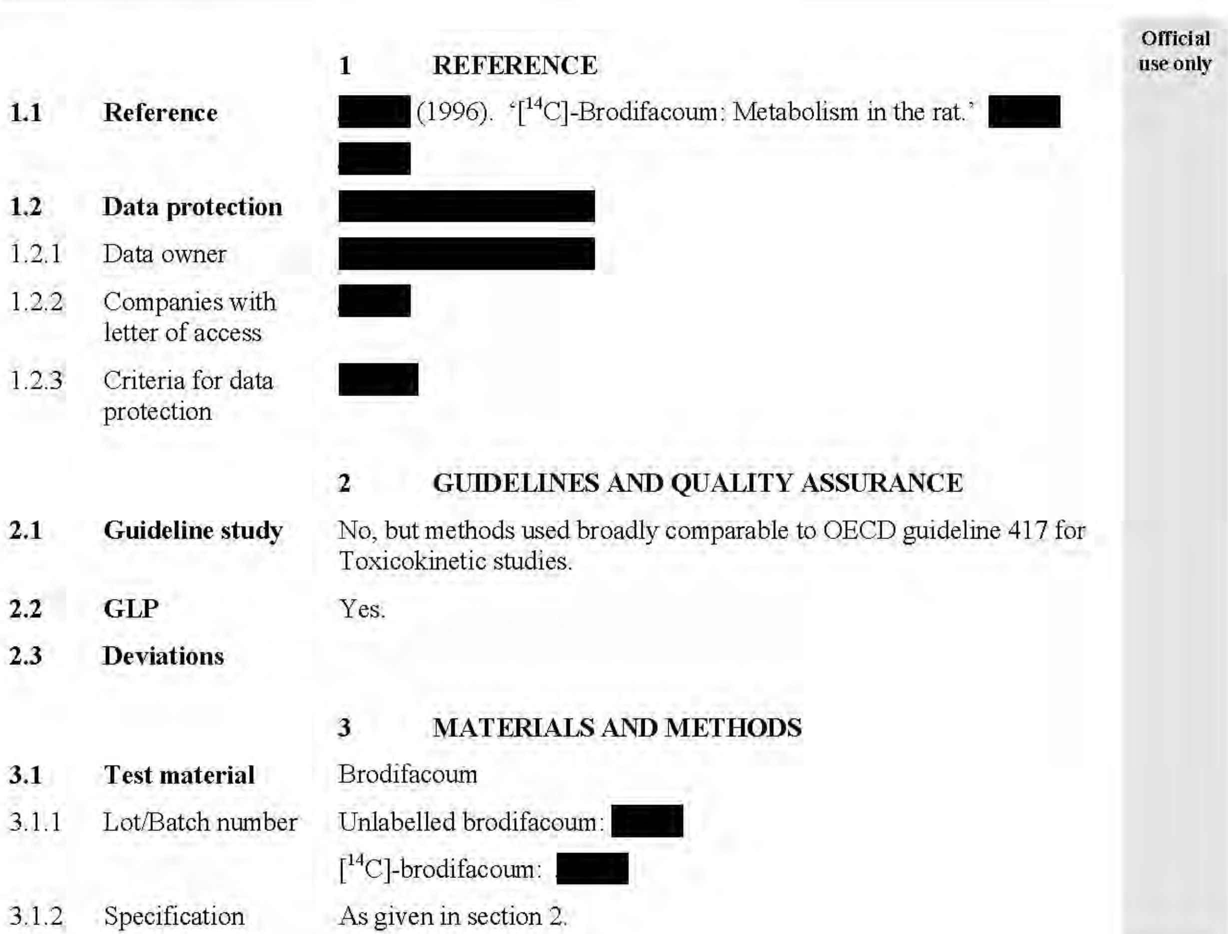

			Official use only
		1 REFERENCE	
1.1	**Reference**	(1996). '[14C]-Brodifacoum: Metabolism in the rat.'	
1.2	**Data protection**		
1.2.1	Data owner		
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection		
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but methods 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: [14C]-brodifacoum:	
3.1.2	Specification	As given in section 2.	

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

3.1.2.1 Description — Off-white powder.

3.1.2.2 Purity — Unlabelled brodifacoum: 95.6% (with *cis:trans* ratio of [redacted]). X

[^{14}C]-brodifacoum (91-J13): radiochemical purity 81.61% (as received).

[^{14}C]-brodifacoum (95-J6): radiochemical purity 91.35% (as received).

The two radiolabelled test substance batches were repurified into two fractions containing predominantly one isomer (*cis* or *trans*):

Batch no.	Isomer fraction	% *cis* isomer	% *trans* isomer	Total
91-J13	*Cis* fraction	85.27	13.25	98.52
	Trans fraction	2.23	97.00	99.23
95-J6	*Cis* fraction	93.37	6.46	99.83
	Trans fraction	6.44	92.98	99.42

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 0.925 GBq/mmole (Batch no: 91-J13) and 2.74 GBq/mmole (Batch no: 95-J6).

3.2 Test Animals

3.2.1 Species — Rat

3.2.2 Strain — Crl:CD(SD)BR

3.2.3 Source — [redacted]

3.2.4 Sex — Male

3.2.5 Age/weight at study initiation — Adult (approximately 6 to 10 weeks old) weighing 249 – 278g (Group A) and 303 – 323g (Group B).

3.2.6 Number of animals per group — Group A: 3 animals (biliary excretion study) .

Group B: 1 animal (*in vitro* liver perfusion study).

3.2.7 Control animals — No

3.3 Administration/ Exposure — Oral

3.3.1 Fasting period — No fasting prior to dosing, but approximately 24 hours prior to surgery, the drinking water was replaced with a solution of Vitamin K_1 and the daily consumption monitored throughout the study.

3.3.2 Metabolic/enzyme inhibitors or inducers — No

Doc IIIA / Section 6.2 **BPD Data Set IIA / Annex Point VI.6.2**		**Toxicokinetics Studies** Excretion and extent of metabolism in male rats following administration of single oral dose
3.3.3	Duration of treatment	Single dose
3.3.4	Frequency of exposure	
3.3.5	Postexposure period	Up to 48 hours
3.3.6	**Oral**	
3.3.6.1	Type	gavage
3.3.6.2	Concentration	Group A: 10 mg/kg bw [^{14}C]-brodifacoum corresponding to a nominal radioactive dose of 50 µCi (1.85 MBq) per animal. The target isomer ratio of radiolabelled brodifacoum in the formulation was 60:40 *cis:trans.* Group B: 10 mg/kg bw unlabelled brodifacoum. A further administration of [^{14}C]-brodifacoum was added directly into the main reservoir of the perfused animal, at a dose level of 10 mg/kg bw corresponding to a nominal radioactive dose of 20 µCi (0.74 MBq) per animal. The target isomer ratio of radiolabelled brodifacoum in the formulation was 60:40 *cis:trans.* Administration of brodifacoum at levels above the documented LD_{50} of 0.3 mg/kg bw was made possible by adding vitamin K_1 to the drinking water to antidote the anticoagulant effects.
3.3.6.3	Vehicle	Polyethylene glycol 600 (PEG 600) for oral dosing. Polyethylene glycol 200 (PEG 200) for *in vitro* liver perfusion.
3.3.6.4	Concentration in vehicle	Group A: 1.7 mg/g Group B: 2.4 mg/g
3.3.6.6	Total volume applied	5 ml dosing solution per kg bw (Groups A and B)
3.3.6.7	Controls	
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, animals were observed throughout the working day as necessary.
3.4.1.2	Mortality	Yes, animals were observed throughout the working day as necessary.
3.4.2	Body weight	Individual bodyweights were recorded within 24 hours of arrival at the laboratory, on the day of dosing, and at necropsy.

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3.4.3 Body fluids/excreta sampled	Yes. Group A: bile, faeces and urine was collected pre-dosing and then at 6, 12, 24, and 48 h post-dosing. Group B: bile was collected pre-dosing, and then at 1, 2, 3, 4, and 6 h post-dosing. Perfusate was collected pre-dosing, and then at 1 min, and 1, 2, 3, 4, and 6 h post-dosing. In addition to the perfusate, the terminal perfusate supernatant, supernatant filtrate	X
3.4.4 Tissues sampled	Yes. Group A: liver and residual carcass. Group B: liver and residual carcass.	
3.4.5 Determination of metabolites	Yes, the bile extracts were investigated for metabolites using the techniques of Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and LC-MS (High Performance Liquid Chromatography-Mass Spectrometry.	
3.4.6 Excretion routes	Yes, bile urine and faeces were collected as follows. Group A: bile, urine and faeces were collected pre-dose, and then 6, 12, 24 and 48 hours after dosing. After each collection of excreta, cage debris was removed and the cages rinsed with water. At the end of the collection period, cages were rinsed thoroughly with water and then methanol. Group B: bile was collected pre-dose, and then 1, 2, 3, 4 and 6 hours after dosing; perfusate was collected pre-dose, 1 min, and then 1, 2, 3, 4 and 6 hours after dosing.	
3.4.7 Other examinations	No.	
3.4.8 Statistics		
3.5 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Observations	In Group A, at approximately 3 hours prior to necropsy, animals were seen to be lethargic, pale and showing signs of pilo-erection. At necropsy massive internal haemorrhaging was observed in all animals, together with a darkening of some of the internal organs. In Group B, no overt pharmacological or toxicological effects were observed.	
4.2 Body weight	Bodyweight loss was noticed in all animals in Group A, but no effects were observed in Group B animals.	
4.3 Absorption		X

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Toxicokinetics Studies

Excretion and extent of metabolism in male rats following administration of single oral dose

4.4	**Distribution**	In Group A (biliary excretion study), the liver accounted for 14.7% of the dose and the residual carcass accounted for 42.9% of the dose. The concentration of radioactivity was higher in the liver at 35.9 µg equivalents/g, as compared to the carcass at 4.8 µg equivalents/g. In Group B (*in vitro* liver perfusion study), the liver accounted for 15.2% of the dose and the recovered perfusate accounted for 59.0%. In the additional experiment to investigate the distribution of radiolabelled material in the perfusate, there were only marginal differences in the amount of radioactivity in the red blood cells (8.4 µg equivalents/g) and the perfusate supernatant (6.4 µg equivalents/g). Further analysis of the supernatant for protein binding of radioactive residues, indicated that all of the radioactivity in the perfusate supernatant was bound to perfusate proteins, with no activity being measured in the aqueous filtrate.
4.5	**Metabolism**	For Group A (biliary excretion study), analysis of neat bile obtained from all time points by HPLC indicated the presence of up to 12 radiolabelled components. Comparisons between animals and time points showed only minor differences between profiles. This system did not resolve the two isomers of brodifacoum. The analytical profile obtained indicated one major metabolite, which accounted for up to 77.3% of the radioactivity in the 12-24 hour bile, corresponding to 6.7% of the total recovered dose over the course of the study. This metabolite was identified as the glucuronide conjugate of brodifacoum. A further radiolabelled peak was identified as unchanged brodifacoum. For Group B (*in vitro* liver perfusion study), the bile collected was pooled across time points due to low levels of radioactivity associated with these samples. Metabolite profiling was performed by HPLC but due to low levels of radioactivity, no meaningful evaluation of chromatograms was possible, although there was some evidence for a radiolabelled component with a similar retention time to brodifacoum. No mass spectral identification was attempted.
4.6	**Elimination and Excretion**	In Group A, the cumulative recovery of administered radioactivity from all excreta was 45.3% of the dose. Faeces represented the predominant route of excretion with 36.1% of the dose being collected over 48 hours; the majority of this was collected between 6 and 24 hours (23.1% of the dose). Only a little radioactivity was associated with the urine. The mean recovery of administered radioactivity in bile was 6.4% - the exclusion of results from one animal which had a reduced bile flow (and consequent reduction in radioactivity elimination), increases the mean recovery bile to 9.5% of the administered radioactivity. See Table A6_2-1 below. In Group B, elimination of radioactivity in the bile during the perfusion accounted for 0.134% of the administered dose at study termination (6 hours after dosing).

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4.7 Recovery of labelled compound

Group A: the mean recovery at study termination was 102.9%.

Group B: the recovery at 6 hours after dosing was 74.3%. This value does not include radioactivity associated with the carcass, residual perfusate in the apparatus, and perfusate that had leaked from the liver perfusion, hence a mass balance was not attempted.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test material: Brodifacoum; Purity:

Unlabelled brodifacoum: 95.6% (with *cis:trans* ratio of 59.3:40.7).

[^{14}C]-brodifacoum (91-J13): radiochemical purity 81.61% (as received).

[^{14}C]-brodifacoum (95-J6): radiochemical purity 91.35% (as received).

The two radiolabelled test substance batches were repurified into two fractions containing predominantly one isomer.

Methods used were broadly comparable to OECD guideline 417 for Toxicokinetic Studies.

The rate of elimination and extent of metabolism of brodifacoum were measured *in vivo* using biliary cannulated male rats and *in vitro* using a liver perfusion system. Administration of brodifacoum at levels above the documented LD_{50} of 0.3 mg/kg bw was made possible by adding vitamin K_1 to the drinking water to antidote the anticoagulant effects.

