Section A1 Applicant

Annex Point IIA1

1.1 Applicant

Contact: [redacted]
Name: PelGar International Ltd.
Address: Midhurst Rd.,
Liphook,
Hants
United Kingdom
Telephone: [redacted]
Fax: [redacted]
Email: [redacted]@pelgar.co.uk

Contact: [redacted]
Name: Activa s.r.l.
Address: Viale Lombardia 22,
20131 Milano,
Italy
Telephone: [redacted]
Fax: [redacted]
Email: activa@activa.it

1.2 Manufacturer of Active Substance (if different)

Name XXXXX
Address: XXXXX

Name: XXXXX
Address: XXXXX

1.3 Manufacturer of Product(s) (if different)

The manufacturers of the product are not different from the manufacturers of the active substance. They are:

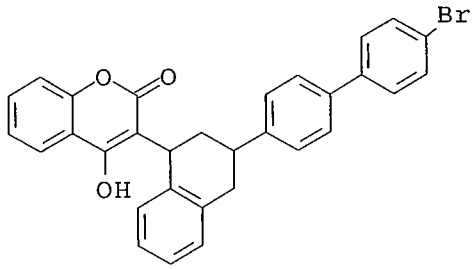
1) Product 1

Name: XXXXX
Address: XXXXX

Name XXXXX
Address: XXXXX

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	Not applicable
Conclusion	The information provided is deemed exhaustive.
Reliability	0
Acceptability	Acceptable
Remarks	On applicant's request, the contact information is to be modified as follows: instead of Midhurst Road, Liphook, PelGar's address is PelGar International Ltd, Unit 13, Newman Lane Industrial Estate, Newman Lane, Alton Hampshire GU34 2QR, UK instead of Viale Lombardia 22, 20131 Milano, Activa's address is: Via Feltre 32, 20132 Milano, Italy
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A2 Identity of Active Substance

Subsection (Annex Point)			Official use only
2.1	**Common name (IIA2.1)**	Brodifacoum	X
2.2	**Chemical name (IIA2.2)**	3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin	X
2.3	**Manufacturer's development code number(s) (IIA2.3)**	None	
2.4	**CAS No and EC numbers (IIA2.4)**		
2.4.1	**CAS-No**	56073-10-0	
2.4.2	**EC-No**	259-980-5	X
2.4.3	**Other**	None	X
2.5	**Molecular and structural formula, molecular mass (IIA2.5)**		
2.5.1	**Molecular formula**	$C_{31}H_{23}BrO_3$	
2.5.2	**Structural formula**		X
2.5.3	**Molecular mass**	523.44 g/mol	X
2.6	**Method of manufacture of the active substance (IIA2.1)**	This information is regarded as commercially sensitive. Please refer to the Confidential Annex to review this information.	X

2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**	g/kg	g/L	% w/w	% v/v
		> 992	-	> 99.2	-

Section A2 Identity of Active Substance

2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	This information is regarded as commercially sensitive. Please refer to the Confidential Annex to review this information.	X
2.9	**The origin of the natural active substance**	Synthesis	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	Dec 2007
Materials and methods	Not applicable.

Section A2 Identity of Active Substance

Conclusion

The information provided is acceptable, but is to be completed as follows:

2.1: CAS 56073-10-0 is registered in Annex I of Directive 67/548/EEC also as *4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin.*

2.2: *3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin* is the chemical name according to IUPAC. CAS-name is *2H-1-Benzopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-.*

2.4.1: number given in EINECS.

2.4.3: CIPAC number and Annex I Index number are also available (370 and 607-172-00-1, respectively).

2.5.2: *cis* and *trans* isomerism:

cis isomer (CA Index name *2H-1-Benzopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-, cis-*, CAS-No. 72654-66-1). *Cis* isomer is a racemic mixture of *3-{(1R,3S)[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]}-4-hydroxycoumarin* and *3-{(1S,3R)[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]}-4-hydroxycoumarin.*

trans isomer (CA Index name *2H-1-Benzopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-, trans-*, CAS-No. 72654-67-2). *Trans* isomer is a racemic mixture of *3-{(1S,3S)[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]}-4-hydroxycoumarin* and *3-{(1R,3R)[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]}-4-hydroxycoumarin.*

2.5.3: 523,64 g/mol.

2.6 and 2.8: information provided by the Applicants in Appendix XIA 'Information claimed as confidential'. Evaluation by RMS in the boxes included therein.

2.7: data from 5-batch analysis are summarized as follows

	Common name	CAS number	Concentration range
Purity of the a.s.	*Brodifacoum*	56073-10-0	99.23-99.91% w/w (mean: 99.46% w/w)
Isomeric composition	*cis* isomer *trans* isomer	72654-66-1 72654-67-2	*Confidential*
Impurity profile	*Confidential*	*Confidential*	*Confidential*

Reliability 0

Acceptability Acceptable

Remarks

COMMENTS FROM ...

Date *Give date of comments submitted*

Section A2 Identity of Active Substance

Results and discussion	*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*
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	

Section A2.10

Annex Point IIA2.10

Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Official use only

Subsection

2.10.1 Human exposure towards active substance

2.10.1.1 Production

i) Description of process

There will be no exposure to operators during the manufacturing process. Operators wear coveralls and gloves.

Closed system;

Full PPE (gloves, coveralls, face-shield, respirator) when filling and for maintenance. No separate cleaning operation since vessel is used only for brodifacoum production

It is only at the last stage of manufacture (see 2.6 in appendix XIA) that an anticoagulant is present.
In addition the active is used only to prepare anticoagulant solutions at 2.5 % or other percentage.
So all production processes, carefully follow three important steps:

1. production of intermediate till last step with no anticoagulant properties
2. coupling of intermediate with 4-hydroxycoumarin to obtain the anticoagulant
3. preparation of solution

Production follows, in sequence, preparation till last intermediate in various quantities.

Right amount of this last intermediate is coupled with 4-hydroxycoumarin, in established conditions, to obtain in situ the anticoagulant needed for 2.5 % or other percentage solution preparation.

Last step, including solution preparation, is achieved without any operator contact excluding only active and solution sampling for quality control, that are made on solution production so no contact with any kind of solid or powder product is possible.

All operations are made inside appropriate closed vessels. Production vessels are always the same.

Production started around 1975 and since over 29 years no accidents of any kind happened: all production processes as well facilities were carefully studied.

ii) Workplace description

The manufacturing and dilution stages are all carried out in closed vessels with mixtures containing the active substance pumped into and out of the reaction vessels and holding containers. Maintenance occurs infrequently as vessels are dedicated to production of the active substance or one of its analogues. There will be no exposure to operators during the manufacturing process..

Section A2.10 Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Annex Point IIA2.10

2.10.1.2 Intended use(s)

1. Professional Users		
	i) Description of application process	**Use in and around buildings:** Loading of bait boxes with wax bait blocks, and placement of the bait boxes. the operator is trained in the correct use of the blocks, i.e. placement, number of bait boxes required based on the infestation rate area, the number of bait blocks per box and safe handling procedures. The use of PPE, i.e. disposable gloves and a face-mask may be used when loading bait boxes and disposing of remaining bait and carcasses. However, when the block is contained within a bait trap there will be no exposure to the product to the operator. For rats each bait box will contain 4 to 5 blocks. A mouse box will only contain one bait block. Boxes for mice should be placed 5 metres apart, although this can be reduced to 2 metres in areas of high infestation and for rats boxes should be 10 metres apart or to 5 metres apart in high infestation areas. Boxes should be checked every day and carcasses removed. Operators should search for all rodent bodies in and around the baited area for disposal. Bait boxes should be removed, in a typical campaign, 6 weeks after initial placement. Sites should not be re-baited until a new infestation is observed. **In sewers:** In sewers, blocks are tied or nailed to stable surfaces above water level. PPE (coverall, boots and gloves) is required as standard when the blocks are used in sewage systems. Blocks placed in sewers are not normally removed. Rodent bodies in sewers will not be collected for disposal.
	ii) Workplace description	The professional pest control worker will work in a variety of places in and around buildings and within the sewerage system. Therefore the workplace environment will vary.
	iii) Inhalation exposure	**In and around buildings**: zero **Sewers**: zero

iv) Dermal exposure	Manufacture: zero Formulation: 4.87×10^{-6} mg/kg bw/day Using an NOAEL of 0.001 mg/kg bw/day, the margin of safety is 205 indicating no cause for concern for the worker during formulation. For professional use two models were used; CEFIC study and a TNsG model (mixing and loading, model 7) CEFIC result: 1.2×10^{-5} mg/kg bw/day TNsG result: 2.67×10^{-07} mg/kg bw/day
2. Non-professional Users including the general public	
i) Description of application process	**Use in and around buildings:** See above
ii) Workplace description	Essentially this would be amateurs such as farmers placing boxes arounds farms and similar locations. Sewers would **not** be included.
iii) Inhalation exposure	There will be no inhalation exposure to the biocidal product from amateur use
iv) Dermal exposure	Two models were used; CEFIC study and a TNsG model (mixing and loading, model 7) CEFIC result: 2.84×10^{-7}mg/kg bw/day TNsG result: 3.33×10^{-8}mg/kg bw/day
2.10.2 Environmental exposure towards active substance	
2.10.2.1 Production	There is no environmental exposure from manufacture and formulation.
2.10.2.2 Intended use(s)	In sewers and in and around buildings
Affected compartment(s):	
From use in sewers:	*PEC in surface water, ground water and sediment* Surface water during emission episode, 2.14 E-06 mg/L Annual average in surface water, 2.1E-07 mg/L Sediment during emission episode, 4.37E-04 mg/kgwwt STP, 2.07E-05 mg/L Surface water during emission episode,STP bypassed, 4.32E-06 mg/L Sediment during emission episode, STP bypassed, 8.83E-04 mg/kg_{wwt} *PEC's in soil* In agricultural soil (total) averaged over 30 d, 8.12E-04 mg/kgwwt

Section A2.10
Annex Point IIA2.10

Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

From use in bait boxes	In agricultural soil (total) averaged over 180 d, 8.11E-03 mg/kgwwt In grassland (total) averaged over 180 d, 3.2E-04 Pore water of agricultural soil, 4.9E-06 mg/L Pore water of grassland, 1.93E-07 mg/L *PEC in air* Annual average in air, 7.22E-24 mg/m^3 *PEC in surface water, ground water and sediment* There is no emission to this compartment from this use-pattern *PEC's in soil* Concentration in soil = 0.011 mg/kg soil Concentration in pore water = 1.13E-04 mg/L *PEC in air* Not expected to be released to air.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of the method include appropriate reliability indicator*
Acceptability	Acceptable / not acceptable *(give reasons if necessary, e.g. if a study is acceptable despite a poor reliability indicator). Discuss the relevance of deficiencies.*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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*

Section A2.10 Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Annex Point IIA2.10

Remarks

Sample table:

Table A2.10: Workplace exposure / Inhalation exposure (use additional terminology from the TNsGs on Human exposure

Exposure scenario	Workplace operation	PPE	Year(s) of measurement	Number of measurements	Type of measurements	Exposure concentration
Production	*Filling, cleaning*	*Full PPE inc respirator*	*None*	*None*	*None*	*None*
Formulation	*Opening concentrate container, or handling finished bait (0.005%)*	*Protective coverall, gloves,*	*None*	*None*	*None*	*None*
Application MG../PT..	*Placing baits, removing residues and decedents*	*Gloves,*	*None*	*None*	*None*	*None*

It is to take in account that the active involved is an anticoagulant: it gains this property when chemical is completed coupling intermediate with 4-hydroxycoumarin. It is to be clear that only at this point the chemical begin an anticoagulant.
In addition the active is used only to prepare anticoagulant solutions at 0.25 % or other percentage.
All staff, composed by 7 operators, is followed from 1975 by a doctor specialised in "hygiene and preventive medicine" and "work medicine".
At beginning in 1975, staff was controlled each 3 months with haematochemical and urine examen.
After a period of ten years in 1985, since no kind of problems rise and all processes were well secured, medical surveillance was changed with:

- six-monthly medical visit made by the competent doctor,
- spyrometric annual control,
- six-monthly haematochemical and urine examen.

In 1995 another change was made: haematochimical and urine examen began annual.
All surveillance plan is made by the upper doctor who inspect also the production facilities with some surprise visit during working.
All upper results control are communicated to local authorities each year.
All documents can be showed on request.
No accidents occur from 1975 till today: this can demonstrate process safety and operator medical surveillance

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point								
Melting pt.	EEC Method A1	100%	235.8°C (pressure: atmospheric)	Material did not appear to melt but was observed to darken and decompose	Y	1	Drake (2003a) Chemex Environmental International, Report No. ENV5808/120140	X
3.1.2 Boiling point								
	EEC Method A2	100%	(pressure: atmospheric)	Not determined as material was a powder and decomposed at 235.8°C	Y	1	Drake (2003a) Chemex Environmental International, Report No. ENV5808/120140	
3.1.3 Bulk density/ relative density								
Relative density	CIPAC MT3.2 OECD 109	> 99%	1.5300 (temperature: 20°C)		Y	1	Garofani (2001a) ChemService s.r.l., Report No. CH-158/2000	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.2 Vapour pressure (IIA3.2)								
Vapour pressure (1)	EEC Method A4 OECD Guideline 104	99.5%	< 0.05 mPa (temperature: 45°C)		Y	1	Fabbrini (1997a) ChemService s.r.l., Report No. CH-14/96-C-BDF	X
Vapour pressure (2)	EEC Method A4	99.7%	1.9E-21 Pa (temperature: 25°C) 2.6E-22Pa (temperature: 20°C)	This result supercedes the Fabbrini (1997a) study.	Y	1	White and Mullee (2006) SarePharm Laboratories Ltd., Report No. 2109/0002	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.2.1 Henry's Law Constant (Pt. I-A3.2)								
Henry's Law (1)	Calculation. The temperature used to calculate Kw is 298K. A result of <0.05mPa (=<5E-05Pa) from study CH-14/96-C was used in the calculation on the reasonable assumption that this was valid at 298K. The water solubility value of <0.1mg/L was taken from study CH-14/96-A.		$<1.06 \times 10^{-4}$ (calculated)	Scarcely volatile	Y	1	Fabbrini (1997b) ChemService S.r.l, Report No. 1	X
Henry's Law (2)	Calculation based on water solutbility and vapour pressure		2.35×10^{-18} Pa.m3.mol^{-1}	This result supercedes the Fabbrini study	Y	1	White and Mullee (2006) SafePharm Laboratories Ltd., Report No. 2109/0002	
3.3 Appearance (IIA3.3)								

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.1 Physical state	98/8/EC. IIA, III, 3.3		Fine powder at 20°C		Y	1	Tremain (2007) Safepharm Laboratories Ltd., Report No. 2109/0006	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.1(a) Dustiness and particle size distribution	*Dustiness:* Storing test material as MT46.1, then using a method based on MT34 of the CIPAC handbook for the analysis of Technical and Formulated Pesticides *Particle size distribution:* Procedure designed to comply with requirements of the OECD guideline 110	99.70% w/w	*Dustiness:* White powder with a few clumps and no signs of caking after storage at 54 ± 2°C for 14 days and under a pressure of 25 g/cm^2. None of the test material became an airborne dust after it was blown with a light jet of nitrogen. *Particle size distribution:* Proportion of test material having an inhalable particle size less than 100 µm = 14.8% (sieve) Proportion of test material having a thoracic particle size less than 10.0 µm = 0.998% (cascade impactor) Proportion of test material having a respirable particle size less than 5.5 µm = 8.14E-02 % (cascade impactor)	The lack of a dusty nature supports the acute toxicity by inhalation waiver in Doc. IIIA6.1.3 The amount of test material that is respirable is indicative that there is little likelihood of acute toxicity by inhalation. This is used to support the waiver in Doc. IIIA6.1.3	Y	1	Woolley and Mullee (2007) SafePharm Laboratories Ltd, Report No. 2109/0003	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.2 Colour	98/8/EC. IIA, III, 3.3		Off-white at 20°C		Y	1	Tremain (2007) SafePharm Laboratories Ltd., Report No. 2109/0006	
3.3.3 Odour				See justification for non-submission				
3.4 Absorption spectra (IIA3.4)								
UV/VIS (1)	OECD Guideline 101	> 99%	Peak maximum wavelength (nm): 265 nm and 307 nm		Y	1	Garofani (2001b) ChemService s.r.l., Report No. CH-157/2000	X
UV/VIS (2)	OECD Guideline 101	99.7%	Peak maximum wavelength (nm): 263 nm (ε = 33601), 266 nm (ε = 37759 and 40191), 308 nm (ε = 14089 and 15629) and 312 nm (ε =16677) ε = molar extinction (absorption) coefficient in l/mol x cm		Y	1	Garofani (2001c) ChemService s.r.l., Report No. CH-133/2001	X
IR (1)	OECD Guideline 101	> 99%	O–H stretching 3398 cm^{-1} C–H stretching 2925 cm^{-1}		Y	1	Garofani (2001b)	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			C = C Ring stretching 1665 – 1430 cm^{-1} C(aryl) – O Stretching 1300 – 1100 cm^{-1}				ChemService s.r.l., Report No. CH-157/2000	
IR (2)	OECD Guideline 101	99.7%	O – H stretching 3398 cm^{-1} C – H stretching 2925 cm^{-1} C = C ring stretching 1665 – 1430 cm^{-1} C (aryl) – O stretching 1300 – 1100 cm^{-1}		Y	1	Garofani (2001c) ChemService s.r.l., Report No. CH-133/2001	X
NMR (1)	OECD Guideline 101	> 99%	^{1}H-NMR δ2.35 ppm – C**H**$_2$CH in aliphatic ring δ2.8 ppm C**H**$_2$CH & CHC**H**2 in aliphatic ring δ2.9-3.2 ppm C**H**$_2$CH in aliphatic ring δ3.4 ppm C**H**CH$_2$ in aliphatic ring δ3.65 ppm C**H**$_2$CH in aliphatic ring δ4.7 ppm CH$_2$C**H** in		Y	1	Garofani (2001b) ChemService S.r.l., Report No. CH-157/2000	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			aliphatic ring δ5 ppm C**H**CH_2 in aliphatic ring δ7.0-7.8 ppm **H** in aromatic rings δ8.15 ppm **H** in aromatic rings ^{13}C-NMR δ35.1 ppm CH_2C**H** in aliphatic ring δ37.5 ppm C**H**CH_2 in aliphatic ring δ38.6 ppm CH_2C**H** in aliphatic ring δ41.5 ppm C**H**CH_2 in aliphatic ring δ116-133 ppm **C**H in aromatic rings δ150 ppm **C**OH in aromatic ring					
NMR (2)	OECD Guideline 101	99.7%	^{1}H-NMR δ2.35 ppm CH_2C**H** in aliphatic ring δ2.8 ppm CH_2C**H** &				Garofani (2001c) ChemService s.r.l., Report No. CH-	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			$CHCH_2$ in aliphatic ring δ2.9 – 3.2 ppm CH_2CH in aliphatic ring δ3.4 ppm $CHCH_2$ in aliphatic ring δ3.65 ppm CH_2CH in aliphatic ring δ 4.7 ppm CH_2CH in aliphatic ring δ5 ppm $CHCH_2$ in aliphatic ring δ7.0 – 7.8 ppm **H** in aromatic rings δ8.15 ppm **H** in aromatic rings ^{13}C-NMR δ35.1 ppm CH_2CH in aliphatic ring δ37.5 ppm $CHCH_2$ in aliphatic ring δ38.6 ppm CH_2CH in aliphatic ring δ41.5 ppm $CHCH_2$ in aliphatic ring				133/2001	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
MS	OECD Guideline 101	99.7%	δ116 – 133 ppm CH in aromatic ring δ150 ppm COH in aromatic ring MS/MS⁻ fragments: 475 371 335 291 256 189 178 174		Y	1	Garofani (2001c) ChemService s.r.l., Report No. CH-133/2001	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.5 Solubility in water (IIA3.5)								
Water solubility (1)	OECD Guideline 105	99.5%	< 0.1 mg/L (temperature: not stated, although column temperature was room temperature) (pH: 5.8 – 6.9)	Very low water solubility. Assumed test run at room temperature on basis of colum temperature.	Y	1	Fabbrini (1997c) ChemService s.r.l., Report No. CH-14/96-A-BDF	X
Water solubility (2)	EEC Method A6	99.7%	5.65E-07 mg/L (at pH5, 10°C) ≤3.17E-06 mg/L (at pH5, 20°C) 6.57E-07 mg/L (at pH5, 30°C) 8.16E-06 mg/L (at pH7, 10°) 5.80E-05 mg/L (at pH7, 20°C) 1.60E-05 mg/L (at pH7, 30°C) 6.27E-04 mg/L (at pH 9, 10°C) 1.86E-03 mg/L (at pH9, 20°C) 7.96E-04 mg/L (at pH 9 , 30°C)	Water solubility was demonstrated to significantly vary with pH. Very low solubility . This study supercedes the Fabbrini (1997) study.	Y	1	White and Mullee (2006) SarePharm Laboratories Ltd., Report No. 2109/0002	X
3.6 Dissociation constant (-)	OECD Guideline 112	99.5%	pKa = 4.50	Calculated pKa	N	1	ACD/I-Website	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.7 **Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)**								
Solubility in organic solvents (1)	OECD Guideline 105	99.5%	(in g/L) Toluene: 6.1 ethyl acetate 10.20 methanol 1.95 n-hexane $< 22 \times 10^{-6}$ acetone 20.10 dichloromethane 60.84 (temperature: 20°C)		Y	1	Staniland (2005) Chemex Environmental International Ltd., Report No. ENV7058/120140	X
Solubility in organic solvents (2)	Flask method: EEC Method A6	99.7%	*Tolulene:* 5.81 g/L (at 10°C) 5.89 g/L (at 20°C) 5.85 g/L (at 30°C) *Dichloromethane:* 28.9 - 33.7 g/L (at 10°C) 29.0 - 33.8 g/L (at 20°C) 40.7 - 50.9 g/L (at 30°C) *n-Hexane:* 7.58E-03 g/L (at 10°C) 8.9E-03 g/L (at 20°C) 7.29E-03 g/L (at 30°C)	The solvent solubility did not vary with temperature except in the dichloromethane assessment. This study supercedes the one above, as it includes effect of temperature on solubility.	Y	1	White and Mullee (2006) SarePharm Laboratories Ltd., Report No. 2109/0002	X X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			Ethyl acetate: 10.2 g/L (at 10°C) 10.1 g/L (at 20°C) 10.8 g/L (at 30°C) *Methanol:* 1.67 g/L (at 10°C) 1.61 g/L (at 20°C) 1.64 g/L (at 30°C) *Acetone:* 20.7 g/L (at 10°C) 21.2 g/L (at 20°C) 21.8 g/L (at 30°C)					
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)				Study not required - see justification for non-submission				

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.9 Partition coefficient n-octanol/water (IIA3.6)								
log Pow (1)	OECD Guideline 117	99.5%	logP = > 4.0 (temperature: not stated) (pH: not stated)	Very low water solubility	Y	1	Fabbrini (1997d) ChemService s.r.l., Report No. CH-14/96-B-BDF	X
log Pow (2)	EEC Method A8	99.7%	6.16 - 6.27 (at pH5, 10°C) 5.99 - 6.13 (at pH5, 20°C) 5.80 - 5.98 (at pH5, 30°C) 5.09 (at pH 7, 10°C) 4.92 (at pH 7, 20°C) 4.78 (at pH 7, 30°C) 4.91 (at pH 9, 10°C) 4.78 (at pH 9, 20°C) 4.58 (at pH 9, 30°C)	pH and temperature affect the partition coefficient of the test material. The temperature effect has been deemed to be insignificant as the log Pow results are within ± 0.5 log units	Y	1	White and Mullee (2006) SarePharm Laboratories Ltd., Report No. 2109/0002	
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	OECD Guideline 113	99.5%	No significant change noted on storage for 14 days at 54°C. Thermally stable below 150°C with no decomposition of transformation noted	It was found to be stable up to its melting point which was noted to be 5°C higher than the manufacturers data.	Y	1	Drake (2005a) Chemex Environmental International Ltd., Report No. ENV7062/120140	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)	Flammability: EC method A.10	>99 %	Non-flammable		Y	1	Garofani (2001d) Chemservice S.r.l, Report No. CH-159/2000	
	Auto-flammability: EC method A.16	99.70 %	None below its melting temperature.		Y	1	Tremain (2007) SafePharm Laboratories Ltd., Report No. 2109/0006	
3.12 Flash-point (IIA3.9)				Study not required - see data waiver				
3.13 Surface tension (IIA3.10)	EC Method A.5	100%	72.9 mN/m (90% dilution saturated at 1.0 g/L in HPLC grade water) at 20°C		Y	1	Sacker (2005a) Chemex Environmental International Ltd., Report No. ENV7164/120140	X
3.14 Viscosity (-)				Study not required - see justification for non-submission				
3.15 Explosive properties (IIA3.11)				Study not required - see justification for non-submission				
3.16 Oxidizing properties				Study not required - see justification for				

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
(IIA3.12)				non-submission				
3.17 Reactivity towards container material (IIA3.13)				Study not required - see justification for non-submission				

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	
Conclusion	General remarks on Section IIIA.3: - All methods submitted have been performed in GLP. - Physical and chemical properties have been tested on different batches of *Brodifacoum.* - The RMS comments have been attached in the following evaluation boxes, including the justifications for non-submission of data submitted by the Applicants.
Reliability	
Acceptability	
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.1.1 Melting point** EEC Method A.1, Capillary method. Batch: not stated. Purity: 100% *Brodifacoum.*
Conclusion	Melting point: *Brodifacoum* did not appear to melt, but was observed to darken and decompose. Decomposition temperature: 235.8°C at atmospheric pressure.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.1.2 Boiling point** EEC Method A.2 not applicable, since *Brodifacoum* turned out to decompose at 235.8°C.
Conclusion	Not applicable. Decomposition temperature: 235.8°C at atmospheric pressure.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.1.3 Bulk density/relative density** CIPAC MT 3.2. OECD 109, Pycnometer method. Batch: L04272. Purity: > 99% *Brodifacoum.*
Conclusion	Relative Density: 1.5300 at T = 20°C
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.2 Vapour pressure 1** OECD 104, EEC Method A.4, Gas saturation method. Batch: 04L950722. Purity: 99.5% *Brodifacoum.*
Conclusion	Vapour pressure: < 0.05 mPa at T = 45°C.
Reliability	4
Acceptability	Not acceptable According to TNsG, vapour pressure is to be studied at 20°C and 25°C. Data are to be expressed as Pa. The provided result lies out of the range recommended for the gas saturation method (E-04 - 1 Pa). Besides, measurements at two different temperatures, at least, are required.
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2006
Materials and methods	**3.2 Vapour pressure 2** OECD 104, EEC Method A.4, vapour pressure balance method from 208 to 218 °C, vapour pressure at 20 and 25 °C estimated by the vapour pressure curve. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	Vapour pressure: 1.9E-21 Pa at 25°C and 2.6E-22 Pa at 20°C (estimated by the vapour pressure curve)
Reliability	2
Acceptability	Acceptable
Remarks	With regard to vacuum conditions before measurements, no information is available in the original study report.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.2.1 Henry's Law Constant 1** Theoretical calculation
Conclusion	Henry's Law Constant: < 1.06E-04
Reliability	4
Acceptability	Not acceptable
Remarks	The Henry's law constant was calculated as reciprocal of the partition coefficient between water and air (Kw) and, as such, turned out to be a pure number. According to TNsG, the Henry's law constant is not adimensional and is to be expressed as $Pa \cdot m^3 \cdot mol^{-1}$, since it depends on the water solubility and vapour pressure of a substance.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.2.1 Henry's Law Constant 2** Theoretical calculation from vapour pressure at 20°C (2.6E-22 Pa, as estimated by the vapour pressure curve) and water solubility at pH 7 and 20°C (5.80E-05 g/l)
Conclusion	Henry's Law Constant: 2.35E-18 Pa m^3 mol^{-1}.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	**3.3.1 Physical state** Visual assessment under natural light on a white background at approximately 20°C. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	Off-white fine powder at 20°C and atmospheric pressure.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of _apporteur member state*
Conclusion	*Discuss if deviating from view of _apporteur member state*
Reliability	*Discuss if deviating from view of _apporteur member state*
Acceptability	*Discuss if deviating from view of _apporteur member state*
Remarks	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	**3.3.1 (a) Dustiness and particle size distribution** *Dustiness:* determined by storing the test material as CIPAC MT46.1, then using a method based on CIPAC MT34. Batch: 69806502. Purity: 99.7% *Brodifacoum.* *Particle size distribution:* data acquired by a procedure designed to comply with requirements of the OECD guideline 110. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	*Dustiness:* White powder with a few clumps and no signs of caking after storage at 54 ± 2°C for 14 days and under a pressure of 25 g/cm^2 . None of the test material became an airborne dust after it was blown with a light jet of nitrogen. *Particle size distribution:* Proportion of test material having an inhalable particle size less than 100 µm = 14.8% (sieve). Proportion of test material having a thoracic particle size less than 10.0 µm = 0.998% (cascade impactor). Proportion of test material having a respirable particle size less than 5.5 µm = 0.0814% (cascade impactor).
Reliability	1
Acceptability	Acceptable
Remarks	No study on dustiness and particle size distribution is required by TNsG to address 3.3.1 requirements.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of _apporteur member state*
Conclusion	*Discuss if deviating from view of _apporteur member state*
Reliability	*Discuss if deviating from view of _apporteur member state*
Acceptability	*Discuss if deviating from view of _apporteur member state*
Remarks	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	**3.3.2 Colour** Visual assessment under natural light on a white background at approximately 20°C. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	Off-white fine powder at 20°C and atmospheric pressure.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of _apporteur member state*
Conclusion	*Discuss if deviating from view of _apporteur member state*
Reliability	*Discuss if deviating from view of _apporteur member state*
Acceptability	*Discuss if deviating from view of _apporteur member state*
Remarks	

Section A3.3.3 Annex Point IIIA *III.2*	Odour	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [X]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The test was not carried out since it was considered that the test material was of a too toxic nature to warrant assessment by nasal inhalation.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Dec 2007	
Evaluation of applicant's justification		
Conclusion	The Applicants' 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		

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date Jan 2006

Materials and methods **3.4 Absorption spectra.**

UV/VIS (1). Original study report not available.

UV/VIS (2). Method: OECD 101. Batch: 00089. Purity: 99.7% *Brodifacoum.*

Conclusion **UV/VIS (2).** The UV/Vis spectra of *Brodifacoum* methanolic solutions obtained at room temperature in the range 220-1100 cm^{-1} were consistent with the accepted structure of *Brodifacoum.* Results are summarized as follows:

Peak maximum Wavelength (nm)	Molar extinction (l $mol^{-1}cm^{-1}$)		
	methanol	10% 1N HCl methanolic solution	10% 1N NaOH methanolic solution
263	-	-	33601
266	37759	40191	-
308	14089	15629	-
312	-	-	16677

Reliability 1

Acceptability Acceptable

Remarks

COMMENTS FROM ...

Date *Give date of comments submitted*

Results and discussion *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

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

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.4 Absorption spectra** **IR (1).** Original study report not available. **IR (2).** Batch: 00089. Purity: 99.7% *Brodifacoum.*
Conclusion	**IR (2).** The IR spectrum obtained at room temperature in the range 4000-400 cm^{-1} was consistent with the accepted structure of *Brodifacoum.*
Reliability	1
Acceptability	Acceptable
Remarks	OECD 101 deals with UV/Vis absorption only.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.4 Absorption spectra** **NMR (1).** Original study report not available. **NMR (2).** Batch: 00089. Purity: 99.7% *Brodifacoum.*
Conclusion	**NMR (2).** Both ^{1}H-NMR and ^{13}C-NMR spectra performed on a sample dissolved in a mix of deuterated acetone and deuterated pyridine were consistent with the accepted structure of *Brodifacoum.* For a correct peak assignment, a bidimensional ^{1}H-NMR spectrum was also performed.
Reliability	1
Acceptability	Acceptable
Remarks	OECD 101 deals with UV/Vis absorption only.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.4 Absorption spectra** **MS.** Batch: 00089. Purity: 99.7% *Brodifacoum.*
Conclusion	The spectrum was consistent with the accepted structure of *Brodifacoum.*
Reliability	1
Acceptability	Acceptable
Remarks	OECD 101 deals with UV/Vis absorption only.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.5 Solubility in water 1** OECD 105, Column elution method with levelling vessel. Batch: 04L950722. Purity: 99.5% *Brodifacoum.*
Conclusion	Solubility in water: <0.1 mg/l, pH: 5.8 – 6.9 (data referring to the collected fractions), temperature: not stated (column temperature at room temperature).
Reliability	4
Acceptability	Not acceptable No data regarding the effect of temperature and pH (5 to 9) on solubility have been submitted.
Remarks	
	COMMENTS FROM …
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of _apporteur member state*
Conclusion	*Discuss if deviating from view of _apporteur member state*
Reliability	*Discuss if deviating from view of _apporteur member state*
Acceptability	*Discuss if deviating from view of _apporteur member state*
Remarks	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2006
Materials and methods	**3.5 Solubility in water 2** EEC Method A.6, Column elution method with re-circulating pump. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	The units quoted by the applicant under the physical-chemical properties table are incorrectly given as mg/L. The actual units are g/L, as follows: at pH5: 5.65E-07 g/L (at 10°C); ≤3.17E-06 g/L (at 20°C); 6.57E-07 g/L (at 30°C) t pH7: 8.16E-06 g/L (at, 10°); 5.80E-05 g/L (at 20°C); 1.60E-05 g/L (at 30°C) At pH9: 6.27E-04 g/L (at 10°C); 1.86E-03 g/L (at 20°C); 7.96E-04 g/L (at 30°C)
Reliability	2 With regard to the preliminary test, deviations from the procedure described in EEC Method A.6 have occurred. These deviations have not affected the quality of the submitted results.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of _apporteur member state*
Conclusion	*Discuss if deviating from view of _apporteur member state*
Reliability	*Discuss if deviating from view of _apporteur member state*
Acceptability	*Discuss if deviating from view of _apporteur member state*
Remarks	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.6 Dissociation constant** QSAR extimation by ACD/I-Lab Web service (ACD/ pKa 8.03). Purity: 99.5% *Brodifacoum.*
Conclusion	pKa = 4.50
Reliability	2
Acceptability	Acceptable
Remarks	The predicted value of pKa was obtained using the ACD/I-Lab Web service (ACD/ pKa 8.03). None of the methods described in OECD 112 was used.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.7 Solubility in organic solvents, including the effect of temperature on solubility 1** OECD 105, Flask method. Batch: not stated. Purity: 99.5% *Brodifacoum*
Conclusion	Solubility at 20°C: toluene 6.10 g/l, ethyl acetate 10.20 g/l, methanol 1.95 g/l, n-hexane <22E-06 g/l, acetone 20.10 g/l, dichloromethane 60.84 g/l.
Reliability	3
Acceptability	Solubility in n-hexane: not acceptable, since the flask method is to be used when solubility is >0.01 g/l. Besides, no data regarding the effect of temperature on solubility have been submitted. Solubility in toluene, ethyl acetate, methanol, acetone, and dichloromethane: not acceptable, as no data regarding the effect of temperature on solubility have been submitted.
Remarks	According to TNsG, results should be stated as mg/l.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2006
Materials and methods	**3.7 Solubility in organic solvents, including the effect of temperature on solubility 2** EEC Method A.6, flask method. MT181 CIPAC method for the assessment of solubility in dichloromethane. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	Agree with the Applicants' version, apart from the data referring to dichloromethane and n-hexane. As for dichloromethane: according to MT181 CIPAC method, results are to be expressed as follows: 29-33 g/l at 10 °C 29-33 g/l at 20 °C 40-50 g/l at 30 °C As for n-hexane: results are to be rejected, since the flask method is not suitable when solubility is <0.01 g/l, as stated in EEC Method A.6. Anyway, no new data are deemed necessary, since TNsG requirements (chapter 3, 3.7) are considered adequately met with solubility data in acetone, dichlorometane, toluene, etyl acetate and methanol.
Reliability	2
Acceptability	Acceptable With regard to the preliminary test, deviations from the procedure described in EEC Method A.6 and MT181 CIPAC method have occurred. These deviations have not affected the quality of the submitted results.
Remarks	According to TNsG, results should be stated as mg/l.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A3.8 Annex Point IIIA *III.2*	Stability in organic solvents used in biocidal products and identity of relevant breakdown products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	As the active substance does not include any organic solvents, according to the TNsG on Data Requirements for the Biocidal Products Directive this study is not necessary. In addition, the biocidal product containing the active substance will be in the form of a wax block that contains no organic solvents.	
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Sept 2005	
Evaluation of applicant's justification		
Conclusion	The Applicants' 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		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.9 Partition coefficient n-octanol/water including effects of pH (5-9) 1** OECD 117, Shake-flask method. Batch: 04L950722. Purity: 99.5% *Brodifacoum.*
Conclusion	LogP = >4.0, temperature: not stated, pH: not stated
Reliability	4
Acceptability	Not acceptable The shake-flask method is applicable to water-soluble substances. No data regarding the effect of temperature and pH on partition coefficient n-octanol/water have been submitted.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2006
Materials and methods	**3.9 Partition coefficient n-octanol/water including effects of pH (5-9) 2** EEC Method A.8, HPLC method. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	Agree with the Applicants' version.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.10 Thermal stability, identity of relevant breakdown products** OECD 113. Batch: 7909101. Purity: 100 % *Brodifacoum*. The active substance content was determined before and after storage (at 54°C in accordance with CIPAC method) using HPLC. The thermal analysis was performed using a combined Thermogravimetric Analyser and a Differential Scanning Calorimeter.
Conclusion	No significant change noted on storage for 14 days at 54°C. *Brodifacoum* was found to be thermally stable below 150°C, with no decomposition or chemical transformation observed.
Reliability	1
Acceptability	Acceptable
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	**3.11 Flammability, including auto-flammability and identity of combustion products - Flammability** EEC method A.10. Batch: LO4272. Purity: > 99 % *Brodifacoum.*
Conclusion	During the preliminary test, the test subtance did not ignite, but melted and decomposed when the bunsen flame was approached. Since the combustion did not propagate, no further test had to be conducted. It can be concluded that *Brodifacoum* is not highly flammable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007
Materials and methods	**3.11 Flammability, including auto-flammability and identity of combustion products – Auto-flammability** EEC method A.16. Batch: 69806502. Purity: 99.7% *Brodifacoum.*
Conclusion	No auto-ignition was observed below the decomposition temperature of the test substance.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A3.12 Annex Point IIA III.3.9	Flash-point	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	Brodifacoum is a solid. Also there is no indication of flammability from flammability studies. On this basis a derogation to perform this study is requested.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Sept 2005	
Evaluation of applicant's justification	Since the flash-point must be provided only for liquids whose vapours can be ignited, no study to address this requirement is necessary for a solid such as *Brodifacoum*.	
Conclusion	The Applicants' 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		

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2006
Materials and methods	**3.13 Surface tension** EEC Method A.5, tensiometer with Platinum-Iridium surface tension ring. Batch: 7909101. Purity: 99.5% *Brodifacoum.*
Conclusion	Surface tension turned out to be 72.9 mN/m at 20°C (9 parts of a *Brodifacoum* solution saturated at 1.0 g/l to 1 part HPLC grade water), but according to EEC method A.5 the test is not necessary for substances such as *Brodifacoum* with solubility in water <1 mg/l.
Reliability	3
Acceptability	Not acceptable The test is for substances which are soluble in water at least at a concentration of 1 mg/l.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A3.14 Annex Point -	Viscosity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Pure active substance is a solid, not soluble in water. On this basis a derogation to perform this study is requested.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Sept 2005	
Evaluation of applicant's justification	Since viscosity data are required only for liquid substances, no study to address this requirement is necessary for a solid such as *Brodifacoum.*	
Conclusion	The Applicants' 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		

Section A3.15 Annex Point IIA 3.11	Explosive properties	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Consideration of structure and physico-chemical properties does not suggest any explosive potential. Widespread experimental and commercial use over many years has not shown any exothermic or explosive activity. On this basis a derogation to perform this study is requested.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Sept 2005	
Evaluation of applicant's justification	On the basis of both strucure and experience in use, no further data are deemed necessary.	
Conclusion	The Applicants' 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		

Section A3.16 Annex Point IIA 3.12	Oxidizing properties	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Consideration of structure and physico-chemical properties does not suggest any oxidising potential. Widespread experimental and commercial use over many years has not shown any signs of oxidising activity. On this basis a derogation to perform this study is requested.	
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Sept 2005	
Evaluation of applicant's justification	On the basis of both strucure and experience in use, no further data are deemed necessary.	
Conclusion	The Applicants' 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		

Section A3.17 Annex Point IIA 3.13	Reactivity towards container material	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Consideration of chemical structure and physico-chemical properties show the compound is stable and largely unreactive. Widespread experimental and commercial use over many years has not shown any signs of reaction with container materials **The pure ac\tive substance does not exist in commerce and is not sold!** **The pure active substance is immediately diluted within manufacturing plant to form 0.25% concentrate.** On this basis a derogation to perform this study is requested.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Nov 2006	
Evaluation of applicant's justification	On the basis of both strucure and experience in use, also considering that the pure *Brodifacoum* is immediately diluted to form a 0.25% concentrate, no further data are deemed necessary.	
Conclusion	The Applicants' 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		

Section A4.1(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1 IIIA-IV.1 *Determination of a.i. content in the technical*

Official use only

1 REFERENCE

1.1	**Reference**	Londyn M (2001) Brodifacoum Five Batch Analysis, Pliva - Lachema a.s., Report No. 01/07/001/PLG
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with letter of access	PelGar International Ltd. Activa s.r.l.
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	None stated
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Not performed
3.1.2	Cleanup	Not performed
3.2	**Detection**	
3.2.1	Separation method	High performance liquid chromatography:

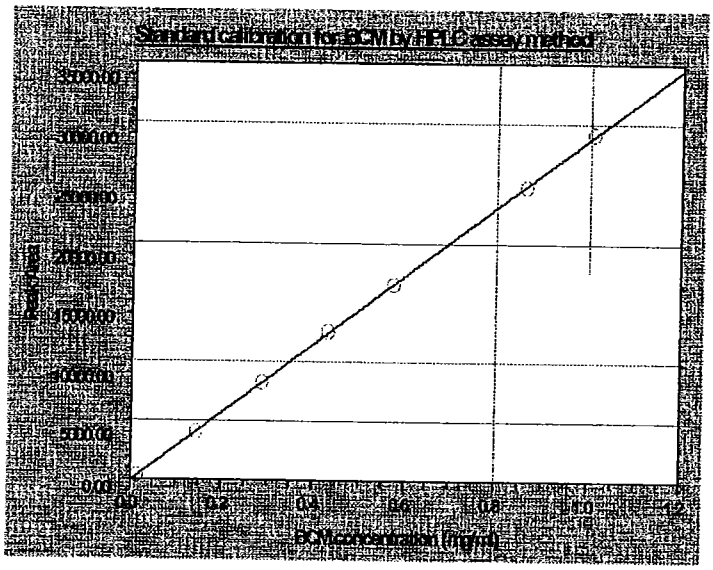
Instrument:	Hewlett Packard 1090 liquid chromatograph
Column:	Zorbax ODS2 (250 mm x 4.5 mm i.d.)
Injection volume:	20.0 µL
Temperature:	ambient
Eluting solvent:	methanol (HPLC grade), distilled water, glacial acetic acid. (94.2: 5: 0.8 v/v/v)
Solvent flow-rate:	1.0 mL/min

3.2.2	Detector	This method of analysis for brodifacoum technical material uses an ultra-violet detector acting at 254 nm
3.2.3	Standard(s)	External standard. 0.0144, 0.144, 0.288, 0.432, 0.576, 0.864, 1.008 mg/mL. The analytical standard was prepared from brodifacoum with a purity of 99.01%, in methanol/dichloromethane (3:2 v/v) solution.
3.2.4	Interfering substance(s)	None identified. The retention time for brodifacoum was 8.30 to 8.34 minutes based on the chromatograms of 5 different batches
3.3	**Linearity**	

Section A4.1(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1 IIIA-IV.1 *Determination of a.i. content in the technical*

3.3.1	Calibration range	14.4 – 1008 µg/mL (mg/L)
3.3.2	Number of measurements	No duplication of measurements, except for the repeatability study below
3.3.3	Linearity	Calibration curve y = 28797.2 *x* + 11.7 r = 0.9999



3.4	**Specificity: interfering substances**	None identified
3.5	**Recovery rates at different levels**	100.65% for both 0.576mg/mL and 0.864mg/mL stds. 101.3% for 1.008mg/mL. Mean recovery = 100.87%
3.5.1	Relative standard deviation	Although not stated in the report or amendment to report, the applicant has calculated the RSD from the 3 different % recoveries given in the amendment to the study report (A 4.1_amendment). Calculated RSD = 0.372% over the range 0.576 mg/mL to 1.008 mg/mL.
3.6	**Limit of determination**	Calculated LOQ at RSD of 30.32% = 0.79µg/mL (see amendment to original report, file name A4.1_amendment.).
3.7	**Precision**	

Section A4.1(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1 IIIA-IV.1 *Determination of a.i. content in the technical*

3.7.1 Repeatability

Repeatability analysis data for brodifacoum batch 01181600 by HPLC.

Sample	% w/w Brodifacoum
1	99.65
2	99.54
3	99.23
4	99.41
5	99.11
6	99.34
7	99.60
8	99.71
9	99.29
10	99.69

Mean = 99.46%
Standard deviation = 0.21
%RSD = 0.21%

X

3.7.2 Independent laboratory validation

No validation has been conducted by an independent laboratory

4 APPLICANT'S SUMMARY AND CONCLUSION

UV detection at 254 nm.

4.1 Materials and methods

The linearity and repeatability of the determination of brodifacoum content in the technical material was performed by HPLC with UV detection, at ambient temperature with a Zorbax OD2 column.

X

4.2 Conclusion

The recovery rates of brodifacoum from blank matrices were determined (see A 4.1_amendment) at different levels were determined. The five batch analysis shows that the range of determined purities was 99.23 to 99.92% w/w, and the linearity and repeatability of the method are good.