For the *in vivo* biliary excretion study, one group of 3 male rats (Crl:CD(SD)BR) weighing 249 – 278g (Group A) were given a single oral dose of 10 mg/kg bw [^{14}C]-brodifacoum. Bile, faeces and urine were collected pre-dosing and then at 6, 12, 24, and 48 h post-dosing. The bile extracts were investigated for metabolites using the techniques of Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and LC-MS (High Performance Liquid Chromatography-Mass Spectrometry.

For the *in vitro* liver perfusion study, one male rat (Crl:CD(SD)BR) weighing 323 g (Group B) was given a single oral dose of 10 mg/kg bw unlabelled-brodifacoum, with a further dose of 10 mg/kg_{bw} [^{14}C]-brodifacoum added directly into the main reservoir of the perfusate. The bile extracts were investigated for metabolites using the technique of High Performance Liquid Chromatography (HPLC).

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5.2 Results and discussion

Administration of brodifacoum at levels above the documented LD_{50} of 0.3 mg/kg was made possible by adding vitamin K_1 to the drinking water to antidote the anticoagulant effects. However, bile duct cannulated animals in group A displayed signs of toxicity prior to termination at 48 h after dosing, probably due to the reduction in the consumption of supplemented drinking water caused by the trauma of surgery. It was assumed that the absorption, distribution , metabolism and excretion of radioactivity was not affected by either the 10 mg/kg dose, or the administration of the vitamin K_1 antidote.

In Group A (biliary excretion study): the cumulative recovery of administered radioactivity from all excreta was 45.3%, with faeces representing the predominant route of excretion, which can be assumed to be unabsorbed material. There was little radioactivity associated with the urine, and this was likely to be contamination by the faeces rather than a route of excretion for this high molecular weight compound. The majority of the single oral radiolabelled dose of brodifacoum (mean 64.0%), was absorbed and could be accounted for in the liver, carcass and bile. The mean recovery of administered radioactivity in bile was 6.4% (+/- 5.454%), but the exclusion of results for one of the animals in this group, which had a reduced bile flow and consequent reduction in radioactivity elimination, increases the mean value to 9.5%.

In Group A, the analytical profile of neat bile displayed one major metabolite, which accounted for up to 77.3% of the radioactivity in the 12-24 h bile, corresponding to 6.7% of the total recovered dose over the course of the study. This metabolite was identified by LC-MS as the glucoronide conjugate of brodifacoum. A further radiolabelled peak was identified as unchanged brodifacoum.

Group B (*in vitro* liver perfusion study): measurement of radioactivity in the bile obtained from the perfused liver preparation, indicated that only 0.134% of the dose added to the perfusate had been eliminated at the 6 h time point in the bile. This low recovery precluded any characterisation of metabolites. However, attempts to refine this test system to improve recoveries were curtailed with the successful identification of metabolites from the *in vivo* biliary excretion study.

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** Excretion and extent of metabolism 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	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and extent of metabolism in male rats following administration of single oral dose

Table A6_2-1 Table for Toxicokinetic Studies:

Excretion Of Radioactivity In Male Rats Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum At 10 mg/kg$_{bw}$ (*Group A: biliary excretion study)*

Time After Dosing	Mean % of administered dose in excreta					
	Urine	SD	Faeces	SD	Bile	SD
0 minutes	ND	NA	-	-	ND	NA
6 hours	0.522	0.145	ND	NA	0.434	0.541
12 hours	0.279	0.087	5.400	8.067	1.225	1.058
24 hours	0.151	0.029	17.74	8.997	2.711	2.314
48 hours	0.431	0.239	12.97	5.075	2.025	1.688
Total	1.384	0.407	36.11	8.826	6.395	5.454

ND = Not Detected

NA = Not Applicable

Doc IIIA/Section 6.3.1 BPD Data Set IIA/Annex Point VI.6.3.1	Short-term repeated dose toxicity (28 days)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:		X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 6.3.2 **BPD Data Set IIA/Annex Point VI.6.3**	**Repeated Dose Toxicity (Dermal)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [✓]	**Technically not feasible** [] **Scientifically unjustified** [✓] **Other justification** [ ]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 6.3.3 BPD Data Set IIA/Annex Point VI.6.3	Repeated Dose Toxicity (Inhalation)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA / Section 6.4.1

BPD Data Set IIA / Annex Point VI.6.4

Subchronic toxicity

Subchronic Oral Toxicity In The Rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	(1984). 'Brodifacoum: 90-Day Feeding Study In Rats'.	
1.2	**Data protection**		
1.2.1	Data owner		
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection		
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Guideline not quoted in report, but study was conducted in general accordance with the principles of OECD Guideline 408: 'Subchronic Oral Toxicity – Rodent: 90-day Study'.	
2.2	**GLP**		
2.3	**Deviations**		X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number		
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	w/w	
3.1.2.3	Stability	Please refer to Section 2 of Doc. IIIA	X
3.2	**Test Animals**		
3.2.1	Species	*Rattus norvegicus* (Norway rat)	
3.2.2	Strain		
3.2.3	Source		
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 .	

Doc IIIA / Section 6.4.1 — **Subchronic toxicity**

Subchronic Oral Toxicity In The Rat

BPD Data Set IIA / Annex Point VI.6.4

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.

3.3.4.2 Concentration — Food: 0.02, 0.08 ppm — X

3.3.4.3 Vehicle

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

3.4.1.2 Mortality — Yes, at same time periods as for clinical signs.

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 approximately at the same time.

Doc IIIA / Section 6.4.1 — **Subchronic toxicity**

Subchronic Oral Toxicity In The Rat

BPD Data Set IIA / Annex Point VI.6.4

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.
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.
3.4.7	Clinical Chemistry	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.
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	No.
3.5.4	Statistics	Bodyweights, food consumption and food utilisation were considered by analysis of variance on a cage basis. Organ weights were considered by analysis of variance and covariance on final bodyweight. Haematological and biochemical parameters 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

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 0.02 ppm brodifacoum (from week 8) and for one rat receiving 0.08 ppm 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.

4.1.2 Mortality

All animals survived until their scheduled termination and were in good clinical condition throughout the study.

4.2 Body weight gain

Bodyweight gains during week 1 of animals fed 0.08 ppm brodifacoum were slightly reduced compared to the controls although this was not statistically significant. There were no differences in bodyweight gain between control and 0.02 ppm dosage groups.

4.3 Food consumption and compound intake

In animals fed diet containing 0.08 ppm 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 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 0.08 ppm brodifacoum. There was no evidence of any effect on these parameters in rats fed 0.02 ppm brodifacoum. ***See Table A6_3-1 below.***

No other statistically significant differences were noted between the

Doc IIIA / Section 6.4.1	**Subchronic toxicity**
BPD Data Set IIA / Annex Point VI.6.4	Subchronic Oral Toxicity In The Rat

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.***

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 0.08 ppm 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

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test substance: brodifacoum; Batch no: [redacted] The study was conducted in general accordance with the principles of OECD Guideline 408, with the following method:

Groups of 15 male Wistar derived rats (Alderley Park strain) were fed diets containing 0, 0.02 or 0.08 ppm brodifacoum. Five rats from each group were sacrificed after 45 days (satellite study) and the remainder after 90 days (main study) dietary administration. At termination, main study rats were given a full post mortem examination with tissue samples from control and top dose (0.08 ppm) groups examined histopathologically . Haematological parameters were determined on all satellite and main study animals at termination (45 and 90 days respectively). Clinical chemistry parameters were determined on all main study animals at termination (90 days).

5.2 Results and discussion

The only significant effect of brodifacoum administration in the diet of rats for up to 90 days, was on blood coagulation parameters which was consistent with the known mode of action of the rodenticides. The increase in blood coagulation time was only observed in rats given 0.08 ppm brodifacoum for 90 days, and not apparent in rats sacrificed after 45 days.

A number of minor effects were also found in the rats given 0.08 ppm brodifacoum. There was a slight decrease in food consumption and consequently a reduction in bodyweight during the first week of the study. In addition, there was an increase in plasma cholesterol concentration after 90 days. These changes were not of sufficient magnitude to be considered toxicologically significant.

Doc IIIA / Section 6.4.1 — **Subchronic toxicity**

Subchronic Oral Toxicity In The Rat

BPD Data Set IIA / Annex Point VI.6.4

None of the effects observed after administration of 0.08 ppm brodifacoum occurred at the lower dietary level of 0.02ppm. Therefore, the only effect associated with dietary administration of brodifacoum for 90 days is an increase in blood coagulation time.

5.3	**Conclusion**		
5.3.1	LO(A)EL		
5.3.2	NO(A)EL	0.02 ppm brodifacoum	X
5.3.3	Other		
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

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

Doc IIIA / Section 6.4.1 **BPD Data Set IIA / Annex Point VI.6.4**	**Subchronic toxicity** Subchronic Oral Toxicity In The Rat
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).

Doc IIIA/Section 6.4.2 BPD Data Set IIA/Annex Point VI.6.4	Subchronic dermal toxicity test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] Scientifically unjustified [✓] Other existing data [✓] Limited exposure [✓]	
Detailed justification:	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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Doc IIIA/Section 6.4.3 BPD Data Set IIA/Annex Point VI.6.4	Subchronic inhalation toxicity test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] Scientifically unjustified [✓] Other existing data [✓] Limited exposure [✓]	
Detailed justification:	[redacted]: [redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 6.5 **BPD Data Set IIA/Annex Point VI.6.5**	**Chronic toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [✓]** **Scientifically unjustified [✓]** **Other justification [ ]**	
Detailed justification:		
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
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Evaluation of applicant's justification		
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA / Section A6.6.1

BPD Data Set IIA / Annex Point VI.6.VI.6.1

Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], (1984), Brodifacoum - An Evalution in the *Salmonella* Mutagenicity Assay. [redacted] (unpublished).	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. The method used was the *Salmonella* mutagenicity assay of Maron and Ames (1983).	x
2.2	**GLP**	Yes.	
2.3	**Deviations**	Yes. The test method of Maron and Ames (1983) was followed, with only certain minor modifications that were based in part on the recommendations of the Arlington workshop (de Serres and Shelby, 1979): ➢ the presence of uvrB deletion was confirmed by testing the sensitivity of each culture to mitomycin C; ➢ when testing for histidine requirement and for reversion properties using diagnostic mutagens, the mutagens were incorporated in the top agar layer as in a normal test rather than spot tested as stated by Ames.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum.	
3.1.1	Lot/Batch number	Batch ref: [redacted].	
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description	Solid.	
3.1.2.2	Purity	[redacted]%.	
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.	
3.2	**Study Type**	Bacterial reverse mutation test.	

Doc IIIA / Section A6.6.1

BPD Data Set IIA / Annex Point VI.6.VI.6.1

Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)

3.2.1 Organism/cell type

Salmonella typhimurium:
TA 1535, TA 1537,TA 98, TA 100, TA 1538.

3.2.2 Deficiencies / Proficiencies

The tester strains of *Salmonella typhimurium* contain mutations in the histidine operon, the deep rough (rfa) mutation, uvrB deletion, DNA repair deficiency and Ampicillin resistant R factors. Each tester strain was screened at weekly intervals for these characteristics.

3.2.3 Metabolic activation system

S9 mix used:

- animal species: Sprague Dawley albino rats;
- organ: liver;
- induction: yes;
- induction substance:Aroclor 1254;
- the S9-mix was prepared using S9 fraction (3ml), Sucrose-Tris-EDTA Buffer (7ml) and Co-factor solution (20ml) for each 30ml volume. In tests without metabolic activation, the S9 fraction was replaced by an equivalent volume of Sucrose-Tris-EDTA Buffer.

3.2.4 Positive control

The following substances were used:

- Acridine Mutagen ICR191,
- 2-Aminoanthracene,
- Daunorubicin,
- 4-Nitro-*o*-phenylenediamine,
- N-Methyl-N'-nitro-N-nitrosoguanidine.