X

4.2.1 Reliability

1

X

4.2.2 Deficiencies

No

X

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Dec 2007

Section A4.1(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1 IIIA-IV.1 *Determination of a.i. content in the technical*

Materials and methods	Test material: technical grade *Brodifacoum*, batch numbers 01181600, 02260900, 04071200, 03221000, 01101201. *Brodifacoum* analytical standard 99,01%; batch number 01220101. The determination of *Brodifacoum* content in technical material was performed by HPLC with UV detection at 254 nm on a Zorbax ODS2 column at ambient temperature, with methanol, distilled water, and glacial acetic acid (94.2: 5: 0.8, v/v/v) as mobile phase at a flow rate of 1 ml/min. Quantitation was performed by the external standard method. *Brodifacoum* standard and sample solutions were prepared in methanol/dichloromethane (3:2, v/v) at approximately 1 mg/ml.
Conclusion	The purity of *Brodifacoum* ranged from 99.23 to 99.91% w/w. Specificity: no interfering peaks observed. Linearity: linear over the range 0.0144-1.008 mg/ml (single determinations at 7 concentration levels), with r = 0.9999. Recovery rates determined at 0.576, 0.867, and 1.008 mg/ml: 100.65%, 100.65%, and 101.30%, respectively (mean recovery: 100.87%, RSD% = 0.372%). Repeatability demonstrated by replicate analysis (n = 10) of one sample from batch 01181600 (mean purity: 99.46% w/w; RSD% = 0.21%). LOQ: 0.79 µg/ml.
Reliability	2
Acceptability	Acceptable
Remarks	Clarification for sub-section 3.7.1: the data presented in the table have been obtained on the same sample (from batch 01181600), which has been injected 10 times.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A4.1(2) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Identification of impurities in Brodifacoum

This information is considered commercially sensitive. Please, refer to the *Confidential Annex_Active Substance*

Section A4.1(3) Analytical Methods for Detection and Identification

Annex Point IIA4.1 IIIA-IV.1

Determination of relative isomer content in technical material

This information is considered commercially sensitive. Please, refer to the *Confidential Annex_Active Substance*

Section A4.2(a) Methods of Identification and Analysis in Soil

Annex Point IIA4.2

IIIA-IV.1

Official use only

1 REFERENCE

4.3	**Reference**	Morlacchini M (2005) Residues Determination of Brodifacoum, Difenacoum and Bromadiolone in Soil, CERZOO, Report No. CZ/05/002/ACTIVA/SOIL
4.4	**Data protection**	Yes
4.4.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
4.4.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

5 GUIDELINES AND QUALITY ASSURANCE

5.1	**Guideline**	96/23/EC
5.2	**GLP**	Yes
5.3	**Deviations**	No

6 MATERIALS AND METHODS

6.1	**Preliminary treatment**	
6.1.1	Enrichment	40.0g of soil is weighted into a series of 500 mL sovirel and 100 mL of 50% acetone/ 50% chloroform extraction solution is added. The soveril is closed and shaken for a minimum of 30 minutes at a rate of 180 movements/ minute on an automatic shaker. The extraction solution is filtered and the another 100 mL quantity of extraction solution is added and shaken again for a minimum of 30 minutes. This process is repeated a third time with 50 mL of extraction solution. The three filtered solutions are combined and evaporated with a rotavapor to 200mm Hg.
6.1.2	Cleanup	The recovery is made with 10 mL of acetone and purified in a glass column with 6 g of florisil and 1 g of anhydrous sodium sulphate. The solution is washed with 40 mL of acetone and evaporated with nitrogen. 1 mL of methanol:water (1:1) is added and centrifuged for 5 minutes at 2000 rpm and the final solution is transferred ready for injection into HPLC.
6.2	**Detection**	
6.2.1	Separation method	HPLC UV-VIS Column type 450x4,60 mm/S/N 224016-2 Volume and type of injection 20µl with autosampler Temp of chiller 25°C Λ of detection 264nm with a window of 4 nm and a reference to 360 with a window of 100 nm

X

Mobile phase was water with 0.1% formic acid and acetonitrile with the following solvent gradient program:

Time (m n)	Flow(mL/min)	Water(0.1%) formic acid %)	Acetonitrile
0.00	1.000	50.0	50.0
5.00	1.000	50.0	50.0
10.00	1.000	10.0	90.0
15.00	1.000	10.0	90.0
18.00	1.000	50.0	50.0
23.00	1.000	50.0	50.0

6.2.2 Detector — Diode array detector (DAD)

6.2.3 Standard(s) — Brodifacoum technical grade Lot Nº L14354

6.2.4 Interfering substance(s) — Non detected. Retention time of brodifacoum was 13.59 minutes.

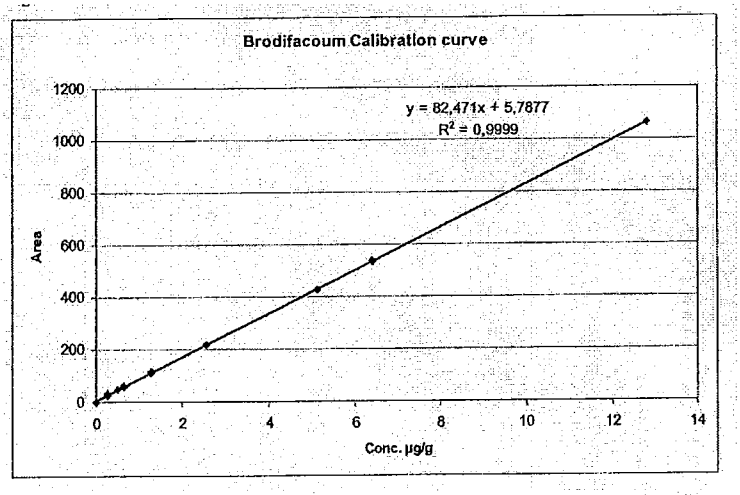
6.3 Linearity

6.3.1 Calibration range — 0.256, 0.512, 0.64, 1.28, 2.56, 5.12, 6.4 and 12.8 µg mL^{-1}

(Conc. Equiv. in soil. 0.006, 0.013, 0.016, 0.032, 0.064, 0.128, 0.160 and 0.320 µg mL^{-1}) X

6.3.2 Number of measurements — 2 measurements for each fortification level X

6.3.3 Linearity



Y = 82.471 x +5.7877

R^2= 0.9999

Section A4.2(a) Methods of Identification and Analysis in Soil

Annex Point IIA4.2

IIIA-IV.1

6.4 Specificity: interfering substances

None detected

A confirmation method was carried out (see A4.2a_amendment) to support the HPLC/DAD method. The method was LC-MS. The method gave a retention time for brodifacoum of 16.30 minutes compared to 13.59 min in other method.

X

6.5 Recovery rates at different levels

X

Sample name	Conc. Add. µg/mL	Conc. equiv. in soil µg/g	Area	Conc. found µg/mL	Recovery %
Rec 1	0.64	0.016	53.4	0.62	97.0
Rec 2	0.64	0.016	50.6	0.59	91.6
Rec 3	0.64	0.016	50.2	0.58	90.6
Rec 4	0.64	0.016	52.1	0.60	94.3
Rec 11	2.56	0.064	187.5	2.31	90.3
Rec 12	2.56	0.064	184.5	2.27	88.8
Rec 13	2.56	0.064	186.5	2.30	89.8
Rec 14	2.56	0.064	187.7	2.31	90.4
Rec 21	6.40	0.160	472.9	5.66	91.2
Rec 22	6.40	0.160	495.5	5.94	89.8
Rec 23	6.40	0.160	495.7	5.94	90.5
Rec 24	6.40	0.160	495.0	5.93	93.9
Blank 1	0.00	0.000	n.r.	0.00	
Blank 2	0.00	0.000	n.r.	0.00	
Blank 3	0.00	0.000	n.r.	0.00	
Blank 2	0.00	0.000	n.r.	0.00	
				Average	91.5
				std. Dev.	2.4

6.5.1 Relative standard deviation

2.15 %

6.6 Limit of determination

LOQ = 0.016 µg/g in soil
(this value was assigned by the Competent Authority based on lowest validated level being for 0.64 µg/mL)

Author of the study states LOQ = 0.006 µg/g in soil, but this is effectively the lowest standard concentration, based on the calculated LOQ being below this standard (see A4.2a_amendment to final report)

In the amendment to the report (A4.2a_amendment) a matrix-matched calibration curve was done covering the range 0.256 -12.8 µg/mL in which an LOQ of 0.016 µg/g was stated.

6.7 Precision

6.7.1 Repeatability

No data

6.7.2 Independent laboratory validation

No data

Section A4.2(a) Methods of Identification and Analysis in Soil

Annex Point IIA4.2

IIIA-IV.1

		7 APPLICANT'S SUMMARY AND CONCLUSION	
7.1	**Materials and methods**	The test method for brodifacoum determination in soil is based on extraction from blank and spiked soil (40.0 g) using chloroform:acetone 1:1 solution. The extract is concentrated by rotary evaporator and recovery with acetone prior to purification with a florisil-sodium sulphate column. The elutes are dried and reconstituted with methanol:water 1:1 and analysed by HPLC UV-VIS	X
7.2	**Conclusion**	The limit of detection, limit of quantisation, recovery rates and linearity suggest that the method is valid for identification and analysis of Brodifacoum in soil	X
7.2.1	Reliability	1	X
7.2.2	Deficiencies	Yes	X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date Dec 2007

Materials and methods

Brodifacoum technical grade from Activa, batch no L14354.

40.0 g soil samples (soil properties reported in paragraph 8.4 in the original report).

Brodifacoum was isolated from spiked soil samples by a three-step extraction with 50% chloroform/ 50% acetone. After filtration, the extracts were combined and evaporated by rotary evaporator. After recovery with acetone, purification was accomplished with a florisil-sodium sulphate column. The eluates were evaporated with nitrogen, then 1 ml of methanol/water (1:1) was added and centrifuged at 200 rpm for 5 min before HPLC/DAD analysis.

HPLC was performed at 25°C on a Synergy 4u Fusion RP 80A Phenomenes column. Mobile phase: water with 0.1% formic acid and acetonitrile according the following gradient program:

Time (min)	Flow rate (ml/min)	Water with 0.1% formic ac. (%)	Acetonitrile (%)
0.00	1.000	50.0	50.0
5.00	1.000	50.0	50.0
10.00	1.000	10.0	90.0
15.00	1.000	10.0	90.0
18.00	1.000	50.0	50.0
23.00	1.000	50.0	50.0

Section A4.2(a) Methods of Identification and Analysis in Soil

Annex Point IIA4.2

IIIA-IV.1

Conclusion	Specificity – the analysis of an untreated soil sample showed that there are no interfering peaks. Since the proposed HPLC/DAD method cannot be considered highly specific, an additional confirmatory method in compliance with SANCO/3029/99 rev.4 11/07/00 and SANCO/825/00 rev.7 17/03/040 is necessary. An LC/MS method is outlined in the first amendment to the original study report. Only experimental conditions (including *Brodifacoum* retention time) are available. Linearity (*Brodifacoum* standard solutions in acetone) – linear over the range 0.256-12.8 µg/ml (equivalent to 0.006-0.32 mg/kg in soil) with R^2 = 0.9999. Single determinations at 8 concentration levels. Accuracy – three fortification levels investigated (0.016, 0.064, 0.16 mg/kg in soil), 4 replicates (instead of 5) per level, overall mean recovery = 92.9% with RSD% = 2.2% (based on the original study report). Repeatability – no information provided in sub-section 3.7.1. Only RSD% based on overall mean recovery is available in sub-section 3.5.1. LOQ = 0.016 mg/kg in soil (lowest validated concentration level).
Reliability	3
Acceptability	Not acceptable. The developed HPLC/DAD method involves the use of chloroform for the extraction of *Brodifacoum* from spiked samples (sub-section 3.3.1). According to SANCO/3029/99 rev. 4 11/07/00, chloroform must not be used and must be substituted by less harmful solvents. According to the first amendment to the original study report, also a matrix-matched calibration curve has been obtained in the range 0.256-12.8 µg/ml (equivalent to 0.006-0.32 mg/kg in soil) and matrix effects have turned out to be negligible. Anyway, no evidence (in particular, the new matrix-matched calibration curve equation and correlation coefficient) has been provided in support of this statement. Recovery rates calculation has been performed on the basis of the calibration curve obtained with *Brodifacoum* standard solutions in water. Only 4 replicates per level have been considered. As for repeatability, only RSD% based on overall mean recovery is available in sub-section 3.5.1. Besides, recovery rates (including raw data) in sub-section 3.5 are different from those in table 5 page 14/41 of the original study report. The LC/MS method summitted for confirmation is not validated. Neither raw data nor experimental results have been provided.

Section A4.2(a) Methods of Identification and Analysis in Soil

Annex Point IIA4.2

IIIA-IV.1

Remarks	The proposed method is to be improved: Chloroform must not be used and must be substituted by less harmful solvents, as required by SANCO/3029/99 rev. 4 11/07/00. Linearity data of the matrix-matched calibration curve are required. Recovery rates assessment is to be accomplished on the basis of the matrix-matched calibration curve. Five replicates per level should be considered. Mean recovery rate and RSD% are to be calculated for each fortification level. A fully-validated confirmatory method is required.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A4.2(b) Annex IIB, IV.4.2.	Analytical methods in Air	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [X]	**Other justification** [ ]	
Detailed justification:	As the active substance has a vapour pressure of <0.05 mPa (Section A3.2, Annex Point IIA, III.3.2.) it is considered to be of low volatility and therefore, in accordance with the TNsG on Data Requirements for the Biocidal Products Directive, analytical methods in air are not required.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Dic 2007	
Evaluation of applicant's justification	The Applicants's justification can be considered acceptable, provided that the vapour pressure value quoted above is replaced by 1.9 E-21Pa (25°C).	
Conclusion	Since *Brodifacoum* is a non-volatile substance and is intended to be used only in solid formulations, a method for the analysis of residues in air is not required.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA4.2 *of Brodifacoum in water*

Official use only

1 REFERENCE

7.3	**Reference**	Martinez PM (2005) Brodifacoum Technical: Validation of the Analytical Method for the Determination of the Residues in Dinking, Ground and Surface Waters, ChemService S.r.l, Report No. CH-289/2005
7.4	**Data protection**	Yes
7.4.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
7.4.2	Companies with letter of access	PelGar International Ltd. Activa s.r.l.
7.4.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

8 GIUDELINE AND QUALITY ASSURANCE

8.1	**Guidelines**	EEC guideline SANCO/3030/99 rev. 4 Directive 91/414/EEC	X
8.2	**GLP**	Yes	
8.3	**Deviations**	No	

9 MATERIALS AND METHODS

9.1	**Preliminary treatment**	
9.1.1	Enrichment	1 l of water is extracted with 3 x 50 mL of dichloromethane and the extract evaporated to dryness by rotary evaporation at 40° C
9.1.2	Cleanup	The residue is redissolved in with 0.5mL of methanol
9.2	**Detection**	
9.2.1	Separation method	Separation by HPLC/MS. HPLC conditions:

HPLC/MS instrument	:	Thermo Finnigan LCQ Advantage
HPLC Column	:	ChemService code No. 131
Teknokroma or equivalent		Tracer Excel 120 ODSB 5 µm, 50 x 2.1 mm i.d.
HPLC pre-column	:	C18 (ODS) 4.0 x 3.0 mm
Interface	:	Electron spray ionization (ESI), negative ions
Detector	:	Mass (Scan in SIM and SRM mode)
Column temperature	:	room temperature
Eluent A	:	water
Eluent B	:	methanol
Eluent C	:	water with 10 mM of TEA
Gradient	:	A:B:C 65:30:5 hold 1 minute from A:B:C 65:30:5 to 0:95:5 in 5 minutes, hold 10 minutes from A:B:C 0:95:5 to 65:30:5 in 2 minutes, hold 10 minutes
Eluent flow	:	0.2 mL/min
Volume of injection	:	10 µL
Brodifacoum ret. Time	:	ca. 12 minutes
Total Analysis Time	:	30 minutes

9.2.2	Detector	DAD detector with an LCQ advantage ionic trap mass detector	X

Mass scan parameters

The mass scan parameters are reported in the following table.

Compound	Ion mode	Collision Energy (%)	m/z Molecular Ion (SIM)	m/z Daughter (SRM)
Brodifacoum	ESI –	60	521	187

9.2.3	Standard(s)	Brodifacoum standard batch no L03950722; purity 99.5%. Stock solution 1 mg/mL in acetone, diluted and working standard solutions in methanol
9.2.4	Interfering substance(s)	None identified.
9.3	**Linearity**	
9.3.1	Calibration range	Brodifacoum standard range: 0.1 – 0.5 µg/mL
9.3.2	Number of measurements	4 measurements of each standard
9.3.3	Linearity	The range tested was from 0.1 to 0.5 $\mu g mL^{-1}$. SIM mode and SRM mode: r = 0.99523 and 0.00747 respectively
9.4	**Specificity: interfering substances**	None specified
9.5	**Recovery rates at different levels**	

X

TABLE 4 Drinking water: recovery at fortification level L1 (0.05 µg/l

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	0.50	1.0	n.d.	-
Blank 2	0	-	0.50	1.0	n.d.	-
Spike L1-1	11597120	0.09	0.50	1.0	0.0426	85.15
Spike L1-2	11864720	0.09	0.50	1.0	0.0436	87.12
Spike L1-3	12407620	0.09	0.50	1.0	0.0456	91.11
Spike L1-4	12535050	0.09	0.50	1.0	0.0460	92.04
Spike L1-5	11365790	0.08	0.50	1.0	0.0417	83.46
				Mean value :	**0.044**	**87.8**
				Standard deviation (S.D.) :	0.0017	3.32
				Coefficient of Variation (C.V. %) :	*3.8%*	*3.8%*

TABLE 5 Drinking water: recovery at fortification level L2 (0.5 µg/L)

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	1.50	1.0	n.d.	-
Blank 2	0	-	1.50	1.0	n.d.	-
Spike L2-1	24604390	0.27	1.50	1.0	0.4022	80.45
Spike L2-2	24492340	0.27	1.50	1.0	0.3995	79.90
Spike L2-3	24530560	0.27	1.50	1.0	0.4004	80.09
Spike L2-4	24033490	0.26	1.50	1.0	0.3883	77.67
Spike L2-5	27425030	0.31	1.50	1.0	0.4709	94.18
				Mean value :	**0.412**	**82.5**
				Standard deviation (S.D.) :	0.0297	5.94
				Coefficient of Variation (C.V. %) :	*7.2%*	*7.2%*

* corrected for mean control residue value
(1) Quantification with the linear calibration curve for fortified samples L2 and with the lowest standard calibration level for fortified samples L1 and for control samples.
n.d. not detected, lower than L.O.D. (0.025 µg/L)

TABLE 6 Drinking water: recovery at fortification level L3 (5.0 µg/l

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	10.00	1.0	n.d.	-
Blank 2	0	-	10.00	1.0	n.d.	-
Spike L3-1	31481060	0.38	10.00	1.0	3.7976	75.95
Spike L3-2	34843780	0.43	10.00	1.0	4.3433	86.87
Spike L3-3	30368010	0.36	10.00	1.0	3.6169	72.34
Spike L3-4	37213640	0.47	10.00	1.0	4.7279	94.56
Spike L3-5	32373260	0.39	10.00	1.0	3.9424	78.85
				Mean value :	**4.086**	**81.7**
				Standard deviation (S.D.) :	0.4005	8.01
				Coefficient of Variation (C.V. %) :	*9.8%*	*9.8%*

TABLE 7 Drinking water: recovery at fortification level L4 (50 µg/L

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	125.00	1.0	n.d.	-
Blank 2	0	-	125.00	1.0	n.d.	-
Spike L4-1	34627770	0.43	125.00	1.0	53.8529	107.71
Spike L4-2	33833760	0.42	125.00	1.0	52.2422	104.48
Spike L4-3	26579380	0.33	125.00	1.0	41.5835	83.17
Spike L4-4	34232860	0.42	125.00	1.0	53.0518	106.10
Spike L4-5	29620930	0.35	125.00	1.0	43.6963	87.39
				Mean value :	**48.885**	**97.8**
				Standard deviation (S.D.) :	5.1681	10.34
				Coefficient of Variation (C.V. %) :	*10.6%*	*10.6%*

* corrected for mean control residue value
(1) Quantification with the linear calibration curve for fortified samples L3 and L4 and with the lowest standard calibration level for control samples.
n.d. not detected, lower than L.O.D. (0.025 µg/L)

TABLE 10 Ground water: recovery at fortification level L1 (0.05 µg/

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	0.50	1.0	n.d.	-
Blank 2	0	-	0.50	1.0	n.d.	-
Spike L1-1	12147077	0.10	0.50	1.0	0.0503	100.61
Spike L1-2	10081474	0.08	0.50	1.0	0.0418	83.50
Spike L1-3	10611774	0.09	0.50	1.0	0.0439	87.90
Spike L1-4	9711174	0.08	0.50	1.0	0.0402	80.44
Spike L1-5	12091538	0.10	0.50	1.0	0.0501	100.15
				Mean value :	**0.045**	**90.5**
				Standard deviation (S.D.) :	0.0042	8.40
				Coefficient of Variation (C.V. %) :	*9.3%*	*9.3%*

TABLE 11 Ground water: recovery at fortification level L2 (0.5 µg/L

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	1.50	1.0	n.d.	-
Blank 2	0	-	1.50	1.0	n.d.	-
Spike L2-1	27918220	0.31	1.50	1.0	0.4691	93.83
Spike L2-2	25800448	0.28	1.50	1.0	0.4228	84.56
Spike L2-3	28042888	0.31	1.50	1.0	0.4719	94.37
Spike L2-4	25352812	0.28	1.50	1.0	0.4130	82.60
Spike L2-5	27743254	0.31	1.50	1.0	0.4653	93.06
				Mean value :	**0.448**	**89.7**
				Standard deviation (S.D.) :	0.0252	5.04
				Coefficient of Variation (C.V. %) :	*5.6%*	*5.6%*

* corrected for mean control residue value
(1) Quantification with the linear calibration curve for fortified samples L2 and with the lowest standard calibration level for fortified samples L1 and for control samples.
n.d. not detected, lower than L.O.D. (0.025 µg/L).

TABLE 12 Ground water: recovery at fortification level L3 (5.0 µg/l

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	10.00	1.0	n.d.	-
Blank 2	0	-	10.00	1.0	n.d.	-
Spike L3-1	37235208	0.45	10.00	1.0	4.4867	89.73
Spike L3-2	33686808	0.40	10.00	1.0	3.9691	79.38
Spike L3-3	38200432	0.46	10.00	1.0	4.6275	92.55
Spike L3-4	33941832	0.40	10.00	1.0	4.0063	80.13
Spike L3-5	38906892	0.47	10.00	1.0	4.7306	94.61
				Mean value :	**4.364**	**87.3**
				Standard deviation (S.D.) :	0.3171	6.34
				Coefficient of Variation (C.V. %) :	7.3%	7.3%

TABLE 13 Ground water: recovery at fortification level L4 (50 µg/L

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	125.00	1.0	n.d.	-
Blank 2	0	-	125.00	1.0	n.d.	-
Spike L4-1	34226360	0.40	125.00	1.0	50.5977	101.20
Spike L4-2	31649700	0.37	125.00	1.0	45.8994	91.80
Spike L4-3	32625908	0.38	125.00	1.0	47.6794	95.36
Spike L4-4	28757250	0.33	125.00	1.0	40.6252	81.25
Spike L4-5	31989852	0.37	125.00	1.0	46.5196	93.04
				Mean value :	**46.264**	**92.5**
				Standard deviation (S.D.) :	3.2490	6.50
				Coefficient of Variation (C.V. %) :	7.0%	7.0%

* corrected for mean control residue value
(1) Quantification with the linear calibration curve for fortified samples L3 and L4 and with the lowest standard calibration level for control samples.
n.d. not detected, lower than L.O.D. (0.025 µg/L)

TABLE 16 Surface water: recovery at fortification level L1 (0.05 µg

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	0.50	1.0	n.d.	-
Blank 2	0	-	0.50	1.0	n.d.	-
Spike L1-1	17870600	0.12	0.50	1.0	0.0607	121.46
Spike L1-2	17060160	0.12	0.50	1.0	0.0580	115.95
Spike L1-3	23033540	0.16	0.50	1.0	0.0783	156.55
Spike L1-4	31036116	0.21	0.50	1.0	0.1055	210.94
Spike L1-5	18286370	0.12	0.50	1.0	0.0621	124.28
				Mean value :	**0.060**	**120.6**
				Standard deviation (S.D.) :	0.0017	3.46
				Coefficient of Variation (C.V. %) :	*2.9%*	*2.9%*

TABLE 17 Surface water: recovery at fortification level L2 (0.5 µg/L

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	1.50	1.0	n.d.	-
Blank 2	0	-	1.50	1.0	n.d.	-
Spike L2-1	26007968	0.27	1.50	1.0	0.4013	80.26
Spike L2-2	25844424	0.27	1.50	1.0	0.3975	79.50
Spike L2-3	27655084	0.29	1.50	1.0	0.4394	87.88
Spike L2-4	27373674	0.29	1.50	1.0	0.4329	86.58
Spike L2-5	27687590	0.29	1.50	1.0	0.4402	88.03
				Mean value :	**0.422**	**84.5**
				Standard deviation (S.D.) :	0.0189	3.77
				Coefficient of Variation (C.V. %) :	*4.5%*	*4.5%*

* corrected for mean control residue value
(1) Quantification with the linear calibration curve for fortified samples L2 and with the lowest standard calibration level for fortified samples L1 and for control samples.
n.d. not detected, lower than L.O.D. (0.025 µg/L)
The values in the grey cells were not considered in the calculation (Dixon Test)

TABLE 18 Surface water: recovery at fortification level L3 (5.0 µg/L

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	10.00	1.0	n.d.	-
Blank 2	0	-	10.00	1.0	n.d.	-
Spike L3-1	40639640	0.49	10.00	1.0	4.9321	98.64
Spike L3-2	34167512	0.39	10.00	1.0	3.9338	78.68
Spike L3-3	36214112	0.42	10.00	1.0	4.2495	84.99
Spike L3-4	35829632	0.42	10.00	1.0	4.1902	83.80
Spike L3-5	37998704	0.45	10.00	1.0	4.5247	90.49
				Mean value :	**4.366**	**87.3**
				Standard deviation (S.D.) :	0.3397	6.79
				Coefficient of Variation (C.V. %) :	7.8%	7.8%

TABLE 19 Surface water: recovery at fortification level L4 (50 µg/L)

Code Number	A_S	C_S (1) (µg/mL)	V_S (mL)	V_W (L)	BDF (µg/L)	Recovery (%) *
Blank 1	0	-	125.00	1.0	n.d.	-
Blank 2	0	-	125.00	1.0	n.d.	-
Spike L4-1	37804220	0.45	125.00	1.0	56.1844	112.37
Spike L4-2	38999050	0.47	125.00	1.0	58.4880	116.98
Spike L4-3	37233090	0.44	125.00	1.0	55.0833	110.17
Spike L4-4	37195208	0.44	125.00	1.0	55.0103	110.02
Spike L4-5	35787232	0.42	125.00	1.0	52.2958	104.59
				Mean value :	**55.412**	**110.8**
				Standard deviation (S.D.) :	2.0019	4.00
				Coefficient of Variation (C.V. %) :	3.6%	3.6%

* corrected for mean control residue value
(1) Quantification with the linear calibration curve for fortified samples L3 and L4 and with the lowest standard calibration level for control samples.
n.d. not detected, lower than L.O.D. (0.025 µg/L)

9.5.1 Relative standard deviation — See tables above (3.5)

9.6 Limit of determination

The limit of quantification (LOQ) of this method is defined as the lowest validated level, i.e. 0.1 µg/mL, corresponding to 0.05 µg/L in the water matrix samples.

The limit of detection (LOD) of this method is defined as 50% of the lowest validated level, i.e. 0.05 µg/mL corresponding to 0.025 µg/L in the water matrix sample.

9.7 Precision

9.7.1 Repeatability

Drinking water: Repeatability and recovery tests.

X

Linear calibration with working standard solutions

Brodifacoum (BDF) (m/z 521)	Standard 1 0.1 µg/mL (peak area)	Standard 2 0.3 µg/mL (peak area)	Standard 3 0.5 µg/mL (peak area)
1st injection	13021630	27784920	39829660
2nd injection	12599960	27066220	37622950

3rd injection	14099590	29509090	39733640
4th injection	15698900	27853220	39086490
5th injection	12675070	26850450	35063780
Mean	13619030	27812780	38267304
SD	1169893	934164	1785493
CV (%)	8.59%	3.36%	4.67%
	Parameter m (slope)	Parameter q (intercept)	Parameter R (correlation
	61620685	8080166	0.99619

Ground water: Repeatability and recovery Tests.

Linear calibration with working standard solutions

Brodifacoum (BDF) (m/z 521)	Standard 1 0.1 µg/mL (peak area)	Standard 2 0.3 µg/mL (peak area)	Standard 3 0.5 µg/mL (peak area)
1st injection	12247585	30294040	45513430
2nd injection	11150964	30229538	39082900
3rd injection	12412624	29687734	37482248
4th injection	12480904	28040110	35897610
Mean	12073019	29862856	39494047
SD	539064	910144	3653208
CV (%)	4.47%	3.08%	9.25%
	Parameter m (slope)	Parameter q (intercept)	Parameter R (correlation
	68552569	6477536	0.98757

Surface water: Repeatability and recovery tests.

Linear calibration with working standard solutions

Brodifacoum (BDF) (m/z 521)	Standard 1 0.1µg/mL (peak area)	Standard 2 0.3 µg/mL (peak area)	Standard 3 0.5 µg/mL (peak area)
1st injection	11549030	27759496	41925412
2nd injection	15276300	29649486	43270552
3rd injection	14769390	28297448	40171696
4th injection	14774080	29137776	38035088
5th injection	14034550	30037916	39838400
Mean	14713580	28976424	40648230
SD	442863	842346	1801066
CV (%)	3.01%	2.91%	4.43%
	Parameter m (slope)	Parameter q (intercept)	Parameter R (correlation
	64836624	8661757	0.99834

9.7.2 Independent laboratory validation — None

10 APPLICANT'S SUMMARY AND CONCLUSION

10.1	**Materials and methods**	Directive 91/414/EEC EEC guideline SANCO/3030/99 rev. 4	X
10.2	**Conclusion**	The analytical method was shown to be specific for brodicacoum residues in each type of water sample. The range tested was from 0.1 to 0.5 µg/mL, corresponding to concentrations from 0.05 to 0.25 µg/L in the water samples and was found to be linear. For precision, the SANCO guideline requires a RSD% lower than 20% for each fortification level; therefore the precision of the analytical method can be considered acceptable. For accuracy, the SANCO guideline requires individual recovery values in the range 70-100% with a mean value 80-100% at each level; some deviation obtained can be accepted because of the very low water solubility of the test substance and the very particular and complex method of analysis; therefore the accuracy of the analytical method can be considered acceptable.	X
10.2.1	Reliability	1	X
10.2.2	Deficiencies	No	X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date Dec 2007

Materials and methods

Type of water: drinking (natural mineral water Fiuggi), ground (sampled from Well SB1 I.Pi.Ci.), surface (sampled at Desenzano, Italy, from lake Garda).

Brodifacoum technical product, batch no L14354; purity 99.9%.

Brodifacoum analytical standard, batch no L03950722; purity 99.5%.

1 l of water is extracted with 3x50 ml of dichloromethane. The extract is evaporated to dryness by rotary evaporator at 40°C. The residue is redissolved in 0.5 ml of methanol for HPLC/MS/MS analysis.

HPLC was performed on a C18 pre-column and a Tracer Excel 120 ODSB column at room temperature, with water (eluent A), methanol (eluent B), and water with 10 mM triethylamine (eluent C) as mobile phase at a flow rate of 0.2 ml/min according to the following gradient program:

A:B:C, 65:30:5 hold 1 min.
A:B:C, from 65:30:5 to 0:95:5 in 5 min, hold 10 min.
A:B:C, from 0:95:5 to 65:30:5 in 2 min, hold 10 min.

Interface: electron spray ionisation, negative ions.

Detector: mass (scan in SIM and SRM mode). Mass scan parameters in the table below:

compound	Ion mode	Collision energy (%)	m/z molecular ion (SIM)	m/z daughter (SRM)
Brodifacoum	ESI	60	521	187

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA4.2 *of Brodifacoum in water*

Conclusion

Agree with Applicants's version.

Specificity: the submitted HPLC/MS/MS method can be considered highly specific.

Linearity: 0.1-0.5 µg/ml (0.05-0.25 µg/l in water), 4 determinations at 5 concentration levels. R = 0.995 (SIM mode), R = 0.997 (SRM mode).

Accuracy and precision:

Sample	Fortification level	Recovery rate (%)		
		Range	Mean	R D %
Drinking water	0.05 µg/l	83.5-92.0	87.8	3.8
	0.5 µg/l	77.7-94.1	82.5	7.2
	5.0 µg/l	72.3-94.6	81.7	9.8
	50 µg/l	83.2-107.7	81.7	10.6
Ground water	0.05 µg/l	80.4-100.6	90.5	9.3
	0.5 µg/l	82.6-94.4	98.7	5.6
	5.0 µg/l	80.1-94.6	87.3	7.3
	50 µg/l	81.3-101.2	92.5	7.0
Surface water	0.05 µg/l	116-124.3	120.6	2.9
	0.5 µg/l	79.5-88.0	84.5	4.5
	5.0 µg/l	78.7-98.6	87.3	7.8
	50 µg/l	104.6-112	110.8	3.6

LOQ: 0.05 µg/l for drinking and ground water; 0.5 µg/l for surface water (since recovery rates and mean recovery rate at 0.05 µg/l are out of the recommended range).

Reliability 2

Acceptability Acceptable.

Remarks

The following deficiencies/mistakes regarding the information reported in the study summary have been observed:
2.1: the SANCO document quoted in this sub-section is related to Technical Material and Formulations. The guidance documents for generating and reporting residue analytical methods are SANCO/3029/99 rev.4 11/07/00 and SANCO/825/00 rev.7 17/03/04.
3.2.2: no DAD detector has been used.
3.3.3: linearity data (including slope and intercept of the curves) are available, but have been erroneously presented in sub-section 3.7.1.
3.7.1: repeatability data related to recovery rates are available in sub-section 3.5.

COMMENTS FROM ...

Date *Give date of comments submitted*

Results and discussion *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

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*

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA4.2 *of Brodifacoum in water*

Remarks

Section A4.2(d)(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Methods of analysis in human and animal body tissues

			Official use only
		1 REFERENCE	
10.3	**Reference**	Turnbull G (2005) Validation of Analytical Methodology to Determine Rodenticides in Food Matrices, Central Science Laboratory, Report No. PGD-180	
10.4	**Data protection**	Yes	
10.4.1	Data owner	Brodifacoum and Difenacoum Task Force	
10.4.2	Companies with letter access	PelGar International Ltd. Activa s.r.l.	
10.4.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		11 GUIDELINES AND QUALITY ASSURANCE	
11.1	**Guideline**	SANCO/825/00 rev. 6	
11.2	**GLP**	Yes	
11.3	**Deviations**	No	
		12 MATERIALS AND METHODS	
12.1	**Preliminary treatment**		X
12.1.1	Extraction	Meat Brodifacoum is extracted from meat by shaking with a mixture of dichloromethane and acetone. The filtered extract is purified by GPC prior to determination by LC-MS-MS.	
12.1.2	Cleanup	Gel permeation chromatography or SPE catridge.	
12.2	**Detection**		
12.2.1	Separation method	HPLC, Phenomenex Luna 150 mm x 2 mm i.d. column packed with 5 µm Phenyl-Hexyl with mobile phase: 10 mM ammonium acetate and methanol.	X
12.2.2	Detector	LC-MS-MS (primary ion m/z: 79)	
12.2.3	Standard(s)	External standard	X
12.2.4	Interfering substance(s)	Analysis of control samples demonstrated that there were no substances which interfered with the detection of Brodifacoum. There were no chromatographic peaks above 30% of the LOQ at the retention time of Brodifacoum.	
12.3	**Linearity**		
12.3.1	Calibration range	0.03 to 1.2 µg/mL	
12.3.2	Number of	Eight	

	measurements		
12.3.3	Linearity	UV detection linearity: r^2 0.997, regression line y = 0.0095x + 0.0368 Fluorescence detection linearity: r^2 0.9994, regression line y = 0.0064x - 0.0019	X
12.4	**Specifity: interfering substances**	Analysis of control samples showed that there were no substances which interfered with the detection of Brodifacoum. The use of LC/MS-MS is considered to be highly specific and self-confirmatory. There were no chromatographic peaks above 30% of the LOQ at the retention time of Brodifacoum.	
12.5	**Recovery rates at different levels**	Recoveries from fortified meat were as follows:	

Matrix	Fortification level (mg/kg)	Recovery (%)	
		range	mean
Meat	0.01	62 – 86	73
	0.10	45 – 87	61

12.5.1	Relative standard deviation	RSD values were as follows:	

Matrix	Fortification level (mg/kg)	RSD (%)
Meat	0.01	13
	0.10	29

12.6	**Limit of determination**	The limit of determination is 0.01 mg/L (defined as the lowest concentration at which acceptable recovery has been demonstrated).	
12.7	**Precision**		
12.7.1	Repeatability	RSD values are presented above in section 3.5.1.	
12.7.2	Independent laboratory validation	N/A	

13 APPLICANT'S SUMMARY AND CONCLUSION

13.1	**Materials and methods**	Meat Brodifacoum is extracted from meat by shaking with a mixture of dichloromethane and acetone. The filtered extract is purified by GPC prior to determination by LC-MS-MS.	X
13.2	**Conclusion**	The report concludes that the methods are acceptable for monitoring purposes on the basis that it is a multi-residue method that allow eight analytes to be determined in the same extract. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the methods are suitable for enforcement purposes.	X
13.2.1	Reliability	2	

Section A4.2(d)(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1 *Methods of analysis in human and animal body tissues*

13.2.2 Deficiencies No X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date Jan 2008

Materials and methods *Brodifacoum* purity = 98.5%; batch No 31126.

Stock solutions of *Brodifacoum* and *Coumatetralyl* (internal standard) in methanol; calibration solutions (*Brodifacoum* 0.03, 0.1, 0.4, 1.2 µg/ml with coumatetralyl 0.4 µg/ml) in methanol; fortification solutions (1 µg/ml and 10 µg/ml) in methanol.

Extraction is performed with dichloromethane:acetone (7:3, v/v). After filtration and change of solvent to cyclohexane:acetate (1:1, v/v), the extract is cleaned by gel permeation chromatography. The cleaned extract is evaporated to dryness; the residue is then re-dissolved in methanol and filtered, prior to LC/MS/MS analysis.

LC was performed on a Phenomenex Luna column packed with 5 µm Phenyl-Hexyl, with 10 mM ammonium acetate (eluent A) and methanol (eluent B) as mobile phase at a flow rate of 0.2 ml/min according to the following gradient program:

Time (min)	%A	%B
0	80	20
5	15	85
17.5	15	85
18	80	20
25	80	20

Detection mode: MS-MS. Ions monitored:

Analyte	Precursor Ion 1, m/z	Product Ion 1, m/z	Precursor Ion 2, m/z	Product Ion 2, m/z
Brodifacoum	521	79	523	81
Coumatetralyl	291	143	291	141

(Product 1 ions used for measurements)

Section A4.2(d)(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Methods of analysis in human and animal body tissues

Conclusion	Specificity: the developed LC/MS/MS method can be regarded to be highly specific, so additional confirmatory methods are not necessary. Linearity: tested in the range 0.03-1.2 µg/ml, 2 determinations at 4 concentration levels. No line equation nor correlation coefficient provided. According to the original study report, all investigated matrices considered, calibration curve correlation values (R^2) ranged from 0.9095 to 0.9963. Accuracy and repeatability: two fortification levels investigated (0.01 and 0.1 mg/kg), with 5 replicates per level. The mean recoveries for *Brodifacoum* were 73% (RSD% = 13%) and 61% (RSD% = 29%), respectively. LOQ: 0.01 mg/kg. The developed method is multi-residue in nature since it allows the determination of 8 analytes – see *Remarks* below – in the same sample extract. Multi-residue methods necessarily involve a compromise in choosing suitable conditions and it is not always possible to obtain high levels of accuracy and/or precision for every analyte included. Therefore, this method can be regarded to be adequately validated at either fortification level, as far as the determination of *Brodifacoum* in the investigated matrices is concerned.
Reliability	2
Acceptability	Acceptable, despite the following deficiencies/mistakes affecting the study summary: 3.1: the preliminary treatment of the fortified samples has been unsatisfactorily summarized (see *Materials and Methods* above). 3.2.1: no information regarding the adopted mobile phase gradient program has been reported (see *Materials and Methods* above). 3.2.3: according to the original study report, an internal standard (*Coumatetralyl*) is used. 3.3.3: the provided results are not acceptable, since not related to the LC-MS-MS method described in this subsection. Linearity data (including line equation and correlation coefficient) are required.
Remarks	The following clarification is necessary: the methods described in "Validation of analytical methodology to determine rodenticides in food matrices" are suitable for the monitoring and control of 8 analytes (*Alphachloralose, Brodifacoum, Bromadiolone, Chlorophacinone, Difenacoum, Difethialone, Flocoumafen,* and *Warfarin*) in cucumber, wheat, meat (muscle), oilseed-rape, and lemon at fortification levels of 0.01 mg/kg and 0.1 mg/kg. Only the data related to *Brodifacoum* residue determination in meat are summarized in this subsection.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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*

Section A4.2(d)(1) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1 *Methods of analysis in human and animal body tissues*

Remarks

Section A4.2(d)(2) **Annex Point IIA IV.4.2**	**Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:** **Animal and human body fluids**	
	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 [x]	*A study has been commissioned at Chemservice S.r.l, protocol number 283/2007, draft report due October 2007.* *The study will validate an analytical method for analysis of brodifacoum in blood serum.*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2008	
Evaluation of applicant's justification	Acceptable	
Conclusion	A validated analytical method for the analysis of brodifacoum in blood serum has beem submitted. RMS is waiting for the relevant study summary.	
Remarks	The robust summary A4.2(d)(3) for the analytical method/validation for the analysis of brodifacoum in blood serum, submitted by the Applicant in March 2009, has been attached as follows.	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4.2 (d) Analytical Methods for Detection and Identification

Annex Point IIA4.2 & IIIA-IV.1

Analytical method for the analysis of brodifacoum in blood.

			Official use only
		1 REFERENCE	
1.1	**Reference**	Garofani S, Validation of the analytical method for the determination of brodifacoum residues in serum blood samples, Chemservice S.r.l., 2008	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with letters of access	N/A	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND GLP	
2.1	**Guideline**	SANCO/3029/99 rev.4. and SANCO 825/00 rev 7	
2.2	**GLP**	Yes	
2.3	**Deviations**	N/A	X1
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Water is added to a sample of blood serum and the total aqueous solution is extracted with dichloromethane after vigorous mixing and separation by centrifuge. A sample of the dichloromethane layer containing the brodifacoum is then taken by syringe for injection into the HPLC system.	
3.1.2	Cleanup	N/A	
3.2	**Detection**		
3.2.1	Separation method	HPLC.	
3.2.2	Detector	Ion-trap mass detector (SIM and SRM modes)	
3.2.3	Standard(s)	Brodifacoum, 99.9%	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.05 µg/ml to 0.40 µg/ml. 5 concentrations were assessed within this range.	X2

3.3.2	Number of measurements	4 measurements per concentration	
3.3.3	Linearity	The calibration equation is y = 924112x + 3156 for the linearity test on brodifacoum working standard solutions (SRM mode) and y = 73661410x – 37556 for brodifacoum working standard solutions (SIM mode) For serum blood, the calibration equation is y = 81165139x – 666940 for three standards; 0.051, 0.206 and 0.412 µg/ml. For all, the correlation coefficient > 0.99, therefore the linearity of the method is considered acceptable.	X3
3.4	**Specificity: interfering substances**	A comparision of the chromatograms of the solvent wash, brodifacoum analytical standard, brodifacoum test substance and control serum blood sample, did not show any interference. The analytical method is therefore considered to be specific to brodifacoum.	
3.5	**Recovery rates at different levels**	Recovery was assessed at two levels; 0.06 µg/ml and 0.30 µg/ml. For 0.06 µg/ml, the accuracy ranged from 81 to 97% with a mean of 92%. For 0.30 µg/ml, the accuracy ranged from 86 to 109% with a mean of 102%. For accuracy the SANCO guideline requires individual recovery values in the range 70-110% with mean value of 80-100% at each level. Some deviation obtained can be accepted because of the very low water solubility of the test substance and the very particular and complex method of analysis; therefore the accuracy of the analytical method can be considered acceptable.	X4
3.5.1	Relative standard deviation	For 0.06 µg/ml, the RSD was 5.98% and for 0.30 µg/ml the RSD was 8.74%	X5
3.6	**Limit of determination**	0.06 µg/ml is the limit of quantification and the limit of detection is defined as 50% of this value i.e. 0.03 µg/ml	
3.7	**Precision**		
3.7.1	Repeatability	The test was performed by spiking a serum blood control sample six times at two levels; 0.06 µg/ml and 0.30 µg/ml. The relative standard deviations (RSD) of the level at 0.06 µg/ml was 6.5% and for 0.30 µg/ml was 8.6%. SANCO guidelines require a figure for RSD less than 20%, so precision of the analytical method is considered acceptable.	
3.7.2	Independent laboratory validation	Not required since the use of HPLC-MS-MS is considered to be a highly specific method.	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	A sample of brodifacoum technical product (99.9%) was supplied to Chemservice. Serum blood was obtained from Sigma-Aldrich. Water is added to a sample of blood serum and the total aqueous solution is mixed well with dichloromethane followed by centrifugation at 12000 rpm for 30 minutes. A sample of the dichloromethane layer (which will contain the brodifacoum) is then taken using a syringe. This sample is injected into a HPLC coupled with an ion-trap mass detector. The method was shown to be specific by determination of the brodifacoum residues using HPLC-MS-MS technique with SIM mode (Selected Ion Monitoring) and SRM mode (Selection Reaction Monitoring) the latter of which is a very specific technique. The presence of brodifacoum in the serum blood samples was identified by comparision of the peak retention time, the SIM and SRM data with those obtained for the known standards.	X
4.2	**Conclusion**	The analytical method was shown to be specific for brodifacoum in the serum blood samples. The range of concentrations tested were found to be linear. The accuracy (recovery) and precision (repeatability) of the method were considered to be acceptable, in accordance with SANCO guidelines.	X
4.2.1	Reliability	1	
4.2.2	Deficiencies	No	X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

Aug 2009

Materials and methods

Agree with the Applicant's version. Additional information is also available in the original study report as follows:

Matrix: serum from Rabbit, lyophilized powder (from clotted whole blood)

Brodifacoum technical product, batch no L04846; purity 99.9%.

Brodifacoum analytical standard, batch no L03950722; purity 99.5%.

0.5 ml of water are added to 0.5 ml of serum blood sample and the total aqueous solution is extracted with 0.5 ml of dichloromethane. Prior to extraction, the total solution is centrifuged at 12000 rpm for 30 min. The extract is injected into the HPLC/MS/MS system.

HPLC was performed on a C18 (ODS) pre-column and a ChemService code No. 183 column at room temperature, with water (eluent A), methanol (eluent B), and water with 10 mM ammonium bicarbonate (eluent C) as mobile phase at a flow rate of 0.2 ml/min according to the following gradient program:

A:B:C, 55:40:5 hold 1 min.
A:B:C, from 55:40:5 to 0:95:5 in 5 min, hold 14 min.
A:B:C, from 0:95:5 to 55:40:5 in 2 min, hold 10 min.

Interface: electron spray ionisation, negative ions.

Detector: mass (scan in SIM and SRM mode). Mass scan parameters in the table below:

compound	Ion mode	Collision energy (%)	m/z molecular ion (SIM)	m/z daughter (SRM)
Brodifacoum	ESI	60	523	187

Conclusion

Agree with the Applicant's version.

The submitted HPLC/MS/MS method can be considered highly specific.

05-0.40 mg/l (equivalent to 0.05-0.40 mg/l in blood serum), 4 determinations at 5 concentration levels. R = 0.99679 (SIM mode), R = 0.99623 (SRM mode).

Accuracy and precision:

Sample	Fortification level	Recovery rate (%)		
		Range	Mean	RSD %
	0.06 mg/l (n = 5)	80.8-96.6	92.1	6.5
	0.3 mg/l (n = 6)	86.2-109.1	101.7	8.6

LOQ: 0.06 mg/l.

Reliability

2

Acceptability	Acceptable, despite the reporting deficiencies affecting the study summary. Furthermore: General remark: data should be express in mg/l. X1 – Deviation: according to SANCO/825/00, LOQ should be set at 0.05 mg/l. X2 – The investigated linearity range corresponds to 0.05-0.40 mg/l in blood serum. X3 – SMR mode (m/z = 187): y = 3156 + 924112x, r = 0.99623; SIM mode (m/z = 523): y = -37556 + 73661410x, r = 0.99679. Linear calibration curve used for quantification at 0.3 mg/l in blood serum (SIM mode, m/z = 523): y = -666940 + 81165139x, r = 0.99575, 3 injections at each concentration level (0.051, 0.206 and 0.412 mg/l). X4 – Number of investigated samples: 5 at 0.06 mg/l in blood serum (1 outlier identified, probably due to injection error); 6 at 0.3 mg/l in blood serum. Quantification was performed with the linear calibration curve mentioned above at 0.3 mg/l in blood serum, with the lowest standard calibration level at 0.06 mg/l in blood serum. Recovery rates were corrected by the mean control residue value. X5 – The reported data are S.D. values. RSD% are 6.5% and 8.6% at 0.06 and 0.3 mg/l in blood serum, respectively.
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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*

Section A4.3 Analytical Methods for Detection and Identification

Annex Point IIIA, IV.1 Brodifacoum residues in treated food and feeding stuffs

Official use only

1 REFERENCE

1.1	**Reference**	Turnbull G (2005) Validation of Analytical Methodology to Determine Rodenticides in Food Matrices, Central Science Laboratory, Report No. PGD-180
1.2	**Data protection**	Yes
1.2.1	Data owner	Brodifacoum Task Force
1.2.2	Companies with letter of access	None
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	SANCO/825/00 rev. 6
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1 Preliminary treatment X

3.1.1 Extraction

Cucumber

Brodifacoum is extracted from cucumber by blending with ethyl acetate. The filtered extract is purified by SPE cartridge and determination is by LC-MS-MS.

Wheat

Brodifacoum is extracted from wheat by blending with ethyl acetate. The filtered extract is purified by gel permeation chromatography (GPC) prior to determination by LC-MS-MS.

Meat

Brodifacoum is extracted from meat by shaking with a mixture of dichloromethane and acetone. The filtered extract is purified by GPC prior to determination by LC-MS-MS.

Oilseed rape

Brodifacoum is extracted from oilseed rape by blending with acetone. The filtered extract is partitioned with hexane and purified by GPC prior to determination by LC-MS-MS.

Lemon

Brodifacoum is extracted from lemon by blending with ethyl acetate. The extract is partitioned with water and purified by SPE cartridge prior to determination by LC-MS-MS.

3.1.2	Cleanup	Gel permeation chromatography or SPE catridge.	
3.2	**Detection**		
3.2.1	Separation method	HPLC, Phenomenex Luna 150 mm x 2 mm i.d. column packed with 5 µm Phenyl-Hexyl with mobile phase: 10 mM ammonium acetate and methanol.	X
3.2.2	Detector	LC-MS-MS (primary ion m/z: 79)	
3.2.3	Standard(s)	External standard	X
3.2.4	Interfering substance(s)	Analysis of control samples demonstrated that there were no substances which interfered with the detection of Brodifacoum. There were no chromatographic peaks above 30% of the LOQ at the retention time of Brodifacoum.	
3.3	**Linearity**		
3.3.1	Calibration range	0.03 to 1.2 µg/mL	
3.3.2	Number of measurements	Eight	
3.3.3	Linearity	R^2 = 0.9095 to 0.9963	X

3.4 Specifity: interfering substances

Analysis of control samples showed that there were no substances which interfered with the detection of Brodifacoum. The use of LC/MS-MS is considered to be highly specific and self-confirmatory. There were no chromatographic peaks above 30% of the LOQ at the retention time of Brodifacoum.