3.3 Administration / Exposure; Application of test substance

Doc IIIA / Section A6.6.1 **BPD Data Set IIA / Annex Point VI.6.VI.6.1**	**Genotoxicity in vitro** *In-vitro* gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)

3.3.1	Concentrations	Experiment 1: 5000, 1000, 200, 40, 8.0 and 1.6 µg/plate for all 5 tester strains, with and without S9-mix. Experiment 2: 5000, 1000, 200, 40, 8.0 and 1.6 µg/plate for all 5 tester strains, with and without S9-mix (ie brodifacoum was assayed twice over this dose range). Experiment 3: 200, 40, 8.0, 1.6, 0.32 and 0.064 µg/plate, with and without S9-mix, for strains TA1538 and TA100. Strain TA1538, without S9-mix, was also tested over the dose range 5000, 1000, 200, 40, 8.0 and 1.6 µg/plate so that the whole extended dose range of 5000-0.064µg/plate was covered. (Brodifacoum was retested over the lower dose range in strains TA1538 and TA100 because of the toxic effects observed over the higher dose range).
3.3.2	Way of application	Solutions of the test material were prepared in dimethylsulphoxide (DMSO) and applied by plate incorporation (mixing with bacterial culture strain, S9-mix or Co-factor/Buffer mix and top agar; then pouring rapidly onto surface of a prepared Vogel Bonner plate and allowing to gel).
3.3.3	Pre-incubation time	Following application of the test substance to the bacterial cultures, the plates were stacked in strain/dose groups, bound with adhesive tape and appropriately labelled before being incubated inverted at 37°C for 64 - 68 hours in the dark.
3.3.4	Other modifications	A solution of histidine and biotin was added to the top agar.
3.4	**Examinations**	
3.4.1	Number of cells evaluated	The spontaneous mutation rate was determined by including 'absolute' negative controls in each test, in addition to the solvent negative controls. This also verified the lack of genotoxicity of the solvent used (DMSO). A positive response in an experiment was achieved when a two-fold or greater increase in the mean number of revertant colonies per test plate occurred (over and above that observed for the solvent control plates). The results tables (Tables A6_6_1-1a, b, c, d and e), therefore give the mutation rate as the ratio of the number of revertant colonies obtained with the test material to that obtained with the solvent control alone. A second criterion for a postive result was the observation of a statistically significant dose-related increase in the number of revertant colonies.
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No.

Doc IIIA / Section A6.6.1 BPD Data Set IIA / Annex Point VI.6.VI.6.1	**Genotoxicity in vitro** *In-vitro* gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)
4.1.2 with metabolic activation	No.
4.2 Cytotoxicity	Yes. Cytotoxicity was indicated in two of the tester strains by a reduction in revertant colony numbers: ■ Strain TA1538 (-S9) at concentrations above 40µg/plate; ■ Strain TA100 (-S9 and +S9) at concentrations above 200µg/plate.

Doc IIIA / Section A6.6.1

BPD Data Set IIA / Annex Point VI.6.VI.6.1

Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test material as a solution in DMSO, was tested in 3 experiments on 5 tester strains (TA1535, TA1537, TA1538, TA98 and TA100), over the concentration range 5000-0.064µg/plate both with and without metabolic activation (S9-mix). Treated plates were incubated inverted at 37°C for 64 - 68 hours in the dark and revertant colonies counted using an automated electronic colony counter. Observed positive plates were validated by replica plating to determine whether true reversion had occurred. The criteria for a positive result were a 2-fold increase in the mean number of revertant colonies compared with solvent control plates for at least one concentration level, and a statistically significant dose related increase in the number of revertants. The results should be reproducible. Both positive and negative controls were used.

Doc IIIA / Section A6.6.1 **BPD Data Set IIA / Annex Point VI.6.VI.6.1**	**Genotoxicity in vitro** *In-vitro* gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)

5.2	**Results and discussion**	With strains TA1535, TA98 and TA100, brodifacoum failed to induce any significant increase in the numbers of observed revertant colonies in experiments 1 and 2, either in the presence or absence of S9. In both experiments significant cytotoxicity was observed with strain TA100 and so this strain was retested over the lower dose range of 200-0.064µg/plate, but again there was no significant increase in revertant colony numbers. With strain TA1537 in the presence of S9, although an increase in colony numbers was observed in experiment 1, the response obtained did not exceed 1.7x the spontaneous mutation rate, showed no dose relationship and was of limited statistical significance ($0.01<P<0.05$). No increase in revertant colony numbers was observed in this strain in experiment 2 in the presence of S9, or in either experiment in the absence of S9. With strain TA1538 in experiment 1, some increases in revertant colony numbers were observed, both in the presence and absence of S9. Although the repsonse with S9 showed a dose related increase, the maximum response did not exceed 1.9x the spontaneous mutation rate, and was of limited statistical significance ($0.01<P<0.05$). The response in the absence of S9 showed no clear dose relationship, and was due to individual 'anomalous' plate counts rather than a more general increase in observed colony numbers with dose. Further examination of these 'positive' plates by the replica plating technique showed that the observed colonies were not formed by protrophic revertants. In experiment 2 with strain TA1538, although no significant increase in colony numbers was observed in the presence of S9, the response showed an inverse dose relationship with the maximum increase being observed at the lowest dose tested (1.6µg/plate). In the absence of S9, the observed reduction in colony numbers at doses above 40µg/plate indicated a toxic effect. In experiment 3 over the lower dose range of 200-0.064µg/plate, with strain TA1538 in the presence of S9, brodifacoum failed to induce any significant increase in revertant colony numbers. In view of the non-reproducibility of the results in strain TA1538 without S9, and of the apparent toxicity at high doses in this strain, the compound was retested over the increased dose range 5000-0.064µg/plate. Although a dose related increase in colony numbers was observed, the maximum repsonse did not exceed 1.97x the background mutation rate (the figure of 2.0x at 40µg/plate is an artefact of rounding to one decimal place and was of limited statistical significance.
5.3	**Conclusion**	Although some increases in revertant colony numbers were observed, these were mainly associated with toxic effects and were of limited significance. Therefore, under the conditions of this assay, brodifacoum gave a negative, ie non-mutagenic, response.
5.3.1	Reliability	1.

Doc IIIA / Section A6.6.1 **BPD Data Set IIA / Annex Point VI.6.VI.6.1**	**Genotoxicity in vitro** *In-vitro* gene mutation study in bacteria (*Salmonella* reverse mutation assay of Maron and Ames, 1983)
5.3.2 Deficiencies	No.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2005
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_6_1-1a **Results of *Salmonella* Reverse Mutation Assay with Strain TA1535**

Strain TA1535				
Concentration [µg/plate]	**Experiment Number**	**Mutation Rate [Ratio of Test:Control]**		**Comments**
		-S9	**+S9**	
1.6	1	0.7	1.1	No cytotoxic effects.
	2	0.9	1.1	
8.0	1	0.7	0.7	
	2	0.8	0.7	
40	1	0.5	0.7	
	2	1.0	0.7	
200	1	0.4	0.5	
	2	0.6	0.7	
1000	1	0.3	0.3	
	2	0.3	0.6	
5000	1	0.3	0.3	
	2	0.3	0.7	

Table A6_6_1-1b Results of *Salmonella* Reverse Mutation Assay with Strain TA1537

Strain TA1537				
Concentration [µg/plate]	**Experiment Number**	**Mutation Rate [Ratio of Test:Control]**		**Comments**
		-S9	**+S9**	
1.6	1	1.2	1.7	No cytotoxic effects.
	2	0.8	0.8	
8.0	1	1.2	1.7	
	2	0.7	0.7	
40	1	0.4	1.4	
	2	0.2	0.7	
200	1	0.7	1.1	
	2	0.2	0.3	
1000	1	0.9	0.7	
	2	0.4	0.5	
5000	1	0.9	0.2	
	2	0.3	0.4	

Table A6_6_1-1c **Results of *Salmonella* Reverse Mutation Assay with Strain TA1538**

Strain TA1538				
Concentration [µg/plate]	**Experiment Number**	**Mutation Rate [Ratio of Test:Control]**		**Comments**
		-S9	**+S9**	
0.064	1			In experiment 1 (-S9), the anomalous high plate counts were examined further by the replica-plating technique, which showed that the observed colonies were not formed by protrophic revertants. In experiment 2 (-S9), the observed reduction in colony numbers at doses above 40 µg/plate indicates a cytotoxic effect.
	2			
	3	1.1	0.9	
0.32	1			
	2			
	3	1.7	1.3	
1.6	1	1.7	1.2	
	2	1.1	1.4	
	3	1.7	1.3	
8.0	1	3.7	1.5	
	2	0.8	1.2	
	3	1.8	0.9	
40	1	0.7	1.9	
	2	0.6	0.9	
	3	2.0	1.2	
200	1	4.9	1.1	
	2	0.5	0.4	
	3	1.9	0.9	
1000	1	1.3	0.8	
	2	0.5	0.9	
	3	1.7		
5000	1	14.6	0.8	
	2	0.2	0.4	
	3	1.3		

Table A6_6_1-1d **Results of *Salmonella* Reverse Mutation Assay with Strain TA98**

Strain TA98				
Concentration [µg/plate]	**Experiment Number**	**Mutation Rate [Ratio of Test:Control]**		**Comments**
		-S9	**+S9**	
1.6	1	1.0	0.8	No cytotoxic effects.
	2	1.1	0.9	
8.0	1	0.9	0.9	
	2	1.1	1.2	
40	1	0.8	0.9	
	2	1.2	0.9	
200	1	0.6	0.7	
	2	0.7	0.8	
1000	1	0.8	0.6	
	2	1.0	0.7	
5000	1	0.7	0.6	
	2	1.0	0.6	

Table A6_6_1-1e **Results of *Salmonella* Reverse Mutation Assay with Strain TA100**

Strain TA100				
Concentration [µg/plate]	**Experiment Number**	**Mutation Rate [Ratio of Test:Control]**		**Comments**
		-S9	**+S9**	
0.064	1			Cytotoxic effects were observed in all 3 experiments and at both high and low concentrations of the test material, but there was no significant increase in revertant colony numbers.
	2			
	3	1.2	1.1	
0.32	1			
	2			
	3	1.0	1.2	
1.6	1	0.9	0.8	
	2	1.0	0.9	
	3	1.1	1.1	
8.0	1	0.9	1.0	
	2	0.8	1.0	
	3	1.0	1.1	
40	1	0.4	0.8	
	2	0.4	0.7	
	3	0.6	1.2	
200	1	0.2	0.4	
	2	0.3	0.4	
	3	0.2	0.8	
1000	1	0.2	0.3	
	2	0.2	0.3	
	3			
5000	1	0.2	0.3	
	2	0.2	0.3	
	3			

Doc IIIA / Section 6.6.2 **BPD Data Set IIA / Annex Point VI.6.VI.6.2**	**Genotoxicity in vitro** *In-vitro* cytogenetic assay in human lymphocytes

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], (1990), Brodifacoum: An Evaluation in the *In Vitro* Cytogenetic Assay in Human Lymphocytes. [redacted], (unpublished). [redacted].	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. Study complied with OECD Guideline 473 (1983).	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum.	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description	Solid.	
3.1.2.2	Purity	[redacted] %.	
3.1.2.3	Stability	Please refer to Section 2of Doc IIIA.	
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test.	
3.2.1	Organism/cell type	Human lymphocytes (male and female).	

Doc IIIA / Section 6.6.2

BPD Data Set IIA / Annex Point VI.6.VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in human lymphocytes

3.2.2	Deficiencies / Proficiencies	
3.2.3	Metabolic activation system	S9-mix used: ■ animal species: male Alpk:APfSD albino rats; ■ organ: liver; ■ induction: yes; ■ induction substance: Aroclor 1254; ■ the S9-mix was prepared using S9 fraction, S9 Buffer (Sucrose, Tris Base and EDTA), and Co-factor solution (Na_2HPO_4 + KCl + Glucose-6-Phosphate + NADP + $MgCl_2$).
3.2.4	Positive control	The following substances were used: ■ Mitomycin C, and ■ Cyclophosphamide.
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	5, 10 and 50μg/ml. These three concentrations were selected for chromosomal aberration analysis from a cytotoxicity test using a range of seven brodifacoum concentrations from 5 to 1000μg/ml. The highest concentration was selected on the basis of a significant depression in mitotic activity compared to the mean solvent control values, and the suitablity of the metaphase preparations for chromosomal aberration analysis.
3.3.2	Way of application	Aliquots of the solutions of brodifacoum in DMSO were administered to the culture medium at a volume of 5µl/ml. Solvent (DMSO) controls were also included (at 5µl/ml) as were duplicate negative (untreated) controls. The negative controls were not analysed in this study.
3.3.3	Pre-incubation time	The cultures were maintained at 37°C for 44 hours prior to application of the test material. The cultures were treated with the test material for a period of approximately 3 hours at 37°C. At the end of the treatment period the growth medium was removed following centrifugation at approximately 400g for 5 minutes and replaced with fresh culture medium. All cultures were maintained at 37°C for the remainder of the 72 hour growth period.
3.3.4	Other modifications	
3.4	**Examinations**	

Doc IIIA / Section 6.6.2

BPD Data Set IIA / Annex Point VI.6.VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in human lymphocytes

3.4.1	Number of cells evaluated	The mitotic index was assessed by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase, for seven concentrations of brodifacoum. From these results, three concentrations were selected for chromosomal aberration analysis. The highest concentration was selected on the basis of a significant depression in mitotic activity compared to the mean solvent control values, and the suitablity of the metaphase preparations for chromosomal aberration analysis. 100 cells in metaphase were analysed from each selected culture for the incidence of structural chromosomal damage, according to the criteria recommended by Scott *et al:* Scott D, Danford N, Dean B J, Kirkland D and Richardson C R (1983). *In Vitro* Chromosome Aberration Assay: In: Brian J Dean (Ed) Report of UKEMS Sub-Committee on Guidelines for Mutagenicity Testing, United Kingdom Environmental Mutagen Society.
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No.
4.1.2	with metabolic activation	No.
4.2	**Cytotoxicity**	No, cytotoxicity was not observed at the concentrations of brodifacoum selected for chromosomal aberration analysis. At the highest brodifacoum concentrations used for the cytotoxicity test (500 and 1000µg/ml), precipitation of the test material was noted in both male and female cultures.