3.5 Recovery rates at different levels

Recoveries from fortified cucumber, wheat, meat, oilseed rape and lemon were as follows:

Matrix	Fortification level (mg/kg)	Recovery (%)	
		range	mean
Cucumber	0.01	82 – 103	91
	0.10	86 – 106	94
Wheat	0.01	88 – 126	107
	0.10	71 – 90	84
Meat	0.01	62 – 86	73
	0.10	45 – 87	61
Oilseed rape	0.01	75 – 99	86
	0.10	110 – 134	119
Lemon	0.01	74 – 93	84
	0.10	62 - 89	76

Section A4.3 Analytical Methods for Detection and Identification

Annex Point IIIA, IV.1 Brodifacoum residues in treated food and feeding stuffs

3.5.1 Relative standard deviation

RSD values were as follows:

Matrix	Fortification level (mg/kg)	RSD (%)
Cucumber	0.01	9
	0.10	9
Wheat	0.01	13
	0.10	9
Meat	0.01	13
	0.10	29
Oil-seed rape	0.01	10
	0.10	8
Lemon	0.01	10
	0.10	13

3.6	**Limit of determination**		The limit of determination is 0.01 mg/L (defined as the lowest concentration at which acceptable recovery has been demonstrated).	
3.7	**Precision**			
	3.7.1	Repeatability	RSD values are presented above in section 3.5.1.	
	3.7.2	Independent laboratory validation	N/A	
			4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**		<u>Cucumber</u> Brodifacoum is extracted from cucumber by blending with ethyl acetate. The filtered extract is purified by SPE cartridge and determination is by LC-MS-MS. <u>Wheat</u> Brodifacoum is extracted from wheat by blending with ethyl acetate. The filtered extract is purified by GPC prior to determination by LC-MS-MS. <u>Meat</u> Brodifacoum is extracted from meat by shaking with a mixture of dichloromethane and acetone. The filtered extract is purified by GPC prior to determination by LC-MS-MS. <u>Oilseed rape</u> Brodifacoum is extracted from oilseed rape by blending with acetone. The filtered extract is partitioned with hexane and purified by GPC prior to determination by LC-MS-MS. <u>Lemon</u> Brodifacoum is extracted from lemon by blending with ethyl acetate. The extract is partitioned with water and purified by SPE cartridge prior to determination by LC-MS-MS.	X
4.2	**Conclusion**		The methods for determination of residues of Brodifacoum in cucumber and wheat have been adequately validated. The methods were successfully evaluated and meet the EU criteria with respect to specificity, linearity and accuracy according to the guidance given in SANCO/825/00. For oilseed rape (at 0.1 mg/kg) the mean recovery exceeds the guideline criteria of 70 to 110%. The report concludes that the methods are acceptable for monitoring purposes on the basis that these are multi-residue methods that allow eight analytes to be determined in the same extract. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the methods are suitable for enforcement purposes.	X
	4.2.1	Reliability	1	X
	4.2.2	Deficiencies	For oilseed rape the mean recovery exceeds the guideline criteria of 70 to 110%. This deviation is not considered to significantly affect the suitability of the method for monitoring purposes.	

Section A4.3 Analytical Methods for Detection and Identification

Annex Point IIIA, IV.1 Brodifacoum residues in treated food and feeding stuffs

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date Jan 2008

Materials and methods

Brodifacoum purity = 98.5%; batch No 31126.

Stock solutions of *Brodifacoum* and *Coumatetralyl* (internal standard) in methanol; calibration solutions (*Brodifacoum* 0.03, 0.1, 0.4, 1.2 µg/ml with coumatetralyl 0.4 µg/ml) in methanol; fortification solutions (1 µg/ml and 10 µg/ml) in methanol.

Cucumber: extraction is performed with ethyl acetate, the extract is loaded onto a SPE column which is eluted with 2 solvents (methanol and 2% v/v formic acid in ethanol) and 2 different fractions (A and B, respectively) are collected. As for *Brodifacoum* determination, fraction B is evaporated to dryness, the residue re-dissolved in methanol and analysed by LC-MS-MS.

Wheat: extraction is performed with ethyl acetate. After filtration and change of solvent to cyclohexane:ethyl acetate (1:1 v/v), the extract is cleaned by gel permeation chromatography. The cleaned extract is evaporated to dryness, re-dissolved in methanol and analysed by LC-MS-MS.

Meat: extraction is performed with with dichloromethane:acetone (7:3v/v). After filtration and change of solvent to cyclohexane:acetate (1:1 v/v), the extract is cleaned by gel permeation chromatography. The cleaned extract is evaporated to dryness, the residue is re-dissolved in methanol and filtered, then analysed by LC-MS-MS.

Oil-Seed Rape: extraction is performed with acetone. The extract is loaded onto a SPE column. The elution step is accomplished using methanol, 2% v/v formic acid in ethanol, and 0.12 M HCl in ethanol (fractions A, B, and C, respectively). For *Brodifacoum* determination, fraction B is evaporated to dryness, the residue re-dissolved in methanol and analysed by LC-MS-MS.

Lemon: extraction is performed with ethyl acetate. The extract is partitioned with water, then loaded onto a SPE column. The elution step is accomplished using methanol, 2% v/v formic acid in ethanol, and 0.12 M HCl in ethanol (fractions A, B, and C, respectively). For *Brodifacoum* determination, fraction B is evaporated to dryness, the residue re-dissolved in methanol and analysed by LC-MS-MS.

HPLC was performed on a Phenomenex Luna column packed with 5 µm Phenyl-Hexyl, with 10 mM ammonium acetate (eluent A) and methanol (eluent B) as mobile phase at a flow rate of 0.2 ml/min according to the following gradient program:

Time (min)	%A	%B
0	80	20
5	15	85
17.5	15	85
18	80	20
25	80	20

Detection mode: MS-MS. Ions monitored :

Analyte	Precursor Ion 1, m/z	Product Ion 1, m/z	Precursor Ion 2, m/z	Product Ion 2, m/z
Brodifacoum	521	79	523	81
Coumatetralyl	291	143	291	141

(Product 1 ions used for measurements)

Section A4.3 Analytical Methods for Detection and Identification

Annex Point IIIA, IV.1 Brodifacoum residues in treated food and feeding stuffs

Conclusion	Specificity: the developed LC-MS-MS methods can be regarded to be highly specific, so additional confirmatory methods are not necessary. Untreated matrices were also used to test specificity. Linearity: tested in the range 0.03-1.2 µg/ml, 2 determinations at 4 concentration levels. Calibration curve correlation (R^2) ranged from 0.9095 to 0.9963. Accuracy and repeatability: two fortification levels investigated (0.01 and 0.1 mg/kg), with 5 replicates per level. The mean recoveries for *Brodifacoum* were in the range 61% to 119%, with corresponding RSD% values in the range 8% to 29%. LOQ: 0.01 mg/kg in all 5 matrices studied. The developed method is multi-residue in nature since it allows the determination of 8 analytes – see *Remarks* below – in the same sample extract. Multi-residue methods necessarily involve a compromise in choosing suitable conditions and it is not always possible to obtain high levels of accuracy and/or precision for every analyte included. Therefore, this methods can be regarded to be adequately validated at either fortification level, as far as the determination of *Brodifacoum* in the investigated matrices is concerned.
Reliability	2
Acceptability	Acceptable, despite the following deficiencies/mistakes affecting the study summary: 3.1: the preliminary treatment of the fortified samples has been unsatisfactorily summarized (see *Materials and Methods* above). 3.2.1: no information regarding the adopted mobile phase gradient program has been reported in the study summary (see *Materials and Methods* above). 3.2.3: according to the original study report, *Coumatetralyl* is used as internal standard. 3.3.3: Linearity data (including line equation and correlation coefficient) are required for every investigated matrix.
Remarks	The following clarification is necessary: the methods described in "Validation of analytical methodology to determine rodenticides in food matrices" are suitable for the monitoring and control of 8 analytes (*Alphachloralose, Brodifacoum, Bromadiolone, Chlorophacinone, Difenacoum, Difethialone, Flocoumafen*, and *Warfarin*) in cucumber, wheat, meat (muscle), oilseed-rape, and lemon at fortification levels of 0.01 mg/kg and 0.1 mg/kg. Only the data related to *Brodifacoum* residue determination in meat are summarized in this sub-section.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Rodenticide	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	Rats and mice; Humans animals and property to be protected	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	Rats and mice Rattus rattus (black rat, ship rat) Rattus norvegicus (brown rat, Norway rat) Mus musculus (house mouse) Mus domesticus (house mouse) *Organisms are widespread throughout European continent and are common to all countries in EC.*	
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	Humans animals and property to be protected	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**		
5.3.1	**Effects on target organisms (IIA5.3)**	Signs of poisoning in rodents and other mammals are those associated with an increased tendency to bleed leading ultimately to profuse haemorrhage. After feeding on bait containing the active ingredient for 2 – 3 days the animal becomes lethargic and slow moving. Signs of bleeding are often noticeable and blood may be seen around the nose and anus. As symptoms develop the animal will loose its appetite and will remain in its burrow or nest for increasingly long periods of time. Death will usually occur within 4-5 days of ingesting a lethal dose and animals often die out of sight in their nest or burrow.	X
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**	The standard concentration at which the second-generation anticoagulants (including bromadiolone, difenacoum and brodifacoum) are typically used in ready for use baits is 0.005% w/w. This concentration has been standardised over the last 25 years as the optimal concentration to deliver the benefits of the active substance. Difenacoum in particular is inherently not very palatable and at concentrations above 50 ppm there is a risk that it can be detected by the target species. Difenacoum and bromadiolone, even at 50 ppm, are, in practice, multi-feed products and if this concentration was lower then the time to control the target population would be extended to several weeks or even months which is unlikely to be acceptable were there is a rodent population that needs to be	

		controlled for public health reasons. In recent years there have been a movement by some formulators (and regulators) to reduce the concentration of brodifacoum to 10 ppm in the final bait. This has potentially serious technical implications since at this level brodifacoum is simply another multi-feed product and the benefit of the single feed kills is lost. This increases the risk of the development of resistance. It would be perhaps preferable to maintain brodifacoum only at 50 ppm and limit its use to situations considered "safe". A disadvantage of reducing the concentration of any of these compounds is that it takes longer to accumulate a lethal dose in the target species such that moribund rodents containing residues of the anticoagulants will be active above ground over a longer period. Because of the poisoning effects of general lethargy these are likely to be the individuals targeted by predators. Maintaining and perhaps limiting the use rate at 50 ppm ensures a lethal dose is quickly ingested and death also follows quickly such that "sick" rodents are available for predators to pick-up for the shortest possible period.	
5.4	**Mode of action (including time delay) (IIA5.4)**		
5.4.1	**Mode of action**	Anticoagulant rodenticides are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin K dependent post translation processing before they are converted into the respective procoagulant zymogens. The specific point of action is thought to be the inhibition of K_1 epoxide reductase. The anticoagulants accumulate and are stored in the liver until broken down. The plasma prothrombin (procoagulant factor II) concentration provides a suitable guide to the severity of acute intoxication and to the effectiveness and required duration of the antidoting therapy (vitamin K_1).	
5.4.2	**Time delay**	Within 24 hrs.	X
5.5	**Field of use envisaged (IIA5.5)**	Include code(s) and term(s)	
	MG03: Pest control	The product is intended for use in domestic, industrial and commercial buildings including in and around farm building but not field use and sewers. For rats each bait box will contain 4 to 5 blocks. A mouse box will only contain one bait block. Boxes for mice should be placed 5 metres apart, although this can be reduced to 2 metres in areas of high infestation and for rats boxes should be 10 metres apart or to 5 metres apart in high infestation areas. –All distances are perimeter distances around the protected building or area. Boxes should be checked every day and carcasses removed. Operators should search for all rodent bodies in and around the baited area for disposal. Bait boxes should be removed, in a typical campaign, 6 weeks after initial placement.	
5.6	**User (IIA5.6)**	Briefly describe the use conditions	
	Professional	In and around buildings including domestic, commercial industrial	

	General public	and institutional; sewers, drains and culverts. Amateur use proposed, in and around buildings including domestic buildings, drains and culverts.
5.7	Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)	
5.7.1	Development of resistance	Resistance to the first generation anticoagulants has been widely reported in both *Rattus norvegicus* and *Mus domesticus* since the late 1950's. The incidence of resistance to first generation anticoagulants in areas in which it is established is commonly 25-85%. Some degree of resistance to difenacoum and bromadiolone has been reported in the UK and Denmark and other European countries but this is usually only found in certain populations of rodents highly resistant to first generation anticoagulants (Greaves et al., 1982a; Lund, 1984; MacNicoll and Gill, 1987). Considerable doubt exists as to the significance of reports of resistance to second-generation anticoagulants and in the UK control failures with the second-generation products are increasingly being attributed to baiting problems rather than physiological resistance (Quy et al. 1992a,b). Mechanisms of Resistance. The biochemical mechanism of warfarin resistance has been studied in four geographic strains of Norway rat. The mechanism appears to differ in each strain, but in each an altered form of vitamin K-epoxide reductase is involved. In two strains (Welsh and Hampshire) the reductase has both decreased activity and a decreased sensitivity to warfarin inhibition whereas in another two strains (Scottish and Chicago) it is reversibly inhibited by warfarin as compared with irreversible inhibition found in susceptible strains. There is some indication that decreased sensitivity of a second enzyme, vitamin K-quinone reductase, to warfarin inhibition may also be significant in certain strains (Misenheimer and Suttie, 1990). There appears to be a consensus amongst biochemists that the variants of at least one of these reductases, by their altered affinities for anticoagulants and vitamin K, and supplemented in some cases by subsidiary mechanisms such as faster microsomal clearance of the anticoagulant, are the biochemical basis of resistance in the Norway rat. Behavioural Resistance Several elements of behaviour such as neophobia and conditioned or unconditioned aversion to bait can help rodents to avoid ingesting a fatal dose and may explain treatment failures that cannot be accounted for by physiological resistance. The enhancement of such behaviour can constitute a novel defence mechanism and was termed behavioural resistance by Humphries et al. (1992) working with mice. Similarly Brunton et al. (1993) cited enhanced neophobia in the Norway rat as an example of behavioural resistance. Resistance is of no importance when it is low compared to the field dosage rate of the anticoagulant. In the UK a small but apparently heritable decrease in susceptibility to brodifacoum was detected by means of laboratory tests with bait containing 10 ppm brodifacoum

but was not known to have a practical effect on field control when using bait of standard concentration (50 ppm), Gill et al., 1992.
In contrast further studies suggested the presence of behavioural resistance (Brunston et al, 1993). Subsequent investigations indicated that the control difficulty was not due to resistance but to the large size of the infestations and the competing attractions for the rats of cereal stored in the infested area (Quy et al, 1992a,b).

References.

Brunton, C.F.A., Macdonald, D.W. and Buckle, A.P. (1993) Behavioural resistance towards poison baits in Brown rats. *Behavioural Processes.*

Gill, J.E., Kerins, G.M. and MacNicoll, A.D. (1992) Inheritance of low grade brodifacoum resistance in the Norway rat. *Journal of Wildlife Management* 56, 809-816.

Greaves, J. H., Shepherd, D. S and Quy, R. (1982). Field trials of second-generation anticoagulants against difenacoum-resistant Norway rat populations. *Journal of Hygiene, Cambridge* 89, 295-301.

Humphries, R.E., Meeham, A.P. and Sibly, R.M. (1992) The characteristics and history of behavioural resistance in inner-city house mice in the UK. In: Borrecco, J.E. and Marsh, R.E. (eds.) *15th Vertebrate Pest Conference.* University of California, Davis, pp. 161-164.

Lund, M. (1984) Resistance to the second-generation anticoagulant rodenticides. In: Clark, D. O. (ed.) *11th Vertebrate Pest Conference.* University of California, Davis pp 89-94

MacNicoll, A. D. and Gill, J. E. (1987) The occurrence and significance of rodenticide resistance in the UK. In : Lawson, T. J. (ed.) *Stored Products Pest Control. British Crop Protection Council Monograph No. 37.* BCPC Publications, Thorton Heath, UK, pp. 89-95

Misenheimer, T.M. and Suttie, J.W. (1990). Warfarin resistance in a Chicago strain of rats. *Biochemical Pharmacology* 40, 2079-2084.

Quy, R. J. , Shepherd, D. S. and Inglis, I. R. (1992a) Bait avoidance and effectiveness of anticoagulant rodenticides against warfarin- and difenacoum-resistant populations of Norway rats. *Crop Protection.* 11, 14-20.

Quy, R. J., Cowan, D. P., Haynes, P., Inglis, I. R. and Swinney, T. (1992b) The influence of stored food on the effectiveness of farm rat control. In: *Pests and Diseases.* British Crop Protection Council Monograph No. 42. BCPC Publications, Thornton Health, UK, pp. 291-300

5.7.2 Management strategies

The immediate aim of resistance management is to prevent or retard the development of resistance to a given anticoagulant while, as far as is not counterproductive, permitting its continued use. The ultimate aim is to reduce or eliminate the adverse consequences of resistance. The use of a suitable arsenal of alternative rodenticides is necessary for the management of resistance. Even out-moded compounds such as zinc phosphide were beneficial when anticoagulant resistance first appeared in the UK. The newer rodenticides to which resistance has not yet developed including the anticoagulants brodifacoum, flocoumafen and difethialone and the non-anticoagulants calciferol and bromethalin, all appear to have a role in resistance management. A consistent selection differential that places resistant individuals at a disadvantage, large or small, is needed to eliminate resistance. The

most practical way to achieve this is first to stop using rodenticides to which the rodenticides are resistant and then to eliminate the resistant population by the exclusive use of non-selective or counter selective control techniques, both chemical and non-chemical.

A contrary strategy is that of withholding or saving effective rodenticides while continuing to use a given anticoagulant until resistance exhausts its usefulness is sometimes put forward as a means of limiting the development of resistance. However it is generally accepted that this strategy is likely to accelerate the development and spread of resistance.
Prevention of Resistance.
The following are considered the most feasible to limit the development of resistance to anticoagulants:

1. Maximum use of non-chemical control techniques.
2. Preferential use of rodenticides and formulations to which resistance rarely develops.
3. Ensure the complete eradication of the target population whenever a rodenticide is used.
4. Avoid the use of first generation anticoagulants, to which resistance develops relatively easily.
5. Maintain uncontrolled, susceptible populations in refugia from which emigration can occur.

5.8 Likely tonnage to be placed on the market per year (IIA5.8)

This information is regarded as commercially sensitive. Please refer to the Confidential Annex to review this information.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2007
Materials and methods	5.3.1 Experimental data of the effectiveness of the active substance against target organisms are reported by XXXXX, 1997. No Study Summary was submitted by the Applicant. However, for the active substance inclusion in Annex I the RMS considers acceptable the information provided and if a complete study summary should be considered need this might be requested at Authorisation product level. 5.4.2 We consider "time delay" as the time after which the first symptoms by the effect of the active substance rise in the rat or mice. As concerns the efficacy trials on against Rattus norvegicus and Mus musculus (see Table 1-2 below) at Authorisation product level, the Applicant should provided more information about the possility to extrapolate the data on Difenacoum to Brodifacoum.
Conclusion	The studies presented by the applicant on the effectiveness of Brodifacoum against target organisms have been carried out according to the standard methods for efficacy testing of active substances (rodenticides) available in Europe. Laboratory tests have been carried out following EPPO guidelines. In conclusion, all bioassays carried out on Lab, on the field or in semi-field

	scenarios, may be considered as exhaustive
Reliability	Appropriate
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	Give date of comments submitted
Results and discussion	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
Conclusion	Discuss if deviating from view of rapporteur member state Conclusions are based on the studies reported in Doc IIIB.
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
Include respective code(s) for function type(s) given in section 5.1	Include respective code(s) for product type(s) given in section 5.5	Describe specification if deviating from that given in section 2	Specify species, strain, sex, weight, growth stage etc. as appropriate	Shortly describe test system and application method used in the tests	Shortly describe test conditions including concentrations applied and exposure time	Describe relevant results; quantify the effects on target organisms; indicate the dependence on the concentrations of the A.S. and the possible existence of a threshold concentration. Also describe if results indicate the mode of action and/or the development of resistance.	Only author(s) and year of publication/ report; full bibliographic data in footnote
No codes	No code	Brodifacoum	rats	Field	Field / various	100% control	XXXXX, 1997[1]
No code	No code	Brodifacoum	mice	Field	Field / various	100% control	XXXXX, 1997

[1] XXXXX, (1997), Efficacy Overview: Vertox™ Rolled oats,
A rodenticide formulation, containing 50ppm Brodifacoum, for the control of rats and mice.
Report EO\VRO\09-97

Unpublished

The potency of brodifacoum, a second-generation anticoagulant, again commensal rodents is well documented. It is well know that both formulation type and age of the formulation can affect palatability and potency. This is particularly true in situations where a control programme is being carried out in a situation where there is an excess of highly attractive, alternative foodstuffs for the rodents to feed on. In this situation control can be extremely difficult to achieve simply because the target rodents do not eat the rodenticide bait preferring other foodstuffs in the vicinity. Because of the over-riding importance of palatability in the ultimate effectiveness of a bait it is normal during the development of bait formulations to carry out either laboratory choice tests or field trials to establish their effectiveness in controlling the target species.

Standard test protocols have been developed for the evaluation of baits. Laboratory test guidelines have been issued by the OEPP/EPPO and US EPA. The standard protocol compares the test bait with a highly palatable standard bait formulation for which the detailed composition and storage conditions are clearly laid down (standard EPA meal). The test bait is then considered effective when acceptance by the target species is not significantly less than 33% compared to the standard EPA meal and mortality in the test is not less than 90%.

The Brodifacoum and Difenacoum Task Force have carried out extensive laboratory and field trials on its standard wax block formulation (20g block containing 0.005% w/w difenacoum). Efficacy reports are presented for the laboratory evaluation of this formulation against *Rattus norvegicus* and *Mus musculus*. The results are summarised below for the analogue difenacoum, and may be extrapolated to brodifacoum:

Table 1. Palatability and Efficacy of a 2 year old Difenacoum (Roban) Wax Block Bait, in Rats

1 SPECIES	Mean Acceptance (%)	Standard Deviation	Confidence Limit P = 0.05	Mean Dose (mg/kg bw)	Mortality (%)
R. norvegicus	37.9	2.5	1.5	7.65	100

The data confirms that even in "old" bait that had been stored under ambient conditions for two years, the formulation remained both palatable and effective in controlling rats.

Table 2. Palatability and Efficacy of a 2 year old Difenacoum (Roban) Wax Block Bait, in Mice

2 SPECIES	Mean Acceptance (%)	Standard Deviation	Confidence Limit P = 0.05	Mean Dose (mg/kg bw)	Mortality (%)
M.musculus	43.9	6.5	12.7	22.06	90

The data confirms that even in "old" bait that had been stored under ambient conditions for two years, the formulation remained both palatable and effective in controlling mice.
It can be concluded that the standard Task Force formulation of difenacoum wax block bait is sufficiently attractive to be effective in the control of rats and mice.

Section A6.1.1a Acute Oral Toxicity

Annex Point IIA6.1 Acute oral toxic class method of brodifacoum in rats

			Official use only
		1 Reference	
1.1	**Reference**	XXXXX (2004) Acute oral toxicty study (Acute toxic class method) of test item brodifacoum technical in rats. XXXXX. Study Code: 04/903-001P	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	**Companies with letter of access**	PelGar International Ltd. Activa srl	
1.2.3	**Criteria for data protection**	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 Guidelines and Quality Assurance	
2.1	**Guideline study**	OECD Guideline 423	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	x
		3 MATERIALS AND MethodS	
3.1	**Test material**	As given in section 2	
3.1.1	**Lot/Batch number**	04359	
3.1.2	**Specification**	As given in section 2	
3.1.2.1	**Description**	White to greyish colour-powder	
3.1.2.2	**Purity**	100%	
3.1.2.3	**Stability**	Store in refrigerator, protected from light.	
3.2	**Test Animals**	Non-entry field	
3.2.1	**Species**	CRL:(WI) BR	
3.2.2	**Strain**	(Wistar) rats	
3.2.3	**Source**	Charles River (Europe) Laboratories Inc.	
3.2.4	**Sex**	Female	
3.2.5	**Age/weight at study initiation**	Young, health adult rats	
3.2.6	**Number of animals per group**	3	

	3.2.7 Control animals	No	
3.3	Administration/ Exposure	Oral	
	3.3.1 Postexposure period	14 days, 4 weeks or other	x
	3.3.2 Type	Gavage	
	3.3.3 Concentration	Gavage 5 mg/kg bw	x
	3.3.4 Vehicle	1% aqueous methylcellulose containing 2% polysorbate 20.	
	3.3.5 Concentration in vehicle	0.5 mg/ml	
	3.3.6 Total volume applied	10 ml/kg bw	
	3.3.7 Controls	None	
3.4	Examinations	Clinical observations, necropsy and body weight measurements	
3.5	Method of determination of LD50	Not known	x
3.6	Further remarks		

4 Results and Discussion

4.1	Clinical signs	Decreased activty, lateral position, decreased righthing reflex, decreased grip and limb tone, paleness, piloerection, dyspnoea and bleeding from the nose were observed. The degree of symptoms was slight moderate and marked. The first symptoms appeared five days after treatment. One rat died after onset of symptoms; one died two days after the first symptoms. The third one died without any clinical signs.
4.2	Pathology	Heamatoma and bleeding were found in various organs, in the thoraic and abdominal cavaties. Clay coloured liver and wqhite spleen occurred as well.
4.3	Other	The body weight decreased in two animals, which showed symptoms for one or two days. The body weight gain of the animal which died without symptomswere less than normal.
4.4	LD50	The acute oral LD_{50} value of the test item Brodifacoum Technical proved to be below 5 mg/kg body weight and ranked into Class 1 of Globally Harmonized Classification System (between 0 and 5).

5 Applicant's Summary and conclusion

5.1	Materials and methods	OECD Guidelines 423. A single oral treatment for each animal was carried out out by gavage after an overnight food withdrawal. The animals were treated with a concentration of 0.5 mg/ml prepared with 1% aqueous methylcellulose containing 2% polysorbate 20.
5.2	Results and discussion	3/3 animals died at the test dose. The LD50 proved to be below 5mg/kg body weight. A necropsy showed effects related to the well-known anticoagulant effect of Brodifacoum technical.

5.3 Conclusion

5.3.1	**Reliability**	1
5.3.2	**Deficiencies**	No

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.03.06*
Guidelines and Quality Assurance	*Include revised text* **2.3 Deviations** No deviations from the OECD TG or affecting the GLP status have been recorded
Materials and Methods	*Include revised text* **3.3.1 Postexposure period** 7 days (3/3 animals were found dead within day 7 after treatment) **3.3.3 Concentration** 5 mg/kg bw **3.5 Method of determination of LD_{50}** Not pertinent (ATC method was used)
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	The reliability indicator is appropriate
Acceptability	*acceptable*
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	

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1 Acute oral toxic class method of Brodifacoum in rats

Table A6_1-1. Table for Acute Toxicity (modify if necessary)

Dose [unit]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
5 mg/kg	3/3	5-7 days	2 out the 32 showed the clinical symtoms described above
x			
xx			
xxx			
LD_{50} value	<5 mg/kg		

Section A6.1.1b Acute Toxicity

Annex Point IIA6.1 Acute Oral LD_{50} in rat

Official use only

1 REFERENCE

1.1	**Reference**	Brodifacoum: Acute Oral Toxicity in Rats of a 0.25% Concentrate – xxxxx – June 1996. Report 14095 TAR Acute oral toxicity study of test substance brodifacoum
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 401
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot / Batch number	TCP 0011/95
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Red liquid
3.1.2.2	Purity	0.262% w/w brodifacoum
3.1.2.3	Stability	Stable under test conditions
3.2	**Test Animals**	
3.2.1	Species	Rats
3.2.2	Strain	Sprague-Dawley ICO: OFA-SD (IOPS Caw)
3.2.3	Source	Iffa Crédo, 69210 L'Arbresle, France
3.2.4	Sex	Male and Female
3.2.5	Age/weight at study initiation	Six weeks Male 180g with standard deviation ± 8g Female 142g with standard deviation ± 8g
3.2.6	Number of animals per group	5 animals/sex/group
3.2.7	Control animals	No

Section A6.1.1b Acute Toxicity

Annex Point IIA6.1 Acute Oral LD_{50} in rat

3.3	**Administration/ Exposure**		Oral	
	3.3.1	Postexposure period	14 days	
			Oral	
	3.3.2	Type	Single dose by gavage	
	3.3.3	Concentration	10 ml/kg	
	3.3.4	Vehicle	Purified water	
	3.3.5	Concentration in vehicle	100, 150, 225, 500 mg/kg (in 5 ml/kg of water)	
	3.3.6	Total volume applied	Single dose of either 100, 150, 225, 500 mg/kg	
	3.3.7	Controls	None	
3.4	**Examinations**		Clinical observations, mortality, body weight, necropsy	
3.5	**Method of determination of LD_{50}**		Probit Method	
3.6	**Further remarks**		None	
			4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**		Hypoactivity, sedation, piloerection, haematoma, and dyspnoea with and without associated bleedings.	X
4.2	**Pathology**		Internal haemorrhages.	
4.3	**Other**		Bodyweight not affected by treatment.	X
4.4	**LD_{50}**		Males 150-225 mg/kg; Females 100-150 mg/kg.	
			5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**		OECD 401 study in rats	
5.2	**Results and discussion**		Acute oral LD_{50} males 150-225 mg/kg and females 100-150 mg/kg	X
5.3	**Conclusion**			X
	5.3.1	Reliability	1	
	5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*

Section A6.1.1b Acute Toxicity

Annex Point IIA6.1

Acute Oral LD_{50} in rat

Materials and Methods	Adopt applicant's version.
Results and discussion	Include revised version. **4.1 Clinical signs** Hypoactivity, sedation, piloerection, haematoma of the ears, and dyspnoea with and without associated bleedings. Clinical signs appeared on day 4. **4.3 Other** Bodyweight not significantly affected by treatment.Among surived animals only one male lost weight during the second week. **5.2 Results and discussion** Animal at 100 (F) and 150 (M) mg/kg survived during the text and at necropsy presented no apparent abnormalities. At higher treatment doses all the animals died within day 10 and presented internal hemorrhages at necropsy. Acute oral LD_{50} in females between 100 and 150 mg/kg Acute oral LD_{50} in males between 150 and 225 mg/kg **5.3 Conclusion** The test substance Brodifacoum 0.25% has to be considered toxic if swalled being the oral LD_{50} between 100 and 150 mg/kg.
Conclusion	Other conclusions: Adopt applicant's version
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	It has to be underlined that the test item is a dilution (0.25% w/w) of the a.s.

	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	

Section A6.1.1b Acute Toxicity

Annex Point IIA6.1 Acute Oral LD_{50} in rat

Table A6_1-1. Table for Acute Toxicity

Dose [unit]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0	-	-	
100 mg/kg	Male 0/5 – Female 0/5	-	
150 mg/kg	Male 0/5 – Female 5/5	Day 5 – Day 10	No apparent abnormalities a necropsy
225 mg /kg	Male 5/5 – Female 5/5	Day 4 – Day 9	Internal haemorrhages in some animals
500 mg/kg	Male 5/5 – Female 5/5	Day 4 – Day 6	Internal haemorrhages in some animals
LD_{50} value	Male between 150 and 225 mg/kg. Female between 100 and 150 mg/kg		

Section A6.1.2a — Acute Dermal Toxicity

Annex Point IIA6.1

Acute dermal toxicty study of the test brodifacoum technical in rats

Official use only

1 REFERENCE

1.1	**Reference**	XXXXX (2005) Acute Dermal Toxicity Study of Test Item Brodifacoum Technical In Rats: XXXXX, Study Code: 04/903-002P
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with access to data	PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 402
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	04359
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White to greyish colour-powder
3.1.2.2	Purity	100%
3.1.2.3	Stability	Keep in refrigerator, protected from light
3.2	**Test Animals**	Non-entry field
3.2.1	Species	CRL:(WI) BR (Wistar) rats
3.2.2	Strain	
3.2.3	Source	Charles River (Europe) Laboratories Inc.
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	young healthy adult rats. Starting bodyweight: female 206-238g male 372-403g
3.2.6	Number of animals per group	5 female animals/sex/group 5 male animals
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal, 24 hours

X

Section A6.1.2a Acute Dermal Toxicity

Annex Point IIA6.1 Acute dermal toxicty study of the test brodifacoum technical in rats

3.3.1	Postexposure period	N/A	X
		Dermal	
3.3.2	Area covered	10 % of body surface	
3.3.3	Occlusion	Occlussive	
3.3.4	Vehicle	None	
3.3.5	Concentration in vehicle	N/A	
3.3.6	Total volume applied	2, 6, 18 mg/kg/day	X
3.3.7	Duration of exposure	24 hr	
3.3.8	Removal of test substance	water	X
3.3.9	Controls	None	
3.4	**Examinations**	Clinical observations, necropsy and body weight measurements.	X
3.5	**Method of determination of LD_{50}**	Finney's method	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No dermal changes were found in either group after 24 hours exposure. Decreased activity, tremor, lateral position, squatting position, paleness, dyspnoea, piloerection, sanguineous fur around the eyes. Clinical symtoms appeared in one animal of 6 mg/kg group and in all animals of 18 mg/kg group. Onset of symptoms was 5 and 6 days after treatment. Mortaliltiy occurred between days 5 and 9,	
4.2	**Pathology**	In animals found dead, bleeding and haematoma in various organs and tissues, clay cloloured liver were observed.	
4.3	**Other**	The body weight decreased in all animals found dead.	X
4.4	**LD_{50}**	7.48 mg/kg in CRL:(WI) BR female rats. Male animals proved to be more sensitive than females.	X

Section A6.1.2a Acute Dermal Toxicity

Annex Point IIA6.1

Acute dermal toxicty study of the test brodifacoum technical in rats

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

OECD 402. 24 hours prior to the start of the treatment period the trunk of animals was shaved. The test item was applied in a ssingle dose uniformly over an approximately 10% area of the total body surface. Sterile gauze pads were placed on the skin of rats. Then the entire trunk of the animals was wrapped with plastic wrap for 24 hours. The gauze pads and plastic wrap were fixed on the skin by a patch with adhesive hypoallergenicplaster. The treated skin surface was washed with body emperature water after the 24 hour exposure.

5 female animals per group were dosed at 2, 6, 18 mg/kg/day. 5 male animals were dosed at 2 mg/kg/day.

5.2 Results and discussion

Mortality

Dose (mg/kg/ bw)	2	6	18
Female treated	5	5	5
Mortalilty	0/5	1/5	5/5
Male treated	5		
Mortality	2/5		

Clinical symptoms and necroscopy findings were related to the well-known anticoagulant effect of brodifacoum. The body weight decreased in dead animals. Male animals proved to be more sensitive than females.

LD_{50} 7.48 mg/kg

5.3 Conclusion

Non-entry field X

5.3.1 Reliability 1

5.3.2 Deficiencies No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *21.11.2006*

Section A6.1.2a Acute Dermal Toxicity

Annex Point IIA6.1

Acute dermal toxicty study of the test brodifacoum technical in rats

Materials and Methods	Include revised version **3.2.6 Number of animals per group** 5 female animals/sex/group. After completing the study, a group of 5 male was also treated at the lowest dose to verify possible gender differences. **3.3.1 Postexposure period** 14 days **3.3.6 Dose applied** 2, 6, 18 mg/kg b.w. **3.3.8 Removal of test substance** Yes, with body temperature water 24 h post exposure. **3.4 Examinations** Clinical observations (1 and 5 h after the treatment and once each day for the 14 days post exposure); body weight measurements (day 0, 7 and 14); necropsy (after death or at study termination)
Results and discussion	Include revised version. **4.3 Other** The body weight decreased in all animals found dead, but was not affected in surviving animals. **4.4 LD_{50}** Dermal LD_{50} =7.48 mg/kg in female rats. Male animals proved to be more sensitive than females, but, being treated with a single dose, the LD_{50} could not be calculated.
Conclusion	Include revised version: **5.3 Conclusion** Considering the LD_{50} value, the test substance has to be considered very toxic after dermal exposure.
Reliability	The reliability indicator is appropriate
Acceptability	Acceptable
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*

Section A6.1.2a Acute Dermal Toxicity

Annex Point IIA6.1

Acute dermal toxicty study of the test brodifacoum technical in rats

Remarks

Table A6_1-1. Table for Acute Toxicity

Dose [mg/kg]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0			
2	0/5		
6	1/5		
18	5/5	Day 6-9	
LD_{50} value	7.48 mg/kg b.w.		

Section A6.1.2b

Annex Point IIA6.1

Acute Toxicity

Acute Dermal LD_{50} in rat

Official use only

1 REFERENCE

1.1	**Reference**	Brodifacoum: Acute Dermal Toxicity in Rats of a 0.25% Concentrate – XXXXX – July 1996. Report 14096 TAR Acute dermal toxicity study of test substance brodifacoum.
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 402
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot / Batch number	TCP 0011/95
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Red liquid
3.1.2.2	Purity	0.262% w/w brodifacoum
3.1.2.3	Stability	Stable under test conditions
3.2	**Test Animals**	
3.2.1	Species	Rats
3.2.2	Strain	Sprague-Dawley ICO: OFA-SD(IOPS Caw)
3.2.3	Source	Iffa Crédo, 69210 L'Arbresle, France
3.2.4	Sex	Male and Female
3.2.5	Age/weight at study initiation	Eight Weeks Male 289g with standard deviation ± 11g Female 227g with standard deviation ± 7g
3.2.6	Number of animals per group	5 animals/sex/group
3.2.7	Control animals	No

Section A6.1.2b Acute Toxicity

Annex Point IIA6.1 Acute Dermal LD_{50} in rat

3.3	**Administration/ Exposure**		Dermal	
	3.3.1	Postexposure period	14 days	
			Dermal	
	3.3.2	Area covered	10 % of body surface	
	3.3.3	Occlusion	Semi-occlusive	
	3.3.4	Vehicle	Purified Water	
	3.3.5	Concentration in vehicle	200, 450, 1000, 2000 mg/kg (in 5 ml/kg of water)	
	3.3.6	Total volume applied	Single dose of either 200, 450, 1000, 2000 mg/kg	
	3.3.7	Duration of exposure	24 hours	
	3.3.8	Removal of test substance	Residual test substance removed using a moistened gauze pad.	
	3.3.9	Controls	None	
	3.3.10			
	3.3.11	Vehicle		X
	3.3.12	Concentration in vehicle		X
	3.3.13	Total volume applied		X
	3.3.14	Controls	None	
3.4	**Examinations**		Clinical observations, mortality, body weight, necropsy	
3.5	**Method of determination of LD_{50}**		Not relevant as no deaths occurred.	
3.6	**Further remarks**		None	
			4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**		No clinical signs and no cutaneous reactions observed. Slight red colouration of skin.	X
4.2	**Pathology**		No death occurred during observation period.	X
4.3	**Other**		Body weight not affected by treatment.	
4.4	**LD_{50}**		No deaths therefore higher than 2000 mg/kg	X
			5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**		OECD 402 study in rats	

Section A6.1.2b Acute Toxicity

Annex Point IIA6.1 Acute Dermal LD_{50} in rat

5.2	**Results and discussion**	No deaths therefore higher than 2000 mg/kg	
5.3	**Conclusion**		X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *21.11.2006*

Materials and Methods Include revised version

3.3.11 Vehicle

Purified water

3.3.12 Concentration in vehicle

0.25% (w/w)

3.3.13 Total volume applied

5 ml/kg

Results and discussion Include revised version.

4.1 Clinical signs

No clinical signs and no cutaneous reactions observed. Slight red colouration of skin occurred between 2 and 5 in all animals.

4.2 Pathology

No death occurred during observation period, nor abnormalities found at necropsy.

4.4 LD_{50}

<2000 mg/kg b.w.

Conclusion Other conclusions:
Include revised version

The test substance brodifacoum 0.25% deserves no classification

Reliability The reliability indicator is appropriate

Acceptability *Acceptable*

Remarks It should be underlined that the test substance is a dilution (0.25%) of the a.s

COMMENTS FROM ...

Date *Give date of comments submitted*

Section A6.1.2b Acute Toxicity

Annex Point IIA6.1

Acute Dermal LD_{50} in rat

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_1-1. Table for Acute Toxicity (modify if necessary)

Dose [unit]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0			
200 mg/kg	0/40	-	No clinical signs nor abnormalities at necropsy
450 mg/kg	0/40	-	No clinical signs nor abnormalities at necropsy
1000 mg/kg	0/40	-	No clinical signs nor abnormalities at necropsy
2000 mg/kg	0/40	-	No clinical signs nor abnormalities at necropsy
LD_{50} value	>2000 mg/kg bw		

Section A6.1.3 Annex Point IIA 6.1.3	Acute Toxicity - Inhalation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	Compound is of very low vapour pressure and stable at NTP. It decomposes without boiling >200°C. It is not applied by vaporisation, spraying or dusting as a fine powder. It is used in the form of a large bait block up to 250g, which is non-friable in nature. The potential for inhalation is therefore negligible. *The sponsor has agreed to perform a dustiness-test and particle size on the active, as requested by the Italian CA, in order to fully accept this waiver.*	
Undertaking of intended data submission []		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*18.2.2009*	
Evaluation of applicant's justification	On the basis of the new results on dustiness and particle size submitted by the applicant on request of the RMS, applicant's justification is considered correct	
Conclusion	The waiving is accepted.	
Remarks	The waiving is accepted but, based on data with other structurally related 2nd generation anticoagulants with the same mechanism of action (i.e.), the a.s. is is considered highly toxic also after inhalation.	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.1.4 (1) Acute Dermal Irritation

Annex Point IIA6.4

Official use only

	1 REFERENCE	
1.1	**Reference**	XXXXX (2004) Acute skin irritation study of test item Brodifacoum Technical in Rabbits. XXXXX. Study code: 04/903-006N.
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2		PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 404
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	04359
3.1.2	Specification	As given in section 2
3.1.2.1	**Description**	White to grayish colour-powder
3.1.2.2	**Purity**	100%
3.1.2.3	**Stability**	Must be kept at a cool temperature 5°C and away from light.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White Rabbits
3.2.3	Source	Ferenc Sandor breeder , 2173 Kartal, Voros Hadsereg street 131, Hungary
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	10 weeks old. 2855-3056g
3.2.6	Number of animals per group	One dose group with animals.
3.2.7	Control animals	Untreated area of animal served as control,

X

Section A6.1.4 (1) Acute Dermal Irritation

Annex Point IIA6.4

3.3	**Administration/ Exposure**	Dermal	
3.3.1	Application		
3.3.1.1	Preparation of test substance	Test substance was used as delivered but moistened sufficiently with water to ensure good contact with the skin.	
3.3.1.2	Test site and Preparation of Test Site	Not less than 24 hours prior to the treatment the back of the experimenta; animals was shaved. The test item was applied to a small, approximately 6 cm^2 area of skin and covered with a gauze patch, which was held loosely in place tape.	X
3.3.2	Occlusion	semiocclusive	
3.3.3	Vehicle	None	
3.3.4	Concentration in vehicle	n/a	
3.3.5	Total volume applied	0.5g	
3.3.6	Removal of test substance	n/a	X
3.3.7	Duration of exposure	4 hours	
3.3.8	Postexposure period	72 hours	
3.3.9	Controls	none	X
3.4	**Examinations**		
3.4.1	Clinical signs	Animals were examined for signs of erythema and oedema.	X
3.4.2	Dermal examination	Yes	X
3.4.2.1	scoring system	*State scoring system*	X
3.4.2.2	Examination time points	60min, /24h, 48h,72h after removal of the patch.	
3.4.3	Other examinations	n/a	
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Average score**		
4.1.1	Erythema	No primary irriation syptoms , like erythema and oedema, or other signs occurred during the observation period. Therefore an average score of zero was given at each time period.	
4.1.2	Edema	No primary irriation syptoms , like erythema and oedema, or other signs occurred during the observation period. Therefore an average score of zero was given at each time period.	

Section A6.1.4 (1) Acute Dermal Irritation

Annex Point IIA6.4

4.2	**Reversibility**	n/a	
4.3	**Other examinations**	During the study the general state and behaviour of animals were normal.	
4.4	**Overall result**	According to EEC directive 2001/59/EEC, the test item has not been classified as irritating for the skin.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 404.	
5.2	**Results and discussion**	No primary irriation syptoms, like erythema and oedema, or other signs occurred during the observation period. Therefore an average score of zero was given at each time period. During the study the general state and behaviour of animals were normal. According to EEC directive 2001/59/EEC, the test item has not been classified as irritating for the skin.	X
5.3	**Conclusion**		X
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*

Section A6.1.4 (1) Acute Dermal Irritation

Annex Point IIA6.4

Materials and Methods	Include revised version **3.2.6 Number of animals per group** 3 animals/group (only one dose was tested) **3.3.1.2 Test site and Preparation of Test Site** Not less than 24 hours prior to the treatment the back of the experimental animals was shaved. The test item was applied to approximately 6 cm^2 area of skin and covered with a gauze patch, which was held loosely in place by not irritating adhesive tape. **3.3.6 Removal of test substance** After the exposure period the test item was removed by body temperature water. **3.3.9 Controls** Non treated skin of each animal served as control. **3.4.1 Clinical signs** Yes. **3.4.2 Dermal examination** Animals were examined for signs of erythema and oedema. **3.4.2.1 Scoring System** Draize scoring system.
Results and discussion	Include revised version. **5.2 Results and discussion** : Delete the last sentence
Conclusion	Other conclusions: According to EEC directive 2001/59/EEC, the test item has not been classified as irritating for the skin.
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
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	

Section A6.1.4 (1) Acute Dermal Irritation

Annex Point IIA6.4

Table A6_1-4S-1. Table for skin irritation study (modify if necessary)

score (average animals investigated)	time	Erythema	Edema
average score Draize scores (0 to maximum 4)	60 min	0	0
	24 h	0	0
	48 h	0	0
	72 h	0	0
other times		0	0
average score	24h, 48h, 72h	0	0
reversibility: *		n/a	n/a
average time for reversibility		n/a	n/a

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

Section 6.1.4 (2) Acute Eye Irritation

Annex Point IIA6.1.4

Acute eye irritation study of Brodifacoum in the Rabbit

Official use only

1 REFERENCE

1.1	**Reference**		XXXXX (2004) Acute eye irritation study of test item Brodifacoum Technical in Rabbits. XXXXX
1.2	**Data protection**		Yes
	1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
	1.2.2	Companies with access to data	PelGar International Ltd. Activa srl
	1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 405
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**		As given in section 2
	3.1.1	Lot/Batch number	04359
	3.1.2	Specification	As given in section 2
3.1.2.1		Description	White to greyish colour-powder
3.1.2.2		Purity	100%
3.1.2.3		Stability	Must be kept at a cool temperature away from light.
3.2	**Test Animals**		Non-entry field
	3.2.1	Species	Rabbit
	3.2.2	Strain	New Zealand White Rabbits
	3.2.3	Source	2173 Kartal, Voros Hadsereg street 131, Hungary
	3.2.4	Sex	male
	3.2.5	Age/weight at study initiation	10 weeks old. 2850-3062 g
	3.2.6	Number of animals per group	3 X
	3.2.7	Control animals	No X

Section 6.1.4 (2) Acute Eye Irritation

Annex Point IIA6.1.4 Acute eye irritation study of Brodifacoum in the Rabbit

3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	Test item applied in pure and unaltered state.
3.3.2	Amount of active substance instilled	0.1 g
3.3.3	Exposure period	The eyesof the test animals were not washed out after the application of test item.
3.3.4	Postexposure period	n/a
3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	Yes
3.4.1.1	Scoring system	Eye irritation scores were calculated according to the scoring system by Draize (1959) and OECD 405 (See Appendix 1 of the report for details. All scores at each of the observation times (24, 48, and 72 hours) for an effect is used in calculating the mean values.
3.4.1.2	Examination time points	60min, 24h, 48h, and 72h.
3.4.2	Other investigations	The bodyweights were recorded at the beginning and at the end of the experiment.
3.5	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	1 hour after treatment every animal had hyperaemic blood vessels in the eye. In two animals the discharge from the eye was "anything different from normal". In one animal discharge with moistening of the lids was found. Every animal was symptom free at 24, 48 and 72 hours after treatment.
4.2	**Average score**	Non-entry field
4.2.1	Cornea	All animals were symptom free at 24, 48, 72 hours after treatment.
4.2.2	Iris	All animals were symptom free at 24, 48, 72 hours after treatment
4.2.3	Conjunctiva	Non-entry field
4.2.3.1	Redness	All animals were symptom free at 24, 48, 72 hours after treatment
4.2.3.2	Chemosis	All animals were symptom free at 24, 48, 72 hours after treatment
4.3	**Reversibility**	All animals were symptom free at 24, 48, 72 hours after treatment
4.4	**Other**	The general state and behaviour of animals were normal during the whole study.

X

Section 6.1.4 (2) Acute Eye Irritation

Annex Point IIA6.1.4

Acute eye irritation study of Brodifacoum in the Rabbit

4.5	**Overall result**	According th EEC directive 2001/59/EEC, the test item Brodifacoum Technical has not been classified as irritating to the eyes. The observed symptoms can be evaluated as fully reversible alteration.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD 405
5.2	**Results and discussion**	After a single application of the test item into the eyes of the rabbit slight redness and slighly or moderately increased discharge excretion were observed in the animals. Chemosis, corneal and iris alteration were noot found during the study. 24 hours after treatment every animal was symtom-free. 72 hours after the treatment the study was terminated, since no primary irritation symptoms occurred.
5.3	**Conclusion**	According th EEC directive 2001/59/EEC, the test item Brodifacoum Technical has not been classified as irritating to the eyes. The observed symptoms can be evaluated as fully reversible alteration.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	Include revised version **3.2.6 Number of animals per group** 3 animals per group (only one dose was tested) **3.2.7 Control animals** The untreated right eye of each animal served as control **3.3.4 Postexposure period** 72 hours
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version or include revised version
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section 6.1.4 (2) Acute Eye Irritation

Annex Point IIA6.1.4 Acute eye irritation study of Brodifacoum in the Rabbit

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	

Appendix

Table A6_1_4E-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to4
60 min	0	0	1	0
24 h	0	0	0	0
48 h	0	0	0	0
72 h	0	0	0	0
Average 24h, 48h, 72h	0	0	0	0
Area effected	0	0	0	0
Maximum average score (including area affected, max 110)	0	0	0	0
Reversibility*	n/a	n/a	n/a	n/a

Section A6.1.4 (3) Acute Dermal Irritation

Annex Point IIA6.4

Acute dermal irritation in rabbits

			Official use only
		1 REFERENCE	
1.1	**Reference**	Brodifacoum Acute Dermal Irritation in Rabbits of a 0.25% Concentrate – XXXXX – July 1996. Report 14094 TAL	
		Acute dermal irritation study in rabbits of test substance brodifacoum.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 404	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	TCP 0011/95	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Red liquid	
3.1.2.2	Purity	0.262% w/w brodifacoum	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	Elevage Cunicole de Val de Selle, 80160 Prouzel, France	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Age – no data Male 2.4 kg with standard deviation ± 0.1 kg	
3.2.6	Number of animals per group	3	X
3.2.7	Control animals	No	X

Section A6.1.4 (3) Acute Dermal Irritation

Annex Point IIA6.4 Acute dermal irritation in rabbits

3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	Non entry field
3.3.1.1	Preparation of test substance	Applied undiluted.
3.3.1.2	Test site and Preparation of Test Site	An area of 6 cm^2 on the flank. X
3.3.2	Occlusion	Semi-occlusive
3.3.3	Vehicle	None
3.3.4	Concentration in vehicle	
3.3.5	Total volume applied	Single dose of 0.5 ml
3.3.6	Removal of test substance	Residual test substance removed using a moistened gauze pad.
3.3.7	Duration of exposure	4 hours
3.3.8	Postexposure period	72 hours
3.3.9	Controls	None X
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Dermal examination	Yes X
3.4.2.1	scoring system	Draize
3.4.2.2	Examination time points	60 min, 24h, 48h, 72h
Other examinations		Observation for erythema/eschar, oedema and any other skin defects or irritation.
3.5	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Average score**	Non-entry field
4.1.1	Erythema	Scores not calculated due to skin colouration.
4.1.2	Oedema	Mean score for all animals at 24, 48, 72 h = 0.0
4.2	**Reversibility**	Yes
		Erythema – disappeared after 9 days
4.3	**Other examinations**	Dryness and red colouration of the skin. No mortalities
4.4	**Overall result**	According to Council Directive 93/21/E.E.C. (27th April 1993) test substance brodifacoum 0.25% should not be classified.

Section A6.1.4 (3) Acute Dermal Irritation

Annex Point IIA6.4

Acute dermal irritation in rabbits

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 404	
5.2	**Results and discussion**	Acute dermal irritation score of 0 – non-irritant. No mortalities	X
5.3	**Conclusion**	Brodifacoum 0.25% is non-irritant and should not be classified.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*21.11.2006*
Materials and Methods	Include revised version **3.2.6 Number of animals per group** 3 animal per group (only one dose was tested) **3.2.7 Control animals** No. Untreated skin from each animal served as control. **3.3.1.2 Test site and Preparation of Test Site** An area of 6 cm^2 on the flank. The day before the treatment the flank of each animal was clipped and examined in order to exclude animals with dermal injuries or irritation from the study. **3.3.9 Controls** Untreated skin of each animal served as control **3.4.2 Dermal examination** Yes. Observation for erythema/eschar, oedema and any other skin defects or irritation..
Results and discussion	Include revised version. **5.2 Results and discussion** Acute dermal irritation score = 0. The test substance resulted as non-irritant. No mortalities nor other clinical signs were recorded.
Conclusion	Adopt applicant's version
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	It has to be underlined that the test item was a dilution (0.25%) of the a.s.

Section A6.1.4 (3) Acute Dermal Irritation

Annex Point IIA6.4 Acute dermal irritation in rabbits

	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_1-4S-1. Table for skin irritation study (modify if necessary)

score (average animals investigated)	Time	Erythema	Oedema
average score Draize scores (0 to maximum 4)	60 min	2§	0
	24 h	2§	0
	48 h	2§	0
	72 h	2§	0
other times	*State time*	-	-
average score	24h, 48h, 72h	2§	0
Reversibility: *		c	c
average time for reversibility		9 days	9 days

* c : completely reversible
n c : not completely reversible
n : not reversible
§ : red coloration of the skin could mask a grade 1-2 erythema. The real evaluation could not be performed.

Section A6.1.4 (4) Acute Eye Irritation

Annex Point IIA6.1.4

Acute eye irritation in rabbits.

Official use only

		1 REFERENCE
1.1	**Reference**	Brodifacoum Acute Eye Irritation in Rabbits of a 0.25% Concentrate – XXXXX – May 1996. Report 14093 TAL Acute eye irritation study of test substance brodifacoum
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD 405
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	TCP 0011/95
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Red liquid
3.1.2.2	Purity	0.262% w/w Brodifacoum
3.1.2.3	Stability	Stable
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	Elevage Cunicole de Val de Selle,80160, Prouzel, France
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age – no data Male 2.6 kg with standard deviation ± 0.1 kg
3.2.6	Number of animals per group	3
3.2.7	Control animals	No (Left eye treated, Right eye used as control)

X

Section A6.1.4 (4) Acute Eye Irritation

Annex Point IIA6.1.4

Acute eye irritation in rabbits.

3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	Applied undiluted	
3.3.2	Amount of active substance instilled	0.1 ml	
3.3.3	Exposure period	72 hours	
3.3.4	Postexposure period	0	
3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	Yes	
3.4.1.1	Scoring system	Draize Technique.	
3.4.1.2	Examination time points	60 min, 24h, 48h, 72h	
3.4.2	Other investigations	Mortality	X
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**		X
4.2	**Average score**		
4.2.1	Cornea	See appendix table A6_1_4E-1	
4.2.2	Iris	See appendix table A6_1_4E-1	
4.2.3	Conjunctiva	Non-entry field	
4.2.3.1	Redness	See appendix table A6_1_4E-1	
4.2.3.2	Chemosis	See appendix table A6_1_4E-1	
4.3	**Reversibility**	Chemosis - Yes Cornea opacity – not affected. Conjunctiva, redness - disappeared by 24 hrs. Conjunctiva, chemosis - disappeared by 24 hrs.	
4.4	**Other**	Conjunctiva, discharge disappeared by 24 hrs.	
4.5	**Overall result**	Non-irritant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 405	

Section A6.1.4 (4) Acute Eye Irritation

Annex Point IIA6.1.4

Acute eye irritation in rabbits.

5.2	**Results and discussion**	Non-irritant when administered by ocular route in rabbit eye No mortalities
5.3	**Conclusion**	Brodifacoum 0.25% is considered non-irritant
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	*21.11.2006*
Materials and Methods	Include revised version **3.2.6 Number of animals per group** 3 animal per group (only one dose was tested). **3.4.2 Other investigations** Clinical signs
Results and discussion	Include revised version **4.1 Clinical signs** Any change in anumals' behaviour or other clinical signs were recorded
Conclusion	Adopt applicant's version
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	It has to be underlined that the test items is a dilution (0.25%) of the a.s.
	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	

Appendix

Table A6_1_4E-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
Score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to 4
60 min	0	0	U	1
24 h	0	0	0	0
48 h	0	0	0	0
72 h	0	0	0	0
Average 24h, 48h, 72h	0	0	0	0
Area effected	0	0	-	
Maximum average score (Draize method - including area affected, max 110)	0	0	0	
Reversibility*	-	-	c	c
average time for reversion	-	-	24 hrs	24 hrs
U *scoring obscured by residual test substance* * *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				

Section A6.1.5 (1) Skin sensitisation

Annex Point IIA6.1.5

Skin sensitisation in guinea pigs:
Guinea pig maximisation test (GPMT), Magnusson & Kligman

Official use only

		1 REFERENCE
1.1	**Reference**	Report: Skin Sensitization Test in Guinea-Pigs of a 0.25% Concentrate. XXXXX – July 1996. XXXXX report 14097 TSG Skin sensitisation study of test substance brodifacoum
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2		PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD 406
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	TCP 0011/95
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Red liquid
3.1.2.2	Purity	0.262% w/w Brodifacoum
3.1.2.3	Stability	Stable
3.1.2.4	Preparation of test substance for application	a) For induction: Intradermal injections: brodifacoum 0.25% at 5% (w/w) in sterile isotonic saline solution (0.9% NaCl) Topical application: brodifacoum 0.25% used undiluted b) For challenge: Topical application: brodifacoum 0.25% at 50% (w/w) in sterile isotonic saline solution (0.9% NaCl)
3.1.2.5	Pretest performed on irritant effects	Yes
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Guinea pigs
3.2.2	Strain	Dunkin-Hartley
3.2.3	Source	Centre d'Elevage Lebeau, 78950 Gambais, France

Section A6.1.5 (1) Skin sensitisation

Annex Point IIA6.1.5 Skin sensitisation in guinea pigs:
Guinea pig maximisation test (GPMT), Magnusson & Kligman

3.2.4	Sex	Male and female (nulliparous and non-pregnant)
3.2.5	Age/weight at study initiation	Approximately 3 months Male 339g with standard deviation ± 20g Female 346g with standard deviation ± 23g
3.2.6	Number of animals per group	Control group 10 (5 male, 5 female) Treatment Group 20 (10 male, 10 female) Magnusson & Kligman
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	State study type: Adjuvant
3.3.1	Induction schedule	Day 0 – intradermal injection Day 7 – topical application Day 8 – topical application *see table in appendix*
3.3.2	Way of Induction	Intradermal or topical – both
3.3.3		Occlusive or semi-occlusive – Occlusive
3.3.4	Concentrations used for induction	5% of brodifacoum 0.25% in sterile isotonic saline
3.3.5	Concentration Freunds Complete Adjuvant (FCA)	10 % or other in water or physiological saline – 5% in saline
3.3.6	Challenge schedule	Day 22 i.e 12 days after last induction
3.3.7	Concentrations used for challenge	50% of brodifacoum 0.25% in sterile isotonic saline
3.3.8	Rechallenge	No
3.3.9	Scoring schedule	24h, 48h after challenge
3.3.10	Removal of the test substance	After 24 hours with dry or moistened gauze pad
3.3.11	Positive control substance	Separate test with 2,4-dinitro chlorobenzene
3.4	**Examinations**	Non-entry field
3.4.1	Pilot study	Yes
3.5	**Further remarks**	No mortalities

Section A6.1.5 (1) Skin sensitisation

Annex Point IIA6.1.5

Skin sensitisation in guinea pigs:
Guinea pig maximisation test (GPMT), Magnusson & Kligman

		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	2 mortalities by intradermal route; well tolerated by cutaneous route
4.2	**Results of test**	
4.2.1	24h after challenge	1 / 20
4.2.2	48h after challenge	1 / 20
4.2.3	Other findings	One mortality **in control group only**
4.3	**Overall result**	Reactions seen in 1/20 animals. Classified as not a sensitiser
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD 406 study
5.2	**Results and discussion**	Reactions seen in 1/20 animals. Classified as not a sensitiser One mortality **in control group only**
5.3	**Conclusion**	Classified as not a sensitiser
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.06*
Materials and Methods	Adopt applicant's version
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	The test is acceptable per se, but it should be underlined that the test item is a dilution (0.25%) of the a.s. The a.s. has not been tested for this end-point and therefore this has to be considered a data gap. A derogation to study conduction and data presentation on the a.s. could be accepted only if the a.s. will be classified as a skin sensitiser.
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.1.5 (1) Skin sensitisation

Annex Point IIA6.1.5

Skin sensitisation in guinea pigs:
Guinea pig maximisation test (GPMT), Magnusson & Kligman

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_1_5-1. Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Inductions	**GPMT**			**Observations/Remarks** *give information on irritation effects*
	day of treatment	Application		
Induction 1	1	Intradermal		No effects noted
Induction 2	8	Topical		Irritation noted in controls and treated on day 10
challenge	20-22	Topical		No effects noted;
scoring 1	21-23			1/20 well-defined erythema
scoring 2	22-24			1/20 well-defined erythema

Table A6_1_5-2. Result of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control***
scored after 24h	**0 / 9**	**1 / 20**	**n/a**
scored after 48h	**0 / 9**	**1 / 20**	**n/a**

* The sensitivity of the test animal was tested with 1% 2,4 dinitrochlorobenzene, which in the same experimental conditions induced a positive response in 75% of the 20 animals dosed (10M+10F) .

Section A6.1.5 (2) Skin sensitisation

Local Lymph Node Assay

Annex Point IIA, VI. 6.1.5

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX (2006) Brodifacoum: Local Lymph Node Assay in the Mouse, XXXXX, Report No. 2109/0004	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa/PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD Guideline 429 Method B42 of Commission Directive 2000/73/EC	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	69806502	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Off white powder	
3.1.2.2	Purity	Not stated	X
3.1.2.3	Stability	Not stated	X
3.1.2.4	Preparation of test substance for application	The test material was freshly prepared in dimethyl formamide. This vehicle was chosen as it produced the highest concentration that was suitable for dosing.	
3.1.2.5	Pretest performed on irritant effects	Yes	

Section A6.1.5 (2) Skin sensitisation

Local Lymph Node Assay

Annex Point IIA, VI. 6.1.5

3.2	**Test Animals**			
3.2.1	Species	Mouse		
3.2.2	Strain	CBA/CA (CBA/CaBkl) CBA/Ca (CBA/Ca CruBR)		
3.2.3	Source	CBA/CA (CBA/CaBkl): B & K Universal Ltd., Hull, UK CBA/Ca (CBA/Ca CruBR): Charles River UK Ltd. Margate, Kent, UK		
3.2.4	Sex	Female (both strains)		
3.2.5	Age/weight at study initiation	Age: not stated Weight: 15 – 23 g	X	
3.2.6	Number of animals per group	4 animals per group	X	
3.2.7	Control animals	Yes – vehicle only		
3.3	**Administration/ Exposure**	Non-Adjuvant		
3.3.1	Induction schedule	Test material administration: days 1, 2 and 3 ^{3}H-methyl thymidine administration: day 6		
3.3.2	Way of Induction	Topical, non-occlusive *Test material administration* The mice were treated by daily application of 25 µl of the appropriate concentration of the test material to the dorsal surface of each ear for three consecutive days. The test material formulation was administered using an automatic micropipette and spread over the dorsal surface of the ear using the tip of the pipette. *^{3}H-methyl thymidine administration:* All mice were injected via the tail vein with 250 µl of phosphate buffered saline (PBS) containing ^{3}-methyl thymidine	X	
3.3.3	Concentrations used for induction	Test material concentration % w/w in dimethyl formamide: 0.001% 0.0005% or 0.00025%		
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	N/A		
3.3.5	Challenge schedule	N/A		
3.3.6	Concentrations used for challenge	N/A		
3.3.7	Rechallenge	N/A		

3.3.8	Scoring schedule	N/A
3.3.9	Removal of the test substance	
3.3.10	Positive control substance	N/A
3.4	**Examinations**	
3.4.1	Pilot study	Yes
3.4.2	Main test	*Clinical observations* All animals were observed twice daily on days 1, 2, and 3 and on a daily basis on days 4, 5 and 6. Any signs of toxicity or signs of ill health during the test were recorded *Bodyweights* The bodyweight of each mouse was recorded on day 1 (prior to dosing) and day 6 (prior to termination)
3.5	**Terminal procedures**	*Termination* Five hours following the administration of ^{3}HTdR all mice were killed by carbon dioxide asphyxiation. The draining auricular lymph nodes from the four mice were excised and pooled for each experimental group. For each group 1 ml of PBS was added to the pooled lymph nodes. *Preparation of Single cell suspension* A single cell suspension of pool lymph node cells was prepared by gentle mechanical disaggregation through a 200-mesh stainless steel gauze. The lymph node cells were rinsed through the gauze with 4 ml of PBS into a Petri dish labelled with the project number and dose concentration. The lymph node cell suspension was transferred to a centrifuge tube. The Petri dish was washed with an additional 5 ml of PBS to remove all remaining lymph node cells and these were added to the centrifuge tube. The pool lymph node cells were pelleted at 1400 rpm (approx. 190 g) for ten minutes. The pellet was re-suspended in 10 ml of PBS and re-pelleted. To precipitate out the radioactive material, the pellet was re-suspended in 3 ml of 5% Trichloroacetic acid (TCA). *Determination of ^{3}HTdR incorporation* After approx. 18 hours incubation at 4°C, the precipitates were recovered by centrifugation at 2100 rpm (approximately 450 g) for tem minutes, re-suspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid (Optiphase 'Trisafe'. ^{3}HTdR incorporation was measured by β-scintillation counting. The "Poly Q™" vials containing the samples and scintillation fluid were placed in the sample changer of the scintillator and left for approximately twenty minutes. The purpose of this period of time in darkness was to reduce the risk of luminescence, which has been shown to affect the reliability of the results. After approximately twenty minutes, the vials were shaken vigorously. The number of radioactive disintegrations per minute was then measured using the Beckman LS6500 scintillation system.

4 RESULTS AND DISCUSSION

4.1 Results of pilot studies

The animals treated with the test material at concentrations of 1%, 0.25%, 0.1%, 0.05% or 0.01 % w/w were killed for human reasons on days 5,6 or 9 due to the approach of the moderate severity limit. Signs of systemic toxicity noted in animals treated with the test material at concentrations of 1%, 0.25%, 0.1%, 0.05% or 0.01% w/w were:

ptosis
emaciation
splayed gait
increased respiratory rate
hypothermia
weight loss

lethargy
hynched posture

No signs of systemic toxicity were noted in the animal treated with the test material at a concentration of 0.001% w/w. Based on this information the dose levels selected for the main test were 0.01%, 0.0005% and 0.00025% w/w in dimethyl formamide.

4.2 Results of test

4.2.1	Proliferative response	A stimulation index of less than 3 was recorded for the three concentrations of the test material (see table A.6.1.5_1)
4.2.2	Clinical observations	No signs of systemic toxicity were noted in the test or control animals during the test
4.2.3	Mortality	There were no deaths
4.2.4	Bodyweight	Bodyweight changes of the test animals between Day1 and Day 6 were comparable to those observed in the corresponding control group animals over the same period.

4.3 Overall result

The test material was considered to be a non-sensitiser under the conditions of the test.

Section A6.1.5 (2) Skin sensitisation

Local Lymph Node Assay

Annex Point IIA, VI. 6.1.5

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Study performed in accordance to OECD Guideline No. 429 and EC Method B42 of the Commission Directive 2004/73/EC.

Following a preliminary test, three groups, each of four animals, were treated with 50 µl (25 µl per ear) of the test material as a solution in dimethyl formamide at concentrations of 0.001%, 0.0005% or 0.00025% w/w. A further group of four animals was treated with dmethyl formadide alone.

5.2 Results and discussion

The Stimulation Index expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group was less than 3 in all 4 groups, and therefore the result for all is negative.

5.3 Conclusion

The test material was considered to be a non-sensitiser under the conditions of the test.

5.3.1 Reliability 1

5.3.2 Deficiencies No

Section A6.1.5 (2) Skin sensitisation

Local Lymph Node Assay

Annex Point IIA, VI. 6.1.5

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *26.02.07*

Materials and Methods

3.1 Test material

Brodifacoum

3.1.2.2 Purity

99.7% w/w , as reported in the certificate of analysis attached to the study.

3.1.2.3 Stability

Preparation date 6/05 - Expiring date 6/07 , as reported in the certificate of analysis attached to the study, if stored well closed at cold temperature (5°C) and away from light.

3.2.5 Age/Weight at study initiation

Age: 8 to 12 weeks old

Weight: 15 – 23 g

3.2.6 Number of animals per group

Preliminary screening test: 6 animals

Main test: 4 animals per group

3.3.2 Way of Induction

Topical, non-occlusive

Test material administration

The mice were treated by daily application of 25 µl of the appropriate concentration of the test material to the dorsal surface of each ear for three consecutive days. The test material formulation was administered using an automatic micropipette and spread over the dorsal surface of the ear using the tip of the pipette. A further group of four mice received the vehicle only

^{3}H-methyl thymidine administration:

All mice were injected via the tail vein with 250 µl of phosphate buffered saline (PBS) containing ^{3}H-methyl thymidine (80 µCi/ml, specific radioactivity 2.0 Ci/mmol), in order to administer a total of 20 µCi to each mouse.

Results and discussion Adopt applicant's version

Conclusion Adopt applicant's version

Reliability The reliability indicator is appropriate

Acceptability Acceptable

Remarks

Section A6.1.5 (2) Skin sensitisation

Local Lymph Node Assay

Annex Point IIA, VI. 6.1.5

	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_1_5-1. Result of skin sensitisation test – Disintegrations per minute, node and stimulation index

Concentration (% w/w) in dimethyl formamide	dpm	dpm/node[a]	Stimulation Index[b]	Result
Vehicle	3866.77	483.35	N/A	N/A
0.00025	4323.52	427.94	0.89	Negative
0.0005	5005.70	625.71	1.29	Negative
0.001	5337.60	667.20	1.38	Negative

dpm: disintegrations per minute

[a] disintegrations per minute/node obtained by dividing the disintegrations per minute value by 8 (total number of lymph nodes)

[b] stimulation Index of 3.0 or greater indicates a positive result

N/A: not applicable

Section A6.2 Annex Point IIA 6.2	Percutaneous absorption (in vivo test)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	A more modern study OECD 428 exists. This is an in-vitro method and therefore is compliant with animal welfare policy. However, it has been decided to use the default data of 10% percutaneous absorption (as indicated in the TGD) for the risk characterisation in the risk assessment document II.	X
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*21.11.2006*	
Evaluation of applicant's justification	If in vitro data are not available, it is not necessary to refer to the OECD guideline. Therefore the first part of the justification should be deleted. The applicant submitted in the metabolism section an in vivo study on flocoumafen in which elimination and retention of the test item after percutaneous administration is carried out. Since the applicant has claimed the 'read across' of metabolism data, this should include for consistency also the percutaneous absorption. The study, described and commented in section A.6.2(2c), have a quite peculiar study design (the test item has not been removed from the application site for the entire duration of the study, i.e. 7 days). Therefore, data on excretion (urine and faeces) and on retention in the tissues cannot be directly used as such. However, they give a clear indication of a substantial percutaneous absorption, supported also by the acute dermal toxicity of the a.s.. The Applicant's decision to adopt the default value is accepted.	
Conclusion	Applicant's justification is in principle acceptable, considering the adoption of the default value.	

Section A6.2 Annex Point IIA 6.2	Percutaneous absorption (in vivo test)
Remarks	18.2.2009 During the commenting time the Applicant change the opinion about the adoption of the default value of 10% for dermal absorption and claimed for reading across from data available for other structurally related 2nd generation anticoagulants and provided in vitro studies on formulations containing difenacoum and bromadiolone. The RMS considers the principle as reasonable, also in view of the fact that it was already accepted to derive other ADME parameters. On this basis the dermal absorption of brodifacoum was re-evaluated. A value of 3%, i.e. the value adopted for difenacoum inclusion in Annex I to Directive 98/8/EC, was considered valid also for brodifacoum. The choice was supported by the 4% dermal absorption adopted for difethialone, by the high acute toxicity following dermal exposure (an average LD_{50}oral/LD_{50}dermal ratio in the range 10-30) and by qualitative data obtained by other ADME studies (as described in the following sections. Therefore, although results on formulations of difenacoum and bromadiolone, containing a very low concentration of the active substance (0.005% w/w) within a complex solid matrix, indicate a low potential for skin penetration, the proposed value represents a reasonable worst case for the absorption of brodifacoum from all products, taking into account the health hazards of brodifacoum (high acute toxicity and low NOAEL values).
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 Annex Point IIA 6.2	Metabolism studies in mammals	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Brodifacoum is a second generation hydroxycoumarin anticoagulant. This group of substances includes brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen that have been reviewed by both the US EPA (1998) and the IPCS (1995). In essence, provided that the metabolisable group is blocked (as with brodifacoum, bromadiolone, difethialone or flocoumafen), then unmetabolised material accumulates in liver and is only very slowly eliminated from it (Parmar et al, 1987; Huckle et al, 1989a; US EPA, 1998). Difenacoum lacks the blocking group on the 4' position of the biphenyl and is metabolised, with the metabolites being retained in the liver (Parmar et al, 1987). For flocoumafen, the 4-trifluoromethyl carries out the same function as the bromo group, in that it exerts a similar blocking effect in terms of preventing metabolism (see Huckle et al, 1989a). Of these hydroxycoumarin anticoagulants, the compound on which a good range of disposition and metabolism studies have been published in the peer-reviewed literature is flocoumafen (Huckle et al, 1988, 1989 a,b). In essence, these studies comply with the requirements of the TNsG within the limits posed when the target species is also the species used as surrogate for human health. In rats, orally administered radiolabelled flocoumafen was relatively rapidly absorbed following oral administration. Elimination of radioactivity was very slow, and almost entirely in faeces. Even at 7 days post dose about 75% of the radioactivity was retained, with approximately half of this being in the liver. At 2 days post dose most of the liver radioactivity comprised unchanged flocoumafen. Multiple oral administration of low or high doses of radioactive flocoumafen once weekly for 14 weeks also resulted in accumulation of unchanged flocoumafen in the liver, which plateaued at 4 weeks. Appreciable penetration of radiolabel occurred though rat skin following percutaneous administration. At 7 days, 12% of the radioactivity remained at the site of administration and 25% in the liver. Urinary excretion (metabolites) was thirty fold greater following percutaneous administration. Biliary excretion was a minor route and the faecal excretion (largely unchanged flocoumafen after percutaneous administration or after repeated, high dose oral administration) included a substantial quantity of non-biliary intestinal excretion. There were three minor metabolites in faeces. Confidence in this 'read across' is enhanced when the US EPA Reregistration Eligibility Review (1998) is examined. The review claims that a large proportion of unmetabolised brodifacoum is retained, particularly in the liver. Radiolabelled brodifacoum, administered orally to biliary cannulated rats, was recovered as follows: faeces (presumed unabsorbed brodifacoum by the EPA, but, in view of the data from flocoumafen, it may have included intestinally excreted material) 36%, liver 15%, rest of carcass 43%, bile 6.4% (as two metabolites, one	

metabolite, a glucuronide, accounted for 39-77% and a second accounted for 1-22%, and unchanged brodifacoum 0-25% of biliary radioactivity).

The half lives of elimination of radioactivity (probably unchanged compound) from blood following single oral doses of radiolabelled flocoumafen were 1.5 and 158 days (Huckle et al, 1989a).

The published data for brodifacoum, although by no means complete, also indicates that it behaves similarly to flocoumafen. In particular, there is substantial evidence that brodifacoum is retained, unchanged, for long periods post dose in rat, dog and sheep (Bachman and Sullivan, 1983; Godfrey et al, 1985; Laas et al, 1985; Ray et al, 1989; US EPA, 1998). Direct comparisons of the terminal phase of elimination from rat liver for brodifacoum and bromadiolone (the two non-metabolised second generation hydroxycoumarin anticoagulants examined by Parmar et al, 1987) indicated that their half lives [218 and 318 days (US EPA, 1998) and 130 and 170 days (Parmar et al., 1987)] were broadly comparable with that for radioactivity from flocoumafen [159 days (US EPA, 1998 or 220 days (Huckle, 1989b)]. The data for brodifacoum indicates that elimination from blood or plasma includes a long (and roughly comparable) terminal half life in studies on rat, rabbit, dog and human (Bachman and Sullivan, 1983; Breckenridge et al, 1985; Donovan et al, 1990; Wetzal et al, 1990; Woody et al, 1992) and, after allowance for the use of radioactively labelled material and the better definition of the kinetics, similar to those for flocoumafen (Huckle et al, 1989a). About 20-30% of orally administered brodifacoum is found as brodifacoum in sheep faeces by 8 days post dose (Laas et al, 1985), which is directly comparable to the amount of flocoumafen found in rat faeces at 7 days following a single oral dose (Huckle et al, 1989a). The dose levels and duration of the study on horses by Boermans et al (1991) prevented definition of this half life.

All of this indicates that the relatively non-metabolisable second generation hydroxycoumarin anticoagulants can be, and have been treated as a group and data 'read across' from one of these substances to the remainder. In view of this, and of the need to minimise experiments in animals, the data on metabolism for flocoumafen should be taken as typical for the group and 'read across' to cover for the data gaps for brodifacoum.

References:

Bachmann K A, Sullivan T J (1983) . Dispositional and pharmacodynamic characteristics of brodifacoum in warfarin-sensitive rats. *Pharmacology*, 27: 281-288.

Boermans H J, Johnstone J, Black W D & Murphy M (1991). Clinical signs, laboratory changes and toxicokinetics of brodifacoum in horses. *Can J Vet Res*, 55: 21-27.

Breckenridge A, Cholerton S, Hart J, Park B, Scott A (1985).A study of the relationship between the pharmacokinetics and the pharmacodynamics of the 4-hydroxycoumarin anticoagulants warfarin, difenacoum and brodifacoum in the rabbit. *Brit J Pharmacol* 84, 81-89

Donovan J W, Ballard J O, Murphy M J (1990) Brodifacoum therapy

Section A6.2 Annex Point IIA 6.2	Metabolism studies in mammals

with activated charcoal: Effect on elimination kinetics. *Vet Hum Toxicol,* 32: 350.

Godfrey M E R, Laas F J, Rammell C G (1985) Acute toxicity of brodifacoum to sheep. *New Zealand Journal of Experimental Agriculture,* 13:23-25.

Huckle, K R, Hutson, D H, Warburton, P A (1988). Elimination and accumulation of the rodenticide flocoumafen in rats following repeated dosing. *Xenobiotica* 18, 1465-1479.

Huckle K R, Hutson, D H, Logan, C J, Morrison, B J, Warburton, P A (1989a). The fate of the rodenticide flocoumafen in the rat: Retention and elimination of a single oral dose. *Pesticide science*, 25: 297-312.

Huckle, K R, Morrison, B J, Warburton, P A (1989b). The percutaneous fate of the rodenticide flocoumafen in the rat: role of non-biliary intestinal excretion. *Xenobiotica* 19, 63-74.

IPCS (1995). Anticoagulant Rodenticides. Envionmental Health Criteria 175. International Programmeon Chemical Safety. Geneva: World Health Organisation.

Laas F J, Forss D A, Godfrey M E R (1985) Retention of brodifacoum in sheep tissues and excretion in faeces. *N Z J Agric Res*, 28: 357-359.

Parmar G, Bratt H, Moore R, Batten P L (1987). Evidence for common binding site in vivo for the retention of anticoagulants in rat liver. *Hum Toxicol*, 6: 431-432.

Ray, A C, Murphy M J, DuVall, M D, Reagor J D (1989). Determination of brodifacoum and bromadiolone residues in rodent and canine liver. *Am J Vet Res,* 50, 546-550.

US EPA (1998). Reregistration Eligibility Decision (RED). Rodenticide Cluster. EPA 738-R-98-007. (http://www.epa.gov/REDs/2100red.pdf).

Woody B J, Murphy M J, Ray A C & Green R A (1992) Coagulopathic effects and therapy of brodifacoum toxicosis in dogs. *J Vet Intern Med*, 6: 23-28

Weitzel J N, Sadowski J A, Furie B C, Moroose R, Kim H, Mount M E, Murphy M J, Furie B (1990) . Surreptitious ingestion of a long-acting vitamin K antagonist/rodenticide, brodifacoum: Clinical and metabolic studies of three cases. *Blood*, 76, 2555-2559.

Undertaking of intended data submission [ ]

Section A6.2 Annex Point IIA 6.2	Metabolism studies in mammals
Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*23.11.06*
Evaluation of applicant's justification	Applicant's justification is reasonable, although it could be formulated in a better way. The request of reading across should be more explicitly expressed and justified on a scientific basis by the similarity in the structure, physico-chemical properties and in the mechanisms of action of the two rodenticides at the very beginning (including the fact, not to be dismissed, that the toxicologically relevant chemical is the parent compound rather than a metabolite). Then the similarity of the submitted results on flocoumafen (which are reported in excess of details) with those cited by EPA, IPCS and/or those present in the open literature and submitted for the evaluation (see the following section) could be used as supporting information.
Conclusion	Applicant's justification is in principle acceptable , and supported by the RMS, although it should be beneficial for the evaluation to reformulate it, according to the comments above.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 (1) METABOLISM

Annex Point IIA6.2 *Rabbit – Warfarin, difenacoum and brodifacoum*

Official use only

			1 REFERENCE	
1.1	**Reference**		A.M. Breckenridge, S. Cholerton, J.A.D. Hart, B.K. Park & A.K. Scott (1985). A study of the relationship between the pharmacokinetics and the pharmacodynamics of the 4-hydroxycoumarin anticoagulants warfarin, difenacoum and brodifacoum in the rabbit. British Journal Pharmac. (1985), 84, 081 -091.	
1.2	**Data protection**		No, published paper.	
	1.2.1	Data owner	Published paper based on a study carried out by Department of Pharmacology and Therapeutics, University of Liverpool.	
	1.2.2	Criteria for data protection	No data protection claimed	
			2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**		The guideline study is not stated in the published paper.	
2.2	**GLP**		The GLP status of the study is not stated in the published paper	
2.3	**Deviations**		No	X
			3 MATERIALS AND METHODS	
3.1	**Test material**		Warfarin, difenacoum and brodifacoum	
	3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.	
	3.1.2	Specification	Warfarin supplied by Ward Blenkinsop Difenacoum and brodifacoum supplied by Sorex Laboratories, Widnes.	X
3.1.2.1	Stability		A specific statement on stability is not provided within the paper.	
3.1.2.2	Test Animals			
	3.1.3	Species	Rabbits	
	3.1.4	Strain	New Zealand White	
	3.1.5	Source	Not stated in published report	
	3.1.6	Sex	Male	
	3.1.7	Age/weight at study initiation	Age not stated in published report. Range from 2.5Kg – 3.0Kg	
	3.1.8	Number of animals per group	Racemic warfarin (high dose) = 8 Racemic warafarin (low dose) = 4 Difenacoum = 4 Brodifacoum = 4	

Section A6.2 (1) METABOLISM

Annex Point IIA6.2

Rabbit – Warfarin, difenacoum and brodifacoum

			R-warfarin = 4 S-warfarin = 4	
	3.1.9	Control animals	no	
3.2	**Administration/ Exposure**		Oral – intravenous injection	X
	3.2.1	Preparation of test site	Not applicable	
	3.2.2	Concentration of test substance	Racemic warfarin (high dose) = 20 µmol/kg Racemic warafarin (low dose) = 4 µmol/kg Difenacoum = 20 µmol/kg Brodifacoum = 20 µmol/kg R-warfarin = 2 µmol/kg S-warfarin = 2 µmol/kg	X
	3.2.3	Specific activity of test substance		
	3.2.4	Volume applied	0.5ml/kg bodyweight	
	3.2.5	Sampling time	Pharmacokinetic study 1-4ml of blood taken at regular intervals up to 384 hours following anticoagulant administration. Exact timings not stated. In addition on one animal given an unknown dose and at an unknown time five plasma samples were taken at regular intervals over a period of 22 days. Pharmacodynamic study Blood samples taken serially – 1ml for prothrombin and 1-4ml for plasma anticoagualnt concentrations. Effect of Phenobarbitone 1ml blood samples taken at regular intervals up to 384 hours.	X
	3.2.6	Samples	Analytical techniques: High performance liquid chromatography For concentrations of difenacoum and brodifacoum in plasma a sensitive reversed phase HPLC assay. For warfarin concentrations in plasma a normal-phase HPLC	X

Section A6.2 (1) METABOLISM

Annex Point IIA6.2

Rabbit – Warfarin, difenacoum and brodifacoum

		4 RESULTS AND DISCUSSION.	
4.1	**Result and discussion**	In the rabbit, the terminal half-lives of difenacoum (83.1 ± 10.3h) and brodifacoum (60.8 ± 1.9h) were approximately ten fold greater than for racemic warfarin (5.6 ± 0.8h). Brodifacoum was cleared from plasma more slowly (0.8 ± 0.02 ml/min/kg) than warfarin (1.33 ± 0.08 ml/min/kg), whereas difenacoum had a far greater apparent volume of distribution (10.4 ± 1.41 l/kg) compared with warfarin (0.65 ± 0.10 l/kg). There was no significant difference between cis- and trans-brodifacoum concentrations up to 240 hours following administration of the isomer mixture.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**		X
5.2	**Results and discussion**	Brodifacoum and difenacoum have the same mechanism of action as warfarin and blood clotting factor synthesis indirectly, by interruption of the vitamin K-epoxide cycle at the epoxide reductase enzyme. However, difenacoum and brodifacoum are far more powerful anticoagulants than warfarin in a number of species, including man, the rat and the rabbit.	X
5.3	**Conclusion**	Brodifacoum and difenacoum have the same mechanism of action as warfarin and blood clotting factor synthesis indirectly, by interruption of the vitamin K-epoxide cycle at the epoxide reductase enzyme. However, difenacoum and brodifacoum are far more powerful anticoagulants than warfarin in a number of species, including man, the rat and the rabbit.	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.06*
Guidelines and Quality Assurance	**2.3 Deviations** Not applicable

Section A6.2 (1) METABOLISM

Annex Point IIA6.2

Rabbit – Warfarin, difenacoum and brodifacoum

Materials and Methods	**Include revised version** **3.1.2 Specifications** Not clearly stated. Brodifacoum and Difenacoum supplied by Sorex Laboratories, Widnes; Warfarin supplied by Ward Blenkinsop. **3.2 Administration/Exposure** Intravenous (i.v.) injection **3.2.2 Concentration of test substance** Brodifacoum (as a mixture of *cis*- and *trans-isomer*)= 20 µmol/kg (PK study); 0.1 µmol/kg (PD study) Difenacoum = 20 µmol/kg (PK study); 0.5-1 µmol/kg (PD study) Racemic warfarin = 4 and 20 µmol/kg (PK study); 2 µmol/kg (PD study) R-warfarin = 2 µmol/kg S-warfarin = 2 µmol/kg **3.2.5 Sampling time** PK study : 1-4ml of blood taken at 10 regular intervals up to 384 hours following anticoagulant administration. PD study: Blood samples taken serially – 1ml for prothrombin and 1-4ml for plasma anticoagualnt concentrations. **3.2.6 Samples** Brodifacoum, difenacoum and warfarin plasma concentrations were detected by means of specific HPLC assay (l.o.d. 50 ng/ml)
Results and discussion	**Include revised version.** **4.1 Results** In the rabbit, the terminal half-life of brodifacoum (60.8 ± 1.9h) was similar to the one of difenacoum (83.1 ± 10.3h) and approximately ten fold greater than the one calcualted for racemic warfarin (5.6 ± 0.8h). Brodifacoum was cleared from plasma more slowly (0.8 ± 0.02 ml/min/kg) than warfarin and difenacoum (1.33 ± 0.08 and 1.43± 0.09 ml/min/kg, respectively). The brodifacoum apparent volume of distribution was 0.41 ± 0.10 l/kg, similar to warfarin (0.65 ± 0.10 l/kg) and relatively small when compared with difenacoum (10.4 ± 1.41 l/kg) There was no significant difference between cis- and trans-brodifacoum concentrations up to 240 hours. The paper reported that the plasma half life of brodifacoum in rat and humans (as determined in one single poisoned patient) is greater than the one measured in rabbits, that is 156 and 487 h, respectively.

Section A6.2 (1) METABOLISM

Annex Point IIA6.2

Rabbit – Warfarin, difenacoum and brodifacoum

Applicant's Summary and Conclusion	**5.1 Materials and methods** The applicant's test is missing **5.2 Results and discussion** In the rabbit, the terminal half-life of brodifacoum was 60.8 ± 1.9h, the plasma clearance was relatively slow (0.8 ± 0.02 ml/min/kg) and the apparent volume of distribution was 0.41 ± 0.10 l/kg. Results clearly indicated toxicokinetics differences among the 3 tested anticoagulants. There was no significant difference between cis- and trans-brodifacoum plasma concentrations up to 240 hours. The PD results indicated that brodifacoum was the most efficient molecule, reaching the complete clotting factor synthesis at the lowest concentrations (0.1 µmol/kg, i.v.).
Conclusion	**Include revised version** Brodifacoum and difenacoum have the same mechanism of action as warfarin, inhibiting blood clotting factor synthesis indirectly, by interruption of the vitamin K-epoxide cycle at the epoxide reductase level. The differences in tokicokinetics parameters may account for the different efficiency of the 3 tested compounds. The paper reported that the plasma half life of brodifacoum in rat and humans (as determined in one single poisoned patient) is greater than the one measured in rabbits, that is 156 and 487 h, respectively.
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	Although acceptable, the study provides limited information on the toxicokinetic of the a.s.. In addition, the route of administration (i.v.) is poorly relevant for human exposure. It can be considered as a supporting study.

	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	

Section A6.2 (2a) METABOLISM

Annex Point IIA6.2

Rat – single dose

			Official use only
		1 REFERENCE	
1.1	**Reference**	Huckle K R et al (1989). The fate of the rodenticide flocoumafen in the rat: Retention and elimination of a single oral dose. Pesticide science, 25: 297-312.	
1.2	**Data protection**	No, published paper.	
1.2.1	Data owner	Published paper based on a study carried out by Shell Research Ltd.	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not stated	
2.2	**GLP**	The GLP status of the study is not stated in the published paper	
2.3	**Deviations**	not stated	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Flocoumafen	
3.1.1	Lot/Batch number	Sample number 720 and 873 and non-radiolabelled flocoumafen	X
3.1.2	Specification	Sample 720: Flocoumafen concentration : 0.14mg ml^{-1} Radiochemical purity : 97.5% Cis:trans : 51:49 Sample 873 Flocoumafen concentration : 0.11mg ml^{-1} Radiochemical purity : 94.5% Cis:trans : 61:39 Non-radiolabelled flocoumafen Isomer ration approximately 1:1	X
3.1.2.1	Description		
3.1.2.2	Purity	Sample 720: Flocoumafen concentration : 0.14mg ml^{-1} Radiochemical purity : 97.5%	X

Section A6.2 (2a) METABOLISM

Annex Point IIA6.2 *Rat – single dose*

		Sample 873
		Flocoumafen concentration : 0.11mg ml^{-1}
		Radiochemical purity : 94.5%
		Non-radiolabelled flocoumafen
		> 99%
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.1.2.4	Radiolabelling	The compound was labelled uniformly with ^{14}C in the aromatic ring of the coumarin moiety.
		Sample 720 had a specific radioactivity of 270 uCimg^{-1} and supplied in acetone.
		Sample 873 had a specific radioactivity of 322 uCimg^{-1} and was supplied in acetonitrile.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	Charles River UK Ltd
3.2.4	Sex	Male and Female
3.2.5	Age/weight at study initiation	Young animals.
		Mean weight of male rats 210g range 187 – 229 and female rats 142g range 130 – 148g
3.2.6	Number of animals per group	**To investigate the effect of flocoumafen on plasma prothrombin time.**
		6 pairs of male rats
		Adsorption-elimination-retention study.
		5 males and 5 females
		Collection of respired CO_2
		2 males and 2 females
		Determination of plasma prothrombin time and half life of ^{14}C flocoumafen in whole blood and plasma
		14 animals
		Measurement of the kinetics of elimination
		30 males
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral intubation
3.3.1	Preparation of test site	None

Section A6.2 (2a) METABOLISM

Annex Point IIA6.2

Rat – single dose

3.3.2	Concentration of test substance	**To investigate the effect of flocoumafen on plasma prothrombin time** 0.025, 0.05, 0.1, 0.2, 0.3 and 0.4 mg of non-radiolabelled flocoumafen per ml^{-1}. **Adsorption-elimination-retention study** 0.14 mg ml^{-1} ^{14}C flocoumafen **Collection of respired CO_2** 0.14 mg ml^{-1} ^{14}C flocoumafen **Determination of plasma prothrombin time and half life of ^{14}C flocoumafen in whole blood and plasma** 0.14 mg ml^{-1} ^{14}C flocoumafen **Measurement of the kinetics of elimination** 0.14 mg ml^{-1} ^{14}C flocoumafen	X
3.3.3	Specific activity of test substance	100%	
3.3.4	Volume applied	**To investigate the effect of flocoumafen on plasma prothrombin time** 1 ml dose per kg body weight. **Adsorption-elimination-retention study** 1 ml dose per kg body weight. **Collection of respired CO_2** 1 ml dose per kg body weight. **Determination of plasma prothrombin time and half life of ^{14}C flocoumafen in whole blood and plasma** 1 ml dose per kg body weight. **Measurement of the kinetics of elimination** 1 ml dose per kg body weight.	X
3.3.5	Size of test site	Whole animal	X
3.3.6	Exposure period	**To investigate the effect of flocoumafen on plasma prothrombin time** 24 hours **Adsorption-elimination-retention study** 7 days **Collection of respired CO_2** 48 hours **Determination of plasma prothrombin time and half life of ^{14}C flocoumafen in whole blood and plasma**	

		4 days Measurement of the kinetics of elimination 373 days
3.3.7	Sampling time	**To investigate the effect of flocoumafen on plasma prothrombin time** 24 hours **Adsorption-elimination-retention study** Urine and faeces daily. Tissues at the end of the study. **Collection of respired CO_2** CO_2 traps replaced each 24 hour period. **Determination of plasma prothrombin time and half life of ^{14}C flocoumafen in whole blood and plasma** 1, 4, 8,and 24 hours, day 2 and day 4. **Measurement of the kinetics of elimination** Day 2, 4, 7, 14, 28, 56, 112, 201, 285 and 373.
3.3.8	Samples	**To investigate the effect of flocoumafen on plasma prothrombin time** 2ml blood directly from the heart. **Adsorption-elimination-retention study** Urine and faeces during the in-life stage. At the end of the study blood sample, liver, kidney, heart, spleen, lungs, brain, gastro-intestinal tract (including contents), skin, gonads and sub-samples of muscle and abdominal fat. **Collection of respired CO_2** Air was drawn out of the metabolism vessel through a CO_2 trap. **Determination of plasma prothrombin time and half life of ^{14}C flocoumafen in whole blood and plasma** Blood via cardiac bleeding. **Measurement of the kinetics of elimination** Blood samples plus liver, kidneys, gastro-intestinal tract (and contents) and sub-samples of muscle and abdominal fat.
		4 RESULTS AND DISCUSSION
4.1	**Result of study**	**To investigate the effect of flocoumafen on plasma prothrombin time** Prothrombin times at 4 and 8 hours after dosing were increased relative to the normal control range of 10-11 seconds. The maximum prothrombin time was recorded at 24 hours after dosing, but the value had returned to normal by 48 hours. Concentration of flocoumafen (and

or metabolites) rose rapidly in whole blood with a maximum value at 4 hours. The values were however very low, 0.025 and 0.04 ug equivalents of flocoumafen per ml of whole blood and plasma respectively. Most of the radioactivity was located in the plasma. Plasma and blood concentrations declined rapidly with time and were less than half the maximum value at 8 hours after dose administration.

Adsorption-elimination-retention study

^{14}C flocoumafen was extensively absorbed. A summary of the daily elimination of radioactivity in the urine and faeces of male and female rats, over 7 days, is attached as Table 1. The elimination of flocoumafen was slow, with no sex difference in rate or route of excretion of radioactivity. The major route of elimination was via the faeces with 26% of the administered radioactivity excreted by males and 23% by females of which half of this appeared in the 0-24 hour sample. The urine accounted for less than 0.5% of the total radioactivity eliminated over the 7 day study. The elimination of $^{14}CO_2$ was below the limit of detection (0.05% of dose).