Doc IIIA / Section 6.6.2 **BPD Data Set IIA / Annex Point VI.6.VI.6.2**	**Genotoxicity in vitro** *In-vitro* cytogenetic assay in human lymphocytes

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Test material: brodifacoum; Lot/Batch number: 0001 (2233); Sponsor ref: AI/90/0053; CTL ref: Y00052/034; Purity: 97.6%. The test material as a solution in DMSO, was tested on male and female human lymphocytes for chromosomal aberrations, at concentrations of 5, 10 and 50μg/ml, both with and without metabolic activation (S9-mix). These three concentrations were selected for chromosomal aberration analysis from a cytotoxicity test using a range of seven brodifacoum concentrations from 5 to 1000μg/ml. Aliquots of the solutions of brodifacoum in DMSO were administered to the culture medium at a volume of 5µl/ml. The cultures were maintained at 37°C for 44 hours prior to application of the test material. The cultures were treated with the test material for a period of approximately 3 hours at 37°C. At the end of the treatment period the growth medium was removed following centrifugation at approximately 400g for 5 minutes and replaced with fresh culture medium. All cultures were maintained at 37°C for the remainder of the 72 hour growth period. The total number of abnormal cells (with chromosomal aberrations), excluding those with only gap-type aberrations, for each of the treatment groups was compared to the appropriate solvent control values using the Fisher's Exact Test (one sided).
5.2	**Results and discussion**	No statistically or biologically significant increases in chromosomal damage above the solvent control values were seen at any of the brodifacoum concentrations tested in either the male or female lymphocytes, either with or without metabolic activation. See Tables A6_6_1-2a and A6_6_1-2b below for a summary of the results.
5.3	**Conclusion**	Brodifacoum is not clastogenic to human lymphocytes *in vitro* under the conditions of the test.
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]

Doc IIIA / Section 6.6.2 **BPD Data Set IIA / Annex Point VI.6.VI.6.2**	**Genotoxicity in vitro** *In-vitro* cytogenetic assay in human lymphocytes
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_1-2a

Results of *in vitro* Cytogenetic Assay in Human Lymphocytes (Male/Donor 1): Chromosomal Analysis

BRODIFACOUM DOSE LEVELS		**control:** **DMSO solvent only**		**low dose:** **5 µg/ml**		**mid dose:** **10 µg/ml**		**high dose:** **50 µg/ml**	
METABOLIC ACTIVATION		**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
CYTOTOXICITY (significant depression in mitotic activity)		no	no	no	no	no	no	yes	yes
CHROMSOME ABERRATIONS:									
gaps	Culture 1	1	4		1	1	1	3	1
	Culture 2	1		2	1	1		3	1
breaks	Culture 1							3	
	Culture 2		1	1			1		
fragments and minutes	Culture 1								
	Culture 2		2	1				1	1
multiple damage	Culture 1								
	Culture 2								
interchanges	Culture 1								
	Culture 2								
others, rearrangements	Culture 1								
	Culture 2								1
MITOTIC INDEX	Culture 1	5.1,	6.1,	4.4,	5.1,	6.9,	4.8,	1.9,	2.7
	Culture 2	5.4	7.5	6.5	5.7	6.3	5.0	2.8	2.7

Table A6_6_1-2b

Results of *in vitro* Cytogenetic Assay in Human Lymphocytes (Female/Donor 2): Chromosomal Analysis

BRODIFACOUM DOSE LEVELS		**control:** DMSO solvent only		**low dose:** 5 µg/ml		**mid dose:** 10 µg/ml		**high dose:** 50 µg/ml	
METABOLIC ACTIVATION		- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
CYTOTOXICITY (significant depression in mitotic activity)		no	no	no	no	no	no	no	yes
CHROMSOME ABERRATIONS:									
gaps	Culture 1	5	3	6	2	4	3	4	3
	Culture 2	6	2	3		1	4	4	2
breaks	Culture 1		1						1
	Culture 2	1				1		1	
fragments and minutes	Culture 1			2	1				
	Culture 2	1				1			
multiple damage	Culture 1								
	Culture 2								
interchanges	Culture 1								
	Culture 2								
others, rearrangements	Culture 1								
	Culture 2								
MITOTIC INDEX	Culture 1	4.9	8.7	7.7	5.6	7.0	5.4	5.3	2.9
	Culture 2	5.4	8.9	6.0	4.8	6.5	5.4	4.8	2.5

Doc IIIA / Section 6.6.3

BPD Data Set IIA / Annex Point VI.6.VI.6.3

Genotoxicity in vitro

In-vitro mammalian cell transformation assay

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1979), An Examination of Brodifacoum for Potential Carcinogenicity Using Two *in vitro* Assays of Potential Carcinogenicity. [redacted] (unpublished).	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. The method used was the mammalian cell transformation assay of Styles (1977): Styles J A (1977), Brit J Cancer, 36, 558.	X
2.2	**GLP**	[redacted]	
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	Solid.	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.	
3.2	**Study Type**	*In vitro* mammalian cell gene mutation test.	x
3.2.1	Organism/cell type	Baby Hamster Kidney Fibroblasts (BHK21/C13).	

Doc IIIA / Section 6.6.3

BPD Data Set IIA / Annex Point VI.6.VI.6.3

Genotoxicity in vitro

In-vitro mammalian cell transformation assay

3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system	S9 mix used: ■ animal species: Sprague Dawley albino rats; ■ organ: liver; ■ induction: yes; ■ induction substance:Aroclor 1254; ■ the S9-mix was prepared using S9 fraction and Co-factor solution in the proportion of approximately 1:9.	
3.2.4	Positive control	Benzidine.	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	Brodifacoum was tested at the following concentrations: 0.12, 1.2, 12, 120 and 1200 μg/ml.	
3.3.2	Way of application	Dissolved in medium	
3.3.3	Pre-incubation time	Following addition of the brodifacoum solution to the test medium, the tubes were incubated at 37°C on a shaker at 140rpm for 3 hours.	
3.3.4	Other modifications		
3.4	**Examinations**		
3.4.1	Number of cells evaluated	A positive result is recorded when the transformation frequency per 10^6 survivors at the LC_{50} exceeds five times the control frequency. The cells used in the study (Baby Hamster Kidney Fibroblasts), had a spontaneous transformation frequency of 35 per 10^6 survivors.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	Not tested without metabolic activation.	
4.1.2	with metabolic activation	No.	
4.2	**Cytotoxicity**	No.	X

Doc IIIA / Section 6.6.3 **BPD Data Set IIA / Annex Point VI.6.VI.6.3**	**Genotoxicity in vitro** *In-vitro* mammalian cell transformation assay

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test material: brodifacoum; Lot/Batch number: PP581, SC 70/77; CTL ref: Y0052/002/001; Purity: 92.5%. The test material as a solution in DMSO, was tested on Baby Hamster Kidney Fibroblasts (BHK21/C13) over the concentration range 0.12, 1.2, 12, 120 and 1200µg/ml with metabolic activation (S9-mix). Following addition of the brodifacoum solution to the test medium, the tubes were incubated at 37°C on a shaker at 140rpm for 3 hours. The positive control substance benzidine was used to validate the test system. A positive result was recorded when the transformation frequency per 10^6 survivors at the LC_{50} exceeds five times the control frequency. The cells used in the study (Baby Hamster Kidney Fibroblasts), had a spontaneous transformation frequency of 35 per 10^6 survivors.	
5.2	**Results and discussion**	Brodifacoum caused an increasing cell mortality with increasing dose with the LC_{50} determined to be 20 µg/ml. The corrected transformation frequency was calculated to be $3.0/10^6$ survivors, which is well below the spontaneous count of $35/10^6$. The results are summarised in Table A6_6_1 below. The positive control substance benzidine gave a positive response in the test.	
5.3	**Conclusion**	Brodifacoum gave a negative or non-mutagenic response in this test system.	x
5.3.1	Reliability	1.	x
5.3.2	Deficiencies	No.	x

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

Doc IIIA / Section 6.6.3

BPD Data Set IIA / Annex Point VI.6.VI.6.3

Genotoxicity in vitro

In-vitro mammalian cell transformation assay

Remarks	**2.1** This is not a guideline study. The reference quoted in 2.1 is not a guideline. **4.2** A cytotoxic effect was actually observed. In 4.2 the cytotoxicity data should be reported, or, briefly, the LC50 = 20.0 µg/ml. **General comment**. The study has a limited biological significance, because of the lack of data in the absence of metabolic activation. Moreover, a general limit of the *in vitro* mammalian cell transformation tests is the unclear association between the tests endpoint and the mechanisms of cancer progression (Dir. 88/303/EEC OJL 133 188). No specific gene mutation event can be associated with the endpoint of this test, therefore the mammalian cell transformation assay can't be defined as a "gene mutation test", and a negative response can't be described as "non-mutagenic".
	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_6_1 Results of Mammalian Cell Transformation Assay with Baby Hamster Kidney Fibroblasts (BHK21/C13)

Concentration [µg/ml]	% Survival of Control	Transformation Frequency		Comments
		-S9	+S9	
0.12	100		8.5	No cytotoxic effects.
1.2	84		11	
12	59		6	
120	8		0	
1200	0		0	

Doc IIIA / Section 6.6.3 BPD Data Set IIA / Annex Point VI.6.VI.6.3	Genotoxicity in vitro *In-vitro* mouse lymphoma gene mutation test	

			Official use only
		1 REFERENCE	
1.1	**Reference**	, (1984), Brodifacoum: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells (unpublished).	
1.2	**Data protection**		
1.2.1	Data owner		
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection		
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No but methods used comparable to OECD Guidelines for Testing of Chemicals, August 1983 (+ 2nd Addendum, August 1984). Section 4 Genetic Toxicology, 476. *In Vitro* Mammalian Cell Gene Mutation Tests.	
2.2	**GLP**	Yes.	
2.3	**Deviations**		
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum.	
3.1.1	Lot/Batch number	.	
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description	White powder.	
3.1.2.2	Purity	96%.	
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.	
3.2	**Study Type**	*In vitro* mammalian cell gene mutation test.	
3.2.1	Organism/cell type	Mouse lymphoma cell line L5178Y (-3.7.2C) (TK +/-).	

Doc IIIA / Section 6.6.3

BPD Data Set IIA / Annex Point VI.6.VI.6.3

Genotoxicity in vitro

In-vitro mouse lymphoma gene mutation test

3.2.2	Deficiencies / Proficiencies	
3.2.3	Metabolic activation system	S9 mix used: ■ Liver from Sprague Dawley rats; ■ Animals induced by ARACLOR 1254; ■ S9 fraction and co-factor mixed in ratio of 1:3. The co-factor solution was made up of Na_2HPO_4 (100mM), KCl (33mM), Glucose-6-phosphate (5mM), NADP (4mM), $MgCl_2$ (8mM). The buffer used for preparation of the liver S9 fraction was Sucrose - Tris Base - EDTA (250, 50 and 1.00mM respectively).
3.2.4	Positive control	The following substances were used: ■ Benzo(α)pyrene (BαP); ■ Ethylmethanesulphonate (EMS).
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Experiment 1: 3.9, 7.8, 15.6, 31.3, 62.5 µg/ml in cell suspension. Experiment 2: 8, 16, 32, 64, 128 µg/ml in cell suspension. Experiment 3: 47.5, 63.3, 84.4, 112.5, 150 µg/ml in cell suspension.
3.3.2	Way of application	200µl of dosing solutions of the test substance in dimethylsulphoxide were added to 20ml of cell culture in exponential growth and incubated for 2 hours. During this period the treated cell culture supsensions were rotated in a test tube holder on a Bellco cell production apparatus to ensure sufficient mixing of the test compound with the cell suspension. The cultures were kept at 37°C under an atmosphere of 5% CO_2 in air.
3.3.3	Pre-incubation time	The exponentially growing mouse lymphoma cells were treated with the test substance for 2 hours at 37°C. After removal of the test medium the cells were washed and a sample diluted to determine survival immediately after treatment. The remaining cells were cultured at 37°C for 48 or 72 hours to allow newly induced mutations to be detected (the expression time). During this time the growth rate was maintained and the cells subcultured when necessary. At the end of the expression time, samples were grown in both selective medium (Trifluorothymidine(TFT) and Ouabain) and non-selective medium and the results used to determine the mutation frequency per viable cell.
3.3.4	Other modifications	
3.4	**Examinations**	

Doc IIIA / Section 6.6.3 **BPD Data Set IIA / Annex Point VI.6.VI.6.3**	**Genotoxicity in vitro** *In-vitro* mouse lymphoma gene mutation test
3.4.1 Number of cells evaluated	For the survival assay, culture samples were diluted to 8 cells/ml directly after treatment with the test substance and then dispensed 200µl per well into one 96 well multiwell plate per dose level. Percentage survival was calculated as cell growth relative to the solvent control growth in the survival plates at approximately 10 days. After the expression time, the post-treated cultures were counted using a haemocytometer and then divided into three series of dilutions: ♦ for dilution 1 (to form cultures for TFT selection), the cultures were diluted to give 50ml of culture at 1 x 10^4 cells/ml. ♦ for dilution 2 (to form cultures for ouabain selection), the cultures were diluted to give 45ml of culture at 2.22 x 10^5 cells/ml. ♦ for dilution 3 (to form cultures for vaibility efficiency), a two step dilution of dilution 2 was made just prior to addition of ouabain to give 8 cells/ml. The cultures were then dispensed into one 96 well multiwell plate at 200µl/well. The data from these plates were used to estimate the number of viable cells available for the mutational event and were used in the calculation of mutation frequency.
	4 RESULTS AND DISCUSSION
4.1 Genotoxicity	
4.1.1 without metabolic activation	No.
4.1.2 with metabolic activation	No.
4.2 Cytotoxicity	Yes, brodifacoum produced relatively high levels of cytotoxicity (>65%) at concentrations greater than 112.5µg/ml. The addition of auxiliary metabolic activation (S9 mix) appeared to slightly decrease the toxicity.