Distribution of radioactivity throughout the tissues 7 days after dosing is summarised and attached as Table 2. The liver contained the highest concentration of radioactivity, which was in the range 1 – 1.7 ug g^{-1}. These are very high residues considering the low dose administered. The kidney contained the next highest concentration (approximately 0.2 ug g^{-1}). Other tissues that contained residues in the range 0.1-0.13 ug g^{-1} were lung, spleen and ovaries. Storage in fat was not significant. Radioactivity in the blood was very low (0.001 ug g^{-1}, limit of detection was 0.0004 ug g^{-1}. This suggests that only a slow redistribution of the residual radioactivity was occurring 7 days after dosing

The retention of ^{14}C flocoumafen-derived radioactivity in the body was considerable at 7 days after dosing (74-76%). The majority (50% of the dose) was located within the liver. The distribution of the major portions of radioactivity throughout the tissues and excreta is summarised and attached in Table 3. Overall recoveries were 100-103% (excluding one female which afforded 64%).

Measurement of the kinetics of elimination

The depletion of residues from tissues was very slow. Liver residues were at a plateau of about 1.2 ug g^{-1} over the first 7 days and depleted slowly with the half life of about 220 days. Flocoumafen depleted from kidney, fat and muscle in a bi-phasic manner. Initially, depletion from those tissues was rapid with a $t_{1/2}$ of 4.5 – 9.8 days (α phase). After 28 days, the depletion had slowed considerably to the β-phase with a $t_{1/2}$ of 187 days for kidney, 241 days for fat and 261 days for muscle. The depletion profile of the low concentrations in blood was similarly bi-phasic. The residues of radioactivity were also persistent in the gastro-intestinal tract, with depletion at a similar rate ($t_{1/2}$ of 220 days to that for liver and the β-phase of the other tissues.

Of the labelled material retained in rat liver at 48 hours after dosing, 81% was extracted with acetonitrile. HPLC and TLC analyses indicated that 83% of the extractable material was parent compound. The cis:trans ratio of the hepatic flocoumafen was found to be 61:39 by HPLC indicating that the trans isomer is cleared or metabolised faster than the trans isomer since the cis:trans ratio before dosing was 51:49.

Section A6.2 (2a) **METABOLISM**

Annex Point IIA6.2 *Rat – single dose*

The remaining extracted material consisted of a mixture of polar metabolites (14%) and non-polar material (3%).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Following oral intubation of a single dose of ^{14}C flocoumafen the distribution, retention and elimination of the compound was studied in rats

X

5.2 Results and discussion

Flocoumafen is efficiently absorbed following a single oral dose and is rapidly distributed from the blood mainly into the liver.

Elimination of radioactivity was very slow with less than 0.5% of the dose appearing in urine over the 7-day study. The main route of excretion is via the faeces (23-26%) with more than half of the radioactivity eliminated within 24 hours of dosing. It is probable that this was unabsorbed compound. Most of the administered radioactivity (74-76%) was still retained in the body 7 days after dosing and 50% of the dose was located in the liver. Unchanged flocoumafen comprised the major portion of the hepatic radioactivity. It was eliminated with a half-life of 220 days. Elimination from other tissues, with the exception of intestine, eas bi-phasic, with β-phase half-lifes being very similar to that of the liver.

Flocoumafen is a very lipophilic molecule and is clearly relatively resistant to metabolism. Given this accumulation in fat would be expected. The accumulation in liver, rather than fat, and its persistence suggests the presence of a high-affinity binding site for flocoumafen in the liver.

5.3 Conclusion

Flocoumafen was rapidly absorbed into the blood from a single oral dose. Elimination was very slow with less than 0.5% of the dose being detected in urine up to 7 days after dosing and 23-26% in the faeces. Approximately half of the dose was found in the liver that was eliminated with a half-life of 220 days.

5.3.1 Reliability 2

5.3.2 Deficiencies No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *21.11.06*

Materials and Methods Insert revised version

3.1.1 Lot/Batch number

Non-radiolabelled flocoumafen supplied by Organic Chemical Division SRC; Radiolabelled ^{14}C- flocoumafen : sample number 720 and 873 dissolved in acetone

3.1.2 Specifications

Section A6.2 (2a) METABOLISM

Annex Point IIA6.2

Rat – single dose

	Sample 720 (after acetone evaporation, the sample was transferred to corn oil): Flocoumafen concentration : 0.14mg ml^{-1} Radiochemical purity : 97.5% ; *Cis:trans* ratio : 51:49 Sample 873 (after acetonitrile evaporation, the sample was transferred to corn oil): Flocoumafen concentration : 0.11mg ml^{-1} Radiochemical purity : 94.5% ; *Cis:trans* ratio: 61:39 Non-radiolabelled flocoumafen Isomer ratio approximately 1:1 **3.1.2.2 Purity** Sample 720: Radiochemical purity = 97.5% Sample 873: Radiochemical purity = 94.5% Non-radiolabelled flocoumafen > 99% **3.3.2 Concentration of test substance** Effect of flocoumafen on plasma prothrombin time: 0.025, 0.05, 0.1, 0.2, 0.3 and 0.4 mg of non-radiolabelled flocoumafen kg^{-1}. TK study : 0.14 mg kg^{-1} [^{14}C] flocoumafen **3.3.4 Volume applied** 1 ml per kg body weight. **3.3.5 Size of test site** Not applicable.
Results and discussion	Adopt applicant's version
Applicant's Summary and conclusion	Include revised version **5.1 Materials and methods** Following oral intubation of a single dose of [^{14}C]- flocoumafen (0.14 mg/kg) the absorption, distribution, retention and elimination of the compound was studied in male and female rats. Sample were taken at sequential time and radioactivity was measured; in liver samples the test substance was determined by HPLC.
Conclusion	**Include revised version** Flocoumafen was rapidly absorbed into the blood from a single oral dose. Based on the levels eliminated via urine (less than 0.5% of the dose) and residues retained within the body up to 7 days after dosing (about 75%), the fraction of oral absorption can be calculated around 75-80% of the administered dose. However the a.s. detected within 24 h in the faeces (23-26%) could represent the sum of unabsorbed material and absorbed material eliminated via the biliary route. In the absence of data on bile cannulated rats, it cannot be ruled out that radioactivity in the faeces accounted just for unabsorbed material.

Section A6.2 (2a) METABOLISM

Annex Point IIA6.2 *Rat – single dose*

	Approximately half of the retained material, accounting for about 75% of the administered dose was found in the liver that was eliminated with a half-life of 220 days. More than 80% of the hepatic residue was identified as the unchanged parent compound, indicating the the flocoumafen is poorly metabolised. However, between the two isomers, trans- flocoumafen is more efficiently metabolised than cis
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	Although conducted with a test substance different from brodifacoum, the similar action and the sufficiently similar chemical structure allow to consider the study acceptable and relevant.
	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	

Section A6.2 (2b) METABOLISM

Annex Point IIA6.2

Rat – multiple dose

			Official use only
		1 REFERENCE	
1.1	**Reference**	Huckle K R, Hutson, D H, Warburton, P A (1988). Elimination and accumulation of the rodenticide flocoumafem in rats following repeated oral administration. *Xenobiotica* 18, 1465-1469	
1.2	**Data protection**	No, published paper.	
1.2.1	Data owner	Published paper based on a study carried out by Shell Research Ltd.	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not stated	
2.2	**GLP**	Not stated (peer-reviewed published paper)	
2.3	**Deviations**	N/A	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Flocoumafen	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Flocoumafen concentration : 0.02 and 0.1 mg ml^{-1}	X
		Radiochemical purity Not stated	
		Non-radiolabelled flocoumafen	
		Isomer ratio 44:43 cis:trans	
3.1.2.1	Description		
3.1.2.2	Purity	Non-radiolabelled flocoumafen	
		> 95%	
3.1.2.3	Stability	Not stated.	X
3.1.2.4	Radiolabelling	The compound was labelled uniformly with ^{14}C in the aromatic ring of the coumarin moiety.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River UK Ltd	
3.2.4	Sex	Male	
3.2.5	Age/weight at study	6-8 weeks; 214-216 g	X

Section A6.2 (2b) METABOLISM

Annex Point IIA6.2 *Rat – multiple dose*

	initiation		
3.2.6	Number of animals per group	Two dose levels n=36 at 0.02 mg/kg/week, n=27 at 0.1 mg/kg/week; control group n=9	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral intubation	
3.3.1	Preparation of test site	None	
3.3.2	Concentration of test substance	0, 0.0.02, 0.1 mg/ml	X
3.3.3	Specific activity of test substance	Not stated	X
3.3.4	Volume applied	1 ml/kg body weight.	
3.3.5	Size of test site	N/A	
3.3.6	Exposure period	10 weeks (0.1 mg/kg bw/week), 14 weeks (0.02 mg/kg bw/week)	
3.3.7	Sampling time	Animals sacrificed at (low dose) 1, 2, 4, 6, 8, 10 and 14 weks, (high dose) 1, 2 4, 6 weeks and (control) 1, 6 and 14 weeks) (3/time point).	X
3.3.8	Samples	Urine and faeces during the in-life stage. At the end of the study blood sample, liver, kidney, fat, and muscle .	X
		4 RESULTS AND DISCUSSION	
4.1	**Result of study**	Clinical signs: none in control and low dose animals; clinical signs consistent with anticoagulant toxicity at high dose became apparent particularly in some animals at 6 weeks. Treatment related histopathological changes occurred in the livers of high dose animals exhibiting symptoms (periportal and/or central atrophy). Total cumulative excretion of ^{14}C flocoumafen in faeces was 28% in the low dose group and 59% in the high dose group. Very litte radioactivity appeared in urine (0.8% in low dose group; 1.6% in high dose group). The elimination in the high dose group increased over the first four weeks (from 18 to 42% of the single dose), and elimination on days 2 and 3 increased relative to that on day 1. Liver was clearly the principal tissue of retention, and the principal material present (~80%) was unchanged flocoumafen. Progressive increases in concentrations in tissues were seen for kidney, skin, fat and muscle; liver concentrations saturated at weeks 4-6. Severe toxic effects became apparent at 8.5-10 weeks in the high dose group. The liver radioactivity appeared to be associated with two possible receptors, a high affinity site and a low affinity site.	X

Section A6.2 (2b) METABOLISM

Annex Point IIA6.2

Rat – multiple dose

Three metabolites were also present in liver (at 1-2%, 8-10% and 0.8-1.2% of liver radioactivity). In faeces the principal radioactive component (44-74%) was flocoumafen, with the same 3 metabolites also being present (5-15%; 4-28%; 0.8-2.9%). The amounts of the first metabolite increased with time in the low dose group, the amounts of the second metabolite increased with time in both groups.

Of the labelled material retained in rat liver at 48 hours after dosing, 81% was extracted with acetonitrile. HPLC and TLC analyses indicated that 83% of the extractable material was parent compound. The cis:trans ratio of the hepatic flocoumafen was found to be 61:39 by HPLC indicating that the trans isomer is cleared or metabolised faster than the trans isomer since the cis:trans ratio before dosing was 51:49. The remaining extracted material consisted of a mixture of polar metabolites (14%) and non-polar material (3%).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The distribution, retention and elimination of ^{14}C flocoumafen was studied following multiple oral doses to rats	X
5.2	**Results and discussion**	Flocoumafen is retained in the liver and the great majority of the material eliminated was eliminated in faeces, mainly as unchanged substance. Three minor metabolites were also present. These three metabolites were also present, but to a lesser extent, in liver. There was evidence of saturation of flocoumafen uptake and metabolism following repeated doses. This saturation occurred at 4-6 weeks in the high dose group. There was evidence of toxic effects at the high dose group; these effects were consistent with the mode of action of flocoumafen.	X
5.3	**Conclusion**	Flocoumafen absorption, liver uptake and metabolism are saturable on multiple dosing. The majority of the administered material was retained, principally in the liver. Elimination from liver was very slow, and was largely of unchanged flocoumafen. Excretion was mainly in faeces.Urine was a very minor route of excretion.	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *22.11.2006*

Materials and Methods Include revised version

3.1.2 Specification

Section A6.2 (2b) METABOLISM

Annex Point IIA6.2 *Rat – multiple dose*

	Flocoumafen concentration : 0.02 and 0.1 mg ml^{-1} Radiolabelled flocoumafen: radiochemical purity : 97.8 ± 1.2 % ; Specific radioactivity : 266 µCi/mg; Isomer ratio 54:46 % cis:trans Non-radiolabelled flocoumafen: Chemical purity > 95%; Isomer ratio 55:45% cis:trans **3.1.2.3 Stability** The radiolabelled compound was radiochemically instable on prolonged storage. During the study, at weekly intervals, aliquots of the stock material were re-purified by HPLC prior to dose formulation. **3.2.5 Age/weight at study initiation** 6-8 weeks; 214-268 g **3.3.2 Concentration of test substance** 0, 0.02, 0.1 mg/ml **3.3.3 Specific activity of test substance** 266 µCi/mg **3.3.7 Sampling time** Animals sacrificed at 1, 2, 4, 6, 8, 10 and 14 weeks (low dose), at 1, 2 4, 6 weeks (high dose) and at 1, 6 and 14 weeks) (control) (3 animal/time point). **3.3.8 Samples** Urine and faeces were collected 3 days post dosing. At the end of the study cardiac and orbital blood samples, liver, kidney, fat, and muscle were collected and used for radioanalysis, clinical chemistry and haematological investigations. Pathological examination were carried out on 3 animal for each treatment group and on muribond or dead animals. Histopatological examination were performed on section taken from the kidneys, liver, skeletal femoral muscle and abdominal adipose tissue.
Results and discussion	Flocoumafen absorption, liver uptake and metabolism are saturable on multiple dosing. The majority of the administered material was retained, principally in the liver. Elimination from liver was very slow, and was largely of unchanged flocoumafen. Excretion was mainly in faeces.Urine was a very minor route of excretion
Applicant's Summary and conclusion	Include revised version **5.1 Materials and methods** Following oral intubation of a repeated doses of [^{14}C]- flocoumafen (0.02 and 0.1 mg/kg per week) the absorption, distribution, retention and elimination of the compound was studied in male rats. Clinical signs, pathological and histopathologiacal examinations were performed. Excreta were collected during the 3 days after each single dose and analysed for radioactivity presence. Blood and tissue samples were taken at sequential time and radioactivity was measured; in liver and faecal samples the test substance was determined by HPLC. **5.2 Results and discussion** There was evidence of saturation of flocoumafen uptake following repeated

Section A6.2 (2b) METABOLISM

Annex Point IIA6.2

Rat – multiple dose

	doses. This saturation occurred at 4-6 weeks in the high dose group. Flocoumafen was highly retained in the liver unchanged (about 80% of the total hepatic radioactivity) and the great majority of the material was eliminated in faeces, mainly as parent compound. Three polar metabolites were identified both in the liver and in the faeces; the amonut of the excreted parent compound decreased during the study (from 74 to 44% of total faecal radioacticity) suggesting possible induction of its own metabolism. There was evidence of toxic effects at the high dose group; these effects were consistent with the mode of action of flocoumafen.
Conclusion	Flocoumafen absorption and liver uptake are saturable on multiple dosing, while metabolism increased. The majority of the administered material was retained, principally in the liver but also in the other tissues where it is distributed. Elimination from the liver was very slow. Excretion was mainly in faeces and was largely of unchanged flocoumafen. Urine was a very minor route of excretion. Three polar metabolites were identified both in the liver and in the feces.
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	Although conducted with a test substance different from brodifacoum, the similar action and the sufficiently similar chemical structure allow to consider the study acceptable and relevant
	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	

Section A6.2 (2c) METABOLISM

Annex Point IIA6.2 *Rat – dermal administration and additional studies*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Huckle K R, Morrison B J, Warburton, P A (1989). The percutaneous fate of the rodenticide flocoumafen in the rat: role of non-biliary intestinal excretion. *Xenobiotica* 19, 63-74	
1.2	**Data protection**	No, published paper.	
1.2.1	Data owner	Published paper based on a study carried out by Shell Research Ltd.	
1.2.2	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not stated	
2.2	**GLP**	Not stated (peer-reviewed published paper)	
2.3	**Deviations**	N/A	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Flocoumafen	
3.1.1	Lot/Batch number	Radiochemical – sample 765, non radiolabelled material – not stated	
3.1.2	Specification	Flocoumafen concentration : 0.02 and 0.1 mg ml^{-1}	X
		Radiochemical purity Not stated	
		Non-radiolabelled flocoumafen – purity >99%	
		Isomer ratio 56:44 cis:trans	
3.1.2.1	Description		
3.1.2.2	Purity	Non-radiolabelled flocoumafen	
		> 99%	
3.1.2.3	Stability	Not stated.	
3.1.2.4	Radiolabelling	The compound was labelled uniformly with ^{14}C in the aromatic ring of the coumarin moiety.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344 (Wistar for intraperitoneal experiment)	X
3.2.3	Source	Charles River UK Ltd	
3.2.4	Sex	Male	
3.2.5	Age/weight at study	Percutaneous study 8-10 weeks, not stated	X

Section A6.2 (2c) METABOLISM

Annex Point IIA6.2 *Rat – dermal administration and additional studies*

		initiation	Intravenous study age not stated; 201-214 g Biliary cannulated rats age not stated, 323-360 g
	3.2.6	Number of animals per group	Total recovery from percutaneous administration – 6 males Recovery following intravenous administration – 3 males Biliary excretion -- 4 males
	3.2.7	Control animals	N/A
3.3	**Administration/ Exposure**		Percutaneous, intravenous, intraperitoneal
	3.3.1	Preparation of test site	Shaved skin (percutaneous) N/A otherwise
	3.3.2	Concentration of test substance	Percutaneous 0.17 mg/kg Intravenous 0.13 mg/kg Intraperitoneal 0.09 mg/kg
	3.3.3	Specific activity of test substance	Not stated
	3.3.4	Volume applied	Percutaneous: 0.1 ml Intravenous: 0.5 ml/kg. I.p.: 0.5 ml/kg
	3.3.5	Size of test site	Percutaneous - 10 cm^2 Otherwise - N/A
	3.3.6	Exposure period	Percutaneous Animals collared to prevent oral ingestion, otherwise substance not removed. Otherwise – N/A
	3.3.7	Sampling time	Percutaneous and intravenous: Urine and faeces collected daily for 7 days. Animals sacrificed at 7 days and blood, brain, testes, heart, gastro-intestinal tract, spleen, lungs, liver and kidney collected. (Whole body autoradiography was performed on three further animals dosed percutaneously). Intraperitoneal - bile collected in 30 minute samples. After 8 h the animals were sacrificed and the liver analysed. Gastrointestinal tract and faeces were alos collected. Urine, faeces and liver samples were extracted and subjected to HPLC and TLC.
	3.3.8	Samples	As stated under sampling time.
			4 RESULTS AND DISCUSSION
4.1	**Result of study**		Percutaneous study: radioactivity was recovered in urine (10.3% of

dose), faeces (30.9%),liver (25.4%), application site (11.5%), carcass (5.0%), tissues other than liver collected separately (4.6%), cage wash 2.7% (total recovery 90.4%).

The liver radioactivity was predominantly (92%) unchanged flocoumafen. Four polar radioactive components were found in urine but not identified further. Faecal radioactivity was predominantly unchaged flocoumafen (70%), with four minor metabolites also present.

Following intravenous administration less than 9% of the administered radioactivity was found in excreta at 7 days post dose. Of this, 7.7% was in faeces. A large portion of the radioactivity (42.9% of administered dose) was found in liver; the rest was distributed generally throughout the body and less than 5% was at the site of administration. Unchanged flocoumafen accounted for 81% of liver radioactivity and a similar percent of early faecal radioactivity. At least four metabolites were found in faeces, hree of which were similar in chromatographic properties to those found following percutaneous administration.

Biliary excretion following intraperitoneal administration of radiolabelled flocoumafen was rapid, peaking at approximately 2.5 h. The total amount eliminated in bile in 8 h was 1.37% of the aministered dose. Unchanged flocoumafen accounted for only 7% of extracted radiolabel and at least 6 metabolites were present in the bile. Liver accounted for 64.2% of the radioactivity administered and total gut redioactivity accounted for 8.15 %, of which 79% was in the intestinal contents. The radioactivity in liver and gastro-intestinal tract were exclusively unchanged flocoumafen (note that bile had not been passed into the gut in these animals).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The distribution, retention and elimination of ^{14}C flocoumafen was studied following single percutaneous and intravenous doses to intact rats and single intraperitoneal doses to biliary cannulated rats.

5.2 Results and discussion

Flocoumafen is retained in the liver unmetabolised and the great majority of the material eliminated was eliminated in faeces, mainly as unchanged substance. In cannulated animals, radioactivity (as unchanged flocoumafen) was present in the intestine and the small amount of radioactivity in the bile was metabolites.

X

5.3 Conclusion

Flocoumafen is absorbed through the skin and is taken up by the liver uptake. The majority of the administered material was retained, principally in the liver. Elimination from liver was very slow, and was largely of unchanged flocoumafen. Excretion was mainly in faeces, and largely by a non-biliary mechanism.

X

5.3.1 Reliability 2

5.3.2 Deficiencies No

Section A6.2 (2c) METABOLISM

Annex Point IIA6.2 *Rat – dermal administration and additional studies*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*22.11.06*
Materials and Methods	Include revised version **3.1.2 Specification** Radiochemical purity: Not stated Specific radioactivity: 266 µCi/mg Non-radiolabelled flocoumafen – purity >99% Isomer ratio 56:44 cis:trans **3.2.2 Strain** Fisher 344 (percutaneous and i.v. administration) Wistar (i.p. injection for bile cannulated rat experiments) **3.2.5 Age/weight at study initiation** Percutaneous study: 8-10 weeks, 173-190 g Intravenous study: age not stated; 201-214 g Biliary cannulated rats: age not stated, 323-360 g **3.2.6 Number of animal per group** Percutaneous administration : n=9 ; at the end of the study (7days) 3 animal were used for whole body autoradiografy; the remaining 6 animals were sacrificed for radioanalysis. Intravenous administration: n= 3 animals Biliary excretion: n= 4 animals **3.3.3 Specific activity of test substance** 266 µCi/mg **3.3.7 Sampling Time** Percutaneous and intravenous: Urine and faeces collected daily for 7 days. Six animals dosed percutaneously and those i.v. injected were sacrificed at 7 days and blood, brain, testes, heart, gastro-intestinal tract, spleen, lungs, liver and kidney collected for radioanalysis together with the apllication site, aliquots of fat and muscle. Whole body autoradiography was carried out on the three remaining animals dosed percutaneously. Intraperitoneal : Bile was collected before test item injection and at in 30 minute interval throughout the study duration. After 8 h the animals were sacrificed and the liver analysed. Gastrointestinal tract and faeces were also collected. Urine, faeces and liver samples were extracted and subjected to HPLC and TLC.
Results and discussion	Include revised version.

Section A6.2 (2c) METABOLISM

Annex Point IIA6.2

Rat – dermal administration and additional studies

	Percutaneous study: A small but consistent amount of the applied dose was absorbed through the skin after a single dermal treatment followed by 7-day holding period. Indeed 1-2% and 3-5% of the dose was eliminated per day in the urine and in the feces, respectively. At the end of the study, the radioactivity recovered in urine was 10.3% of dose, in the faeces 30.9%. The test items was mainly distributed to the liver, but also to other organs; after 7 days the radioactivity was recovered in the liver (25.4%), at the application site (11.5%), in the carcass (5.0%), organs other than liver (4.6%), in the cage wash (2.7%) accounting for a total recovery of 90.4%. The hepatic radioactivity was predominantly (92%) unchanged flocoumafen. Faecal radioactivity was predominantly unchaged flocoumafen (70%), with four more polar metabolites also present. The same four polar radioactive components were found in urine but not identified further. Following i.v. administration about 7.7% of the administered radioactivity was found in faeces at 7 days post dose and less than 1% in the urine. About 1-2% of the dose was eliminated daily in the faeces during days1-4, the amount decreased to <1% during the last 3 days. A large portion of the radioactivity (42.9% of administered dose) was found in liver; the rest was distributed throughout the body and less than 5% was at the site of administration. Unchanged flocoumafen accounted for 81% of liver radioactivity and a similar percent of early faecal radioactivity. At least four metabolites were found in faeces, three of which were similar in chromatographic properties to those found following percutaneous administration. Biliary excretion following i.p. administration of radiolabelled flocoumafen was rapid, peaking at approximately 2.5 h. The total amount eliminated in bile in 8 h was about 1.4% of the aministered dose. Unchanged flocoumafen accounted for only 7% of extracted radiolabel and at least 6 polar metabolites were present in the bile. Liver accounted for 64.2% of the radioactivity administered and total gut redioactivity accounted for 8.15 %, of which 79% was in the intestinal contents. The radioactivity in liver and gastro-intestinal tract were exclusively unchanged flocoumafen. In conclusion, after a single subletal percutaneous treatment, flocoumafen was extensively but slowly absorbed, as indicated by the daily recovery of radioacticivity in the faeces (3-5%) and to a lesser extent in the urine (about 1%). After 7-day holding period, without removing the test item, 25% of the applied dose was accumualted in the liver. Since the urine contained mainly polar metabolites, the extent of metabolism seemed to be higher after percutaneous treatemnt than after oral administration. On the contrary the great majority of radioactivity in the faeces was represented by the unchanged compound. Results from the i.v. administration study (7.7 % of the dose present in the faeces mainly as unchanged compound) indicated that the a.s. may be excreted in the faeces without any biotrasformation. The study with bile cannulated rats indicating that 1.4% of the dose after i.p. administration was excreted in 8 h via the bile mainly as conjugates and that about 8% of the dose was recovered in the g.i. tract as unchanged compound, suggesting that faecal excretion of unmetabolized flocoumafen after oral administration not necessarily correspond to unabsorbed material.
Applicant's Summary and conclusion	**Include revised version**

Section A6.2 (2c) METABOLISM

Annex Point IIA6.2

Rat – dermal administration and additional studies

	5.2 Results and discussion After a single subletal percutaneous treatment, flocoumafen was extensively but slowly absorbed, as indicated by the daily recovery of radioacticivity in the faeces (3-5%) and to a lesser extent in the urine (about 1%). After 7-day holding period, without removing the test item, 25% of the applied dose was accumualted in the liver. The great majority of radioactivity in the liver as well as in the faeces was represented by the unchanged compound. The i.v. administration study showed that 7.7 % of the dose was ecreted in the faeces mainly as unchanged compound, 7 days post treatment. The study with bile cannulated rats indicated that 1.4% of the dose after i.p. administration was excreted in 8 h via the bile mainly as conjugates and that about 8% of the dose was recovered in the g.i. tract as unchanged compound. In both cases the majority of radioactivity was bioaccumulated in the liver.
Conclusion	Other conclusions: *Include revised version* Flocoumafen is slowly but substantially absorbed through the skin, as indicated by data on excretion via urinary and faecal route (about 17% during the first 3 days after treatemnt). The majority of the administered material was retained, mainly in the liver as unchanged flocoumafen. Excretion was in faeces was largely due to a non-biliary mechanism, as indicated by the studies after i.v. and i.p. administration of flocoumafen to bile-cannulated rats. As a consequence, faecal excretion of unmetabolized flocoumafen after oral administration not necessarily correspond to unabsorbed material.
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	Although conducted with a test substance different from brodifacoum, the similar action and the sufficiently similar chemical structure allow to consider the study acceptable and relevant. With respect to percutaneous absorption, data may be used only partially due to the unusual experimental design: indeed, the a.s. was not removed after application for the following 7 days and the levels of tissue residues are available only at study termination.
	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	

Section A6.2 (3) METABOLISM

Annex Point IIA6.2 *Rat liver- Brodifacoum, difenacoum, bromadiolone and coumatetralyl*

Official use only

1 REFERENCE

1.1	**Reference**	G. Parmar, H. Bratt, R. Moore & P.L.Batten (1985). Evidence for common binding site *in vivo* for the retention of Anticoagulants in Rat Liver. Hum Toxicol, 6: 431-432.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Published paper based on a study carried out by Imperial Chemical Industries plc.
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Difenacoum, brodifacoum, bromadiolone and coumatetralyl
3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
3.1.2	Specification	Not stated in published report.
3.1.2.1	Description	Not stated
3.1.2.2	Purity	Not stated
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.1.2.4	Radiolabelling	^{14}C-labelled compound
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Alp:AP (Wistar derived)
3.2.3	Source	Not stated in published report
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age not stated in published report. Weight range from 180g – 240g
3.2.6	Number of animals per group	Up to 24 per group

	3.2.7	Control animals	no
3.3	**Administration/ Exposure**		Oral
	3.3.1	Preparation of test site	Not applicable
	3.3.2	Concentration of test substance	Difenacoum = 2.70 µmol/kg Brodifacoum = 0.67 µmol/kg Bromadiolone = 1.76 µmol/kg Coumatetralyl = 20.55 µmol/kg
	3.3.3	Specific activity of test substance	Not relevant
	3.3.4	Volume applied	Not stated in published report
	3.3.5	Sampling time	Three animals were killed at days 1, 4, 8, 14, 28, 56, 84, 133 and 182 after dosing
	3.3.6	Samples	Hepatic concentration of radioactivity and prothrombin and kaolin cephalin times.
			4 RESULTS AND DISCUSSION
4.1	**Result of study**		For all anticoagulants the elimination of radioactivity from the liver was biphasic. Initial rapid phase (T½ approx 2 days) lasting up to 8 days. Slower terminal phase with elimination half lives of: brodifacoum – 130 days; bromadiolone – 170 days and difenacoum – 120 days. Coumatetralyl was much lower at 55 days.
			5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**		Test substance was administered orally and liver concentrations monitored for radioactivity.
5.2	**Results and discussion**		For all anticoagulants the elimination of radioactivity from the liver was biphasic. Thin layer chromatographic analysis of solvent extracts of livers from rats killed 1 and 14 days post dosing showed that for brodifacoum, bromadiolone and coumatetralyl most of the radioactivity was present as the unchanged parent compound , whereas for difenacoum the bulk of the radioactivity consisted of metabolites
5.3	**Conclusion**		Despite the differences in hepatic radioactivity concentration at day 1, the similarity in liver concentrations of the different anticoagulants during the slow, terminal phase suggests that they all interact at a common, saturable binding site.
	5.3.1	Reliability	2
	5.3.2	Deficiencies	No

X

Section A6.2 (3) METABOLISM

Annex Point IIA6.2 *Rat liver- Brodifacoum, difenacoum, bromadiolone and coumatetralyl*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	The applicants version is acceptable
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	Based on the assessment of materials and methods the appropriate reliability indicator is 3.
Acceptability	*Acceptable*
Remarks	The study gives very limited information on the TK of the a.s.. The only relevant result refers to the $t_{1/2}$ of brodifacoum in rats, which is consistent with other papers.
	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	

Section A6.2 (4) METABOLISM

Annex Point IIA6.2

Rat brodifacoum

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bachmann KA & Sullivan TJ (1983) – Dispositional and pharmacodynamic characteristics of brodifacoum in warfarin-sensitive rats. *Pharmacology*, 27: 281-288.	
1.2	**Data protection**	No, published paper.	
1.2.1	Data owner	Public domain	
1.2.2			
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Reasons unknown	X
2.2	**GLP**	The GLP status of the study is not stated in the published paper	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	94% pure	
3.1.2.1	Description	*No information*	
3.1.2.2	Purity	94% pure	
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.	
3.1.2.4	Radiolabelling	Not radiolabelled	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Harlan Sprague-Dawley	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Age unstated. Mean weight of male rats 165-425.	
3.2.6	Number of animals per group	Not stated. 3-5 animals / group used to investigate pharmacodynamics	X

Section A6.2 (4) METABOLISM

Annex Point IIA6.2 *Rat brodifacoum*

3.2.7	Control animals	Yes		X
3.3	**Administration/ Exposure**	Oral intubation		
3.3.1	Preparation of test site	None		
3.3.2	Concentration of test substance	0.1, 0.15, 0.2 and 0.33 mg/kg in PEG		X
3.3.3	Specific activity of test substance	100%		
3.3.4	Volume applied	1 ml dose per kg body weight.		
3.3.5	Size of test site	Whole animal		X
3.3.6	Exposure period	0.4,6,8, 12, 24, 48 72, 96 and 120 hours		X
3.3.7	Sampling time	0.4,6,8, 12, 24, 48 72, 96 and 120 hours		
3.3.8	Samples	Blood taken directly from the heart.		X
		4 RESULTS AND DISCUSSION		
4.1	**Results**	The general patterns of hypothrombinaemia were similar for warfarin and brodifacoum. Disappearance from serum was slow, half life of 156 hrs for brodifacoum; in intestine it showed a rapid initial decline with a gradual increase in concentration from 24 to 72 hrs. Liver concentrations rose abruptly and remained relatively constant for at least 96 hrs..		X
		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**	Rats dosed with 0.1-0.33 mg/kg and blood sampled at intervals thereafter		X
5.2	**Results and discussion**	The general patterns of hypothrombinaemia were similar for warfarin and brodifacoum. Disappearance from serum was slow, half life of 156 hrs for brodifacoum; in intestine it showed a rapid initial decline with a gradual increase in concentration from 24 to 72 hrs. Liver concentrations rose abruptly and remained relatively constant for at least 96 hrs..		
5.3	**Conclusion**	Brodifacoum exhibited a remarkably steep dose-response curve, and hypothrombinaemia was mediated by brodifacoum rather than by metabolites. Disappearance from serum was slow, half life of 156 hrs for brodifacoum; in intestine it showed a rapid initial decline with a		

		gradual increase in concentration from 24 to 72 hrs. Liver concentrations rose abruptly and remained relatively constant for at least 96 hrs.. Sustained liver concentrations may account for the apparent relative potency of brodifacoum.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Guidelines and Quality Assurance	Include revised version **2.1 Guideline study** No **2.3 Deviations** Not applicable
Materials and Methods	**3.2.6 Number of animals per group** 3-5 animals/group in the toxicokinetics study. 3-4 animals/group for the dose-response assay. **3.2.7 Control animals** No (in the TK study) Yes (in the dose-response assay) **3.3.2 Concentration of test substance** 0.2 mg/kg in PEG (in the TK study); 0.1, 0.15, 0.2 and 0.33 mg/kg in PEG (in the dose-response assay) **3.3.5 Size of the test site** Not applicable **3.3.6 Exposure period** One single administration **3.3.8 Samples** Blood (withdrawn by cardiac puncture). Liver and small intestine (immediately excised after animal decapitation) Brodifacoum was determined by an HPLC method.

Section A6.2 (4) METABOLISM

Annex Point IIA6.2 *Rat brodifacoum*

Results and discussion	*Include revised version.* **4.1 Results** Brodifacoum exibited a steep dose-response curve; although brodifacoum was more efficient than warfarin, the pattern of hypothrombinaemia of the two anticoagulant agents were similar. Since brodifacoum activity was decreased and enhanced by phenobarbital and SKF525 pretreatment , respectively, the brodifacoum-induced anticoagulant response is very likely due to the parent compound rather than to a metabolite. When rats were orally dosed with 0.2 mg/kg brodifacoum, disappearance from serum was slow, with a half life of 156 hrs; in intestine it showed a rapid initial decline with a gradual increase in concentration from 24 to 72 hrs, very likely due to enterohepatic circulation. Liver concentrations rose abruptly, with a liver:blood ratio higher than 20, and remained relatively constant for at least 96 hrs . The apparent volume of distribution was 0.985 l/kg (about 6 time larger than warfarin) and systemic clearance was 4.45 ml $kg^{-1}h^{-1}$.
Applicant's Summary and conclusion	**5.1 Materials and Methods** In the dose –response assay, rats were dosed with 0.1-0.33 mg/kg brodifacoum and blood sampled at intervals thereafter (up to 5 days after dosing) In the toxicokinetics study, rats were dosed with 0.2 mg/kg brodifacoum and blood sampled by cardiac puncture at intervals up to 120 hours. At each time, animal were sacrified by decapitation, and liver and small intestine were excised and processed in order to measure by HPLC brodifacoum levels in the tissues. **5.2 Results and discussion** The general mode of action were similar for warfarin and brodifacoum. Brodifacoum activity was decreased and enhanced by phenobarbital and SKF525 pretreatment , respectively, The brodifacoum plasma half life was 156 hrs; in intestine it showed a rapid initial decline with a gradual increase in concentration from 24 to 72 hrs. Liver concentrations rose quickly and remained relatively constant for at least 96 hrs, with a liver:serum mean ratio of 21.2.
Conclusion	Insert revised version **5.3 Conclusion** Brodifacoum exhibited a remarkably steep dose-response curve, and hypothrombinaemia was mediated by brodifacoum itself rather than by metabolites. Disappearance from serum was slow ($t_{1/2}$= 156 hrs); brodifacoum is persistent within the organism: high liver concentrations remained relatively constant for at least 96 hrs, also due, at least partially, to enterohepatic circulation. Sustained liver concentrations may account for the apparent relative potency of brodifacoum.
Reliability	The reliability indicator is appropriate
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.2 (4) METABOLISM

Annex Point IIA6.2

Rat brodifacoum

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	

Section A6.2 (5) METABOLISM

Annex Point IIA6.2 *Brodifacoum in Sheep*

Official use only

1 REFERENCE

1.1	**Reference**	F.J. Laas, D.A. Forss & M.E.R. Godfrey. Retention of brodifacoum in sheep tissues and excretion in faeces. New Zealand Journal of Agricultural Research, 1985, Vol 28: 357-359.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Published paper based on a study carried out by Invermay Agricultural Research Centre.
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Not stated in the published paper.
3.1.2	Specification	0.005% Brodifacoum
3.1.2.1	Description	
3.1.2.2	Purity	Not stated in the published paper.
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.2	**Test Animals**	
3.2.1	Species	Sheep
3.2.2	Strain	New Zealand grazing sheep
3.2.3	Source	Not stated in published report
3.2.4	Sex	Not stated in published report
3.2.5	Age/weight at study initiation	Not stated in published report
3.2.6	Number of animals per group	7 per group (2 wethers and 5 ewes)
3.2.7	Control animals	Yes – 1 ewe
3.3	**Administration/ Exposure**	Oral

X

Section A6.2 (5) METABOLISM

Brodifacoum in Sheep

Annex Point IIA6.2

3.3.1	Preparation of test site	Not applicable	
3.3.2	Concentration of test substance	0.2mg/kg & 2.0mg/kg liveweight	
3.3.3	Specific activity of test substance	Not relevant	
3.3.4	Volume applied	0.2 and 2.0 mg/ml	X
3.3.5	Sampling time	Ewes allocated randomly to sampling times of 2,4,8,64 or 128 days. Wethers were retained in pens for 10 days for collection of faeces then returned to grazing	
3.3.6	Samples	One wether form each group was slaughtered at day 15 and the other at day 32. One ewe was slaughtered at each sampling time.	X

4 RESULTS AND DISCUSSION

4.1 Result of study

Faeces

About 33% of the brodifacoum administered to the sheep dosed at 2.0mg/kg had been excreted in faeces 8 days after dosing compared to 20 % in the 0.2mg/kg group.. By day 32 faecal levels of brodifacoum were below detection limits in both groups (<0.05mg/kg)

Carcass, liver and omental fat

Brodifacoum could not be detected in omental fat in both groups from sheep killed 8 days after dosing. By day 15 0.1mg/kg was detected in the high dose group

Brodifacoum was still present in the livers of both dose groups in sheep killed at 128 days. Peaks levels in the liver were detected at day 2 in the high dose group and day 8 in the low dose group.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Single dose administered to 2 groups of sheep. Animal killed at 2,4,8,15,32,64 or 128 days after treatment. Faeces were collected daily and liver, carcass and omental fat sampled at slaughter and analyses by HPLC.

5.2 Results and discussion

Faeces: About 33% of the brodifacoum administered to the sheep dosed at 2.0mg/kg had been excreted in faeces 8 days after dosing compared to 20 % in the 0.2mg/kg group. By day 32 faecal levels of brodifacoum were below detection limits in both groups (<0.05mg/kg)

Carcass, liver and omental fat: Brodifacoum could not be detected in omental fat in both groups from sheep killed 8 days after dosing. By day 15 0.1mg/kg was detected in the high dose group

Brodifacoum was still present in the livers of both dose groups in sheep killed at 128 days. Peaks levels in the liver were detected at day 2 in the high dose group and day 8 in the low dose group.

5.3 Conclusion

From the study it appears that brodifacoum is rapidly excreted from all sheep tissues except the liver, which is the major site of the synthesis of

Section A6.2 (5) **METABOLISM**

Annex Point IIA6.2 *Brodifacoum in Sheep*

		blood clotting factors.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.06*
Materials and Methods	Insert revised version **3.1.2 Specification** 2.5 % solution of brodifacoum in propylene glycol/polyethylene glycol 200/triethanolamine (94:3:3 v/v/v) was supplied by Imperial Chem.Industried LTD, Plant Protection Division, Berkshire, England. The stock solution was diluted in the same solvent mixture at concentration of 0.2 and 2 mg/ml. **3.3.4 Volume applied** Not stated **3.3.6 Samples** One wether form each group was slaughtered at day 15 and the other at day 32. One ewe was slaughtered at each sampling time. The control sheep was slaughtered after 2 days. Faeces were collected; the liver, the omental fat and blood were removed, the carcaas was minced and freeze-dried for brodifacoum analysys by HPLC.
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	The appropriate reliability indicator is 3
Acceptability	*Acceptable as supporting study*
Remarks	Although acceptable, the study provides very limited information on the toxicokinetics of brodifacoum (used as a 0.005% dilution), just supporting the accumulation in the liver of an animal species (sheep), not usually considered for regulatory purposes

	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	

Section A6.2 (5) METABOLISM

Annex Point IIA6.2

Brodifacoum in Sheep

Section A6.2 (6) METABOLISM

Annex Point IIA6.2 *Brodifacoum in Dogs*

Official use only

1 REFERENCE

1.1	**Reference**	Benny J. Woody , Michael J. Murphy, Allen C. Ray & Robert A. Green. Coagulopathic effect and therapy of brodifacoum toxicosis in dogs. Journal of Veterinary Internal Medicine 1992; 6:23-28.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Published paper based on a study carried out by College of Veterinary Medicine, Texas A&M University and Texas Veterinary Medical Diagnostics Laboratory.
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Not stated in the published paper.
3.1.2	Specification	0.005% Brodifacoum
3.1.2.1	Description	Commercial bait formulation
3.1.2.2	Purity	Not stated in the published paper.
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.2	**Test Animals**	
3.2.1	Species	Dogs
3.2.2	Strain	Mixed breeds
3.2.3	Source	Not stated in published report
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age - Not stated in published report Weight – 12.3 to 15.4 kg
3.2.6	Number of animals per group	4 (2 male – 2 female)
3.2.7	Control animals	No

Section A6.2 (6) METABOLISM

Annex Point IIA6.2

Brodifacoum in Dogs

3.3	**Administration/ Exposure**		Oral	
	3.3.1	Preparation of test site	Not applicable	
	3.3.2	Concentration of test substance	1.1 mg/kg bw	X
	3.3.3	Specific activity of test substance	Not relevant	
	3.3.4	Volume applied	1.1 mg/kg bw	X
	3.3.5	Sampling time	Hourly post dosing to confirm total consumption of dose in food 4 times a day throughout the trial period	X
	3.3.6	Samples	Animals were monitored for : One-stage prothrombin time (OSPT) Activated partial thromboplastin time (APTT) Activated coagulation time (ACT) Complete blood counts Thrombocyte counts Serum chemistry	X
	3.3.7	Therapy	Vitamin K1 therapy was initiated when the OSPT was greater than 40 seconds. Dosage was 0.83 mg/kg bw three times a day for 5 days.	

4 RESULTS AND DISCUSSION

4.1 Result of study

Clinical observations: By day 6, three of the four dogs exhibited poor or selective appetite that progressed to complete anorexia by day 8 or 9 in two of the four animals. Prolonged bleeding or hematoma formation after venipuncture was observed on day 8 or 10 in al dogs. Melena in one dog on day 11 and vomition occurred in three dogs post exposure but was variable.

Vitamin K1 therapy was initiated in all dogs by day 10.

Within 2 days of initiating Vitamin K1 therapy all prolonged coagulation times had returned to the normal reference range.

The brodifacoum concentration in sera was highest on days 4 to 6. Serum brodifacoum concentration ranged from 37.5 to 83.0 ng/ml at the initiation of the Vitamin K1 therapy (day 10) and from 8.5 to 21 ng/ml at the cessation of the therapy (day 14). By day 24 it was down to 3.0 to 7.5 ng/ml and by day 30 were below the limit of detection at 2 ng/ml in all animals.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Dogs were given oral dose of 0.005% bait twice daily for 3 consecutive days and observed for clinical signs. Blood chemistry was monitored at 48 hour intervals for 21 days. Dogs were given antidotal therapy subcutaneously on the first day and orally thereafter for 5 days. Brodifacoum content was assessed by HPLC.

Section A6.2 (6) METABOLISM

Annex Point IIA6.2 *Brodifacoum in Dogs*

5.2	**Results and discussion**	A mean brodifacoum elimination half-life of 6 ± 4 days was observed. Vitamin K1 therapy when initiated was successful in neutralising the effects of the anticoagulant poisoning effects of brodifacoum	
5.3	**Conclusion**	Based on data from this study the recommendation for the management of brodifacoum poisoned animals is administration of vitamin K1 (0.83 mk/kg bw TID) for a minimum of 5 days with monitoring for a further 14 days. In clinical cases however it may be prudent to continue therapy for 14 days since brodifacoum is detectable for 3 weeks after an LD_{50} dose.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *21.11.2006*

Materials and Methods Include revised version

3.3.2 Concentration of the test substance

1.1. mg/kg bw (as cumulative dose divided in 6 aliquots admistered twice a day for 3 consecutive days)

3.3.4 Volume applied

Not stated

3.3.5 Sampling time

Brodifacoum levels in serum: serum was collected at 48h intervals starting from day 0 to day 30.

3.3.6 Samples

Blood samples were processed for brodifacoum levels analysis by HPLC.

Aniamal were also monitored for: *One-stage prothrombin time (OSPT); Activated partial thromboplastin time (APTT); Activated coagulation time (ACT); Complete blood counts; Thrombocyte counts; Serum chemistry.*

Results and discussion Adopt applicant's version

Conclusion Adopt applicant's version

Reliability The appropriate reliability indicator is 3

Acceptability *Not acceptable*

Remarks The study gives no relevant indication about the metabolism and disposition of the a.s.; in addition it has been carried out with the commercial biocidal product

COMMENTS FROM ...

Date *Give date of comments submitted*

Section A6.2 (6) METABOLISM

Annex Point IIA6.2

Brodifacoum in Dogs

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	

Section A6.2 (7) METABOLISM

Annex Point IIA6.2 *Horse brodifacoum*

Official use only

1 REFERENCE

1.1 Reference Boermans HJ, Johnstone J, Black WD & Murphy M (1991) – Clinical signs, laboratory changes and toxicokinetics of brodifacoum in horses. *Can J Vet Res*, 55: 21-27.

1.2 Data protection No, published paper.

1.2.1 Data owner: Public domain

1.2.2 Criteria for data protection: No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No

Reasons unknown

2.2 GLP The GLP status of the study is not stated in the published paper

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Test material Brodifacoum X

3.1.1 Lot/Batch number: Not stated

3.1.2 Specification: 0.005% Bait X

3.1.2.1 Description: *No information*

3.1.2.2 Purity: 0.005% Bait X

3.1.2.3 Stability: A specific statement on stability is not provided within the paper.

3.1.2.4 Radiolabelling: Not radiolabelled

3.2 Test Animals

3.2.1 Species: Horse

3.2.2 Strain: Standard bred

3.2.3 Source: Unstated

3.2.4 Sex: 4 Mares, 2 geldings

3.2.5 Age/weight at study initiation: Adult, 4-8 yrs

Mean weight 426.3 +/- 25.1kg

3.2.6 Number of animals per group: Not stated.

Section A6.2 (7) METABOLISM

Annex Point IIA6.2

Horse brodifacoum

3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral gavage	
3.3.1	Preparation of test site	None	
3.3.2	Concentration of test substance	0.125mg brodifacoum/kg bw in 1l water	
3.3.3	Specific activity of test substance	100%	
3.3.4	Volume applied	1l	
3.3.5	Size of test site	Whole animal	X
3.3.6	Exposure period	0.4,6,8, 12, 24, 48 72, 96 and 120 hours	
3.3.7	Sampling time	24 and 48 hours before and 1,2,5,6,8,12,16,19 and 23 days after dosing	
3.3.8	Samples	Blood taken directly from jugular catheter.	