Doc IIIA / Section 6.6.3 **BPD Data Set IIA / Annex Point VI.6.VI.6.3**	**Genotoxicity in vitro** *In-vitro* mouse lymphoma gene mutation test

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Test material: Brodifacoum; Purity: [redacted]%; [redacted] The test material as a solution in DMSO, was assessed for mutagenic potential by its ability to induce forward mutation in the L5178Y (TK +/-) mouse lymphoma cell line as monitored by cell growth in multi-well plates, assessed by resistance to trifluorothymidine (TFT) and ouabain in the presence or absence of an auxiliary metabolic activation system. A large population of exponentially growing L5178Y mouse lymphoma cells in the presence or absence of a metabolic activation system (S9 mix), was treated with brodifacoum in dimethylsulphoxide (DMSO) for 2 hours. After removal of the test substance the cells were washed and a sample diluted to determine survival immediately after treatment. The remaining cells were cultured for sufficient time to allow newly induced mutations to be detected (the expression time). During this time the growth rate was maintained and the cells subcultured when necessary. At the end of the expression time (48 or 72 hours), samples were grown in selective medium (Trifluorothymidine (TFT) and Ouabain) and non-selective medium to determine the mutation frequency per viable cell.
5.2	**Results and discussion**	Brodifacoum produced relatively high levels of cytotoxicity (>65%) at concentrations greater than 112.5µg/ml. The addition of auxiliary metabolic activation (S9 mix) appeared to slightly decrease the toxicity. The positive and negative controls responded as expected, except in the case of BαP where too few cells survived to allow selection in ouabain (+S9) and so this part of the assay was invalid. Two non-consecutive concentrations of brodifacoum in experiment one (ouabain selection only) and a single concentration in experiment three, gave an approximately three times background increase in mutation frequency. As these results were not reproduced in parallel assays (ie non-equivalent results with paired selective agents), or repeat experiments, and were not dose related, they were considered not to be biologically significant. Therefore, brodifacoum did not induce forward mutation in L5178Y mouse lymphoma cells. The results are given below in Tables A6_6_1-1a, A6_6_1-1b, and A6_6_1-1c.
5.3	**Conclusion**	Brodifacoum in non-mutagenic in this L5178Y mouse lymphoma cell mutation assay.
5.3.1	Reliability	1.
5.3.2	Deficiencies	No.

Doc IIIA / Section 6.6.3	**Genotoxicity in vitro**
BPD Data Set IIA / Annex Point VI.6.VI.6.3	*In-vitro* mouse lymphoma gene mutation test

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

Table A6_6_1-1a RESULTS OF *IN VITRO* MOUSE LYMPHOMA GENE MUTATION TEST WITH BRODIFACOUM

EXPERIMENT 1

Concentration of brodifacoum in cell suspension [µg/ml]	Mutation Frequency x 10^{-4} with 48 hour expression time			Comments
	Trifluorothymidine Medium		Ouabain Medium	
	-S9	+S9	-S9	
0 (Solvent Control)	4.6	3.7	4.1	
3.9	4.1	6.7	3.2	
7.8	4.6	6.0	13.3	
15.6	10.0	4.1	2.6	
31.3	5.5	3.4	13.7	
62.5	4.0	4.7	7.1	

Table A6_6_1-1b RESULTS OF *IN VITRO* MOUSE LYMPHOMA GENE MUTATION TEST WITH BRODIFACOUM

EXPERIMENT 2				
Concentration of brodifacoum in cell suspension [µg/ml]	Mutation Frequency x 10^{-4} with 48 hour expression time			Comments
	Trifluorothymidine Medium		Ouabain Medium	
	-S9	+S9	-S9	
0 (Solvent Control)	5.5	3.1	3.1	
8	4.1	4.5	2.4	
16	6.5	3.9	0.9	
32	6.9	4.4	2.7	
64	4.2	4.4	1.3	
128	4.4	5.7		

Table A6_6_1-1c RESULTS OF *IN VITRO* MOUSE LYMPHOMA GENE MUTATION TEST WITH BRODIFACOUM

EXPERIMENT 3

Concentration of brodifacoum in cell suspension [µg/ml]	**Mutation Frequency x 10^{-4} with 72 hour expression time**			**Comments**
	Trifluorothymidine Medium		**Ouabain Medium**	
	-S9	**+S9**	**-S9**	
0 (Solvent Control)	1.7	1.5	8.3	
47.5	1.6	1.1	19.2	
63.3	1.3	1.4	14.4	
84.4	1.1	1.6	6.0	
112.5	4.8	2.1		
150		2.8		

Doc IIIA / Section 6.6.5

BPD Data Set IIA / Annex Point VI.6.VI.6.5

Genotoxicity in vitro

In-vitro unscheduled DNA synthesis assay in mammalian cells (hela cells).

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1 REFERENCE

1.1 Reference

[redacted], (1984), Study of the capacity of the test article brodifacoum to induce unscheduled DNA synthesis in cultured hela cells (autoradiographic method). Istituto Di Ricerche Biomediche "Antione Marxer" SpA (Italy) [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

No specific guideline was quoted, but the following methods were referenced:

- J E Cleaver (1977) - Handbook of Mutagenicity Test Procedures, B. Kilbey *et al* eds., Elsevier, Amsterdam 19-4B;
- A Abbondandolo *et al* (Roma 1979) - Mutagenesi ambientale, Metodiche di analisi ed. CNR 223-236;
- Benigni *et al.*, Mutation Res. 103 (1982) 385-390;
- Ames B N, McCann J, Yamasaki E, Mut. Res. 31 (1975) 347-364;
- Snedecor C W, Statistical Methods, Iowa State College Press, Ames, 5th Edition, 1956.

2.2 GLP Yes.

2.3 Deviations

3 MATERIALS AND METHODS

3.1 Test material Brodifacoum.

3.1.1 Lot/Batch number

3.1.2 Specification As given in section 2.

Doc IIIA / Section 6.6.5 **BPD Data Set IIA / Annex Point VI.6.VI.6.5**	**Genotoxicity in vitro** *In-vitro* unscheduled DNA synthesis assay in mammalian cells (hela cells).

3.1.2.1	Description	White powder.
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.
3.2	**Study Type**	Unscheduled DNA synthesis in mammalian cells *in vitro*.
3.2.1	Organism/cell type	Hela cells (human).
3.2.2	Deficiencies / Proficiencies	
3.2.3	Metabolic activation system	S9 mix used: ■ animal species: Sprague Dawley (Charles River CD) rats; ■ organ: liver; ■ induction: yes; ■ induction substance:Aroclor 1254; ■ the S9-mix was prepared using S9 fraction (0.5ml), Hanks Balanced Salt Solution (HBSS) (0.36ml), $MgCl_2$ 20mM (40µl), NADP (3.94mg), Glucose-6-phosphate (6.08mg).
3.2.4	Positive control	The following substances were used: ■ Methylmethane sulphonate (MMS), ■ Cyclophosphamide (CP).
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	1, 10, 100 and 1000µg/ml.
3.3.2	Way of application	Solutions of the test material were prepared in dimethylsulphoxide (DMSO). The cultured Hela cells were rinsed with Hanks Balanced Salt Solution (HBSS), and then treated with the test solutions of brodifacoum in 5ml of HBSS buffered with Hepes. The treated cells were incubated for 60 minutes at 37°C. The experiment was carried out in both the presence and absence of S9-mix. Following treatment with the test material, the Hela cells were exposed to labelled nucleic acid precursors (tritiated thymidine), and then processed for autoradiography, enabling a distinction to be made between heavily labelled (S-phase cells) and lightly labelled (UDS cells).
3.3.3	Pre-incubation time	The Hela cells were seeded on 20x40 coverslips in 6cm diameter tissue-culture dishes. The dishes were inoculated with about 200,000 cells/ml in 5ml of Dulbecco Modified Eagle Medium (DMEM). The cells were incubated for 18-24 hours at 37°C in a 5% CO_2 incubator.

Doc IIIA / Section 6.6.5 BPD Data Set IIA / Annex Point VI.6.VI.6.5	Genotoxicity in vitro *In-vitro* unscheduled DNA synthesis assay in mammalian cells (hela cells).
3.3.4 Other modifications	
3.4 Examinations	
3.4.1 Number of cells evaluated	100 (or as close to as possible). The cells were evaluated as follows: ■ <10 grains per cell = negative, ■ >10 to <100 grains per cell = UDS, ■ >100 grains per cell = S-phase cells.
	4 RESULTS AND DISCUSSION
4.1 Genotoxicity	
4.1.1 without metabolic activation	No.
4.1.2 with metabolic activation	No.
4.2 Cytotoxicity	Yes. Cytotoxicity was indicated at the higher dose levels (100 and 1000μg/ml) by an inhibition of S-phase.

Doc IIIA / Section 6.6.5 **BPD Data Set IIA / Annex Point VI.6.VI.6.5**	**Genotoxicity in vitro** *In-vitro* unscheduled DNA synthesis assay in mammalian cells (hela cells).

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test material: brodifacoum; Purity: [redacted]. The test material as a solution in DMSO, was tested on cultured human Hela cells over the concentration range 1-1000µg/ml, both with and without metabolic activation (S9-mix). Treated cell cultures were incubated at 37°C for 60 minutes. Following treatment with the test material, the Hela cells were exposed to labelled nucleic acid precursors (tritiated thymidine), and then processed for autoradiography, enabling a distinction to be made between heavily labelled (S-phase cells) and lightly labelled (UDS cells).	
5.2	**Results and discussion**	Brodifacoum was found not to induce statistically significant increases in the incorporation of tritiated thymidine in cultured human Hela cells, either in the presence of absence of metabolic activation up to a dosage concentration of 1000µg/ml (see Table A6_6_1-3 below). The positive and negative control treatments performed as expected, proving the validity of the test results. There were no significant differences with respect to unscheduled DNA synthesis between the control and brodifacoum incubations, although at higher dose levels there was inhibition of the S-phase. This was probably due to cytotoxicity.	
5.3	**Conclusion**	The results of this assay show that brodifacoum does not induce unscheduled DNA synthesis in cultured human Hela cells either in the presence or absence of metabolic activation up to a dosage concentration of 1000µg/ml.	
5.3.1	Reliability	1.	x
5.3.2	Deficiencies	No.	x

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

Doc IIIA / Section 6.6.5 **BPD Data Set IIA / Annex Point VI.6.VI.6.5**	**Genotoxicity in vitro** *In-vitro* unscheduled DNA synthesis assay in mammalian cells (hela cells).
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_6_1-3 Results of Unscheduled DNA Synthesis Assay in Human Hela Cells *in vitro* with Brodifacoum

Concentration of brodifacoum	Solvent Control Group		1 µg/ml		10 µg/ml		100 µg/ml		1000 µg/ml	
Metabolic activation	**+ S9**	**-S9**	**+ S9**	**-S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**
No. of cells evaluated	100	86	86	93	93	90	83	92	92	86
No. of nuclei with <10 grains (negative)	72	50	53	65	56	60	61	79	87	83
No. of nuclei with >10 to <100 grains (UDS cells)	0	1	0	0	0	0	3	2	3	1
No. of nuclei with >100 grains (S-phase cells)	28	35	33	28	37	30	19	11	2	2

Doc IIIA / Section 6.6.4	Genotoxicity in vivo
BPD Data Set IIA / Annex Point VI.6.VI.6.4	Mouse Micronucleus Test

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1 REFERENCE

1.1 Reference — [redacted], (1984), An Evaluation of Brodifacoum in the Mouse Micronucleus Test, [redacted]

1.2 Data protection — [redacted]

1.2.1 Data owner — [redacted]

1.2.2 Companies with letter of access — [redacted]

1.2.3 Criteria for data protection — [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study — Yes, the following guidelines were used:

- OECD guidlines for testing of chemicals (1983). *In vivo* mammalian bone marrow cytogenetic tests - Mouse Micronucleus Test No 474;
- Jenssen D and Ramel C, (1980). The micronucleus test as part of a short-term mutagenicity test programmed for the prediction of carcinogenicity evaluated by 153 agents. Mutat Res 75 191-202.
- Salamone M F, Heddle J A and Katz M, (1981). Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. In an evaluation of short-term tests for carcinogens. Edited by Frederick J de Serres and John Ashby. P686-697.

2.2 GLP — Yes.

2.3 Deviations

3 MATERIALS AND METHODS

Doc IIIA / Section 6.6.4 **BPD Data Set IIA / Annex Point VI.6.VI.6.4**		**Genotoxicity in vivo** Mouse Micronucleus Test
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	Off-white powder.
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.
3.1.2.4	Maximum tolerable dose	The highest dose used in the study was 80% of the median lethal dose (MLD) calculated on the deaths over a seven day period (MLD/7). A second dose level of 50% of the MLD/7 was also used to allow the observation of a dose response, should the higher dose give a positive result.
3.2	**Test Animals**	
3.2.1	Species	Mouse.
3.2.2	Strain	C57BL/6J.
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study initiation	8-12 weeks old for both phase I (determination of the MLD) and phase II (dosing for micronucleus test) of the study.
3.2.6	Number of animals per group	5 male + 5 female per dose and sampling time (24, 48 and 72 hours; ie a total of 15 of each sex per dose level).
3.2.7	Control animals	Yes, 5 male and 5 female for each sampling time (ie 15 of each sex in total, given corn oil only).
3.3	**Administration/ Exposure**	Intraperitoneal dosing.
3.3.1	Number of applications	1.
3.3.2	Interval between applications	
3.3.3	Postexposure period	24, 48, 72 hours after treatment.
		Intraperitoneal/Intravenous/Intratracheal instillation
3.3.10	Vehicle	Corn oil.
3.3.11	Concentration in vehicle	
3.3.12	Total volume applied	

Doc IIIA / Section 6.6.4 BPD Data Set IIA / Annex Point VI.6.VI.6.4	Genotoxicity in vivo Mouse Micronucleus Test

3.3.13	dose applied	Two dose levels of 0.187 and 0.30 mg/kg bw (single doses). The lower dose level was used to allow the observation of a dose response, if the higher dose were to give a positive result.	
3.3.14	Substance used as Positive Control	Cyclophophamide at a dose level of 65 mg/kg bw.	
3.3.15	Controls	Vehicle (corn oil).	
3.4	**Examinations**		
3.4.1	Clinical signs	No.	
3.4.2	Tissue	Bone marrow.	
		Number of animals:	All animals.
		Number of cells:	500.
		Time points:	24, 48, 72 hours after treatment.
		Type of cells	Polychromatic and mature erythrocytes in bone marrow.
		Parameters:	Number of micronuclei.
			% Polychromatic erythrocytes: mature erythrocytes.
3.5	**Further remarks**	Although 500 cells were examined, the results were expressed as number of micronuclei per 1000 cells.	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**		

Doc IIIA / Section 6.6.4

BPD Data Set IIA / Annex Point VI.6.VI.6.4

Genotoxicity in vivo

Mouse Micronucleus Test

4.2	**Haematology / Tissue examination**	No statistically significant increase in frequency of micronuclei were observed at any dose level of brodifacoum at any of the three sampling times investigated. The test system positive control (cyclophosphamide) gave an elevated and statistically significant increase in micronuclei at the 24 hour and 48 hour sampling times, but by 72 hours the incidence of micronuclei had fallen considerably. The results of the incidence of micronuclei are given below in Table A6_6_4-1. No statistically significant reduction in polychromatic erythrocytes was observed when comparing the ratio of polychromatic erythrocytes to mature erythrocytes for brodifacoum. The test system positive control (cyclophosphamide) gave a small non-statistically significant reduction in polychromatic erythrocytes at all sampling times. The results of the comparison of the ratios of polychromatic erythrocytes to mature erythrocytes are given below in Table A6_6_4-1.
4.3	**Genotoxicity**	No.
4.4	**Other**	

Doc IIIA / Section 6.6.4

BPD Data Set IIA / Annex Point VI.6.VI.6.4

Genotoxicity in vivo

Mouse Micronucleus Test

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test material: Brodifacoum; Purity: [redacted]%; Batch no: [redacted]; Division reference no: [redacted]; [redacted] reference no: [redacted]. Guidelines: (1) OECD guidlines for testing of chemicals (1983). In vivo mammalian bone marrow cytogenetic tests - Mouse Micronucleus Test No 474; (2) Jenssen D and Ramel C, (1980). The micronucleus test as part of a short-term mutagenicity test programmed for the prediction of carcinogenicity evaluated by 153 agents. Mutat Res <u>75</u> 191-202. (3) Salamone M F, Heddle J A and Katz M, (1981). Mutagenic activity of 41 compounds in the in vivo micronucleus assay. In an evaluation of short-term tests for carcinogens. Edited by Frederick J de Serres and John Ashby. P686-697. The test material as a solution in corn oil, was administered as a single intraperitoneal dose at dose levels of 0.187 and 0.30 mg/kg to 3 groups of 5 male and 5 female C57BL/6J mice. After 24, 48, and 72 hours, the bone marrow of the animals were examined for the number of micronuclei per 1000 polychromatic erythrocytes. Cytotoxicity was assessed by determining the ratio of the percentage of polychromatic erythrocytes to mature erythrocytes.	
5.2	**Results and discussion**	No statistically significant increase in incidence of micronuclei was seen with brodifacoum at any dose level or sampling time, even though the dose levels were equivalent to 80% and 50% of the MLD/7. The positive control substance (cyclophosphamide) gave an elevated and statistically significant increase in micronuclei 24 and 48 hours after being dosed. This is the type of response normally seen with the positive control and shows the test system to be sensitive. The small non-statistically significant reduction in polychromatic erythrocytes observed with the positive control (cyclophosphamide) at all sampling times, is not usually seen with this compound. Normally a much larger reduction in the ratio of polychromatic to mature erythrocytes would have been expected. The results are summarised below in Table A6_6_4-1.	
5.3	**Conclusion**	Brodifacoum did not induce an increase in frequency of micronuclei seen at the dose levels tested, which were close to an expected lethal dose. It is therefore concluded that there is no evidence for clastogenic acitivity of brodifacoum in this test system.	
5.3.1	Reliability	1.	x
5.3.2	Deficiencies	No.	x

Doc IIIA / Section 6.6.4

BPD Data Set IIA / Annex Point VI.6.VI.6.4

Genotoxicity in vivo

Mouse Micronucleus Test

	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	

Doc IIIA / Section 6.6.4

BPD Data Set IIA / Annex Point VI.6.VI.6.4

Genotoxicity in vivo

Mouse Micronucleus Test

Table A6_6_4-1. Results of Mouse Micronucleus Test *In Vivo* with Brodifacoum

State mean ± standard deviation state individual numbers for critical findings	control group			0.187 mg/kg$_{bw}$			0.30 mg/kg$_{bw}$		
Number of cells (polychromatic erythrocytes) evaluated	500			500			500		
Sampling time (hours)	24	48	72	24	48	72	24	48	72
Number of polychromatic erythrocytes with micronuclei per 1000 cells	1.2	3.2	3.4	1.8	1.7	1.8	2.2	2.4	1.1
% Polychromatic erythrocytes	48.3	43.6	42.5	51.0	38.6	49.1	40.0	44.9	36.6

Doc IIIA/Section 6.7 BPD Data Set IIA/Annex Point VI.6.7	Carcinogenicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [✓] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA / Section 6.8.1
BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study
Teratogenicity Study in the Rat

Official use only

1 REFERENCE

1.1 REFERENCE	(1980). Brodifacoum: Teratogenicity Study in the Rat (unpublished).
1.2 DATA PROTECTION	
1.2.1 Data owner	
1.2.2 Companies with letter of access	
1.2.3 Criteria for data protection	

1 GUIDELINES AND QUALITY ASSURANCE

1.1 GUIDELINE STUDY	Guideline not quoted in report, but study conducted in accordance with the principles of OECD 414.
1.2 GLP	Yes.
1.3 DEVIATIONS	No.

2 MATERIALS AND METHODS

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rat
3.1 TEST MATERIAL	Brodifacoum.
3.1.1 Lot/Batch number	Batch number: [redacted]. [redacted].
3.1.2 Specification	As given in section 2.
3.1.2.1 Description	Off-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	Rat (*Rattus norvegicus*).
3.2.2 Strain	Pathogen-free Wistar-derived.
3.2.3 Source	[redacted].
3.2.4 Sex	Nulliparous females.
3.2.5 Age/weight at study initiation	13 – 14 weeks of age/ 205 – 274 g.
3.2.6 Number of animals per group	30.
3.2.7 Control animals	Yes.
3.2.8 Mating period	The female rats were paired overnight with 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 0 of pregnancy, and on this same day the successfully mated females were delivered to the study laboratory.
3.3 ADMINISTRATION/ EXPOSURE	Oral.

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rat

3.3.1 Duration of exposure	
	rat: day 6-15 (inclusive) post mating
3.3.2 Postexposure period	6 days.
	Oral
3.3.3 Type	Gavage.
3.3.4 Concentration	0.001, 0.01, 0.02 $mg/kg_{bw}/day$
3.3.5 Vehicle	10 % v/v aqueous ethanol.
3.3.6 Concentration in vehicle	10 ml of dosing solution per kg bodyweight.
3.3.7 Total volume applied	Total volume of dosing solution given to each animal was adjusted daily according to bodyweight.
3.3.8 Controls	Vehicle.
3.4 EXAMINATIONS	
3.4.1 Body weight	Yes.
3.4.2 Food consumption	No.
3.4.3 Clinical signs	Yes.
3.4.4 Examination of uterine content	Gravid uterine weight.
	Number of corpora lutea.
	Number of implantations. Number of resorptions.

Doc IIIA / Section 6.8.1
BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study
Teratogenicity Study in the Rat

3.4.5 Examination of foetuses

3.4.5.1 General Number of live foetuses, mean foetal weight, mean litter weight, sex ratio.

3.4.5.2 Skelet Yes.

3.4.5.3 Soft tissue Yes.

3.5 FURTHER REMARKS

4 RESULTS AND DISCUSSION

4.1 MATERNAL TOXIC EFFECTS

The uteri of three females at the top dose (0.02 mg/kg bw/day) and one female at the middle dose (0.01 mg/kg bw/day), contained blood. Also, one female at the top dose was found to have red fluid in the bladder and pale kidneys, with slight pelvic dilatation. Microsopic examination identified these findings as a severe haemorrhagic cystitis and ascending pyelonephritis.

See Table A6_8-1 below.

4.2 TERATOGENIC / EMBRYOTOXIC EFFECTS

There were no adverse effects on litter data parameters. The uterus of one control female contained nine late resorptions and only three live foetuses, and this caused a slight reduction in mean gravid uterus weight and mean litter weight. See Table A6_8-2 below.

There were few external foetal abnormalities. One hind limb in each of two foetuses (0.01 and 0.02 kg/mg/day dose groups) was malrotated. The cranium of one foetus (0.02 mg/kg bw/day) was flattened and one foetus (0.01 mg/kg bw/day) was grossly abnormal (agnathia). The viability of three control foetuses from the same litter was reduced.

There was some pelvic dilatation of the kidneys in foetuses of all groups but this was not related to treatment. The incidence of skeletal abnormalities or degree of ossification of the foetuses examined was not adversely affected by the administration of brodifacoum.

4.3 OTHER EFFECTS

5 APPLICANT'S SUMMARY AND

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rat

CONCLUSION

5.1 MATERIALS AND METHODS

Test material: brodifacoum; Purity: 92.5 %w/w; Batch no: 2,3,4,5 R1; Guidelines: study conducted in accordance with the principles of OECD 414.

Groups of 30 pregnant rats were dosed orally by gavage with brodifacoum solution in 10 %v/v aqueous ethanol at levels of 0.001, 0.01 and 0.02 mg/kg bw/day. Dosing to place daily from Days 6 to 15 inclusive of pregnancy which covered the period of major organogenesis.

On day 21 of pregnancy, the females were killed and their uteri examined for resorptions and live implantations. The foetuses were weighed, sexed and examined for gross abnormalities. From approximately 20 litters per group, half the foetuses were processed for skeletal examination and the remainder for soft tissue examination. Any additional litters containing abnormal foetuses were also processed in a similar way, but other litters were discarded. Liver, kidney and any abnormal tissues from 20 pregnant females per group were submitted for hisopathological examination.

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rat

5.2 RESULTS AND DISCUSSION

No clinical findings were recorded which could be attributed to the administration of brodifacoum.