4 RESULTS AND DISCUSSION

4.1	**Results**	Somnolence and anorexia seen in 4 horses by day 4, persisting to day 8. Significant weight loss on day 7, returning to baseline by day 14. One animal received vitamin k therapy. No significant changes seen in haematology and serum biochemical variables in any animal. Five horses had unchanged TCT ratios. A half-life of 1.2 days was seen, with considerable variation. Maximum brodifacoum concentrations were observed in serum from 2-3 hours after dosing. It was detectable for 4-9 days.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Horses dosed with 0.125 mg/kg and blood sampled at intervals thereafter
5.2	**Results and discussion**	Somnolence and anorexia seen in 4 horses by day 4, persisting to day 8. Significant weight loss on day 7, returning to baseline by day 14. No significant changes seen in haematology and serum biochemical variables in any animal. Five horse had unchanged TCT ratios. A half-life of 1.2 days was seen, with considerable variation. Maximum brodifacoum concentrations were observed in serum from 2-3 hours after dosing. It was detectable for 4-9 days.
5.3	**Conclusion**	Half-life of 1.22+/- 0.22 days. Maximum plasma concentration seen in horse with most severe clinical signs. Single exposure to brodifacoum

Section A6.2 (7) METABOLISM

Annex Point IIA6.2 *Horse brodifacoum*

		has the potential to cause illness and possibly death. 0.125mg brodifacoum/kg corresponds to 6.25 g of 0.005% bait/kg	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	Insert revised version **3.1 Test material** Commercial product containing 0.005% brodifacoum **3.1.2 Specifications** Talone® rodenticide bait minipellets containing 0.005% brodifacoum supplied by Chipman, Stoney Creek, Ontario. **3.1.2.2 Purity** Not stated **3.3.5 Size of test site** Not applicable
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	The appropriate reliability indicatoris 3
Acceptability	*Not acceptable*
Remarks	The study gives no relevant indication about the metabolism and disposition of the a.s.; in addition it has been carried out with the commercial biocidal product in an animal species not usually considered for regulatory purposes.
	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	

Section A6.2 (7) **METABOLISM**

Annex Point IIA6.2 *Horse brodifacoum*

Section A6.2 (8) METABOLISM

Human - brodifacoum

Annex Point IIA6.2

Official use only

1 REFERENCE

1.1	**Reference**	Weitzel JN, Sadowski JA, Furie BC, Moroose R, Kim H, Mount ME, Murphy MJ & Furie B (1990) – Surreptitious ingestion of a long-acting vitamin K antagonist/rodenticide, brodifacoum: Clinical and metabolic studies of three cases. Blood, 76(12): 2555-2559.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Published paper based on a study carried out by Invermay Agricultural Research Centre.
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Not stated in the published paper.
3.1.2	Specification	Not stated in the published paper.
3.1.2.1	Description	
3.1.2.2	Purity	Not stated in the published paper.
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.2	**Test Animals**	
3.2.1	Species	Humans
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	2 male 1 female
3.2.5	Age/weight at study initiation	20 year Female 37 and 48 year Male
3.2.6	Number of animals per group	1

X

3.2.7	Control animals	No		
3.3	**Administration/ Exposure**	Oral		
3.3.1	Preparation of test site	Not applicable		
3.3.2	Concentration of test substance			X
3.3.3	Specific activity of test substance			
3.3.4	Volume applied	Female 17.5mg D-Con 48 year male (2 weeks after ingestion 270.7 nmol/l brodifacoum) 37 male (2759 nmol/l)		X
3.3.5	Sampling time			
3.3.6	Samples			
		4 RESULTS AND DISCUSSION		
		Describe findings. If appropriate, include table. Sample tables are given below.		
4.1	**Result of study**	Female: Showed clinical signs: abdominal pains, melena, menorrhagia and gross hematuria on admission to hospital. Also vitamin K dependant proteins were deficient. Three weeks after ingestion blood clotting in the leg. 48 year male: Admitted to hospital with severe epitaxis and abnormal coagulation parameters. Two weeks later had acute bleeding in the left calf. 37 year male: initial conditions showed gross hematuria and deficiency iof the vitamin K-dependant coagulant proteins.		X
		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**			
5.2	**Results and discussion**	In all case the long term treatment with a vitamin K_1 therapy was seen to correct the effects of anticoagulants such as warfarin and brodifacoum		
5.3	**Conclusion**	Long term treatment with a vitamin K_1 is an effective antidote for long-acting vitamin K antagonists such as brodifacoum and warfarin.		X
5.3.1	Reliability	2		X
5.3.2	Deficiencies	No		

Section A6.2 (8) METABOLISM

Annex Point IIA6.2 *Human - brodifacoum*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	Include revised version **3.2.6 Number of animal per group** Not applicable **3.3.2 Concentration of the test substance** Unknown **3.3.4 Volume applied** Unknown.
Results and discussion	Include revised version. After ingestion of unknow quantities of commercial products containing brodifacoum, three humans presented severe clinical signs due to the anticoagulant activity of the test substance. They required long term therapy with large doses of vit. K_1. The serum elimination half-life for brodifacoum ranged from 16 to 36 days in these patients.
Conclusion	Include revised version The serum elimination half-life of unknown quantities of brodifacoum ranged from 16 to 36 days in human patients; the need for chronic therapy with vit. K_1, suggestes that residues of the a.s. may still be present within the body
Reliability	The appropriate reliability indicator is 3.
Acceptability	*Not acceptable*
Remarks	Although the study refers to humans, it provides only qualitative indication of brodifacoum absorption after ingestion and on its persistence within the body. These data are not useful for risk assessment.
	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	

Section A6.2 (8) METABOLISM

Annex Point IIA6.2

Human - brodifacoum

Section A6.2 (9) METABOLISM

Annex Point IIA6.2

Human - brodifacoum

Official use only

1 REFERENCE

1.1	**Reference**	Donovan JW, Ballard JO & Murphy MJ Brodifacoum therapy with activated charcoal: effect on elimination kinetics (1990). Vet Hum Toxicol (1990), 32: 350
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Published paper based on a study carried out by Capital Area Poison Center, Penn State University and College of Veterinary Medicine, University of Minnesota.
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
3.1.2	Specification	Not stated in the published paper
3.1.2.1	Description	Not stated in the published paper
3.1.2.2	Purity	Not stated in the published paper
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.2	**Test Animals**	
3.2.1	Species	Humans
3.2.2	Strain	Adults
3.2.3	Source	Not stated in published report
3.2.4	Sex	Not stated in published report
3.2.5	Age/weight at study initiation	Not stated in published report
3.2.6	Number of animals per group	2 Adults

X

3.2.7	Control animals	no		
3.3	**Administration/ Exposure**	Oral		
3.3.1	Preparation of test site	Not applicable		
3.3.2	Concentration of test substance	Not stated in published report		
3.3.3	Specific activity of test substance	Not stated in published report		
3.3.4	Volume applied	Not stated in published report		
3.3.5	Sampling time	Both patients received 25gm RDAC q4h for one day, the first at 33 days post-ingestion and again 1 day and 3 days later. Brodifacoum serum levels were measured twice daily.		
3.3.6	Samples	Analytical techniques. Serum measured by HPLC	X	
		4 RESULTS AND DISCUSSION *Describe findings. If appropriate, include table. Sample tables are given below.*		
4.1	**Result of study**	Brodifacoum levels ranged from 3.2 to 219.4 ng/ml, and βt½ was 399 hrs. Bt½ shortened during the rdac, but brodifacoum levels rebounded and both patients required large doses of vitamin k for over 3 months.		
		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**		X	
5.2	**Results and discussion**	Brodifacoum levels ranged from 3.2 to 219.4 ng/ml, and βT½ was 399 hrs. βT½ shortened during the RDAC, but Brodifacoum levels rebounded and both patients required large doses of Vitamin K for over 3 months		
5.3	**Conclusion**	It was concluded that even low brodifacoum levels in serum are associated with toxicity, that serum βT½ in human overdose exceeds 16 days, and that a one day course of RDAC may shorten the βT½ but does not eliminate the need for prolonged Vitamin K therapy		
5.3.1	Reliability	2	X	
5.3.2	Deficiencies	No		

Section A6.2 (9) METABOLISM

Annex Point IIA6.2 *Human - brodifacoum*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	**Include revised version.** **3.2.3 Source** Not applicable **3.3.6 Samples** Brodifacoum levels in serum were measured by HPLC.
Applicant's Summary and conclusion	**5.1 Materials and Methods** *The applicant's text is missing*
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	The paper is an abstract; therefore materials and methodsand results are poorly reported and the validity could not be assessed. The appropriate reliability indicator is 4.
Acceptability	Not acceptable Although the study refers to humans, its quality could not be assessed (see the poor reliability indicator).
Remarks	Although the study refers to humans, its quality could not be assessed (see the poor reliability indicator).
	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	

Section A6.2 (10) METABOLISM

Annex Point IIA6.2

Sheep - Brodifacoum

Official use only

1 REFERENCE

1.1	**Reference**	M.E.R. Godfrey, F.J.Laas and C.G. Rammell (1985) Acute toxicity of brodifacoum to sheep. New Zealand Journal of Experimental Agriculture, 1985, Vol. 13:23-25.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	© Crown copyright 1985
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
3.1.2	Specification	Brodifacoum supplied by ICI Tasman Ltd.
3.1.2.1	Description	
3.1.2.2	Purity	Not stated in the published paper
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.1.2.4	Radio labelling	Not radiolabelled
3.2	**Test Animals**	
3.2.1	Species	Sheep
3.2.2	Strain	Not stated in the published paper
3.2.3	Source	Not stated in the published paper
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	Mature ewes
3.2.6	Number of animals per group	5 per group
3.2.7	Control animals	No

Section A6.2 (10) METABOLISM

Annex Point IIA6.2

Sheep - Brodifacoum

3.3	**Administration/ Exposure**		Oral
	3.3.1	Preparation of test site	Not applicable
	3.3.2	Concentration of test substance	1.56, 3.13, 6.25, 12.5 and 25.0 mg/kg bw
	3.3.3	Specific activity of test substance	Technical grade brodifacoum prepared in propane-1,2-diol/polyethylene-glycol/triethanolamine (94/3/3, v/v)
	3.3.4	Volume applied	1 ml/kg
	3.3.5	Sampling time	Sheep that died were examined to establish the cause of death and liver samples taken for analysis. Sheep that survived dosing were sacrificed at monthly intervals starting one month after the last death for examination of liver.
	3.3.6	Samples	The levels of brodifacoum in the liver were measured.

4 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

4.1	**Result of study**	The mean brodifacoum level in livers was 1.44 mg/kg. There was no correlation between liver brodifacoum levels and dose level or time from dosing to death. Over all dose groups the LD50 was 33 mg/kg with 95% confidence limits of 5-210 mg/kg. The wide confidence limits reflects the low response at the 25 mg/kg dosing group, which was attributed to the observed precipitation of brodifacoum from the 2.5% solution on contact with the saliva. If only the lower dose rates are used the LD50 becomes 11 mg/kg and the 95% limits are 4-36 mg/kg.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Metabolism study in sheep; Oral administration with observation for toxic effects; Sheep that died were examined to establish the cause of death and liver samples taken for analysis. Sheep that survived dosing were sacrificed at monthly intervals starting one month after the last death for examination of liver. The levels of brodifacoum in the liver were measured
5.2	**Results and discussion**	The mean brodifacoum level in livers was 1.44 mg/kg. There was no correlation between liver brodifacoum levels and dose level or time from dosing to death. Over all dose groups the LD50 was 33 mg/kg with 95% confidence limits of 5-210 mg/kg. The wide confidence limits reflects the low response at the 25 mg/kg dosing group, which was attributed to the observed precipitation of brodifacoum from the 2.5% solution on contact with the saliva. If only the lower dose rates are used the LD50 becomes 11 mg/kg and the 95% limits are 4-36 mg/kg.
5.3	**Conclusion**	The acute oral toxicity of brodifacoum to sheep was examined in a trial of 40 sheep. There was a low mortality in the high dose group attributed

Section A6.2 (10) METABOLISM

Annex Point IIA6.2 *Sheep - Brodifacoum*

		to the precipitation of insoluble brodifacoum in the sheep's alimentary canal. Therefore the LD50 of 11mg/kg was calculated from the lower dose groups only. The lack of correlation of the level of brodifacoum in the liver suggests that the liver is not suitable tissue for quantifying exposure of an animal to brodifacoum. The liver may be a metabolic site that is saturable.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	Not acceptable The study was not considered relevant for the assessment of the end-point. Therefore it was not subject to any comment by the RMS
Remarks	The description of the study should be removed from Doc.IIIA
	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	

Section A6.2 (11) METABOLISM

Annex Point IIA6.2 Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum

Official use only

1 REFERENCE

1.1	**Reference**	Allen C. Ray, Michael J. Murphy, Michael D. DuVall and John C. Reagor (1989). Determination of brodifacoum and bromadiolone residues in rodent and canine liver. Am J Vet Res, Vol 50, No. 4, April 1989, 546-550.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Public domain
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	The guideline study is not stated in the published paper.
2.2 GLP	The GLP status of the study is not stated in the published paper
2.3 Deviations	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum and bromadiolone
3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
3.1.2	Specification	Not stated in the published paper
3.1.2.1	Description	Baits containing 0.005% a.i
3.1.2.2	Purity	Not stated in the published paper
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.1.2.4	Radio labelling	No
3.2	**Test Animals**	
3.2.1	Species	Rats and dogs
3.2.2	Strain	Sprague-Dawley Rats – Mixed breed dogs
3.2.3	Source	Not stated in the published paper
3.2.4	Sex	Rats – 8 male and 4 female Dogs – male and female
3.2.5	Age/weight at study initiation	Rats 400 to 450 g Dogs 11 to 20 kg
3.2.6	Number of animals per group	Brodifacoum dosing: Single dose group – 3 rats

X

Section A6.2 (11) METABOLISM

Annex Point IIA6.2 Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum

		Multiple dose group – 4 rats Single dose – 9 dogs Bromadiolone dosing: Single dose group – 3 rats Multiple dose group – 2 rats
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Preparation of test site	None
3.3.2	Concentration of test substance	Brodifacoum Rats: Single dose - 0.28 mg/kg bw Mulitple dose – (5 doses) total dose 7.5 to 11.25 mg/kg bw Dogs single dose – 1.1mg Bromadiolone Rats: Single dose – 1.25mg/kg bw Multiple dose (5 doses) total dose 6.75 to 10.6 mg/kg bw
3.3.3	Specific activity of test substance	Rats: Commercial bait containing 50µg/g of either brodifacoum or bromadiolone Dogs: 1.1 mg of technical grade brodifacoum in polyethylene glycol 400/kg, PO, via gastric tube.
3.3.4	Volume applied	
3.3.5	Sampling time	Rats every 2 to 6 hours for clinical signs up to 7 days. Dogs every 2 to 4 hours.
3.3.6	Samples	Liver tissue

4 RESULTS AND DISCUSSION

4.1 Result of study

Identification of methylated products of chromic acid oxidisation of brodifacoum and bromadiolone was accomplished by gas chromatography/mass spectrometry. The primary oxidisation product 4-bromobenzoic acid was identified after trimethylanilinium hydroxide methylation by matching its retention time (4.8 mins) and mass spectrum with that of an authentic standard.

Rats given low doses of either brodifacoum or bromadiolone had no outward signs of clinical illness. At necropsy, lesions were minimal, but 2 rats given brodifacoum had small amounts of blood in the thoracic cavity and 1 rat given bromadiolone had pulmonary congestion. In rats given the higher doses of both substances lesions were more pronounced and were confined to pulmonary congestion and abdominal haemorrhages. Bait intake decreased by the 4th day and signs of clinical toxicosis were not apparent until at least the 5th day. Dogs given brodifacoum also had lesions confined to pulmonary congestion and

Section A6.2 (11) METABOLISM

Annex Point IIA6.2 Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum

abdominal haemorrhages. Analysis of hepatic tissue from these dogs and rats indicated that residues from bromadiolone were usually less than those for brodifacoum and that he kidney may be as suitable a sample for analysis as the liver.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the study was to simulate natural poisoning. Rats were fed commercial bait with 50 µg/g of either brodifacoum or bromadiolone to create a single dose equivalent to 0.28 mg/kg body weight of active substance or for multiple dose (up to 30g of bait every 24 hours for 5 days) giving a total dosage of between 7.5 to 11.25 mg/kg bodyweight. Once the animals showed clinical signs of poisoning they were terminated and tissue samples taken. These samples were analysed for active substance residues in the liver or kidney.

For dogs a single dose was administered equivalent to 1.1 mg of technical grade material. When clinical signs were seen the dogs were given treatment with vitamin K_1. Livers were examined on those that died during the experiment.

5.2 Results and discussion

Rats given low doses of either brodifacoum or bromadiolone had no outward signs of clinical illness. At necropsy, lesions were minimal, but 2 rats given brodifacoum had small amounts of blood in the thoracic cavity and 1 rat given bromadiolone had pulmonary congestion. In rats given the higher doses of both substances lesions were more pronounced and were confined to pulmonary congestion and abdominal haemorrhages. Bait intake decreased by the 4th day and signs of clinical toxicosis were not apparent until at least the 5th day. Dogs given brodifacoum also had lesions confined to pulmonary congestion and abdominal haemorrhages.

Analysis of hepatic tissue from these dogs and rats indicated that residues from bromadiolone were usually less than those for brodifacoum and that the kidney may be as suitable a sample for analysis as the liver.

5.3 Conclusion

Analysis by gas chromatography/mass spectrometry after chromic acid oxidisation of liver extracts does not differentiate between brodifacoum and bromadiolone because they yield the same product, but conversely offers the advantage of screening with one protocol for the 2 most commonly used rodenticides. This technique also offers comparable sensitivity and much improved selectivity as contrasted with existing HPLC methods. X

Bromadiolone was less persistent in liver and may be eliminated more rapidly than brodifacoum.

In dogs the selected dose was in the lower end of the reported spectrum. Because the monitoring and therapy were aggressive, unknown predisposing factors may have contributed to the demise of the 3 dogs during the study.

5.3.1 Reliability 2 X

5.3.2 Deficiencies No

Section A6.2 (11) METABOLISM

Annex Point IIA6.2 Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21.11.2006*
Materials and Methods	Include revised version **3.1.2. Specification** Commercial baits (Talone G, ICI Americas Inc. Wilmington, Del.) containing 0.005% a.i. (rat Study) Technical grade Brodifacoum in polyethylene glycol 400 (dog study)
Results and discussion	Adopt applicant's version
Conclusion	Include revised version Brodifacoum was present at higher levels and was more persistent in liver than bromadiolone.
Reliability	The appropriate reliability indicator is 3
Acceptability	*Not acceptable*
Remarks	The study in the rat has been performed with commercial baits and not with the a.s.; in addition the study provides no quantitative information which may be useful for risk assessment.
	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	

Section A6.3.1 Annex Point IIA 6.3	Short-term repeated dose toxicity, oral (28 days)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Not required as Subchronic oral toxicity test (90 day repeated dose) available in Section 6.4.1. Data available on a 90-day study can be used as a conservative model for any operator exposure risk assessment scenario.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*21.11.2006*	
Evaluation of applicant's justification	Applicant's justification is reasonable, although reference to TGD requirement would be appreciated	
Conclusion	Applicant's justification is substantially 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		

Section A6.3.2 Annex Point IIA VI.6.3	Short-term repeated dose toxicity (dermal) 28 days	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Brodifacoum is a well-known compound, which has been used extensively for many years. Brodifacoum is a second generation hydroxycoumarin anticoagulant. This group of substances includes brodifacoum, bromodiolone, difenacoum, difethialone and flocoumafen (see IPCS, 1995). Mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested. A first generation coumarin anticoagulant is administered to humans for therapeutic purposes (warfarin). There are no other significant toxic effects. Mode of action is well understood and documented, and is common among all mammalian species. The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Single dose dermal absorption is less than oral administration. The oral LD_{50} (rat) is <5 mg/kg [Section A6.1.1(first summary)]. By multiplying out the value in Section A6.1.1(second summary) by the concentrations present the actual oral LD_{50} is 0.25-0.56 mg/kg, in good agreement with the value (0.26 mg/kg) in IPCS 1995. The dermal LD_{50} is 7.48 mg/kg [Section A6.1.2(first summary)] or >5 mg/kg [Section A6.1.2(second summary, multiplied out)]. This suggests that there is 10-30 fold less absorption by the dermal route. Flocoumafen is the type compound for examining the disposition and metabolism of non-metabolisable second generation hydroxycoumarin anticoagulants (see justification at section A6.2). Based on amounts of radiolabel (largely parent compound) retained in the liver following oral (37.5%) and dermal (25%) administration, absorption is approximately two thirds oral absorption. Comparison of the acute LD_{50} values in IPCS (1995) (oral: 0.46 mg/kg; dermal: 0.54 mg/kg) suggests that dermal absorption for flocoumafen is approximately 80% of oral absorption. This data gives confidence that the LD_{50} comparisons are an appropriate way of comparing absorption following oral and dermal absorption, and therefore that brodifacoum is poorly absorbed dermally. The 90 day repeated dose oral toxicity studies with brodifacoum (section A6.4) indicates that there are no signs of toxicity outside those associated with mode of action. The IPCS review of anticoagulant rodenticides (IPCS 1995) indicates that this is a general phenomenon. The data in the the metabolism section suggests that, like the other largely non-metabolised second generation hydroxycoumarin anticoagulants brodifacoum is not reailly metabolised following oral ingestion. Thus the systemic effects seen are probably those for parent substance. Under these circumstances, a dermal 28-day study on brodifacoum is considered to be of very limited value and, on animal welfare grounds, should not be undertaken.	

Section A6.3.2 Annex Point IIA VI.6.3	Short-term repeated dose toxicity (dermal) 28 days
	IPCS (1995). Anticoagulant Rodenticides. Envionmental Health Criteria 175. International Programmeon Chemical Safety. Geneva: World Health Organisation.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*23.11.06*
Evaluation of applicant's justification	Applicant's justification is weak and not correct . As an example it is stated that dermal absorption of the a.s. is lower than the oral one, but it is concluded that the a.s. is poorly absorbed trough the skin. The dermal LD_{50} is 7.48 mg/kg in females, but it was not possibile to derive the value in males, which are more sensitive than females: therefore the exact dermal LD_{50} is < 7.48 mg/kg. It is opinion of the RMS (for a number of reasons) that it not correct to conclude that on the basis of differences in acute toxicity *via* the two routes of exposure (which we do not know), it is expected that absorption by the dermal route is 10-30 fold less than the oral. The a.s. is highly toxic via both route of exposure and on this basis no difference in the absorption can be claimed. In addition the RMS disagrees also with some of the considerations by the Applicant with respect to the study on flocoumafen: only data on residues in the liver have been considered as accounting for the 'absorbed fraction' to support the Applicant considerations that '*LD_{50} comparisons are an appropriate way of comparing absorption following oral and dermal absorption, and therefore that brodifacoum is poorly absorbed dermally*'. If data on excreta were also summed up, as it should be, different results were obtained.
Conclusion	Applicant's justification is not acceptable, also in view of the fact that the dermal exposure could be the relevant for human exposure, expecially for the operators. Therefore data of short term (28 days) on structurally related compound should be presented (if not available on the a.s.) in order to consider a possible read across. Alternatively a derogation to conduct a short term toxicity test could be acceptable if data on dermal subchronic toxicity (also on structurally related compounds) become available (see also the related section on subchronic toxicity).
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3.3 Annex Point IIA VI.6.3	Short-term repeated dose toxicity (inhalation) 28 days)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum is a well-known compound, which has been used extensively for many years. Brodifacoum is a second generation hydroxycoumarin anticoagulant. This group of substances includes brodifacoum, bromodiolone, difenacoum, difethialone and flocoumafen (see IPCS, 1995). The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested (see IPCS, 1995 and US EPA, 1998).There are no other significant toxic effects. Mode of action is well understood and documented, and is common among all mammalian species. Inhalation is not considered to be a significant potential route of entry due to the substance low vapour pressure and the very low exposure likely to occur from handling of solid bait and during the manufacturing and formulation processes, which are strictly controlled. Under these circumstances, a 28-day inhalation study on brodifacoum is considered to be of very limited value and, on animal welfare grounds, should not be undertaken. IPCS (1995). Anticoagulant Rodenticides. Envionmental Health Criteria 175. International Programmeon Chemical Safety. Geneva: World Health Organisation. US EPA (1998). Reregistration Eligibility Decision (RED). Rodenticide Cluster. EPA 738-R-98-007. (http://www.epa.gov/REDs/2100red.pdf).	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*23.11.06*	

Section A6.3.3 Annex Point IIA VI.6.3	Short-term repeated dose toxicity (inhalation) 28 days)
Evaluation of applicant's justification	Applicant's justification can be considered in principle reasonable. . However, with reference to TGD requirements, the properties of the a.s. (vapor pressure, stability, etc) supporting the fact that the potential for inhalation is negligible should be cited in more details, including knowledge of dustiness, the possible particle size and the new value of vapore pressure (on which studies are being carried out on request of the RMS) in order to fully accept the waiving (see justification for acute and subchronic inhalation toxicity, which should be consistent each other more than they are in the present version).
Conclusion	Pending submission of data on dustiness, particle size and vapor pressure, which will be ready in the near future, as declared by the Applicant, and pending results will confirm that potential for inhalation is actually negligible, the applicant's justification will be considered acceptable Alternatively, data of short term toxicity on structurally related compounds should be submitted, if available. In case data on subchronic toxicity structurally related compounds were submitted, a derogation for presenting data on short term toxicity is accepted.
Remarks	Data on dustiness, particle size and vapor pressure have to be submitted to give the final conclusion. These data can be crucial in order to understand the possibility for human exposure.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.4 Subchronic toxicity

Annex Point IIA. VI.6.4

90-day feeding study in rats

				Official use only
			1 REFERENCE	
1.1	**Reference**		Report: BRODIFACOUM 90-day Feeding Study in the Rat. XXXXX – September 1995. XXXXX report MLS/10020 Repeated dose toxicity study of test substance brodifacoum.	
1.2	**Data protection**		Yes	
	1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
	1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl	
	1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
			2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**		OECD 408	
2.2	**GLP**		Yes	
2.3	**Deviations**		No	X
			3 MATERIALS AND METHODS	
3.1	**Test material**		As given in section 2	
	3.1.1	Lot/Batch number	TCP 01/95	
	3.1.2	Specification	As given in section 2	
3.1.2.1	Description		Off-white powder	
3.1.2.2	Purity		Brodifacoum 99.1%	
3.1.2.3	Stability		Stable	X
3.2	**Test Animals**			
	3.2.1	Species	Rats	
	3.2.2	Strain	Wistar	
	3.2.3	Source	Charles River Laboratories, Wilmington, Mass., U.S.A.	
	3.2.4	Sex	Male and female	
	3.2.5	Age/weight at study initiation	Age not stated Male 201 - 223g Female 170 - 197g	
	3.2.6	Number of animals per group	10 animals/sex/group	

Section A6.4 Subchronic toxicity

Annex Point IIA. VI.6.4 90-day feeding study in rats

3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	By gavage	
3.3.4.2	Concentration	1 ml/100g bw	X
3.3.4.3	Vehicle	Polyethylene glycol 300	
3.3.4.4	Concentration in vehicle	0, 0.01, 0.02 0.04 and 0.08 mg/kg	X
3.3.4.5	Total volume applied	0, 0.9, 1,8, 3.6 and 7.2 mg/kg	X
3.3.4.6	Controls	Vehicle	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes - Start and then twice daily	
3.4.1.2	Mortality	Yes - Daily	
3.4.2	Body weight	Yes - Daily	
3.4.3	Food consumption	No - Free access to food	
3.4.4	Water consumption	No - Unlimited water	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	Yes 1 ml of blood from tail vein from all animals on days 1, 15, 30, 50, 80 and 90 prior to termination. Parameters: Haematocrit and platelet count, haemoglobin, total white cell count, red cell count, mean cell volume, mean cell haemoglobin and concentration. A differential white cell count on a Romanowsky-stained blood film and the appearance of red cells examined in all groups.	
3.4.7	Clinical Chemistry	Yes All animals at end of study after termination. By cardiac puncture and collected in tubes containing lithium heparin. Parameters: sodium, potassium, glucose, total cholesterol, plasma urea,	

		total bilirubin, creatinine, creatinine kinase, total protein, albumin, alanine transaminase, aspartate transaminase, plasma alkaline phosphatase, gamma glutamyl transferase activity, triglycerides, calcium, chloride,, phosphorus (as phosphate).
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, brain
3.5.2	Gross and histopathology	Yes; all dose groups organs: brain, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, urinary bladder, lymph nodes, epididymus, caecum, colon, duodenum, ileum, jejunum, rectum, salivary gland, stomach, testis.
3.5.3	Other examinations	None
3.5.4	Statistics	Bodyweights, food consumption, haematology, blood chemistry and organ weights were considered by analysis of variance. Least-square means for each group using LSMEAN option in SAS PROC GLM. All statistical tests were two-sided.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Piloerection, salivation and signs of diarrhoea were seen in all groups, including control, and were therefore considered unrelated to the treatment with Brodifacoum.
4.1.2	Mortality	No mortalities at any dose
4.2	**Body weight gain**	No significant differences in weight gain across the treatment groups and control.
4.3	**Food consumption and compound intake**	No effects
4.4	**Ophthalmoscopic examination**	
4.5	**Blood analysis**	
4.5.1	Haematology	All animals other than the highest dose group of 0.08 mg/kg/day revealed normal coagulation and haematology compared to the control group. At 0.08 mg/kg/day there was a slight elevation of the KCT time.
4.5.2	Clinical chemistry	No effects
4.5.3	Urinalysis	No data
4.6	**Sacrifice and pathology**	

Section A6.4 Subchronic toxicity

Annex Point IIA. VI.6.4 90-day feeding study in rats

4.6.1	Organ weights	No effects
4.6.2	Gross and histopathology	Increased incidence of haemorrhaging in males that received the 0.08 mg/kg/day dose rate.
4.7	**Other**	Macroscopic post mortem showed that there were no treatment-related effects in animals of any treatment dose group.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	OECD 408
5.2	**Results and discussion**	Oral administration of brodifacoum 0.01,0.02, 0.04 mg/kg/day in male and female rats and 0.08 mg/kg/day in female rats had no effect on clinical, haematological or pathological parameters measured. Oral administration of brodifacoum at 0.08 mg/kg/day in male rats resulted in a slight increased incidence of haemorrhage in two animals and slight increase in clotting times indices.
5.3	**Conclusion**	
5.3.1	LO(A)EL	0.08mg/kg/day
5.3.2	NO(A)EL	Males 0.04mg/kg/day Females 0.08mg/kg/day
5.3.3	Other	No deaths occurred.
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	*21.11.2006*
Guidelines and Quality Assurance	**2.3 Deviations** No ophtalmoscopic neither urinanalysis performed.

Section A6.4 Subchronic toxicity

Annex Point IIA. VI.6.4

90-day feeding study in rats

Materials and Methods	Include revised version **3.1.2.3 Stability** Stable. Stability was checked in the dosing formulation at regualr interval up to day 100 (exceeding the dosing period) **3.3.4.1. Concentration** 0, 0.01, 0.02, 0.04 and 0.08 mg/kg **3.3.4.4 Concentration in the vehicle** 1, 2, 4, 8 µg/ml **3.3.4.5 Total volume applied** 1 ml/100g bw
Results and discussion	Adopt applicant's version
Conclusion	LO(A)EL: 0.08mg/kg/day NO(A)EL: 0.04mg/kg/day Other conclusions: Adopt applicant's version
Reliability	The reliability indicator is appropriate
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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4-1. Results of clinical chemistry haematology and urinalysis

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

parameter changed	Unit	Controls			low dose			medium dose			high dose		
	Days												
PT / KCT	1	0	0		0	↓		0	0		0	↓	
PT / KCT	15	0	0		↓	↓		0	↓		↑	↑	
PT / KCT	30	0	0		0	↓		0	↓		↑	↑	
PT / KCT	50	0	0		↑	↓		0	0		↑	↑	
PT / KCT	80	0	0		0	↑		0	↑		↑	↑	
PT / KCT	90	0	0		0	↑		0	↑		↑	↑	

* $p < 0,05$

Give only those parameters, which are changed in at least one dose group compared to control. Usually only statistically significant effects

Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_4-1. Results of repeated dose toxicity study

Parameter	Control		low dose		medium dose		high dose		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	10	10	10	10	10	10	10	10	10	10
Mortality	0	0	0	0	0	0	0	0	0	0
clinical signs*	0	0	0	0	0	0	0	0	0	0
body weight	0	0	0	0	0	0	0	0	0	0
food consumption	-	-	-	-	-	-	-	-	-	-
clinical chemistry*	0	0	0	0	0	0	0	0	0	0
haematology*	0	0	0	0	0	0	↑	↑	+	+
urinalysis*	-	-	-	-	-	-	-	-	-	-
organ weight*	0	0	0	0	0	0	0	0	0	0
gross pathology*	0	0	0	0	0	0	0	0	0	0
microscopic pathology*	10	10	10	10	10	10	10	10	10	10

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

Section A6.4.1 Annex Point IIA VI.6.4	Subchronic oral test second species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum is a well-known compound, which has been used extensively for many years. Brodifacoum is a second generation hydroxycoumarin anticoagulant. This group of substances includes brodifacoum, bromdiolone, difenacoum, difethialone and flocoumafen (see IPCS, 1995). Mode of action is by inhibition of blood clotting and is seen in all the mammalian species tested.A limited sub chronic study on bromodiolone in dogs that confirms this position has been reported in the US EPA Reregistration Eligibility Decision (US EPA 1998). A first generation coumarin anticoagulant is administered to humans for therapeutic purposes (warfarin). There are no other significant toxic effects. Mode of action is well understood and documented, and is common among all mammalian species (see e.g IPCS, 1995; US EPA, 1998). The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. The data on section A6.2 for brodifacoum indicates that elimination from blood or plasma includes a long (and roughly comparable) terminal half life in studies on rat, rabbit, dog and human and, after allowance for the use of radioactively labelled material and the better definition of the kinetics, similar to those for flocoumafen. This suggests that inter species differences in toxicity are likely to be minimal. The 90 day repeated dose oral toxicity studies with brodifacoum (section A6.4) indicates that there are no signs of toxicity outside those associated with mode of action. The limited information available from acute studies on brodifacoum in species other than rat and rabbit (namely dog and horse) also indicate that the primary toxic effect is that due to the mode of action of brodifacoum (Boermans et al., 1990; Woody et al,1992). This also suggests that interspecies differences in toxicity are likely to be minimal. Under these circumstances, a sub chronic study in a second species is considered to be of very limited value and, on animal welfare grounds, should not be undertaken. Boermans H, Johnstone I, Black W D, Murphy, M (1991) Clinical signs, laboratory changes and toxicokinetics of brodifacoum in the horse. Can J Vet Res 55, 21-27 IPCS (1995). Anticoagulant Rodenticides. Envionmental Health Criteria 175. International Programmeon Chemical Safety. Geneva: World Health Organisation. US EPA (1998). Reregistration Eligibility Decision (RED). Rodenticide Cluster. EPA 738-R-98-007. (http://www.epa.gov/REDs/2100red.pdf).	

Section A6.4.1 Annex Point IIA VI.6.4	Subchronic oral test second species
	Woody B J, Murphy M J, Ray A C, Green R A Coagulopathic effects and therapy of brodaficoum toxicosis in dogs. J Veterin Intern Med 6, 23-28,
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*23.11.06*
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	

Section A6.4.2 Annex Point IIA VI.6.4	Subchronic dermal	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum is a well-known compound, which has been used extensively for many years. Brodifacoum is a second generation hydroxycoumarin anticoagulant. This group of substances includes brodifacoum, bromdiolone, difenacoum, difethialone and flocoumafen (see IPCS, 1995). Mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested and there are no other significant toxic effects (see IPCS, 1995; US EPA, 1998). A first generation coumarin anticoagulant is administered to humans for therapeutic purposes (warfarin). There are no other significant toxic effects. Mode of action is well understood and documented, and is common among all mammalian species. The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Single dose dermal absorption is less than oral administration. The oral LD_{50} (rat) is <5 mg/kg [Section A6.1.1(first summary)]. By multiplying out the value in Section A6.1.1(second summary) by the concentrations present the actual oral LD_{50} is 0.25-0.56 mg/kg, in good agreement with the value (0.26 mg/kg) in IPCS 1995. The dermal LD_{50} is 7.48 mg/kg [Section A6.1.2(first summary)] or >5 mg/kg [Section A6.1.2(second summary, multiplied out)]. This suggests that there is 10-30 fold less absorption by the dermal route. Flocoumafen is the type compound for examining the disposition and metabolism of non-metabolisable second generation hydroxycoumarin anticoagulants (see justification at section A6.2). Based on amounts of radiolabel (largely parent compound) retained in the liver following oral (37.5%) and dermal (25%) dermal absorption is approximately two thirds oral absorption. Comparison of the acute LD_{50} values in IPCS (1995) (oral: 0.46 mg/kg; dermal: 0.54 mg/kg) suggests that dermal absorption for flocoumafen is approximately 80% of oral absorption. This data gives confidence that the LD_{50} comparisons are an appropriate way of comparing absorption following oral and dermal absorption, and therefore that brodifacoum is poorly absorbed dermally. The 90 day repeated dose oral toxicity studies with brodifacoum (section A6.4) indicates that there are no signs of toxicity outside those associated with mode of action. The IPCS and US EPA reviews of anticoagulant rodenticides (IPCS 1995, US EPA, 1998) indicates that this is a general phenomenon. The data in the the metabolism section suggests that, like the other largely non-metabolised second generation hydroxycoumarin anticoagulants brodifacoum is not readily metabolised following oral ingestion. Thus the systemic effects seen are probably those for parent substance. Under these circumstances, a sub chronic dermal study on brodifacoum is considered to be of very limited value and, on animal welfare grounds,	

Section A6.4.2 Annex Point IIA VI.6.4	Subchronic dermal
	should not be undertaken. IPCS (1995). Anticoagulant Rodenticides. Envionmental Health Criteria 175. International Programmeon Chemical Safety. Geneva: World Health Organisation. US EPA (1998). Reregistration Eligibility Decision (RED). Rodenticide Cluster. EPA 738-R-98-007. (http://www.epa.gov/REDs/2100red.pdf).
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24.11.06*
Evaluation of applicant's justification	Applicant's justification is weak and not correct. About 80% of the text overlaps with the justification for non submission of data on short term dermal toxicity and is based on considerations on acute dermal toxicity, on which the RMS disagrees. See evaluation of that justification in section A.6.3.2. The RMS fully agree that the sign of toxicity are consistent with the mode of action independently from the route of administration and also in considering the parent compound as the toxicologically relevant molecule rather than metabolite(s). In view of these consideration, taking into account that dermal absorption cannot be disregarded, it is expected that repeated dermal administration could lead to substantial toxicity (as a 'very worst case' similar to those ones identified after oral administration). Since dermal exposure could be relevant for humans, it is not possible to state that it is not justified to present data on this end-point.
Conclusion	Applicant's justification is not acceptable, also in view of the fact that the dermal exposure could be the relevant for human exposure, expecially for the operators. Therefore data of dermal subchronic toxicity on structurally related compounds should be presented (if not available on the a.s.) in order to consider a possible read across. Alternatively, a derogation to conduct a subchronic dermal toxicity test could be acceptable if ,as a worst case, it is assumed that the NO(A)EL coming from the oral study is considered valid also for the dermal one.
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*

Section A6.4.2 Subchronic dermal Annex Point IIA VI.6.4
Remarks

Section A6.4.3 Annex Point IIA VI.6.4	Subchronic inhalation toxicity test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	Test is only required for volatile substances and gases which have a vapour pressure greater than $1x10^{-2}$ Pa. The vapour pressure of Brodifacoum is 5×10^{-1} Pa (Fabbrini 1997). Therefore a test is not justified. Further note: the above vapour pressure has been queried by the RMS, and therefore a vapour pressure study is being carried out at SafePharm. This waiver will then require re-writing on the basis of that result.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*23.11.06*	
Evaluation of applicant's justification	Applicant's justification can be considered in principle reasonable. . However, with reference to TGD requirements, the properties of the a.s. (vapor pressure, stability, etc) supporting the fact that the potential for inhalation is negligible should be cited in more details, including knowledge of dustiness, the possible particle size and the new value of vapore pressure (on which studies are being carried out on request of the RMS) in order to fully accept the waiving (see justification for acute and short term inhalation toxicity, which should be consistent each other more than they are in the present version).	
Conclusion	Pending submission of data on dustiness, particle size and vapor pressure, which will be ready in the near future, as declared by the Applicant, and pending results will confirm that potential for inhalation is actually negligible, the applicant's justification will be considered acceptable Alternatively, data of short term toxicity on structurally related compounds should be submitted, if available. In case data on subchronic toxicity structurally related compounds were submitted, a derogation for presenting data on short term toxicity is accepted	
Remarks	Data on dustiness, particle size and vapor pressure have to be submitted to give the final conclusion. These data can be crucial in order to understand the possibility for human exposure	
	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*	

Section A6.4.3 **Subchronic inhalation toxicity test** **Annex Point IIA VI.6.4**
Remarks

Section A6.5 Annex Point IIA VI.6.5	Chronic toxicity –both species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [X] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	Brodifacoum is a well-known compound, which has been used extensively for many years. Brodifacoum is a second generation hydroxycoumarin anticoagulant. This group of substances includes brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen (see IPCS, 1995). Mode of action is by inhibition of blood clotting and is seen in all the mammalian species tested. There are no other significant toxic effects (see IPCS, 1995; US EPA, 1998). A first generation coumarin anticoagulant is administered to humans for therapeutic purposes (warfarin). Mode of action is well understood and documented, and is common among all mammalian species (see e.g IPCS, 1995). The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. The data on section A6.2 for brodifacoum indicates that elimination from blood or plasma includes a long (and roughly comparable) terminal half life in studies on rat, rabbit, dog and human. The liver is the major site of retention and retains parent moiety. Repeated dose studies (weekly dosing) indicated that accumulation of parent substance took place over the first four weeks. This suggests that inter species differences in toxicity are likely to be minimal and that saturation of metabolism was clearly achieved in sub chronic studies. The 90 day repeated dose oral toxicity studies in a rodent with brodifacoum (section A6.4) indicates that there are no signs of toxicity outside those associated with mode of action. The limited information available from acute studies on brodifacoum in species other than rat and rabbit (namely dog and horse) also indicate that the primary toxic effect is that due to the mode of action of brodifacoum (Boermans et al., 1990; Woody et al, 1992). This suggests that interspecies differences in toxicity are likely to be minimal. The IPCS review of anticoagulant rodenticides (IPCS 1995) states that long term studies with second generation anticoagulants are difficult to carry out for more than a few weeks due to the rapid acute effects (the mode of action) and the extremely low concentrations at which these effects are seen. This is exemplified in the extremely low dose levels used in the sub-chronic toxicity test in rat (Section 6.4) and the effects became apparent as early as 6 weeks in some animals in the 'high dose' (0.1 mg/kg bw, once per week) rats in the repeated dose disposition and metabolism study on flocoumafen [Section 6.2.1(2b)]. The substance is not genotoxic (Section 6.6) and is therefore unlikely to be a genotoxic carcinogen. Under these circumstances, chronic toxicity studies are considered to be	

Section A6.5 Annex Point IIA VI.6.5	Chronic toxicity –both species
	of very limited value and, on animal welfare grounds, should not be undertaken. Boermans H, Johnstone I, Black W D, Murphy, M (1991) Clinical signs, laboratory changes and toxicokinetics of brodifacoum in the horse. Can J Vet Res 55, 21-27 IPCS (1995). Anticoagulant Rodenticides. Envionmental Health Criteria 175. International Programmeon Chemical Safety. Geneva: World Health Organisation. US EPA (1998). Reregistration Eligibility Decision (RED). Rodenticide Cluster. EPA 738-R-98-007. (http://www.epa.gov/REDs/2100red.pdf). Woody B J, Murphy M J, Ray A C, Green R A Coagulopathic effects and therapy of brodaficoum toxicosis in dogs. J Veterin Intern Med 6, 23-28,
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*23.11.06*
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	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 Reverse mutation assay "Ames Test" using *Salmonella typhimurium*

Official use only

1 REFERENCE

1.1	**Reference**	Report: Brodifacoum: Reverse mutation assay, Ames Test, using *Salmonella typhimurium*. XXXXX, October 2002, XXXXX report 1558/006
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2		PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 471
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	04355
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.9% (brodifacoum)
3.1.2.3	Stability	Stable under test conditions
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100, TA 102
3.2.2	Deficiencies / Proficiencies	*Not stated Select / delete as appropriate:* DNA-Polymerase-A- proficient DNA-Polymerase-A-deficient Acetyltransferase proficient Nitroreductase proficient
3.2.3	Metabolic activation system	S9 mix

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 Reverse mutation assay "Ames Test" using *Salmonella typhimurium*

	3.2.4	Positive control	N-ethyl-N'-nitro-N-nitrosoguanidine (EENG) – 3 µg/plate for T100 and 5 µg/plate for TA1535 9-Aminoacridine (9AA) – 80 µg/plate for TA1537 Mitomycin C (MMC) – 0.5 µg/plate for TA102 4-Nitroquinoline-1-oxide (4NQO) – 0.2 µg/plate for TA98	
3.3	**Administration / Exposure; Application of test substance**			
	3.3.1	Concentrations	TA100, TA1535, TA1537 (without S9): 0.15, 0.5, 1.5, 5, 15, 50 µg/plate TA100, TA1535, TA1537 (with S9): 0.5, 1.5, 5, 15, 50, 150 µg/plate TA102, TA98 (without S9): 50, 150, 500, 1500, 5000 µg/plate TA102, TA98 (with S9): 15, 50, 150, 500, 1500, 5000 µg/plate	
	3.3.2	Way of application	Dissolved in dimethyl sulphoxide	
	3.3.3	Pre-incubation time	10 hours at 37°C	
	3.3.4	Other modifications		
3.4	**Examinations**		None	
			4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**			
	4.1.1	without metabolic activation	Negative	
	4.1.2	with metabolic activation	Negative	
4.2	**Cytotoxicity**		No	X
			5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**		OECD 471	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 Reverse mutation assay "Ames Test" using *Salmonella typhimurium*

5.2	**Results and discussion**	The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level although reductions in the frequency of revertant colonies were observed in the majority of the tester strains at the upper dose levels both with and without S9-mix. The test material tested upto maximum recommended dose level of 5000µg/plate, A whitish, opaque film was noted at and above 1500µg/plate and a white powdery precipitate was observed at 5000µg/plate. There were no significant increases in the frequency of revertant colonies recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation. All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.
5.3	**Conclusion**	The test material brodifacoum is considered to be non-mutagenic.
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	February 2006
Materials and Methods	Adopt applicant's version
Results and discussion	**Cytotoxicity:** Cytotoxic at 15 µg/plate w/o S9 and at 50 µg/plate with S9 in strains TA100, TA1535 and TA1537. Cytotoxic at 5000 µg/plate in strains TA102 and TA98
Conclusion	Adopt applicant's version
Reliability	Reliability indicator 1
Acceptability	The study is acceptable
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*

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 Reverse mutation assay "Ames Test" using *Salmonella typhimurium*

Remarks

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 Reverse mutation assay "Ames Test" using *Salmonella typhimurium*

Table A6_6_1-1. Mean revertants/plate (±SD) in the absence of S-9

Treatment (µg/plate)	Strain				
	TA100	TA1535	TA102	TA98	TA1537
Experiment 1					
0	81 (5.7)	20 (5.5)	341 (8.4)	19 (4.5)	14 (2.6)
50	76 (14)	24 (3.2)	324 (18)	15 (2.3)	9 (2.6)
150	67 (10)	14 (2.5)	331 (10.5)	18 (2.5)	4 (1)
500	56 (10.6)	16 (2.5)	319 (7.5)	15 (4.9)	4 (2)
1500	59 (15.7)	19 (5.1)	325 (15.5)	18 (6.2)	8 (4)
5000	52 (4.4)	12 (4)	255 (49.4)	10 (1.5)	4 (1)
Positive control Name	ENNG	ENNG	MMC	4NQO	9AA
Concentration (µg/plate)	3	5	0.5	0.2	80
No. Colonies/plate	461 (19.5)	277 (35.8)	1005 (34.7)	171 (3)	3813 (66.2)
Experiment 2					
0	82 (2)	18 (4.4)	287 (14.6)	13 (6.2)	10 (3.2)
50	66 (3.5)	13 (4.5)	272 (5.9)	16 (1.7)	6 (1.7)
150	53 (1.5)	16 (0.6)	258 (13.6)	11 (3.5)	3 (1.5)
500	49 (9.5)	9 (4)	256 (5.1)	10 (5.7)	3 (1)
1500	44 (10.3)	11 (2.1)	269 (22.5)	17 (1.5)	4 (1.5)
5000	44 (5)	11 (5.2)	244 (11.1)	12 (2)	3 (0.6)
Positive control Name	ENNG	ENNG	MMC	4NQO	9AA
Concentration (µg/plate)	3	5	0.5	0.2	80
No. Colonies/plate	350 (19.1)	261 (106.7)	890 (35.1)	100 (9.5)	2691 (390.8)

ENNG – N-ethyl-N'-nitro-N-nitrosoguanidine, 4NQO – 4-Nitroquionoline-1-oxide, 9AA – 9-Amintoacridine, MMC – Mitomycin C, P- Precipitate,

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 Reverse mutation assay "Ames Test" using *Salmonella typhimurium*

Table A6_6_1-2. Mean revertants/plate (±SD) in the presence of S9

Treatment (µg/plate)	Strain				
	TA100	TA1535	TA102	TA98	TA1537
Experiment 1					
0	79 (1.7)	15 (1.2)	378 (26.7)	31 (5)	13 (7)
50	86 (5.2)	15 (3.5)	402 (10.8)	29 (5.2)	13 (4.5)
150	69 (8.1)	12 (1.2)	363 (17)	29 (2.5)	6 (0.6)
500	66 (7.5)	11 (5.5)	355 (10.8)	21 (2.5)	11 (2)
1500	61 (8.1)	7 (0.6)	343 (5.1)	18 (4.6)	4 (1.5)
5000	50 (4.9)	5 (1.2)	257 (35.9)	12 (2.9)	4 (0.6)
Positive control Name	2AA	2AA	DAN	BP	2AA
Concentration (µg/plate)	1	2	10	5	2
No. Colonies/plate	2120 (54.5)	260 (50.1)	1048 (34.5)	218 (18.3)	495 (28.6)
Experiment 2					
0	89 (15.2)	12 (3.5)	313 (12.9)	32 (5.8)	18 (1.5)
50	82 (14.4)	14 (2.6)	324 (6.7)	27 (10.2)	10 (7.6)
150	57 (10.8)	11 (2)	344 (14.1)	22 (0.6)	4 (1.2)
500	62 (8.5)	10 (2.3)	322 (21.4)	21 (2.3)	5 (4)
1500	51 (9)	7 (3.5)	301 (24.4)	16 (4.4)	5 (3.2)
5000	57 (0.6)	6 (3)	256 (29.5)	15 (4.6)	4 (2.1)
Positive control Name	2AA	2AA	DAN	BP	2AA
Concentration (µg/plate)	1	2	10	5	2
No. Colonies/plate	2641 (75.2)	157 (27.8)	715 (45.1)	218 (32.4)	493 (10.5)

2AA- 2-Aminoanthracene, BP – Benzo(a)pyrene, DAN – 1,8-Dihydroxyanthraquinone, P- Precipitate,

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6.2 Chromosome aberration test in human lymphocytes *in vitro*

Official use only

1 REFERENCE

1.1	**Reference**	Report: BRODIFACOUM: Chromosome aberration test in human lymphocytes *in vitro*. XXXXX – January 2003. XXXXX report 1558/003
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 473
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	04355
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.9% (brodifacoum)
3.1.2.3	Stability	Stable under test conditions
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	mammalian cell lines: other primary cultures: lymphocytes or other
3.2.2	Deficiencies / Proficiencies	
3.2.3	Metabolic activation system	S9 mix
3.2.4	Positive control	In the absence of S9 – mitomycin at 0.4 µg/ml dissolved in Minimal Essential Medium In the presence of S9 – cyclophosphamide at 10 µg/ml dissolved in dimethyl sulphoxide

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6.2 Chromosome aberration test in human lymphocytes *in vitro*

3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	0, 18.75, 37.5, 75, 150, 225 and 300 µg/ml	
3.3.2	Way of application	Dissolved in dimethyl sulphoxide	
3.3.3	Pre-incubation time	Not stated	
3.3.4	Other modifications		
3.4	**Examinations**	*see tables in appendix for examinations and results*	
3.4.1	Number of cells evaluated	2000 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**	No

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

OECD 473

5.2 Results and discussion

Preliminary test:

The test material showed some evidence of toxicity in all three groups. A precipitate was observed in the cultures at the end of the exposure, at 500µg/ml in the 4(20)-hour pulse exposure groups and in the continuous exposure group. The maximum dose with metaphases present in the 24-hour continuous exposure was 5µg/ml.