Macrosopic examination of the females showed that the uteri of three females at the top dose (0.02 mg/kg bw/day) and one female at the middle dose (0.01 mg/kg bw/day), contained blood. Also, one female at the top dose was found to have red fluid in the bladder and pale kidneys, with slight pelvic dilatation. Microsopic examination identified these findings as a severe haemorrhagic cystitis and ascending pyelonephritis.

The lungs of several females from all groups were patchy and not deflated at autopsy, and the animals examined microscopically showed haemorrhages. These findings were considered to be the results of the method of humane killing.

None of these findings, with the possible exception of the presence of blood in the uteri, were considered to be related to the administration of brodifacoum.

There were no adverse effects on litter data parameters. The uterus of one control female contained nine late resorptions and only three live foetuses, and this caused a slight reduction in mean gravid uterus weight and mean litter weight.

There were few external foetal abnormalties. One hind limb in each of two foetuses (0.01 and 0.02 mg/kg bw/day) was malrotated. The cranium of one foetus (0.02 mg/kg bw/day) was flattened and one foetus (0.01 mg/kg bw/day) was grossly abnormal (agnathia). The viability of three control foetuses from the same litter was reduced. There was some pelvic dilatation of the kidneys in foetuses of all groups but this was not related to treatment. The incidence of skeletal abnormalities or degree of ossification of the foetuses examined was not adversely affected by the administration of brodifacoum.

Therefore, brodifacoum was found to be neither teratogenic, embryotoxic nor foetotoxic in the rat at levels up to 0.02 mg/kg bw/day, and it appears unlikely that sublethal levels would affect embryonic or foetal development.

5.3 CONCLUSION

5.3.1 LO(A)EL maternal toxic effects

0.01 mg/kg bw/day.

5.3.2 NO(A)EL maternal toxic effects

0.001 mg/kg bw/day

5.3.3 LO(A)EL embryotoxic / teratogenic effects

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rat
5.3.4 NO(A)EL embryotoxic / teratogenic effects	0.02 mg/kg bw/day.
5.3.5 Reliability	1.
5.3.6 Deficiencies	No.

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	[redacted]
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	

Doc IIIA / Section 6.8.1

Teratogenicity Study

Teratogenicity Study in the Rat

BPD Data Set IIA / Annex Point VI.6.8.1

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Parameter	control data		low dose	medium dose	high dose	
	historical	study	0.001 mg/kg bw/day	0.01 mg/kg bw/day	0.02 mg/kg bw/day	
Number of dams examined		25	26	27	24	
Clinical findings during application of test substance		One female had rapid respiration and dark eyes on Day 10 only.	One female littered on Day 21 and was excluded from pregnancy and litter results.	-	One female lacked upper incisors and was killed on Day 6.	
Mortality of dams (intercurrent deaths)		0 % (0/30)	0 % (0/30)	0 % (0/30)	3 % (1/30)	
Body weight gain (day 0 – end of test)		157.7 g	157.9 g	160.6 g	161.3 g	
Pregnancies		25/30	26/30	27/30	24/30	
Necropsy findings in dams dead before end of test		-	-	-	Female killed on Day 6 not examined.	

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rat

Table A6_8-2. Table for Teratogenic effects (separate data for all dosage groups)
Litter response (Caesarean section data)

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	
	historical	study	0.001 mg/kg bw/day	0.01 mg/kg bw/day	0.02 mg/kg bw/day	
Corpora lutea (mean no.)		13.5	12.9	12.8	13.0	
Implantations (mean no.)		12.3	12.2	12.3	12.3	
Resorptions		18/25	6/26	11/27	8/24	
total number of live fetuses		290	311	321	288	
pre-implantation loss (mean %)		8.6	6.6	5.8	6.8	
post-implantation loss (mean %)		5.8	2.6	3.3	2.7	
total number of litters		25	26	27	24	
litter weight (mean) *[g]*		58.8	61.1	62.2	62.7	
live fetuses / litter (mean)		11.6	12.0	11.9	12.0	
fetus weight (mean) *[g]*		5.08	5.11	5.25	5.25	
gravid uterus weight (mean) *[g]*		80.4	82.7	83.6	85.2	
Fetal sex ratio *[ratio m/f]*		145/145	162/149	163/158	144/144	

Doc IIIA / Section 6.8.1	**Teratogenicity Study**
BPD Data Set IIA / Annex Point VI.6.8.1	Teratogenicity Study in the Rabbit

1 REFERENCE

1.1 REFERENCE	(1980). Brodifacoum: Teratogenicity Study in the Rabbit.
1.2 DATA PROTECTION	Yes.
1.2.1 Data owner	Syngenta Limited.
1.2.2 Companies with letter of access	
1.2.3 Criteria for data protection	.

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

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rabbit
3.1 TEST MATERIAL	Brodifacoum.
3.1.1 Lot/Batch number	Batch number: [redacted] [redacted].
3.1.2 Specification	As given in section 2.
3.1.2.1 Description	Off-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	Rabbit (*Oryctolagus cuniculus*)
3.2.2 Strain	Dutch.
3.2.3 Source	Ranch Rabbits, Crawley Down, Sussex, UK.
3.2.4 Sex	Nulliparous females.
3.2.5 Age/weight at study initiation	13 – 14 weeks of age/ 205 – 274 g.
3.2.6 Number of animals per group	15.
3.2.7 Control animals	Yes.
3.2.8 Mating period	Females were mated with untreated males each day from Monday to Thursday over a two week period. The fertility of each male was confirmed by the presence of live sperm in the ejaculate, as shown by the vaginal smear examination of a recently-mated female. Approximately one hour after mating, each doe was given an intravenous injection of chorionic gonadotrophin in order to promote ovulation. The day of mating was considered to be Day 0 of pregnancy.
3.3ADMINISTRATION /EXPOSURE	Oral

Doc IIIA / Section 6.8.1
BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study
Teratogenicity Study in the Rabbit

3.3.1 Duration of exposure	
	rabbit: day 6-18 (inclusive) post mating
3.3.2 Postexposure period	11days.
	Oral
3.3.3 Type	Gavage.
3.3.4 Concentration	0.001, 0.002, 0.005 mg/kg$_{bw}$/day.
3.3.5 Vehicle	5 % v/v aqueous ethanol.
3.3.6 Concentration in vehicle	2 ml of dosing solution per kg bodyweight.
3.3.7 Total volume applied	Total volume of dosing solution given to each animal was adjusted daily according to bodyweight of individual animals.
3.3.8 Controls	Vehicle.
3.4 EXAMINATIONS	
3.4.1 Body weight	Yes.
3.4.2 Food consumption	No.
3.4.3 Clinical signs	Yes.
3.4.4 Examination of uterine content	
	Number of corpora lutea. Number of implantations. Number of resorptions.

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

3.4.5 Examination of foetuses

3.4.5.1 General — Number of live foetuses, number of male foetuses, number of female foetuses, mean foetal weight, mean litter weight, sex ratio.

3.4.5.2 Skelet — Yes.

3.4.5.3 Soft tissue — Yes.

3.5 FURTHER REMARKS

4 RESULTS AND DISCUSSION

4,1 MATERNAL TOXIC EFFECTS — At the top dose (0.005 mg/kg bw/day) there was a high proportion of maternal deaths. These animals which died or were killed were all found to have internal haemorrhage; generally from the gravid uterus and/or thoracic contents.

See Table A6_8-1 below.

4.2 TERATOGENIC / EMBRYOTOXIC EFFECTS — There were no adverse effects on any of the litter parameters measured, apart from the number of pregnant does surviving to Day 29 in the 0.005 mg/kg bw/day group. See Table A6_8-2 below.

4.3 OTHER EFFECTS

5 APPLICANT'S SUMMARY AND CONCLUSION

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rabbit	
5.1 MATERIALS AND METHODS	▇▇▇Groups of 15 pregnant rabbits were dosed orally by gavage with brodifacoum solution in 5%v/v aqueous ethanol, at levels of 0.001, 0.002 and 0.005 mg/kg bw/day. Dosing to place daily from Days 6 to 18 inclusive of pregnancy which covered the period of major organogenesis. On Day 29 of pregnancy, the females were killed and their uteri examined for resorptions and live implantations. The foetuses were removed, weighed, and examined for gross abnormalities. They were sexed internally on later examination. Half the foetuses from each litter were processed for skeletal examination and the remainder for soft tissue examination.	X
5.2 RESULTS AND DISCUSSION	The administration of brodifacoum had no adverse effect on any of the litter parameters measured, apart from the number of pregnant does surviving to Day 29 in the 0.005 mg/kg bw/day group. At the top dose (0.005 mg/kg bw/day) there was a high proportion of maternal deaths. These animals which died or were killed were all found to have internal haemorrhage; generally from the gravid uterus and/or thoracic contents. No maternal treatment related effects were seen at the two lower dose levels (0.002 and 0.001 mg/kg bw/day), with the possible exception of a small subcutaneous hemorrhage in one doe receiving 0.002 mg/kg bw/day. Mean bodyweight gain during pregnancy was low in all groups, but there was no evidence of any group differences. Maternal pathology of those does surviving to Day 29 did not reveal any treatment related effects. However, the mortalities at the top dose rate indicated that brodifacoum caused haemorrhage, particularly from the gravid uterus. One foetus exposed to 0.001 mg/kg bw/day had malrotated hind limbs and flexed forelimbs. Other than that, the only other external foetal abnormalities were found in control animals. The incidence of soft tissue abnormalities was not influenced by treatment with brodifacoum. There were no trends towards either reduced ossification or increased numbers of abnormalities found on skeletal examination. Therefore, brodifacoum, when dosed to rabbits at 0.005 mg/kg bw/day, caused a high proportion of maternal deaths. Despite the maternal toxicity there was no evidence of any teratogenic, embryotoxic or foetotoxic effect at this dose level. No treatment related effects were seen in either the mother or the developing foetus at the two lower dose levels.	X
5.3 CONCLUSION		
5.3.1 LO(A)EL maternal toxic effects	0.005 mg/kg bw/day.	

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rabbit
5.3.2 NO(A)EL maternal toxic effects	0.002 mg/kg bw/day.
5.3.3 LO(A)EL embryotoxic / teratogenic effects	
5.3.4 NO(A)EL embryotoxic / teratogenic effects	0.005 mg/kg bw/day.
5.3.5 Reliability	1.
5.3.6 Deficiencies	No.

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
2.1	EVALUATION BY RAPPORTEUR MEMBER STATE
2.2 DATE	Give date of action
2.3 MATERIALS AND METHODS	Although the study is adequately reported the group size at intermediate and low dose levels (as well as in controls) is low as compared to the requirements of OECD 414. Number of animals with implantation sites should ideally be 20; groups with fewer than 16 animals may be Inappropriate for a full effect evaluation.
2.4 RESULTS AND DISCUSSION	The results support that Brodifacoum does not elicit a marked developmental toxicity in the rabbit. Nevertheless, the group size at low and intermediate dose levels as well as in controls may be inappropriate for a full evaluation of effects.
2.5 CONCLUSION	The group size at low and intermediate dose levels as well as in controls may be inappropriate for a full evaluation of effects. The study provides supportive evidence about the absence of a marked developmental toxicity of Brodifacoum at non-maternally toxic doses
2.6 RELIABILITY	Parly reliable (supportive evidence)
2.7 ACCEPTABILITY	Number of animals with implantation sites is low as compared to the requirement of OECD 414
2.8 REMARKS	The study can be considered as supportive evidence about the absence of an evident teratogenic potential of Brodifacoum
2.9	3 COMMENTS FROM ...
3.1 DATE	*Give date of comments submitted*

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

3.2 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*
3.3 RESULTS AND DISCUSSION	*Discuss if deviating from view of rapporteur member state*
3.4 CONCLUSION	*Discuss if deviating from view of rapporteur member state*
3.5 RELIABILITY	*Discuss if deviating from view of rapporteur member state*
3.6 ACCEPTABILITY	*Discuss if deviating from view of rapporteur member state*
3.7 REMARKS	

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Parameter	control data		low dose	medium dose	high dose	
	historical comparison control (0.5%v/v Tween 80)	study control (5%v/v ethanol)	0.001 mg/kg bw/day	0.002 mg/kg bw/day	0.005 mg/kg bw/day	
Number of females mated	15	15	15	15	15	
Number of dams examined (pregnant females surviving to Day 29)	11	8	10	11	3	
Clinical findings during application of test substance	-	-	-	Small subcutaneous haemorrhage in one doe	-	
Mortality of dams (number of intercurrent deaths)	17% (2)	38% (3)	31% (4)	15% (2)	83% (10)	
Body weight gain (day 0 – end of test/Day 29)	0.00g	0.16g	0.07g	0.10g	0.06g	
Pregnancies	12/15	11/15	13/15	13/15	12/15	
Necropsy findings in dams dead before end of test	Deaths or reasons for premature killing were unrelated to treatment	Deaths or reasons for premature killing were unrelated to treatment	Deaths or reasons for premature killing were unrelated to treatment	Deaths or reasons for premature killing were unrelated to treatment	The 10 dams which died were all found to have internal haemorrhage (generally from the gravid uterus and/or thoracic contents).	