Dose selection for experiment 1 was based on toxicity and was 300µg/ml for both 4-hour exposure groups.

Experiment 1

In the absence of metabolic activation (S9) the maximum test material dose level with scorable metaphases was at the intermediate dose level of 225µg/ml. No precipitate of test material was observed at the dose levels. The near optimum 50% mitotic inhibition was observed at 150µg/ml. In the presence of S9, 50% mitotic inhibition was observed at 150µg/ml.

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6.2 Chromosome aberration test in human lymphocytes *in vitro*

		The test material induced a statiscally significant increase in the frequency of cells with aberrations at 75µg/ml in the absence of S9 only. The increase was small, not dose-related and was observed at an intermediate dose level that had only 12% mitotic inhibition. The increase was observed against on a concurrent negative control that had no background aberrant cells. Therefore, the increase was considered spurious and of no toxicological significance. The test material did not induce a statistically significant increase in the numbers of polyploidy cells at any dose level in either of the exposure groups. Experiment 2 50% mitotic inhibition was achieved at 37.5 and 50µg/ml in the absence of S9. In the presence of S9, an approximate 50% mitotic inhibition was not achieved at 150µg/ml, a dose level that did demonstrate growth inhibition in experiment 1. The maximum dose level selected for Metaphase analysis was the same as Experiment 1 for the metabolic activation exposure group and was based on toxicity for the without-S9 group (37.5 µg/ml). The test material did not induce any statistically significant increases in the frequency of cells with chromosome aberrations either in the absence or presence of metabolic activation. The test material did not induce a significant increase in the numbers of polyploidy cells at any dose level in either of the exposure groups.
5.3	**Conclusion**	The test material brodifacoum is considered to be non-clastogenic to human lymphocytes
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	February 2006
Materials and Methods	Adopt applicant's version
Results and discussion	**Cytotoxicity**: mitotic index decrease at ≥ 50 µg/ml with and w/o S9.
Conclusion	Adopt applicant's version
Reliability	Reliability indicator 1
Acceptability	The study is acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6.2 Chromosome aberration test in human lymphocytes *in vitro*

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_2-1. Table for Cytogenetic *in vitro*-Test: Chromosomal Analysis (-S9) – Experiment 1

Treatment Group	Replicate	Mitotic index (%)	No of Cells Scored	Number of Aberrations						Total No of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosomes		Others X	(+ Gap)	(-Gap)	(+ Gap)	(-Gap)
					Breaks	Exchanges	Breaks	Exchanges					
Vehicle control	A	3.20	100	3	0	0	0	0	0	3	0	3	0
	B	3.70	100	0	0	0	0	0	0	0	0	0	0
	Total		200	3	0	0	0	0	0	3	0	3 (1.5)	0 (0)
37.5 µg/ml	A	2.65	100	2	1	0	0	0	0	3	1	3	1
	B	3.30	100	0	0	0	0	0	0	0	0	0	0
	Total		200	2	1	0	0	0	0	3	1	3 (1.5)	1 (0.5)
75 µg/ml	A	2.95	100	3	2	0	0	0	0	5	2	5	2
	B	3.10	100	2	2	0	2	0	0	6	4	6	4
	Total		200	5	4	0	2	0	0	11	6	11(5.5)	6* (3)
150 µg/ml	A	1.50	100	2	0	0	1	0	0	3	1	3	1
	B	1.85	100	5	2	0	0	0	0	7	2	7	2
	Total		200	7	2	0	1	0	0	10	3	10 (5.0)	3 (1.5)
Positive Control 0.4 MMC µg/ml	A	2.15	100	4	19	19	3	0	0	45	41	36	33
	B	1.85	100	8	34	16	7	0	0	65	57	40	37
	Total		200	12	53	35	10	0	0	110	98	76 (38)	70*** (35)

X = > 10 aberrations per cell (not included in total aberrations), (figures in brackets) = aberrations per 100 cell, MMC = Mitomycin C, * = P<0.05, *** = P<0.001

Table A6_6_2-2. Table for Cytogenetic *in vitro*-Test: Chromosomal Analysis (+S9) – Experiment 1

Treatment Group	Replicate	Mitotic index (%)	No of Cells Scored	Number of Aberrations						Total No of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosomes		Others	(+ Gap)	(-Gap)	(+ Gap)	(-Gap)
					Breaks	Exchanges	Breaks	Exchanges	X				
Vehicle control	A	4.20	100	0	2	1	0	0	0	3	3	3	3
	B	4.30	100	0	2	0	0	0	0	2	2	2	2
	Total		200	0	4	1	0	0	0	5	5	5 (2.5)	5 (2.5)
37.5 µg/ml	A	3.70	100	0	1	0	1	0	0	2	2	2	2
	B	3.75	100	0	1	0	0	1	0	2	2	2	2
	Total		200	0	4	0	1	1	0	4	4	4 (2.0)	4 (2.0)
75 µg/ml	A	2.25	100	0	0	0	0	0	0	0	0	0	0
	B	2.70	100	0	2	0	0	0	0	2	2	2	2
	Total		200	0	2	0	0	0	0	2	2	2 (1.0)	2 (1.0)
150 µg/ml	A	1.65	100	1	7	0	0	0	0	8	8	5	5
	B	1.85	100	0	4	2	0	1	0	7	7	6	6
	Total		200	1	11	2	0	1	0	15	15	11 (5.5)	11 (5.5)
Positive Control 10 CP µg/ml	A	2.40	100	0	14	2	2	0	0	18	18	15	15
	B	1.75	100	1	12	5	0	0	0	18	18	17	16
	Total		200	1	26	7	2	0	0	36	36	32 (16.0)	31*** (15.5)

CP – Cyclophosphamide, *** = P <0.001, X = > 10 aberrations per cell (not included in total aberrations) (Figures in brackets) = aberrations per 100 cell

Table A6_6_2-3. Table for Cytogenetic *in vitro*-Test: Chromosomal Analysis (-S9) – Experiment 2

Treatment Group	Replicate	Mitotic index (%)	No of Cells Scored	Number of Aberrations						Total No of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosomes		Others X	(+ Gap)	(-Gap)	(+ Gap)	(-Gap)
					Breaks	Exchanges	Breaks	Exchanges					
Vehicle control	A	4.40	100	2	2	0	0	0	0	3	1	4	2
	B	3.25	100	0	0	0	2	0	0	2	2	1	1
	Total		200	2	2	0	2	0	0	5	3	5 (2.5)	3 (1.5)
12.5 µg/ml	A	4.35	100	0	0	0	0	0	0	0	0	0	0
	B	4.00	100	0	0	0	0	0	0	0	0	0	0
	Total		200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
25 µg/ml	A	3.30	100	0	0	0	1	0	0	1	1	1	1
	B	4.20	100	0	0	0	0	0	0	0	0	0	0
	Total		200	0	0	0	1	0	0	1	1	1(0.5)	1 (0.5)
37.5 µg/ml	A	1.50	100	0	0	0	1	0	0	1	1	1	1
	B	1.90	100	1	0	0	1	0	0	2	1	2	1
	Total		200	1	0	0	2	0	0	3	2	3 (1.5)	2 (1.0)
Positive Control 0.2 MMC µg/ml	A	1.00	50[a]	4	23	9	1	0	0	37	33	28	26
	B	1.40	50[a]	13	15	14	6	0	0	48	35	29	25
	Total		200	17	38	23	7	0	0	85	68	57 (57.0)	51*** (51.0)

X = > 10 aberrations per cell (not included in total aberrations), (figures in brackets) = aberrations per 100 cell, MMC = Mitomycin C, * = P<0.05, *** = P<0.001, a = slide evaluation terminated at 50 cells because approximately 50% cells with aberrations had been observed

Table A6_6_2-4. Table for Cytogenetic *in vitro*-Test: Chromosomal Analysis (+S9) – Experiment 2

Treatment Group	Replicate	Mitotic index (%)	No of Cells Scored	Number of Aberrations						Total No of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosomes		Others X	(+ Gap)	(-Gap)	(+ Gap)	(-Gap)
					Breaks	Exchanges	Breaks	Exchanges					
Vehicle control	A	5.65	100	1	0	0	0	0	0	1	0	1	0
	B	4.65	100	1	2	0	1	0	0	4	3	4	3
	Total		200	2	2	0	1	0	0	5	3	5 (2.5)	3 (1.5)
37.5 µg/ml	A	4.55	100	2	0	0	0	0	0	2	0	2	0
	B	4.45	100	1	0	0	0	0	0	1	0	1	0
	Total		200	3	0	0	0	0	0	3	0	3 (1.5)	0 (0.0)
75 µg/ml	A	3.30	100	1	1	0	0	0	0	2	1	2	1
	B	4.05	100	4	0	0	0	0	0	4	0	2	0
	Total		200	5	1	0	0	0	0	6	1	4 (2.0)	1 (0.5)
150 µg/ml	A	3.60	100	0	1	1	0	0	0	2	2	2	2
	B	3.90	100	5	2	0	3	0	0	10	5	8	4
	Total		200	5	3	1	3	0	0	12	7	10 (5.0)	6 (3.0)
Positive Control 10 CP µg/ml	A	0.95	100	13	24	9	2	0	0	48	35	29	21
	B	1.45	50[a]	18	31	8	3	2	0	62	44	32	25
	Total		200	31	55	17	5	2	0	110	79	61 (30.5)	46*** (23.0)

CP – Cyclophosphamide, *** = P <0.001, X = > 10 aberrations per cell (not included in total aberrations) (Figures in brackets) = aberrations per 100 cell

Table A6_6_2-5. Mean Frequency of Polyploid Cells (%) – Experiment 1

Dose Level 1 µg/ml	Harvest Time 24 Hours	
	4 hours without S9	4 hours with S9
0	0.0	0.0
37.5	0.0	0.0
75	0.5	0.5
150	1.5	1.5
MMC 0.4	0.0	NA
CP 10	NA	0.5

Table A6_6_2-6. Mean Frequency of Polyploid Cells (%) – Experiment 1

Dose Level 1 µg/ml	Harvest Time 24 Hours	
	4 hours without S9	4 hours with S9
0	0.0	0.5
12.5/56.25*	0.0	0.0
25/75*	0.0	0.0
37.5/150*	0.0	0.5
MMC 0.2	0.0	NA
CP 10	NA	0.0

MMC = Mitomycin C
CP = Cyclophosphamide
NA = Not applicable

* = Dose levels for tthe wih S9 exposure group

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 Mouse Lymphoma Assay

Official use only

	1 REFERENCE	
1.1	**Reference**	Report: BRODIFACOUM: L5178Y TK+/- Mouse Lymphoma Assay. XXXXX – XXXXX report 1558/004
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
1.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 476
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	04355
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.9% (brodifacoum)
3.1.2.3	Stability	Stable under test conditions
3.2	**Study Type**	Mouse lymphoma assay
3.2.1	Organism/cell type	Mouse lymphoma L5178Y cells
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	In the absence of S9 – ethylmethanesulphonate at 800 µg/ml for 3 hour exposure and 150 µg/ml for 24 hour exposure dissolved in dimethyl sulphoxide In the presence of S9 – cyclophosphamide at 5 µg/ml dissolved in dimethyl sulphoxide
3.3	**Administration / Exposure; Application of test substance**	

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 Mouse Lymphoma Assay

3.3.1	Concentrations	0, 3.13, 6.25, 12.5, 25, 37.5 and 50 µg/ml
3.3.2	Way of application	Dissolved in dimethyl sulphoxide
3.3.3	Pre-incubation time	0
3.3.4	Other modifications	*none*
3.4	**Examinations**	*see tables in appendix for examinations and results*
3.4.1	Number of cells evaluated	*give number (i.e. for micronucleus test, chromosome aberrations)*

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

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	OECD 476

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 Mouse Lymphoma Assay

5.2	**Results and discussion**	Experiment 1 There was evidence of a dose-related reduction in % RSG and RTG values in cultures dosed with the test material in the absence and presence of metabolic activation. The test material induced a small but statistically significant dose related increases in the mutant frequency in the presence of metabolic activation. In the absence of metabolic activation there was no evidence of a mutagenic response. The response observed in the presence of S9 was very modest, the maximum mutant frequency value was well within the acceptable range and less than 3-fold over the control value. The biological relevance of the response was uncertain. The increase in mutant frequency was partly due to small colony formation suggesting clastogenic activity. Experiment 2 There was evidence of a dose-related reduction in % RSG and RTG values in cultures dosed with the test material in the absence and presence of metabolic activation. There was evidence of a reduction in Day 2 (%V) viability in the absence of metabolic activation. The toxicity observed at and above 37.5µg/ml in the absence of metabolic activation exceeded the upper acceptable limit of 90% therefore both dose levels were excluded from the statistical analysis. The test material did not induce any significant or dose-related increases in the mutant frequency in the absence of metabolic activation. In the presence of metabolic activation, a weak but significant linear-trend response was observed. It was therefore considered that the responses were of little or no biological relevance and to have no toxicological significance.
5.3	**Conclusion**	The test material brodifacoum is considered to be non-mutagenic.
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	February 2006
Materials and Methods	Adopt applicant's version
Results and discussion	**Cytotoxicity**: Cytotoxic at ≥ 150 µg/ml with and w/o S9.
Conclusion	Adopt applicant's version
Reliability	Reliability indicator 1
Acceptability	The study is acceptable
Remarks	
	COMMENTS FROM ...

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 Mouse Lymphoma Assay

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_3-1. Table for cell gene mutation test In-Vitro-Test (Expt.1)

Treatment (µg/ml)	3- Hour - S9			Treatment (µg/ml)	3- hour +S9		
	% RSG	RTG	MF		% RSG	RTG	MF
0	100	1.00	48.89	0	100	1.00	45.92
3.13	111	1.19	45.01	3.13	109	0.97	54.46
6.25	108	1.16	44.64	6.25	109	0.90	60.02
12.5	113	1.17	51.45	12.5	97	0.84	54.74
25	88	0.98	63.98	25	80	0.71	48.14
37.50	70	0.74	67.19	37.50	51	0.45	77.86
50	28	0.35	57.66	50	23	0.15	122.98
EMS 800	84	0.48	1367.42	CP 4	41	0.09	1044.38

Table A6_6_3-2 Table for cell gene mutation test In-Vitro-Test (Expt. 2)

Treatment (µg/ml)	24 Hour - S9			Treatment (µg/ml)	3hour +S9		
	% RSG	RTG	MF		% RSG	RTG	MF
0	100	1.00	84.64	0	100	1.00	49.45
3.13	98	1.14	70.46	10	106	0.95	70.68
6.25	97	0.85	85.80	20	94	0.95	67.37
12.5	58	0.55	89.80	30	96	0.94	60.36
25	31	0.27	82.23	40	48	0.43	56.54
37.50	4	0.03	215.98	50	33	0.32	72.94
50	0	0.00	691.27	60	20	0.20	80.60
EMS 150	80	0.50	1580.56	CP 4	81	0.46	679.84

% RSG = Relative suspension Growth
RTG = Relative Total Growth corrected for post treatment toxicity
EMS = Ethylmethanesulphonate
CP = Cyclophosphamide
MF = 5-TFT resistant mutants/10^6 viable cells 2 days after treatment

Section A6.6.4 Annex Point IIA 6.6.4	Genotoxicity in vivo mutagenicity (bone marrow assay for chromosomal damage or a micronucleus test)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	No positive findings seen in *in vitro* studies	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	February 2006	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The 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		

Section A6.6.5 Annex Point IIA 6.6.5	Genotoxicity in vivo mutagenicity or evidence of DNA damage in tissue other than bone marrow	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	No positive findings in *in vitro* genotox studies	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	February 2006	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The 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		

Section A6.6.6 Annex Point IIA 6.6.6	Genotoxicity in vivo (germ cell effects)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X] **Other justification** [ ]	
Detailed justification:	No positive findings in *in vitro* genotox studies	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	February 2006	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The 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		

Section A6.6.7 Annex Point -	Genotoxicity in vivo (further test if metabolites of concern are formed in mammals)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	No positive findings in *in vitro* genotox studies. No metabolites of concern are noted for Brodifacoum in the literature or for any other analogue.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	February 2006	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The 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		

Section A6.7 Annex Point IIA 6.7	Carcinogenicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [X]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The compound belongs to a well-known and closely analogous group of anticoagulants with very similar properties. All studies on vertebrates show the same effects, primarily loss of blood coagulation, and these are shown clearly in acute studies. There is little species differentiation in effects or dose response, and there are no positive findings in genotox studies. To avoid acute effects, doses in repeat dose studies must be kept very low and it is considered infeasible to keep alive animals receiving any appreciable dose for more than a few months. The potential for exposure to rodenticides is limited by the nature of their use, and there is no exposure as a result of residues of the substance, or as a result of long-term exposure to vapour. A carcinogenicity study is therefore considered unjustified.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	March 2006	
Evaluation of applicant's justification	The applicant's justification is acceptable	
Conclusion	The 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		

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	Report: BRODIFACOUM: Development toxicity to the rat. XXXXX – September 1995. XXXXX. report MLS/10025 Repeated dose oral developmental toxicity study of test substance brodifacoum.	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with letter of access	PelGar International Ltd. Activa srl	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 414	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	TCP 003/95	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	Brodifacoum 98.8%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rats	
3.2.2	Strain	Wistar	
3.2.3	Source	Charles River Laboratories, Wilmington, Mass., U.S.A.	
3.2.4	Sex	Female (virgin)	
3.2.5	Age/weight at study initiation	12 weeks Female 237 – 293g	
3.2.6	Number of animals per group	20	

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

3.2.7	Control animals	Yes
3.2.8	Mating period	Overnight
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Rat: day 7-16 post mating
3.3.2	Postexposure period	6 days
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	0, 0.01, 0.02 and 0.04 mg/kg/day
3.3.5	Vehicle	Polyethylene glycol 300
3.3.6	Concentration in vehicle	0, 1, 2 and 4 µg/ml
3.3.7	Total volume applied	1 ml/100g bw
3.3.8	Controls	Vehicle
		Inhalation
3.3.9	Concentrations	
3.3.10	Particle size	
3.3.11	Type or preparation of particles	
3.3.12	Type of exposure	
3.3.13	Vehicle	
3.3.14	Concentration in vehicle	
3.3.15	Exposure period / day	
3.3.16	Controls	
		Intraperitoneal
3.3.17	Vehicle	
3.3.18	Concentration in vehicle	
3.3.19	Total volume applied	
3.3.20	Controls	

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

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 and position of implantations Individual foetal weights
3.4.5	Examination of fetuses	
3.4.5.1	General	Litter size, no. of dead fetuses, foetal weight, sex
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**	NOEL: No effects at 0.04 mg/kg/day
4.2	**Teratogenic / embryotoxic effects**	NOEL: No effects at 0.04 mg/kg/day.
4.3	**Other effects**	No evidence of teratogenicity or other indications of developmental toxicity were seen.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD 414
5.2	**Results and discussion**	No signs of toxicity or evidence of teratogenicity or other indications of developmental toxicity were seen.
5.3	**Conclusion**	No evidence of teratogenicity or other development effects seen.
5.3.1	LO(A)EL maternal toxic effects	Maximum dose tested was below toxic limit
5.3.2	NO(A)EL maternal toxic effects	0.04 mg/kg/day of brodifacoum
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Maximum dose tested was without effect

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

5.3.4	NO(A)EL embryotoxic / teratogenic effects	0.04 mg/kg/day of brodifacoum
5.3.5	Reliability	1
5.3.6	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	*January 2007*
Materials and Methods	The study is overall adequate.
Results and discussion	The assesment of results performed by the applicant is overall adequate.
Conclusion	The conclusions drawn by the applicant as regards the study resuts are overall adequate. It is unclear wether OECD 414 in rat is a proper study protocol to evaluate developmental toxicity of anticoagulant rodenticides.
Reliability	The study is reliable.
Acceptability	The study is overall accetable.
Remarks	Tte actual relevance of OECD 414 to risk assessmeent of anticoagulant rodenticides is unclear.
	COMMENTS FROM ...
Date	*January 2007*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Maternal effects

Modify if necessary and give historical data if available

Parameter	control data	low dose	medium	high	dose-

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1

A6.8.1 Oral developmental toxicity to the rat

	historical	study		dose	dose	response + / -
Number of dams examined		20	20	20	20	
Clinical findings during application of test substance		0	0	0	0	
Mortality of dams *state %*		0	0	0	0	
Abortions		0	0	0	0	
Body weight gain *day 0-end of test,*	-	262g – 396g	264g – 398g	264g – 399g	264g – 394g	
Food consumption	-	-	-	-	-	
Water consumption	-	-	-	-	-	
Pregnancies *pregnancy rate or %*		20	20	20	20	
Necropsy findings in dams dead before end of test		-	-	-	-	

Table A6 8-2. Table for Teratogenic effects (separate data for all dosage groups)
Litter response (Caesarean section data)
Modify if necessary and give historical data if available

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	study				
Corpora lutea *Mean number/number of dams*		16.3/20	15.6/20	16.6/20	16.3/20	
Implantations *Mean number/number of dams*		14.5/20	14.8/20	14.2/20	13.9/20	
Resorptions *state total/number of dams*		-	-	-	-	
total number of fetuses		248	254	250	256	
pre-implantation loss *state %*		8.0	5.1	14.5	14.7	
post-implantation loss *state %*		9.0	8.4	7.0	4.7	
total number of litters		20	20	20	20	
fetuses / litter		12.4	12.7	12.5	12.8	
live fetuses / litter *state ratio (mean)*		12.4/12.4	12.7/12.7	12.5/12.5	12.8/12.8	
dead fetuses / litter *state ratio*		0/12.4	0/12.7	0/12.5	0/12.8	
fetuses weight (mean) *[g]*		4.77	4.97	5.18	4.64	

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

placenta weight (mean) *[g]*		-	-	-	-	
crown-rump length (mean) *[mm]*		-	-	-	-	
Fetal sex ratio *[state ratio m/f]*		126/122	122/132	128/122	133/123	

Table A6_8-3. Table for Teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Modify if necessary and give historical data if available

Parameter	control data		low dose	Medium dose	high dose	dose-response + / -
	historical	study				
External malformations* *[%]*						
External anomalies* *[%]*						
Skeletal malformations* *[%]*						
Skeletal anomalies* *[%]*						
Skeletal variants* *[%]*						
Visceral malformations* *[%]*						
Visceral anomalies* *[%]*						
Variants visceral* *[%]*						

Section A6.8.1 (2) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rabbit

Official use only

1 REFERENCE

1.1 Reference

Report: BRODIFACOUM: Development toxicity to the rabbit. XXXXX – July 1995. XXXXX. report MLS/10019

Repeated dose oral developmental toxicity study of test substance brodifacoum.

1.2 Data protection Yes

1.2.1 Data owner: Activa / PelGar Brodifacoum and Difenacoum Task Force

1.2.2 Companies with letter of access: PelGar International Ltd.; Activa srl

1.2.3 Criteria for: Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

	data protection	the purpose of its [entry into Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD 414
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	TCP 0007/94
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Off-white powder
3.1.2.2	Purity	Brodifacoum 99.1%
3.1.2.3	Stability	Stable
3.2	**Test Animals**	
3.2.1	Species	Rabbits
3.2.2	Strain	New Zealand White
3.2.3	Source	Charles River Laboratories, Wilmington, Mass., U.S.A.
3.2.4	Sex	Female (virgin)
3.2.5	Age/weight at study initiation	Female 2.27 – 2.71 kg
3.2.6	Number of animals per group	20
3.2.7	Control animals	Yes
3.2.8	Mating period	Animals mated at suppliers - designated as day 1 of gestation. Rabbits delivered to laboratory on day 2 of gestation.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Rabbit: day 6-18 inclusive post mating
3.3.2	Postexposure period	11 days
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	0, 0.001, 0.002 and 0.004 mg/kg/day
3.3.5	Vehicle	Polyethylene glycol 300

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

3.3.6	Concentration in vehicle	0, 0.5, 1.0 and 2.0 µg/ml
3.3.7	Total volume applied	2 ml/kg bw
3.3.8	Controls	Vehicle
		Inhalation
3.3.9	Concentrations	
3.3.10	Particle size	
3.3.11	Type or preparation of particles	
3.3.12	Type of exposure	
3.3.13	Vehicle	
3.3.14	Concentration in vehicle	
3.3.15	Exposure period / day	
3.3.16	Controls	
		Intraperitoneal
3.3.17	Vehicle	
3.3.18	Concentration in vehicle	
3.3.19	Total volume applied	
3.3.20	Controls	
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 and position of implantations Individual foetal weights
3.4.5	Examination of fetuses	
3.4.5.1	General	Litter size, no. of dead fetuses, foetal weight, sex

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

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**	NOEL: No effects at 0.004 mg/kg/day
4.2	**Teratogenic / embryotoxic effects**	NOEL: No effects at 0.004 mg/kg/day.
4.3	**Other effects**	No evidence of teratogenicity or other indications of developmental toxicity were seen.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD 414
5.2	**Results and discussion**	No signs of toxicity or evidence of teratogenicity or other indications of developmental toxicity were seen.
5.3	**Conclusion**	No evidence of teratogenicity or other development effects seen.
5.3.1	LO(A)EL maternal toxic effects	Maximum dose tested was below toxic limit
5.3.2	NO(A)EL maternal toxic effects	0.004 mg/kg/day of brodifacoum
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Maximum dose tested was without effect
5.3.4	NO(A)EL embryotoxic / teratogenic effects	0.004 mg/kg/day of brodifacoum
5.3.5	Reliability	1
5.3.6	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	*Give date of action*
Materials and Methods	The study is overall adequate.

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

Results and discussion	The assessment of result performed by the applicant is overall adequate.
Conclusion	The conclusion made by the applicant are overall adequate.
Reliability	The study is overall reliable.
Acceptability	The study is overall accetable.
Remarks	NO
	COMMENTS FROM ...
Date	*January 2007*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Maternal effects

Modify if necessary and give historical data if available

Parameter	control data		low dose	medium dose	high dose	dose-response + / -
	historical	study				
Number of dams examined		20	20	20	20	
Clinical findings during application of test substance		0	0	0	0	
Mortality of dams *state %*		0	0	0	0	
Abortions		0	0	0	0	
Body weight gain *day 0-end of test,*	-	2.44 kg – 2.54 kg	2.49 kg – 2.60 kg	2.47 kg – 2.57 kg	2.47 kg – 2.57 kg	
Food consumption	-	-	-	-	-	
Water consumption	-	-	-	-	-	
Pregnancies *pregnancy rate or %*		20	20	20	20	
Necropsy findings in dams dead before end of test		-	-	-	-	

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

Table A6 8-2. Table for Teratogenic effects (separate data for all dosage groups)
Litter response (Caesarean section data)
Modify if necessary and give historical data if available

Parameter	control data		low dose	medium dose	high dose	dose-response + / -
	historical	study				
Corpora lutea *Mean number/number of dams*		11.5/20	12.2/20	11.7/20	11.9/20	
Implantations *Mean number/number of dams*		11.0/20	11.5/20	11.4/20	11.5/20	
Resorptions *state total/number of dams*		-	-	-	-	
total number of fetuses		193	204	184	197	
pre-implantation loss *state %*		11.7	10.4	12.0	10.6	
post-implantation loss *state %*		11.5	8.7	9.9	10.4	
total number of litters		20	20	20	20	
fetuses / litter		9.65	10.2	9.2	9.85	
live fetuses / litter *state ratio (mean)*		9.65/9.65	10.2/10.2 .	9.2/9.2	9.85/9.85	
dead fetuses / litter *state ratio*		0/9.65	0/10.2	0/9.2	0/9.85	
fetus weight (mean) *[g]*		39.19	39.21	43.64	39.48	
placenta weight (mean) *[g]*		-	-	-	-	
crown-rump length (mean) *[mm]*		-	-	-	-	
Fetal sex ratio *[state ratio m/f]*		94/99	104/100	95/89	96/101	

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

Table A6_8-3. Table for Teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Modify if necessary and give historical data if available

Parameter	control data		low dose	Medium dose	high dose	dose-response +/-
	historical	study				
External malformations* *[%]*						
External anomalies* *[%]*						
Skeletal malformations* *[%]*						
Skeletal anomalies* *[%]*						
Skeletal variants* *[%]*						
Visceral malformations* *[%]*						
Visceral anomalies* *[%]*						
Variants visceral* *[%]*						

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 A6.8.2 Two-generation reproduction toxicity study in rats

Official use only X

1 REFERENCE

1.1 Reference Report: BRODIFACOUM: Two-generation reproduction toxicity study of test item brodifacoum technical in rats. Study Director - XXXXX XXXXX. report 03/737-202P.

1.2 Data protection Yes

1.2.1 Data owner: Activa / PelGar Brodifacoum and Difenacoum Task Force

1.2.2 Companies with letter of access: PelGar International Ltd.; Activa srl

1.2.3 Criteria for data protection: Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study OECD 416

2.2 GLP Yes

2.3 Deviations No

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	04359
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White to greyish colour powder
3.1.2.2	Purity	100%
3.1.2.3	Stability	Stable
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar
3.2.3	Source	Charles River Laboratories, Wilmington, Mass., U.S.A.
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Male Age – 6 weeks Mean body weight approx 204g Female Age - 6 weeks Mean body weight approx 207g
3.2.6	Number of animals per group	25 rats/sex/group
3.2.7	Mating	See table below
3.2.8	Duration of mating	2 weeks
3.2.9	Deviations from standard protocol	None
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	*See table below*
3.3.2	Duration of exposure before mating	10 weeks

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

3.3.3	Duration of exposure in general P, F1, F2 males, females	Daily dosing of the Parent (P) males began 10 weeks prior to the mating period, throughout 2 weeks mating period until termination. Dosing of the F1 male animals selected for mating began at weaning and it was continued (throughout mating period) until termination. Daily dosing of the parent (P) females began 10 weeks prior to the mating period, it was continued during the mating period, throughout pregnancy and up to the weaning of the F1 offspring (until termination on postpartal day 29) Dosing of the F1 female animals selected for mating began at weaning (postnatal day 29), and it was continued during the mating period, throughout pregnancy and up to the weaning of the F2 offspring (until termination on postpartal day 22). The animals were treated once daily, at similar times each day , in the morning. Animals were not treated on the day of termination.
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	Gavage 5 ml/kg bw
3.3.6	Vehicle	Ethanol with distilled water
3.3.7	Concentration in vehicle	1 mg/ml then diluted with distilled water to create concentrations of 0, 1, 3 and 10 µg/kg/day 10 µg/kg/day was reduced to 6 µg/kg/day on day 40 due to death of some animals Study was completed with levels 0, 1 and 3 µg/kg/day
3.3.8	Total volume applied	Females (P) 565 ml/kg maximum (depending on mating period) Males (P) 460 ml/kg
3.3.9	Controls	Vehicle
		Inhalation
3.3.10	Concentrations	
3.3.11	Particle size	
3.3.12	Type or preparation of particles	
3.3.13	Type of exposure	
3.3.14	Vehicle	
3.3.15	Concentration in vehicle	
3.3.16	Duration of exposure/day	

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

3.3.17	Frequency of exposure	
3.3.18	Controls	
3.4	**Examinations**	
3.4.1	Clinical signs	Clinical observations were made twice daily in treated and control animals during the study. Detailed examination made weekly
3.4.2	Body weight	P – (Male and female) once per week prior to and during mating
		P – (Female) postnatal days 1, 5, 8, 15, 22, 29 and then weekly.
		F1 – (Male) once per week prior to and during mating, on gestation days 1, 8, 15 and 21 and on postpartal days 1, 8, 15, 22 and 29.
		F1 – (female)postnatal days 1, 5, 8, 15, 22, 29 and then once a week prior to and during mating, on gestation days 1, 8, 15 and 21 and on postpartal days 1, 5, 8, 15 and 22.
3.4.3	Food/water consumption	Mean daily food consumption calculated weekly
3.4.4	Oestrus cycle	Smears prepared daily during premating period and during mating period.
3.4.5	Sperm parameters	Total number of cells
		Sperm with separated head and tail
		sperm motility
		sperm morphology
3.4.6	Offspring	number and sex of pups
		stillbirths
		live births
		presence of gross anomalies
		weight gain
		physical or behavioural abnormalities
		or other
3.4.7	Organ weights P and F1	Uterus
		ovaries
		testes
		epididymides
		prostate
		seminal vesicles
		brain
		liver
		kidneys
		spleen
		pituitary
		thyroid
		adrenal glands

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

3.4.8	Histopathology P and F1	uterus ovaries kidney liver spleen adrenal gland stomach seminal vesicle	
3.4.9	Histopathology F1 not selected for mating, F2	uterus ovaries kidney liver spleen adrenal gland stomach seminal vesicle	

3.5 Further remarks

4 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

4.1 Effects

4.1.1	Parent males	Deaths in high dose group caused reduction in dose levels. Reproductive performance, organ weights and body weight unaffected.	
4.1.2	Parent females	Deaths in high dose group caused reduction in dose levels. Reproductive performance, organ weights, body weight and sex ratio of pups unaffected.	
4.1.3	F1 males	No effects to postnatal development. Reproductive performance unaffected	
4.1.4	F1 females	No effects to postnatal development. Reproductive performance unaffected	
4.1.5	F2 males	No effects to postnatal development	
4.1.6	F2 females	No effects to postnatal development	

4.2 Other

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Rats / OECD 416	X
5.2	**Results and discussion**	Reproductive performance of males and females was unaffected. No effect on postnatal development of pups in either F1 or F2 generations. At 10 µg/kg/day dose level no adverse effect was observed in the P, F1 and F2 generations	
5.3	**Conclusion**	Brodifacoum technical induced adverse parental effects at 6 (10)	X

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

	µg/kg/day dose level in CRL:(W1) BR rats. This included clinical symptoms and death caused by the general haemorrhagic diathesis. Female animals proved to be more sensitive than male animals in the P generation. Reproductive performance of males and females were unaffected by the treatment with Brodifacoum technical. There was no effect on postnatal development of pups either in F1 or in F2 generations. At 1 µg/kg/day dose level no adverse effects in the examined parameters in the P, F1 and F2 generations
5.3.1 LO(A)EL	
5.3.1.1 Parent males	3 µg/kg/day
5.3.1.2 Parent females	3 µg/kg/day
5.3.1.3 F1 males	3 µg/kg/day
5.3.1.4 F1 females	3 µg/kg/day
5.3.1.5 F2 males	3 µg/kg/day
5.3.1.6 F2 females	3 µg/kg/day
5.3.2 NO(A)EL	
5.3.2.1 Parent males	3 µg/kg/day
5.3.2.2 Parent females	1 µg/kg/day
5.3.2.3 F1 males	3 µg/kg/day
5.3.2.4 F1 females	3 µg/kg/day
5.3.2.5 F2 males	3 µg/kg/day
5.3.2.6 F2 females	3 µg/kg/day
5.3.3 Reliability	1
5.3.4 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	*January 2007*
Materials and Methods	The study design and protocol is overall adequate
Results and discussion	The evaluation of results performed by the applicant is overall adequate
Conclusion	The conclusion made by the applicant are accetable. A two-generation study is not normally required for anticoagulant rodenticides. However the results of this study are relevant to the establishment of a NOAEL for impaired coagulation in rodents.
Reliability	The study is reliable.

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

Acceptability	Acceptable.
Remarks	Altough a two-generation study is not normally required for anticoagulant rodenticides, the study is relevant for the enstablishment of an overall NOAEL for anticoagulant effects in rodents.
	COMMENTS FROM ...
Date	*January 2007*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8_2-1. Table for animal assignment for mating (modify as appropriate)

		Number of animals			
		Controls	**Low Dose**	**Medium Dose**	**High Dose**
Parents	**m**	25	25	25	25
	f	25	25	25	25
F_1	**m**	1 per litter	1 per litter	1 per litter	1 per litter
	f	1 per litter	1 per litter	1 per litter	1 per litter

Table A6_8_2-2. Table for reproductive toxicity study (modify if appropriate)

If effects are found in one generation, the figures for the other generation(s) should be given as well (as shown as an example for mortality). Give only information on endpoints with effects, delete other endpoints.

Parameter			**control**		**low dose**		**medium dose**		**High dose**			
		Genera-tion	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	incidence	**P**	**0**	**0**	**0**	**0**	**0**	**1**	**12**	**19**		
		F_1	**0**	**0**	**0**	**1**	**0**	**0**	**-**	**-**		
		F_2	**0**	**0**	**0**	**0**	**0**	**0**	**-**	**-**		
Food consumption	% of control		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		
Body weight gain	% of control		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		
Clinical Observations *specify effects*	Incidence											
Organ weights	% of control		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		
Pathology												

Section A6.8.1 (1) Teratogenicity Study

Annex Point IIA6.8.1 A6.8.1 Oral developmental toxicity to the rat

Histopathologic examination *specify effects*	Incidence											
Reproductive Performance												
Mating index			**100**		**88**		**96**					
Fertility index			**88**	**88**	**91**	**91**	**100**	**100**				
Number of implantation sites	Mean											
Duration of pregnancy	Mean			**22**		**22**		**22**				
Birth index												
Live birth index				**100**		**100**		**100**				
Gestation index				**100**		**100**		**96**				
Litter size	Mean		**12**		**10**		**13.6**					
Litter weight	Mean		**6.6**		**6.6**		**6.6**					
Pup weight	Mean		**79.2**		**66**		**89.8**					
Sex ratio	Male/female		**143**	**155**	**136**	**116**	**183**	**158**				
Survival index												
Viability index			**98**		**99**		**99**					
Lactation index			**98**		**100**		**96**					
Sperm characterization												
Number	% of control		**100**	**-**	**-**	**-**	**-**	**100**	**-**			
Deformations	% of control		**100**	**-**	**-**	**-**	**-**	**100**	**-**			

Section A6.9 Annex Point *IIIA VI.1*	Neurotoxicity study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	No evidence of neurotoxic effects in any study. Consideration of chemical structure does not suggest neurotoxic effects. No neurotoxic effects shown by analogues in any species.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*21.11.2006*	
Evaluation of applicant's justification	Applicant's justification for not conducting any neurotoxic study is scientifically based.	
Conclusion	Applicant's justification is acceptable . No neurotoxic data have to be provided	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.10 Annex Point *IIIA VI.1*	**Mechanistic study – any studies necessary to clarify effects seen in toxicity studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum is an anticoagulant with a well-known mode of action,due to the inhibition of blood coagulation by blocking clotting-factors synthesis, as indicated by studies on different animal species, including humans. References: Boermans H J, Johnstone J, Black W D & Murphy M (1991). Clinical signs, laboratory changes and toxicokinetics of brodifacoum in horses. *Can J Vet Res*, 55: 21-27. Breckenridge A, Cholerton S, Hart J, Park B, Scott A (1985).A study of the relationship between the pharmacokinetics and the pharmacodynamics of the 4-hydroxycoumarin anticoagulants warfarin, difenacoum and brodifacoum in the rabbit. *Brit J Pharmacol* 84, 81-89 Godfrey M E R, Laas F J, Rammell C G (1985) Acute toxicity of brodifacoum to sheep. *New Zealand Journal of Experimental Agriculture,* 13:23-25. Laas F J, Forss D A, Godfrey M E R (1985) Retention of brodifacoum in sheep tissues and excretion in faeces. *N Z J Agric Res*, 28: 357-359. Ray, A C, Murphy M J, DuVall, M D, Reagor J D (1989). Determination of brodifacoum and bromadiolone residues in rodent and canine liver. *Am J Vet Res,* 50, 546-550. Woody B J, Murphy M J, Ray A C & Green R A (1992) Coagulopathic effects and therapy of brodifacoum toxicosis in dogs. *J Vet Intern Med*, 6: 23-28 Weitzel J N, Sadowski J A, Furie B C, Moroose R, Kim H, Mount M E, Murphy M J, Furie B (1990) . Surreptitious ingestion of a long-acting vitamin K antagonist/rodenticide, brodifacoum: Clinical and metabolic studies of three cases. *Blood*, 76, 2555-2559.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A6.10 Annex Point *IIIA VI.1*	**Mechanistic study – any studies necessary to clarify effects seen in toxicity studies**
Date	*21.11.2006*
Evaluation of applicant's justification	Applicant's justification is reasonable
Conclusion	Applicant's justification is acceptable
Remarks	A note should be added in the justification text, indicating that the cited studies have been described insection 6.2
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.11 Annex Point *IIIA III-0§*	Studies on other routes of administration (parental routes)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Compound is highly toxic by the oral route to all mammalian species. It is a large, lipophilic molecule, which is poorly absorbed through the skin. It is of very low water solubility and low vapour pressure. The mode of action is common to all mammals and is well understood as a vitamin K antagonist, without secondary effects. It is only used as baits for the control of rodents. Manufacturing takes place in closed or controlled environments with full protective clothing and use as a rodenticide necessarily involves wearing gloves, overalls and other protective clothing because of the biological hazards involved and associated hygiene requirements. Data on other routes of administration are considered an unjustifiable waste of experimental animals since the compound is shown to be highly toxic by the oral route and other routes of administration are not relevant to the current and proposed uses of the compound. However the parental route is summarised in section A6.8	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*21.11.2006*	
Evaluation of applicant's justification	Applicant's justification is reasonable. However some statements should be change being not correct, i.e. *'It is a large, lipophilic molecule, which is poorly absorbed through the skin'.* In addition reference to the study described in the metabolism section (A.6.2.2c) in which the a.s. is administered i.v. could be cited to support the justification	
Conclusion	Applicant's justification is acceptable, pending amendements suggested above.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.12.1 Annex Point IIA VI.6.9.1	Medical surveillance data on manufacturing plant personnel if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Variation: It is to take in account that the active involved is an anticoagulant: it gains this property when chemical is completed coupling intermediate with 4-hydroxycoumarin. It is to be clear that only at this point the chemical begins as an anticoagulant. In addition the active is used only to prepare anticoagulant solutions at 2,5% or other percentage. All staff, composed by 7 operators, is followed from 1975 by a doctor specialised in "hygiene and preventive medicine" and "work medicine". At beginning in 1975, staff was controlled each 3 months with haematochemical and urine examination. After a period of ten years in 1985, since no kind of problems rise and all processes were well secured, medical surveillance was changed with: - six-monthly medical visit made by the competent doctor, - spyrometric annual control, - six-monthly haematochemical and urine examination. In 1995 another change was made: haematochimical and urine examination began annual. All surveillance plans aremade by the upper doctor who inspect also the production facilities with some surprise visit during working. All upper results control are communicated to local authorities each year. All documents can be showed on request. No accidents occur from 1975 till today: this can demonstrate process safety and operator medical surveillance. These records are covered by personnel privacy provisions.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*November 2006*	

Section A6.12.1 Annex Point IIA VI.6.9.1	Medical surveillance data on manufacturing plant personnel if available
Evaluation of applicant's justification	
Conclusion	The provided information has been considered
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	

A large amount of data exists in the public domain relating to the metabolism of anticoagulants especially with regard to warfarin as a result of its therapeutic use in humans. Since the anticoagulants are a closely related group of analogues with clear and similar physico-chemical and toxicological properties, it is considered valid to consider that data on one compound as being applicable to others.

Section A6.12.2
Annex Point IIA VI.6.9.2

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

Human- Brodifacoum

Official use only

1 REFERENCE

1.1	**Reference**	Weitzel JN, Sadowski JA, Furie BC, Moroose R, Kim H, Mount ME, Murphy MJ & Furie B (1990) – Surreptitious ingestion of a long-acting vitamin K antagonist/rodenticide, brodifacoum: Clinical and metabolic studies of three cases. Blood, 76(12): 2555-2559.
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Public domain
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Warfarin sodium
3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
3.1.2	Specification	Not stated in the published paper
3.1.2.1	Description	*Not described*
3.1.2.2	Purity	Not stated in the published paper
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.1.2.4	Radio labelling	No
3.2	**Test Animals**	
3.2.1	Species	Human
3.2.2	Strain	n/a
3.2.3	Source	Patients
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Aged 20 to 48 years
3.2.6	Number of animals per group	3
3.2.7	Control animals	No

Section A6.12.2 Direct observation, e.g. clinical cases, poisoning incidents if available
Annex Point IIA VI.6.9.2

Human- Brodifacoum

3.3	**Administration/ Exposure**		Oral or i.v injection
	3.3.1	Preparation of test site	n/a
	3.3.2	Concentration of test substance	n/a
	3.3.3	Specific activity of test substance	Not relevant
	3.3.4	Volume applied	Not relevant or stated
	3.3.5	Sampling time	
	3.3.6	Samples	

4 RESULTS AND DISCUSSION

4.1 Result of study

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

3 cases of patients with severve bleeding disorders due to deficiency of the vitamin K-dependent blood clotting proteins were reported on. Symptoms were caused by ingestion of Brodifacoum. No other anticoagulants were detected in any of the patients. Patients were treated with long-term administration of high-dose vitamin K1 therapy.

5.2 Results and discussion

CASE 1

A 20-year-old female was hospitalised with abdominal pain, melena, menorrhagia, and gross heamturia. She was previously well apart from a history of anorexia nervosa and depression.

The follwing coagulation studies were performed on the patient.(normal values are in brackets).