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

Table A6_8-2. Table for Teratogenic effects (separate data for all dosage groups)
Litter response (Caesarean section data)

Parameter	control data		low dose	medium dose	high dose	
	historical comparison control (0.5%v/v Tween 80)	study control (5%v/v ethanol)	0.001 mg/kg bw/day	0.002 mg/kg bw/day	0.005 mg/kg bw/day	
Corpora lutea (mean no.)	8.4	8.0	7.9	7.4	7.7	
Implantations (mean no.)	7.3	7.1	6.5	6.4	7.3	
Resorptions (total)	8.8%	14.1%	20.0%	11.4%	9.1%	
total number of live fetuses	73	49	52	62	20	
pre-implantation loss (mean %)	14.8	14.7	19.1	16.7	5.6	
post-implantation loss (mean %)	8.7	12.7	24.6	11.9	7.9	
total number of litters	11	8	10	11	3	
litter weight (mean) *[g]*	218.3	221.3	191.6	208.0	238.7	
live fetuses / litter (mean)	6.6	6.1	5.2	5.6	6.7	
fetus weight (mean) *[g]*	32.8	37.7	36.9	37.6	35.3	
Fetal sex ratio *[ratio m/f]*	37/36	27/22	30/22	38/24	10/10	

Detailed justification:	
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	The Applicant's justification is based on the following considerations: a) *Brodifacoum presents no reproductive risks*: the teratogenicity studies in rats and rabbits indicate that Brodifacoum does not induce prenatal developmental toxicity, although it induces a marked effect in the pregnant female, that is related to anticoagulant action. Data from a two-generation shall provide a more comprehensive picture, in particular whether a long-term exposure to the test compound would impair male or female fertility and whether the immature organism would be equally or more sensitive than the adult one. The relevant NOEL could be used for risk assessment of Brodifacoum. b) *Long-term human data*. Long term human data are available for Warfarin, but not for Brodifacoum. Warfarin is a recognized human teratogen inducing a characteric embryopathy as well as fetal hemorrhages in late pregnancy. Thus, warfarin data cannot be used to justify waiving. c) *Technical feasibility*. It is recognized that is difficult to perform conventional reproductive toxicology testing on rodenticides, since guidelines require tests to be performed in rodents, i.e., the target species. Nevertheless, this should be possible by testing possible effects on reproduction and offspring at dose levels that are arounfd the NOEL/LOEL for the supposedly primary (anticoagulant) effect; thus a MTD can be derived. Moreover, technical feasibility *is shown by the rat 2-generation study performed on Brodifacoum, notified by Activa\PelGar Consortium.*

Conclusion	The request for waiving of the 2-generation study for Brodifacoum is unjustified. In particular, a) available data doi not allow to rule out a potential for Brodifacoum to affect reproducing or immature animals at lower dose levels, or with diverse effects; b) long term human data are available for Warfarin, but not for Brodifacoum; Warfarin is a regonized embryofetotoxic agent in humans, thus, warfarin data cannot be used to justify waiving; c) a two-generation study should be technically feasible by testing possible effects on reproduction and offspring at dose levels that are arounfd the NOEL/LOEL for the supposedly primary (anticoagulant) effect. Furthermore, it has to be emphasized that two-generation study is the only protocol that allows the identification of potential hazards upon exposure during the whole development (from early embryo through to sexual maturity); thus it represents a key study in risk assessment. Therefore, a properly conducted two-generation study on Brodifacoum must be provided.
Remarks	*No further remarks*
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

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BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

1 REFERENCE

1.1 REFERENCE	[redacted] (1980). Brodifacoum: Teratogenicity Study in the Rabbit. [redacted] (unpublished).
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	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

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rabbit
3.1 TEST MATERIAL	Brodifacoum.
3.1.1 Lot/Batch number	Batch number: ■■■
3.1.2 Specification	As given in section 2.
3.1.2.1 Description	Off-white powder.
3.1.2.2 Purity	■■■
3.1.2.3 Stability	Please refer to Section 2 of Doc IIIA.
3.2 TEST ANIMALS	
3.2.1 Species	Rabbit (*Oryctolagus cuniculus*)
3.2.2 Strain	Dutch.
3.2.3 Source	Ranch Rabbits, Crawley Down, Sussex, UK.
3.2.4 Sex	Nulliparous females.
3.2.5 Age/weight at study initiation	13 – 14 weeks of age/ 205 – 274 g.
3.2.6 Number of animals per group	15.
3.2.7 Control animals	Yes.
3.2.8 Mating period	Females were mated with untreated males each day from Monday to Thursday over a two week period. The fertility of each male was confirmed by the presence of live sperm in the ejaculate, as shown by the vaginal smear examination of a recently-mated female. Approximately one hour after mating, each doe was given an intravenous injection of chorionic gonadotrophin in order to promote ovulation. The day of mating was considered to be Day 0 of pregnancy.
3.3ADMINISTRATION /EXPOSURE	Oral

Doc IIIA / Section 6.8.1 — **Teratogenicity Study**
Teratogenicity Study in the Rabbit

BPD Data Set IIA / Annex Point VI.6.8.1

3.3.1 Duration of exposure	
	rabbit:
3.3.2 Postexposure period	11days.
	Oral
3.3.3 Type	Gavage.
3.3.4 Concentration	0.001, 0.002, 0.005 mg/kg$_{bw}$/day.
3.3.5 Vehicle	5 % v/v aqueous ethanol.
3.3.6 Concentration in vehicle	2 ml of dosing solution per kg bodyweight.
3.3.7 Total volume applied	Total volume of dosing solution given to each animal was adjusted daily according to bodyweight of individual animals.
3.3.8 Controls	Vehicle.
3.4 EXAMINATIONS	
3.4.1 Body weight	Yes.
3.4.2 Food consumption	No.
3.4.3 Clinical signs	Yes.
3.4.4 Examination of uterine content	
	Number of corpora lutea. Number of implantations. Number of resorptions.

Doc IIIA / Section 6.8.1
Teratogenicity Study
Teratogenicity Study in the Rabbit

BPD Data Set IIA / Annex Point VI.6.8.1

3.4.5 Examination of foetuses

3.4.5.1 General Number of live foetuses, number of male foetuses, number of female foetuses, mean foetal weight, mean litter weight, sex ratio.

3.4.5.2 Skelet Yes.

3.4.5.3 Soft tissue Yes.

3.5 FURTHER REMARKS

4 RESULTS AND DISCUSSION

4,1 MATERNAL TOXIC EFFECTS

At the top dose (0.005 mg/kg bw/day) there was a high proportion of maternal deaths. These animals which died or were killed were all found to have internal haemorrhage; generally from the gravid uterus and/or thoracic contents.

See Table A6_8-1 below.

4.2 TERATOGENIC / EMBRYOTOXIC EFFECTS

There were no adverse effects on any of the litter parameters measured, apart from the number of pregnant does surviving to Day 29 in the 0.005 mg/kg/day group. See Table A6_8-2 below.

4.3 OTHER EFFECTS

5 APPLICANT'S SUMMARY AND CONCLUSION

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

5.1 MATERIALS AND METHODS

Test material: brodifacoum; Purity: 92.5 %w/w; Batch no: 2,3,4,5 R1; Guidelines: study conducted in accordance with the principles of OECD 414.

Groups of 15 pregnant rabbits were dosed orally by gavage with brodifacoum solution in 5%v/v aqueous ethanol, at levels of 0.001, 0.002 and 0.005 mg/kg bw/day. Dosing to place daily from Days 6 to 18 inclusive of pregnancy which covered the period of major organogenesis.

On Day 29 of pregnancy, the females were killed and their uteri examined for resorptions and live implantations. The foetuses were removed, weighed, and examined for gross abnormalities. They were sexed internally on later examination. Half the foetuses from each litter were processed for skeletal examination and the remainder for soft tissue examination.

5.2 RESULTS AND DISCUSSION

The administration of brodifacoum had no adverse effect on any of the litter parameters measured, apart from the number of pregnant does surviving to Day 29 in the 0.005 mg/kg bw/daygroup.

At the top dose (0.005 mg/kg bw/day) there was a high proportion of maternal deaths. These animals which died or were killed were all found to have internal haemorrhage; generally from the gravid uterus and/or thoracic contents.

No maternal treatment related effects were seen at the two lower dose levels (0.002 and 0.001 mg/kg bw/day), with the possible exception of a small subcutaneous hemorrhage in one doe receiving 0.002 mg/kg bw/day. Mean bodyweight gain during pregnancy was low in all groups, but there was no evidence of any group differences. Maternal pathology of those does surviving to Day 29 did not reveal any treatment related effects. However, the mortalities at the top dose rate indicated that brodifacoum caused haemorrhage, particularly from the gravid uterus.

One foetus exposed to 0.001 mg/kg bw/dayhad malrotated hind limbs and flexed forelimbs. Other than that, the only other external foetal abnormalities were found in control animals. The incidence of soft tissue abnormalities was not influenced by treatment with brodifacoum. There were no trends towards either reduced ossification or increased numbers of abnormalities found on skeletal examination.

Therefore, brodifacoum, when dosed to rabbits at 0.005 mg/kg bw/day, caused a high proportion of maternal deaths. Despite the maternal toxicity there was no evidence of any teratogenic, embryotoxic or foetotoxic effect at this dose level. No treatment related effects were seen in either the mother or the developing foetus at the two lower dose levels.

5.3 CONCLUSION

Doc IIIA / Section 6.8.1 **BPD Data Set IIA / Annex Point VI.6.8.1**	**Teratogenicity Study** Teratogenicity Study in the Rabbit
5.3.1 LO(A)EL maternal toxic effects	0.005 mg/kg bw/day
5.3.2 NO(A)EL maternal toxic effects	0.002 mg/kg bw/day
5.3.3 LO(A)EL embryotoxic / teratogenic effects	
5.3.4 NO(A)EL embryotoxic / teratogenic effects	0.005 mg/kg bw/day
5.3.5 Reliability	1.
5.3.6 Deficiencies	No.

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	[redacted]
DATE	[redacted]
MATERIALS AND METHODS	[redacted]
GUIDELINES AND QUALITY ASSURANCE	[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*

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

ACCEPTABILITY	*Discuss if deviating from view of rapporteur member state*
REMARKS	

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)
Maternal effects

Parameter	control data				high dose	
	historical comparison control (0.5%v/v Tween 80)			0.002 mg/kg/day	0.005 mg/kg/day	
Number of females mated	15			15	15	
Number of dams examined (pregnant females surviving to Day 29)	11			11	3	
Clinical findings during application of test substance	-			Small subcutaneous haemorrhage in one doe	-	
Mortality of dams (number of intercurrent deaths)	17% (2)			15% (2)	83% (10)	
Body weight gain (day 0 – end of test/Day 29)	0.00g			0.10g	0.06g	
Pregnancies	12/15			13/15	12/15	
Necropsy findings in dams dead before end of test	Deaths or reasons for premature killing were unrelated to treatment			Deaths or reasons for premature killing were unrelated to treatment	The 10 dams which died were all found to have internal haemorrhage (generally from the gravid uterus and/or thoracic contents).	

Doc IIIA / Section 6.8.1

BPD Data Set IIA / Annex Point VI.6.8.1

Teratogenicity Study

Teratogenicity Study in the Rabbit

Table A6_8-2. **Table for Teratogenic effects (separate data for all dosage groups)**
Litter response (Caesarean section data)

Parameter	control data				high dose	
	historical comparison control (0.5%v/v Tween 80)			0.002 mg/kg/day	0.005 mg/kg/day	
Corpora lutea (mean no.)	8.4			7.4	7.7	
Implantations (mean no.)	7.3			6.4	7.3	
Resorptions (total)	8.8%			11.4%	9.1%	
total number of live fetuses	73			62	20	
pre-implantation loss (mean %)	14.8			16.7	5.6	
post-implantation loss (mean %)	8.7			11.9	7.9	
total number of litters	11			11	3	
litter weight (mean) *[g]*	218.3			208.0	238.7	
live fetuses / litter (mean)	6.6			5.6	6.7	
fetus weight (mean) *[g]*	32.8			37.6	35.3	
Fetal sex ratio *[ratio m/f]*	37/36			38/24	10/10	

Doc IIIA/Section 6.8.2 BPD Data Set IIA/Annex Point VI.6.8.2	**Two generations reproduction study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [✓]** **Scientifically unjustified [✓]**	
Detailed justification:		
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks	*No further remarks*	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 6.9 BPD Data Set IIIA/ Annex Point VI.I	Neurotoxicity study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [✓] **Other existing data** [✓] **Limited exposure** [✓]	
Detailed justification:	1. [redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 6.10 BPD Data Set IIIA/ Annex Point VI.7	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [✓] **Other existing data** [✓] **Limited exposure** [✓]	
Detailed justification:		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 6.11	Studies on other routes of administration (parenteral routes)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [✓] **Other existing data** [✓] **Limited exposure** [✓]	
Detailed justification:	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*May 2007*	
Evaluation of applicant's justification	Applicant's justification is reasonable	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		