Prothrombin time (s) (10.8-13.2) **>50**

PTT (s) (29.2-40.4) **98**

The patient was treated for 4 days with fresh frozen plasma and oral vitamin K_1 resolution of the bleeding and normalisation of the coagulation studies. Three weeks later she representedwith recurrent menorrhagia and a compartment syndrome from hemorrhage into the left calf requiring surgical drainage. The prothrombin time was greater than 50 seconds and the PTT was 98.9 seconds. Bleeding decreased after transfusion with frozen plasma and oral vitamin K_1, with partial correction of her prothrombin time to 16.7 seconds and her partialthromboplastin time to 37.8 seconds. Vitamin K_1 (5 to 10 mg orally) was ineffective, and was substituted by subcutaneous and intravenous administration supplemented with fresh frozen plasma therapy.

Two months later, the prothrombin time was 44.5 seconds, PTT was 68

Section A6.12.2
Annex Point IIA VI.6.9.2

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

Human- Brodifacoum

seconds. A search for vitamin K antagonists in her serum showed the presence of brodifacoum at 382 nmol/L, asa measured by immunoassay, and 289 nmol/L as determined by HPLC.

The patient admitted ingesting seven packs of D-Con (17.5 mg), a rodenticide, just before her intial presentation.. Serial serum brodifacoum concentrations showed a serum half disappearance time of approximately 34 days. Serum brodifacoum was 7.6 nmol/L 8 months after her intial presentation, but not detectable at 12 months despite evidence for continued inhibition of the vitamin K cycle.

CASE 2

A 48-year-old businessman was hospitalised with severve epistaxis and abnormal coagulation parameters.

Prothrombin time (s) (10.8-13.2) **> 40**

PTT (s) (29.2-40.4) **> 100**

Treatedwith fresh frozen plasma and parenteral vitamin K_1 his epistaxis subsided. Two weeks later he represented with acute bleeding into the left calf resulting in compartment syndrome. There was no past history of bleeding. After treatment with fresh frozen plasma and vitamin K_1 he underwent a fasciotomy for decompression of the compartment syndrome. Postoperatively, his coagulopathy persisted until the vitamin K_1 dosage was increased to 100 mg daily.

The presence of vitamin K antagonist was confirmed by the ratio of vitamin K epoxide to vitamin K of 5.9. Screening of the serum for vitamin K epoxide to vitamin K antagonists showed brodifacoum(270.7 nmol/L); levels decreased over the ensuing 2 months. Oral vitamin K_1 administration for 100 days was necessary to correct his coagulopathy. His residence contained numerous packages of Contrac, a brodifacoum containing rodenticide, but he denied ingesting the rodenticide.

CASE 3

A 37-year-old shirt manufacturer was in good health except for mild hypertension controlled with hydrochlorthizide. He initially presented with gross haemturia and deficiency of the vitamin K dependent coagulation proteins.

Prothrombin time (s) (10.8-13.2) **> 60**

PTT (s) (29.2-40.4) **> 100**

Past history was negative and he had undergone vasectomy and dental extractions without problem. He denied ingestion of any anticoagulants or rodenticides. Warfarin was detected in his serum by photometric assay at 4.4 µg/mL, but was negative for warfarin by HPLC. He was treated with fresh frozen plasma and parenteral vitamin K_1 which controlled his bleeding disorder. After being to follow-up, he represented with hematuria, epstaxis, and hematomas due to deficiency of the vitamin K-dependent blood clotting proteins. He was restarted on vitamin K_1 and fresh frozen plasma. He required 150 to 280 mg per day of vitamin K_1 to achieve partial correction of his prothrombin time and PTT. Studies of Vitamin Kmetabolism confirmed blockade in the

Section A6.12.2 Annex Point IIA VI.6.9.2	Direct observation, e.g. clinical cases, poisoning incidents if available Human- Brodifacoum
	vitamin K cycle with an elevated K epoxide of 298 nmol/L and vitamin K_1 of 122 nmol/L (ratio 2.4). Screening for anticoagulants revealed brodifacoum at 2759 nmol/L.
Conclusion	The analysis of vitamin K metabolites in serum is a sensitive method for detecting the presence of vitamin K antagonists. With these studies one can make a presumptive diagnosis of anticoagulant poisoning, allowing rational vitamin K_1 therapy. In general, the vitamin K epoxide to vitamin K_1 ratio reflect the degree of inhibition of vitamin K action. Vitamin K_1 is the treatment of choice for cases of anticoagulant rodenticide poisoning . Except in the face of serious bleeding fresh frozen plasma should be avoided due to the risk of transmission of viral diseases. The amount of vitamin K_1 administered varies, but doses excess of 100 mg daily may be necessary to obtain and maintain a normal prothrombin time. Although initial parenteral vitamin K is often indicated, the long-term administration of vitamin K is prefereably given by the oral route. Treatment is usually necessary for many months, but the dosages of vitamin K should be the minimum necessary to maintain a normal prothrombin time.
5.2.1 Reliability	2
5.2.2 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	*November 2006*
Materials and Methods	
Results and discussion	
Conclusion	The provided information has been considered
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 apporteur member state*
Results and discussion	*Discuss if deviating from view of apporteur member state*
Conclusion	*Discuss if deviating from view of apporteur member state*

Section A6.12.2
Annex Point IIA VI.6.9.2

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

Human- Brodifacoum

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

Section A6.12.7
Annex Point IIA VI.6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known

Human- Brodifacoum

Official use only

		6 REFERENCE
6.1	**Reference**	Donovan, J.W. Ballard J.O Murphy, M.J (1990) Brodifacoum therapy with activated charcoal: Effect on elimination kinetics. Capital area poison center, Penn State University.
6.2	**Data protection**	No, published paper.
6.2.1	Data owner	Public domain
6.2.2		
6.2.3	Criteria for data protection	No data protection claimed
		7 GUIDELINES AND QUALITY ASSURANCE
7.1	**Guideline study**	The guideline study is not stated in the published paper.
7.2	**GLP**	The GLP status of the study is not stated in the published paper
7.3	**Deviations**	No
		8 MATERIALS AND METHODS
8.1	**Test material**	Brodifacoum
8.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
8.1.2	Specification	Not stated in the published paper
8.1.2.1	Description	*Not described*
8.1.2.2	Purity	Not stated in the published paper
8.1.2.3	Stability	A specific statement on stability is not provided within the paper.
8.1.2.4	Radio labelling	No
8.2	**Test Animals**	
8.2.1	Species	Human
8.2.2	Strain	n/a
8.2.3	Source	Patients
8.2.4	Sex	Not stated in report
8.2.5	Age/weight at study initiation	Not stated in report
8.2.6	Number of animals per group	2
8.2.7	Control animals	No

Section A6.12.7
Annex Point IIA VI.6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known

Human- Brodifacoum

8.3	**Administration/ Exposure**		Oral or i.v injection
	8.3.1	Preparation of test site	All humans tested for health and fitness
	8.3.2	Concentration of test substance	1.5 mg/kg bodyweight
	8.3.3	Specific activity of test substance	Not relevant
	8.3.4	Volume applied	Not relevant or stated
	8.3.5	Sampling time	n/a
	8.3.6	Samples	Serum

9 RESULTS AND DISCUSSION

9.1 Result of study

10 APPLICANT'S SUMMARY AND CONCLUSION

10.1 Materials and methods

In 2 adults who intentionally ingested Brodifacoum, an attempt was made to enhance elimination and shorten treatment time by administering repeated doses of activated charcoal (RDAC). Both patients received 25 gm RDAC q4h for one day, the first at 33 days post-ingestion (PI) and the second at 1 and again at 3 days PI. Brodifacoum levels were measured twice daily and analysed by HPLC. A best-fit curve of plasma concentrations was plotted by linear regression, and terminal elimination half-lives (BT1/2) were calculated.

10.2 Results and discussion

Brodifacoum levels ranged from 3.2 to 219.4ng/ml, and βT1/2 was 399 hrs, comparable to a previously reported βT1/2 of 487 hrs.

The βT1/2 shortened during RDAC, but Brodifacoum levels reboundec and both patients required large doses of vitamin K for over 3 months.

10.3 Conclusion

It was councluded that even low B levels in serum are associated with toxicity, that serum βT1/2 in human overdose exceeds 16 days, and that a one-day course of RDAC may shorten the βT1/2 but does not eliminate the need for prolonged vitamin K therapy.

10.3.1	Reliability	2
10.3.2	Deficiencies	Ages and sex of patients are not known.

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

Section A6.12.7
Annex Point IIA VI.6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known

Human- Brodifacoum

Date	*November 2006*
Materials and Methods	
Results and discussion	
Conclusion	The provided information has been considered
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 apporteur member state*
Results and discussion	*Discuss if deviating from view of apporteur member state*
Conclusion	*Discuss if deviating from view of apporteur member state*
Reliability	*Discuss if deviating from view of apporteur member state*
Acceptability	*Discuss if deviating from view of apporteur member state*
Remarks	

Section A6.12.8 Annex Point IIA VI.6.9.8	Prognosis following poisoning	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum is an indirect anit-coagulant. Vitamin K1 is antidotal. In the case of suspected poisoning, determine prothrombin times not less than 18 hours after consumption. If elevated, administer vitamin K1 and continue until prothrombin times normalise. Continue determination of prothrombin time for three days after withdrawal of antidote and resume treatment if elevation recurs in that time.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*November 2006*	
Evaluation of applicant's justification		
Conclusion	The provided information has been considered	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.13 Toxic effects on livestock and pets
Annex Point IIIA VI.2 *Sheep-Brodifacoum*

Official use only

		1 REFERENCE
1.1	**Reference**	M.E.R. Godfrey, F.J.Laas and C.G. Rammell (1985) Acute toxicity of brodifacoum to sheep. New Zealand Journal of Experimental Agriculture, 1985, Vol. 13:23-25
1.2	**Data protection**	No, published paper.
1.2.1	Data owner	Public Domain
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	The guideline study is not stated in the published paper.
2.2	**GLP**	The GLP status of the study is not stated in the published paper
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Batch numbers not stated in the published paper.
3.1.2	Specification	Not stated in the published paper
3.1.2.1	Description	Not stated in the published paper
3.1.2.2	Purity	Not stated in the published paper
3.1.2.3	Stability	A specific statement on stability is not provided within the paper.
3.1.2.4	Radio labelling	No
3.2	**Test Animals**	
3.2.1	Species	Sheep
3.2.2	Strain	Not stated in published paper
3.2.3	Source	Not stated in published paper
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	Report refers to ewes as being 'mature.'
3.2.6	Number of animals per group	8
3.2.7	Control	No

Section A6.13 **Toxic effects on livestock and pets**
Annex Point IIIA VI.2 *Sheep-Brodifacoum*

		animals	
3.3	**Administration/ Exposure**		Oral
	3.3.1	Preparation of test site	Not applicable
	3.3.2	Concentration of test substance	1.56, 3.13, 6.25, 12.5 and 25.0 mg/kg-1
	3.3.3	Specific activity of test substance	
	3.3.4	Volume applied	1ml/kg-1 bw
	3.3.5	Sampling time	Sheep that survived dosing were sacrificed at monthly intervals, starting one month after the last death, for analysis of brodifacoum levels in the liver.
	3.3.6	Samples	Liver samples.

4 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

4.1 Result of study

One sheep in the 1.56 mg/lkg-1 group was removed because of unrelated health problems. Eleven of the remaining 39 dosed sheep died after 17-36 days, giving an LD50 of 33 mg/kg-1 with a 95% confidence interval of 5-210 mg/kg-1. The wide confidence limits reflected the low response at 25 mg/kg-1, which was attributed to the observed precipitation of brodifacoum from the 2.5% solution on contact with the saliva. Similar precipitation had been noted previously with 4 and 8% brodifacoum solutions. The LD50 should probably be based on the lower dose rates, because precipitated brodifacoum is less likely to be absorbed. On this basis, the LD50 was 11mg kg-1 with a 95% confidence interval of 4-36mg kg-1. Limited field experience with sheep accidentally poisoned during rabbit poisoning trials supports the lower LD50 estimate. Brodifacoum thus appears to be more toxic to sheep than originally thought. The oral toxicity of brodifacoum is probably partly dependent upon particle size, because brodifacoum is virtually insoluble in water. This dependence of toxicity on particle size has been noted with arsenic trioxide, which is sparingly soluble in water, where the lethal dose for a rabbit may range from 20 mg/kg-1 for a fine powder to 200 mg/kg-1 for a coarse powder.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Solutions of 2.5, 1.25, 0.625, 0.313, and 0.156% (w/v) technical grade brodifacoum were prepared in propane-1,2-diol/polyethylene-glycol/triethanolamine (94/3/3, v/v). Forty mature ewes were allocated at random to one of 5 groups and orally dosed with one of the 5 solutions at a rate of 1ml kg-1 body weight. The sheep were housed indoors on a concrete floor and fed hay and commercial nuts from hoppers. The floor was cleaned daily to minimise re-ringestion of excreted brodifacoumas well as for hygiene purposes. Water was freely

Section A6.13 Toxic effects on livestock and pets
Annex Point IIIA VI.2 *Sheep-Brodifacoum*

		available.
5.2	Results and discussion	One sheep in the 1.56 mg/lkg-1 group was removed because of unrelated health problems. Eleven of the remaining 39 dosed sheep died after 17-36 days, giving an LD50 of 33 mg/kg-1 with a 95% confidence interval of 5-210 mg/kg-1. The wide confidence limits reflected the low response at 25 mg/kg-1, which was attributed to the observed precipitation of brodifacoum from the 2.5% solution on contact with the saliva. Similar precipitation had been noted previously with 4 and 8% brodifacoum solutions. The LD50 should probably be based on the lower dose rates, because precipitated brodifacoum is less likely to be absorbed. On this basis, the LD50 was 11mg kg-1 with a 95% confidence interval of 4-36mg kg-1. Limited field experience with sheep accidentally poisoned during rabbit poisoning trials supports the lower LD50 estimate. Brodifacoum thus appears to be more toxic to sheep than originally thought. The oral toxicity of brodifacoum is probably partly dependent upon particle size, because brodifacoum is virtually insoluble in water. This dependence of toxicity on particle size has been noted with arsenic trioxide, which is sparingly soluble in water, where the lethal dose for a rabbit may range from 20 mg/kg-1 for a fine powder to 200 mg/kg-1 for a coarse powder.
5.3	Conclusion	See above.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2006*
Materials and Methods	Adopt applicant's version
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	The reliability indicator is appropriate
Acceptability	Acceptable
Remarks	None
	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*

Section A6.13 Toxic effects on livestock and pets

Annex Point IIIA VI.2 *Sheep-Brodifacoum*

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

Section A6.14 Annex Point IIIA *III-XI.2*	Other test(s) related to the exposure of humans	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Current exposure estimates do not suggest any routes of exposure, which are not already satisfactorily covered by existing data. No further studies have been conducted for this reason and no studies are planned or scheduled which might be relevant to this area. Information regarding exposure to humans from anticoagulants are already well researched and fully elucidated, for this reason it is deemed to be scientifically unjustified to conduct a study for which the end points have been reasonable determined by previous studies conducted on analogous substances (bromadiolone). Hence, all endpoints have been reasonably assessed by the analogous substances. The technical active ingredient has a high level of purity and there are no other substances that are of concern included as impurities or additives. There are also no other known significant toxic effects. In addition, based on animal welfare grounds other test related to the exposure of humans is considered to be of no value as this additional animal testing would not provide any additional relevant data than is not already available from the analogous substances.	
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	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	

Section A6.14 Annex Point IIIA *III-XI.2*	Other test(s) related to the exposure of humans
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.15.2 **Annex Point *IIIA XI.1.2, 1.3, 1.5, 1.6***	**Food and feedingstuffs - Behaviour of the residues of the active substance, its degradation and reaction products and where relevant, its metabolites on the treated or contaminated food or feedingstuffs including the kinetics of disappearance**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.15.3 Annex Point *IIIA XI..1.4*	**Food and feedingstuffs - Estimation of potential or actual exposure of the active substance to humans through diet and other means**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.15.4 Annex Point *IIIA XI.1.7*	**Food and feedingstuffs - Proposed acceptable residues and the justification of their acceptability**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Brodifacoum will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.15.5 Annex Point *IIIA XI.1.8*	**Food and feedingstuffs - Any other available information that is relevant**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.15.6 Annex Point *IIIA XI.1.9*	**Food and feedingstuffs - Summary and evaluation of data submitted under point 6.15**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Current exposure estimates do not suggest any routes of exposure, which are not already satisfactorily covered by existing data. No further studies have been conducted for this reason and no studies are planned or scheduled which might be relevant to this area. Information regarding exposure to humans from anticoagulants are already well researched and fully elucidated, for this reason it is deemed to be scientifically unjustified to conduct a study for which the end points have been reasonable determined by previous studies conducted on analogous substances (bromadiolone). Hence, all endpoints have been reasonably assessed by the analogous substances. The technical active ingredient has a high level of purity and there are no other substances that are of concern included as impurities or additives. There are also no other known significant toxic effects. In addition, based on animal welfare grounds other test related to the exposure of humans is considered to be of no value as this additional animal testing would not provide any additional relevant data than is not already available from the analogous substances.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	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*	

Section A6.15.6 Annex Point *IIIA XI.1.9*	Food and feedingstuffs - Summary and evaluation of data submitted under point 6.15
Remarks	

Section A6.16 Annex Point *IIIA VI.3.5, XI.2*	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Current exposure estimates do not suggest any routes of exposure, which are not already satisfactorily covered by existing data. No further studies have been conducted for this reason and no studies are planned or scheduled which might be relevant to this area	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*April 2007*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.17 Annex Point *IIIA VI.6*	**If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [X] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Product is not used in products for action against plants **<u>BRODIFACOUM IS A RAT POISON, NOT A WEED KILLER</u>** RATS ARE NOT PLANTS	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	Applicant's justification is considered acceptable	
Conclusion	Adopt applicant's version	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 6.18
Annex Point *IIA 6.18*

Summary of mammalian toxicology and conclusions

Official use only

Summary

1.Anticoagulant rodenticides are rapidly absorbed via the gastro-intestinal tract. Oral absorption is therefore assumed to be 100%. The major route of elimination of anticoagulants after oral administration is via the faeces, primarily as metabolites rather than parent compound. The active substance is mainly distributed in the liver with minor concentrations in the kidney. Elimination processes are very slow with approximately 50% of the administered dose being retained.	X
2.Brodifacoum is highly toxic in acute toxicity studies by the oral and dermal routes of exposure.	
3.A 90-day repeat oral dose study in the rat indicated that males were more sensitive to the active substance than females. The toxicity observed in high dose group males was consistent with the properties of an anti-coagulant. The overall NOAEL for sub-chronic toxicity is 0.04 mg/kg/day.	X
4. The technical concentrate containing 0.25% w/v of the active substance is not a skin or eye irritant in the rabbit.	X
5.The technical concentrate containing 0.25% w/v of the active substance is not a skin sensitiser (1/29 sensitisation rate) in the guinea pig.	X
6.Brodifacoum is not mutagenic or clastogenic in in vitro Ames test, in vitro chromosome aberration study and in vitro mouse lymphoma test.	
7. A carcinogenicity studies were considered unnecessary for this type of compound and use, and are not considered to be technically feasible due to the required action of the active substance on the test species used for the performance of these studies	X
8. There is no evidence of embryotoxic or teratogenic potential in rabbits or rats. The NOAEL for teratogenic effects is 0.004 mg/kg/day.	
9. The active substance does not have an effect on reproduction below the level of toxicity. The NOAEL for reproduction is 3 µg/kg/day (0.003 mg/kg/day). The NOAEL for female in the two-generation study is 1 µg/kg/day (0.001 mg/kg/day).	
10.The Acceptable Operator Exposure Level (AOEL) is therefore the lowest sub-chronic NOAEL, in this case the NOAEL for females (0.001 mg/kg/day) divided by an Assessment Factor of 100 (x 10 for inter-species effects and x 10 for inter-individual effects). The AOEL is therefore 1.0E-05 mg/kg/day. It should be noted that the NOAEL is via the oral route, whereas the main route of exposure will be via the dermal route, therefore the AOEL is overestimated.	
11. An Acceptable Daily Intake (ADI) value is applicable for the active substance use as a biocide as there is potential exposure via food and feedstuffs. The ADI is set in the same manner as the AOEL again using the most sensitive NOAEL value (NOAEL for female 0.001 mg/kg/day) with an Assessment factor of 100. Therefore the ADI is 1.0E-05 mg/kg/day. It should however be noted that exposure is unintentional as the product is not applied to food crops. Baits containing it are kept secure from foodstuffs, usually in bait boxes, and frequently in locations where foods are not present. Dead rats and mice are collected for disposal along with unused bait remains. Therefore the possibilities of the material entering the food chain are minimal.	
12. The acute reference dose (ARfD) is based upon the acute oral toxicity	

Section 6.18 **Summary of mammalian toxicology and conclusions** Official use only
Annex Point *IIA 6.18*

LD50 (100 mg/kg) with a 100-fold assessment factor giving an ARfD of 1 mg/kg.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 24.11.06

Evaluation of applicant's summary

Include revised version of paragraphs (tagged with X)

1. Anticoagulant rodenticides including brodifacoum are rapidly absorbed via the gastro-intestinal tract and oral absorption is therefore assumed to be 100%, on the basis of amount of radioactivity recovered in the excreta and retained in the tissues. The major route of elimination after oral administration is via the faeces, both as polar metabolites and parent compound. The active substance is widely distributed and bioaccumulates in the liver with minor concentrations in the kidney. Elimination processes are very slow with 50-75% of the administered dose being retained in the liver ($t_{1/2}$ for hepatic residues more than 200 days). The metabolism of the a.s. is limited, although in repeated dose studies, evidence of induction of metabolism was reported, with increasing levels of radioactivity associated to polar metabolites recovered in the urine. The toxicologically relevant chemical species is the parent compound.

The a.s. on the basis of results on a structurally related compound is expected to be slowly but substantially absorbed through the skin, due to the lipophilicity of the molecule, allowing passive transport through the membrane

3.A 90-day repeated oral dose study in the rat indicated that clinical signs and toxicity are consistent with the mode of action of the rodenticide and its properties of anti-coagulant agent. The overall NOAEL for subchronic oral toxicity is 0.04 mg/kg/day. No data have been submitted on dermal repeated toxicity, but it can be anticipated on the basis of both physico-chemical and kinetics properties and mode of action of the a.s. that subchronic effect due to prolonged skin contact should not be disregarded and are as a 'worst case' comparable to those seen after oral administration.

4. Both the pure a.s. and the technical concentrate containing 0.25% w/v of the a.s. are not skin or eye irritants in the rabbit.

5.The technical concentrate containing 0.25% w/v of the active substance is not a skin sensitiser (1/29 sensitisation rate) in the guinea pig. However, no conclusion can be reached on the active substance.

7. Chronic/carcinogenicity long term studies were not considered to be technically feasible due to the specific action of the active substance on the test/target species. Repeated toxicity studies with second generation anticoagulants cannot be carried out for more than a few weeks due to the rapid acute effects and the extremely low concentrations at which these effects are seen.

12. The acute reference dose (ARfD) is based upon the NOAEL from the reproduction study (3 µg/kg/d) with a 100-fold assessment factor giving an ARfD of 0.03 µg/kg/d.

Remarks

Section 6.18 Summary of mammalian toxicology and conclusions

Official use only

Annex Point *IIA 6.18*

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

				Official use only
			6 REFERENCE	
6.1	**Reference**		Report: BRODIFACOUM: Determination of abiotic degradation hydrolysis as a function of pH. Study Director – Dr. R. Fabbrini – April 1997. ChemService S.p.A. report CH-15/96-B-BDF	
6.2	**Data protection**		Yes	
	6.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
	6.2.2	Companies with Access to data	PelGar International Ltd. Activa srl	
	6.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
			7 GUIDELINES AND QUALITY ASSURANCE	
7.1	**Guideline study**		OECD 111	
7.2	**GLP**		Yes	
7.3	**Deviations**		No	
			8 MATERIALS AND METHODS	
8.1	**Test material**		As given in section 2	
	8.1.1	Lot/Batch number	03940709	X
	8.1.2	Specification	As given in section 2	
	8.1.3	Purity	99.0% brodifacoum	X
	8.1.4	Further relevant properties	None	
8.2	**Reference substance**		No	
	8.2.1	Initial concentration of reference substance		
8.3	**Test solution**		See table A7_1_1_1_1-1 See table A7_1_1_1_1-2	
8.4	**Testing procedure**			
	8.4.1	Test system	See table A7_1_1_1_1-3	
	8.4.2	Temperature	50°C.	
	8.4.3	Ph	Actual pH not measured. Nominal values only.	
	8.4.4	Duration of	5 days.	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

	the test	
8.4.5	Number of replicates	1
8.4.6	Sampling	Sampling at 0, 2.5 and 5 days
8.4.7	Analytical methods	10 ml samples were taken and analysed. The test item content was determined by the following conditions: Detector UV/V is 254 nm Column Lichrospher 100 RP-18, 5µm 250 x 4.6 mm Column temperature Room temperature Eluent Methanol + water + acetic acid: 94.2 + 5 + 0.8 ml Eluent Flow 0.8 ml/min Retention time for Brodifacoum 8.5 min ca.
8.5	**Preliminary test**	No
	9 RESULTS	
9.1	**Concentration and hydrolysis values**	Compound is of extremely low water solubility (1.2 ppm) and is hydrolytically stable (>1year) at pH7 and 9 at only concentration tested (2 mg/l, limit of solubility).
9.2	**Hydrolysis rate constant (k_h)**	Not stated
9.3	**Dissipation time**	> 2.5 hours
9.4	**Concentration – time data**	No reaction, no degradation seen..
9.5	**Specification of the transformation products**	No degradation and no transformation products produced.
	10 APPLICANT'S SUMMARY AND CONCLUSION	
10.1	**Materials and methods**	OECD 111
10.2	**Results and discussion**	Brodifacoum can be considered to be stable to hydrolysis under conditions tested.
10.2.1	k_H	
10.2.2	DT_{50}	>2.5 hours
10.2.3	r^2	
10.3	**Conclusion**	Brodifacoum is hydrolytically stable under conditions tested
10.3.1	Reliability	1
10.3.2	Deficiencies	No

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*8/11/06*
Materials and Methods	
Results and discussion	
Conclusion	*RMS can conclude that Brodifacoum is hydrolytically stable.*
Reliability	
Acceptability	
Remarks	*8.1.1 and 8.1.3 The Lot/Batch number 03940709 corresponds to difenacoum at a purity of 99.0% the correct lot btach number is 04L950722 brodifacoum at a purity of 995.5%*
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
4	-	30 ml of H_3PO_4 0.1N added to 100 ml of KH_2PO_4 0.1 N (1.36 g of product in 100 ml of water) then diluted to 200 ml with water
7	-	59.00 ml of NaOH 0.1 N added to 100 ml of KH_2PO_4 0.1 N (1.36 g of product in 100 ml of water) then diluted to 200 ml with water
9	-	21 ml of NaOH 0.1 N added to 50 ml of 0.1 M H_3BO_3 (0.63 g in 100 ml) then diluted to 100 ml with water

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Freshly boiled distilled water
Preparation of test medium	A solution of 1 mg/ml of brodifacoum in acetone was prepared (20.0 mg in 20 ml), then diluted 1:10 with acetone.
Test concentrations (mg a.i./L)	150µl of medium corresponding to 15µg of brodifacoum added to each of the 3 pH solutions
Temperature (°C)	50°C
Controls	None
Identity and concentration of co-solvent	
Replicates	Not stated

Table A7_1_1_1_1-3: Description of test system

Glassware	Normal laboratory glassware
Other equipment	Analytical balance, Constant temperature bath with thermometer, technical balance, pH meter, HPLC system.
Method of sterilization	Not stated

Table A7_1_1_1_1-4: **Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9.** ***(one table for each pH value; adjust table size as required)***

Compound pH 4	Sampling times (*days, hours, or other time period*)							
	0	2.5d	5d	t_3	t_4	t_5	t_6	t_n
Parent compound mg/l	0.15	0.15	0.15	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	100	100	-	-	-	-	-

Compound pH 7	Sampling times (*days, hours, or other time period*)							
	0	2.5d	5d	t_3	t_4	t_5	t_6	t_n
Parent compound mg/l	0.13	0.14	0.13	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	108	100	-	-	-	-	-

Compound pH 9	Sampling times (*days, hours, or other time period*)							
	0	2.5d	5d	t_3	t_4	t_5	t_6	t_n
Parent compound	0.15	0.15	0.15	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	100	100	-	-	-	-	-

Table A7_1_1_1_1-5: **Dissipation times of parent compound, transformation products and reference compound at pH 4, pH 7 and pH 9**

	pH 4		pH 7		pH 9	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Parent compound	> 2.5hrs	> 2.5hrs	> 2.5hrs	> 2.5hrs	> 2.5hrs	> 2.5hrs
Transformation product 1	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-

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

CAS-Number	*CAS and/or IUPAC* Chemical Name*(s)*	Amount [%] of parent compound measured at		
		pH 4	pH 7	pH 9
	None – compound was stable	0	0	0

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products

Determination of the direct photolysis rate in water by sunlight

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11 REFERENCE

11.1	**Reference**	Drake R.M (2004) Determination of the direct photolysis rate in water by sunlight of Brodifacoum. Chemex Environmental Internation Ltd. Reference ENV6768/120140
11.2	**Data protection**	Yes
11.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
11.2.2	Companies with letter of access	PelGar International Ltd. Activa srl
11.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

12 GUIDELINES AND QUALITY ASSURANCE

12.1	**Guideline study**	Yes OPPTS 835 2210
12.2	**GLP**	Yes
12.3	**Deviations**	Yes

13 MATERIALS AND METHODS

13.1	**Test material**	Brodifacoum
13.1.1	Lot/Batch number	7909101
13.1.2	Specification	As given in section 2
13.1.3	Purity	100%
13.1.4	Radiolabelling	N/A
13.1.5	UV/VIS absorption spectra and absorbance value	Brodifacoum showed three absorbance maxima in the region 190 to 340nm only one of which was above 290nm. No absorbance was detected (above the base line) for wavelengths above 340 nm
13.1.6	Further relevant properties	N/A
13.2	**Reference substances**	Methanol was used as a reference substance.
13.3	**Test solution**	Brodifacoum was prepared as a 155 mg/l dosing solution in acetonitrile. 1ml of the dosing solution was added to a 100ml volumetric flask and made to volume with 0.2µm filtered deionised water (1.55mg/l – 0.00000296M).
13.4	**Testing procedure**	

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

13.4.1	Test system	Ten tubes (2 off pyrex and 8 off quartz) were filled with the above solution. The pyrex tubes were placed in boiling tubes and covered in aluminium foil which formed a light proof jacket (control). The remaining tubes were placed in sunlight inclined at angle of about 30° with the tops facing magnetic north. The test was set up at 12.00 on 31 March 2004. The test site is located at a latitude of 52° north. Two samples were taken from the tubes every hour for 6 hours. The samples were analysed using the HPLC conditions below. All samples were injected in triplicate.
13.4.2	Properties of light source	N/A
13.4.3	Determination of irradiance	Brodifacoum was prepared in the same way as for Tier 2 phase 1 of this test. A stock of PNAP was prepared by making 0.165g to 100 ml in acetonitrile (0.01M). An intermediate stock was prepared by diluting 10ml of this stock to 100ml with distilled water (0.001M). 17.40g of pyridine was weighed into a 100ml volumetric flask and was partially filled with 0.2µm filtered deionised water. 1ml of the intermediate PNAP stock was added and the flask made to volume with further deionised water.
13.4.4	Temperature	N/A
13.4.5	pH	N/A
13.4.6	Duration of the test	Exposure period was 6 hours for the tier 1 test and 5 hours for the tier 2 test.
13.4.7	Number of replicates	Each sample was tested 3 times.
13.4.8	Sampling	N/A
13.4.9	Analytical methods	The samples were analysed using HPLC which were all run in triplicate. The conditions were as follows: Chromotography System: Perkin Elmer Quaternary System Mobile phase: Methanol: distilled water: Aecetic acid Flow rate: 1.5ml/min Injection volume 250µl
13.5	**Transformation products**	No
13.5.1	Method of analysis for transformation	N/A

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

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

products

14 RESULTS

14.1 Screening test The maximum absorbance between 290 and 800 nm was at 290nm.

14.2 Actinometer data N/A

14.3 Controls Control loss for Brodifacoum was not considered to be significant.

14.4 Photolysis data

14.4.1 Concentration values

Fraction of day	Molar concentration Run 1	Run 2
0.000	0.00916	0.00945
0.078	0.00567	0.00582
0.155	0.00238	0.00230
0.233	0.00092	0.00088
0.310	-	-
0.388	-	-
0.465	-	-

14.4.2 Mass balance N/A

14.4.3 k^c_p 10.30 day $^{-1}$ (3 hours exposure)

14.4.4 Kinetic order N/A

14.4.5 k^c_p / k^a_p 0.481 (first 60 minutes)

1.232 (60 to 180 minutes)

14.4.6 Reaction quantum yield (ϕ^c_E) 1.28×10^{-3} (first 60 minutes)

3.29×10^{-3} minutes (60 to 180 minutes)

14.4.7 k_{pE} 4.68 day^{-1}.

14.4.8 Half-life ($t_{1/2E}$) Half life in minutes:

$\phi^c_E = 1.28 \times 10^{-3}$

Summer = 60

Winter = 366

Spring = 78

$\phi^c_E = 3.29 \times 10^{-3}$

Summer = 23

Winter = 143

Spring = 30

14.5 Specification of the transformation N/A

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

products		
		15 APPLICANT'S SUMMARY AND CONCLUSION
15.1	**Materials and methods**	Test guidelines follwed were OPPTS 835 2210
15.2	**Results and discussion**	
15.2.1	k^c_p	10.30 day^{-1} (3 hours exposure)
15.2.2	K_{pE}	4.68 day^{-1}
15.2.3	ϕ^c_E	1.28 x 10^{-3} (first 60 minutes)
		3.29 x 10^{-3} minutes (60 to 180 minutes)
15.2.4	$t_{1/2E}$	Half life in minutes:
		ϕ^c_E = 1.28 x 10^{-3}
		Summer = 60
		Winter = 366
		Spring = 78
		ϕ^c_E = 3.29 x 10^{-3}
		Summer = 23
		Winter = 143
		Spring = 30
15.3	**Conclusion**	Photolysis of Brodifacoum was fast with 38 % removal in the first hour of exposure. Greater than 89 % photolysis was noted to have occurred by around three hours. Futhermore, whatever the season the half life of Brodifacoum is less than a day. In the laboratory the substance completes photolyses.
15.3.1	Reliability	1
15.3.2	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	*08/11/06*
Materials and Methods	
Results and discussion	
Conclusion	*Agreed with notifier conclusion*
Reliability	*1*

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

Acceptability	*acceptable*
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	

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

Ready biodegradability

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16 REFERENCE

16.1	**Reference**	Report: Determination of the ready biodegradability of BRODIFACOUM TECHNICAL. Study Director – R.M.Drake – June 2003. Chemex Environmental International Ltd report ENV5807/120140
16.2	**Data protection**	Yes
16.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
16.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
16.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

17 GUIDELINES AND QUALITY ASSURANCE

17.1	**Guideline study**	OECD 301B
17.2	**GLP**	Yes
17.3	**Deviations**	No

18 MATERIALS AND METHODS

18.1	**Test material**	As given in section 2
18.1.1	Lot/Batch number	7909101
18.1.2	Specification	As given in section 2
18.1.3	Purity	100 % brodifacoum
18.1.4	Further relevant properties	Not applicable
18.1.5	Composition of Product	Not applicable
18.1.6	TS inhibitory to microorganisms	Yes/No *(If yes, specify, e.g. results of respiration inhibition test)*
18.1.7	Specific chemical analysis	Not specified
18.2	**Reference substance**	Yes – sodium acetate
18.2.1	Initial concentration of reference substance	Not stated

Section A7.1.1.2.1 Biodegradability (ready)
Annex Point IIA7.6.1.1 Ready biodegradability

18.3 Testing procedure

18.3.1	Inoculum / test species	See table A7_1_1_2-2
18.3.2	Test system	See table A7_1_1_2-3
18.3.3	Test conditions	See table A7_1_1_2-4
18.3.4	Method of preparation of test solution	No specific preparation
18.3.5	Initial TS concentration	21.1 mg in 1.5l of mineral medium
18.3.6	Duration of test	29 days
18.3.7	Analytical parameter	Carbon dioxide (determined as dissolved inorganic carbon)evolved within 28 days.
18.3.8	Sampling	Sampling on days 0, 3, 5, 8, 11, 14, 18, 21, 24, 28 and 29
18.3.9	Intermediates/ degradation products	Not identified
18.3.10	Nitrate/nitrite measurement	No
18.3.11	Controls	Reference: sodium acetate 102.4 mg in 1.5l mineral medium Toxicity :sodium acetate 102.4 mg in 1.5l mineral medium and test material at 15.2 mg.
18.3.12	Statistics	Calculations according to OECD Guideline 301 B

X

19 RESULTS

19.1 Degradation of test substance

19.1.1 Table

Table of % degradation v time

Days	Reference	Test	Toxicity Control
3	37	-3	34
5	50	-4	45
8	60	-5	53
11	65	-5	57
14	69	-6	60
18	73	-6	62
21	75	-7	63
24	77	-8	64
28	79	-9	63
29	81	-10	40
29	91	-5	42

Section A7.1.1.2.1 Biodegradability (ready)
Annex Point IIA7.6.1.1

Ready biodegradability

19.1.2	Degradation	No plateau observed At the end of incubation -9 % degradation at 15.2 mg per 1.5l
19.1.3	Other observations	
19.1.4	Degradation of TS in abiotic control	No abiotic control with TS
19.1.5	Degradation of reference substance	79% degradation after 28 days and 60% degradation after 8 days
19.1.6	Intermediates/ degradation products	No intermediate or degradation product identified

20 APPLICANT'S SUMMARY AND CONCLUSION

20.1	**Materials and methods**	OECD 301 B
20.2	**Results and discussion**	The test substance failed to meet the requirements for a pass in this test (>60% degradation relative to the $ThCO_2$ value) with a maximum of – 3% recorded on day 3. However, because of the stringency of the test, this does not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates that more work would be necessary to establish biodegradability.
20.3	**Conclusion**	The test substance is not considered to be significantly inhibitory (degradation of toxicity control was greater than 25% by day 14)
20.3.1	Reliability	1
20.3.2	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	*08/11/06*
Materials and Methods	
Results and discussion	
Conclusion	*Brodifacoum is not ready biodegradable.*
Reliability	*1*
Acceptability	*acceptable*
Remarks	*Initial TS concentration 14.1 mg/l*

COMMENTS FROM ...

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

Ready biodegradability

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

Table A7_1_1_2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

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

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

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not stated
Strain	Not stated
Source	Cambridge Sewage Treatment Works
Sampling site	Cambridge Sewage Treatment Works
Laboratory culture	No - activated sludge plant for domestic sewage
Method of cultivation	Uncultivated
Preparation of inoculum for exposure	Sieved (500µm) settled and decanted. Centrifuged @ 4000 rpm for 5 minutes, decanted and resuspended in mineral media. This was repeated and sludge was centrifuged and decanted.
Pretreatment	
Initial cell concentration	30 mg/l

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	
Number of culture flasks/concentration	Reference =1, Test Substance = 2
Aeration device	Carbon Dioxide free air at a controlled rate in the dark at 22 ± 2°C
Measuring equipment	UV-Persulfate Analyser
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Not stated
Additional substrate	No
Test temperature	22 ± 2°C
pH	Not measured
Aeration of dilution water	Inoculated mineral medium aerated by passage of carbon dioxide free air at controlled rate. Rate not specified.
Suspended solids concentration	6.0%
Other relevant criteria	-

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

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%		
Percentage of removal of reference substance reaches pass level by day 14		

1.1.1.1 Criteria for poorly soluble test substances	**1.1.1.2**	**1.1.1.3**
1.1.1.4	1.1.1.5	1.1.1.6
1.1.1.7	1.1.1.8	1.1.1.9

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

	fulfilled	not fulfilled
Pass levels		
20% removal (DOC or COD);		
Pass values reached within 10-d window (within 28-d test period)	No	
Removal of reference substance (DOC or COD) > 70 % within 14 d	No (69%)	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)	No (69%)	
Percentage of DOC-removal of reference compound ≥ 40 % within 7 days and ≥ 65 % within 14 days Average residual amount of test compound in blank tests ≥ 40 % (OECD 302 C)	Not applicable	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)		

Criteria for poorly soluble test substances	**1.1.1.10**	**1.1.1.11**
	1.1.1.12	1.1.1.13
	1.1.1.14	1.1.1.15

Section A7.1.1.2.2 Annex Point IIA7.6.1.1 — Inherent Biodegradability

Determination of the inherent biodegradability of Brodifacoum

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		21 REFERENCE
21.1	**Reference**	Drake RM, 2005, Determination of the inherent biodegradability of Brodifacoum, Chemex Environmental International Limited, Chemex Ref: ENV7146/120140.
1.2	**Data protection**	Yes
1.2.1	Data owner	Bromadiolone Task Force PelGar International Limited Babolna Bioenvironmental Centre Limited Activa s.r.l Laboratories Agrochem S.L
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes- Degradation of test compound according to OECD Guidelines for Testing of Chemicals Proposal for a New Guideline 302D. Inherent biodegradability CONCAWE test.
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	7909101
3.1.2	Specification	As given in section 2
3.1.3	Purity	100% (w/w)
3.1.4	Further relevant properties	
3.1.5	Composition of Product	n/a
3.1.6	TS inhibitory to microorganisms	No
3.1.7	Specific chemical analysis	
3.2	**Reference substance**	Yes- Hexadecane
3.2.1	Initial concentration of reference	

Section A7.1.1.2.2 Annex Point IIA7.6.1.1 — Inherent Biodegradability

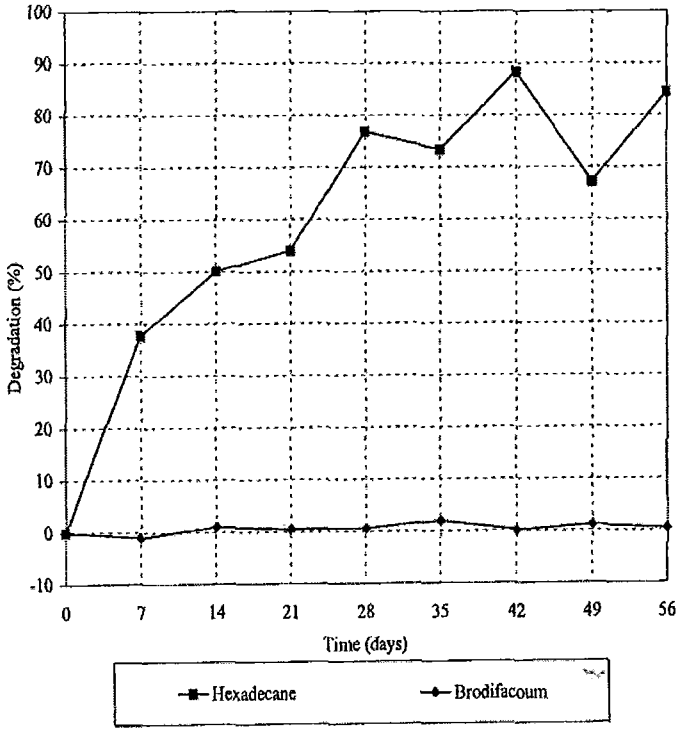
Determination of the inherent biodegradability of Brodifacoum

	substance	
3.3	**Testing procedure**	*Non-entry field*
3.3.1	Inoculum / test species	A composite microbial inoculum derived from soil and a wastewater treatment plant that has been pre-exposed to the test substance.
3.3.2	Test system	see table A7_1_1_2-3
3.3.3	Test conditions	see table A7_1_1_2-4
3.3.4	Method of preparation of test solution	*Describe if appropriate, e.g. in case of poorly soluble test substance*
3.3.5	Initial TS concentration	Quantity of Brodificoum (day 0)- 5.6 mg/l Quantity of Brodificoum (day 7)- 11.7 mg/l Quantity of Brodificoum (day 11)- 11.5 mg/l
3.3.6	Duration of test	56 days
3.3.7	Analytical parameter	CO_2 evolution
3.3.8	Sampling	7 days
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Yes- inoculum medium only
3.3.12	Statistics	None performed
		4 RESULTS
4.1	**Degradation of test substance**	*Non-entry field*

Section A7.1.1.2.2 Annex Point IIA7.6.1.1 — Inherent Biodegradability

Determination of the inherent biodegradability of Brodifacoum

4.1.1 Graph



4.1.2	Degradation	Brodificoum failed to meet the requirements for a pass in this test ((2% degradation relative to the ThIC value) with a maximum of 2% recorded on day 35).	X
4.1.3	Other observations	None	
4.1.4	Degradation of TS in abiotic control		
4.1.5	Degradation of reference substance	77% degradation after 28 days.	
4.1.6	Intermediates/ degradation products	n/a	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Degradation of test compound according to OECD Guidelines for Testing of Chemicals Proposal for a New Guideline 302D. Inherent biodegrability CONCAWE Test.	
5.2	**Results and discussion**	Brodificoum failed to meet the requirements for a pass in this test (2% degradation relative to the ThIC value) with a maximum of 2% recorded on day 35).	X
5.3	**Conclusion**	The test is considered valid if:	

Section A7.1.1.2.2 Annex Point IIA7.6.1.1 — Inherent Biodegradability

Determination of the inherent biodegradability of Brodifacoum

		The mean percentage biodegradation of hexadecane reaches 60% by the end of the test. A value of 84% was recorded. The mean amount of IC produced from the blanks at the end of the test is 15% of the organic carbon added initially as the test substance (15% of 20mg/lC-3mg). A value of 1.9mg/l C was recorded. Both the validity criteria were met for the this met for this test.
5.3.1	Reliability	2
5.3.2	Deficiencies	The data for the IC content of the blank runs are not included in the report, so validity criteria can not be confirmed.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*08/11/06*
Materials and Methods	
Results and discussion	
Conclusion	*Brodifacoum is not inherently biodegradable.*
Reliability	*2*
Acceptability	*acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_2-1: Guidline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

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

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

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	*e.g. activated sludge*
Species	
Strain	
Source	*e.g. sewage treatment plant treating predominantly domestic sewage*
Sampling site	
Laboratory culture	Yes/No *(If no, specify)*
Method of cultivation	
Preparation of inoculum for exposure	*give details, e.g. on washing, centrifugation*
Pretreatment	*e.g. adaptation*
Initial cell concentration	*include data as mg suspended solids/l, mg effluent/l or approx. number of cells/l depending on test method*

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	*e.g. respirometer*
Number of culture flasks/concentration	
Aeration device	
Measuring equipment	
Test performed in closed vessels due to significant volatility of TS	Yes/No *(If yes, specify)*

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	*Give details e.g. on added mineral medium*
Additional substrate	Yes/No *(If yes, specify: e.g. peptone)*
Test temperature	*Give measurements conducted during test*
pH	*Give measurements conducted at start and end of test*
Aeration of dilution water	Yes/No *(If yes, specify: e.g. air-flow)*
Suspended solids concentration	
Other relevant citeria	*e.g. stirring of test solution*

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

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%		
Percentage of removal of reference substance reaches pass level by day 14		

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

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

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

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

Section A7.1.1.2.3 Annex Point *IIIA XII.2.1*	**Biodegradation in seawater**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Compound is of low water solubility and shown to be negligible biodegradability in ready biodegradation study. Normal rodenticide use practice is to remove product residues in order to minimise possibility of ingestion by non-target organisms. Product is not applied to seawater since rats can swim and do not feed in the sea	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification	*Product is not applied to seawater*	
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		

Section A7.1.2.1.1 Annex Point *IIIA XI.2.1*	**Aerobic biodegradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Compound is of low water solubility and shown to be negligible biodegradability in ready biodegradation study. Normal rodenticide use practice is to remove product residues in order to minimise possibility of ingestion by non-target organisms. As a study into the determination of abiotic degradation and hydrolysis as a function of pH has been conducted (Section A7.1.1.1.1, Annex Point IIA VII.7.6.2.1), an aerobic biodegradadtion study is not required.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier 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		

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

Official use only

		22 REFERENCE
22.1	**Reference**	Drake, R.M (2005) Determination of the anaerobic biodegradability of Brodifacoum. Chemex Environmental Ltd. Ref:ENV7145/120140
22.2	**Data protection**	Yes
22.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
22.2.2		PelGar International Ltd. Activa srl
22.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		23 GUIDELINES AND QUALITY ASSURANCE
23.1	**Guideline study**	The method followed was method 3 of ECETOC Report 28 and ISO DIS 11734
23.2	**GLP**	Yes
23.3	**Deviations**	None
		24 METHOD
24.1	**Test material**	As given in section 2
24.1.1	Lot/Batch number	7909101
24.1.2	Specification	As given in section 2
24.1.3	Purity	100%
24.1.4	Further relevant properties	Must be kept at room temperature and in the dark.
24.1.5	Composition of Product	N/A
24.1.6	TS inhibitory to microorganisms	No
24.1.7	Specific chemical analysis	N/A
24.2	**Reference substance**	Sodium benzoate
24.2.1	Initial concentration of reference substance	24.035 g/l
24.3	**Testing procedure**	

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

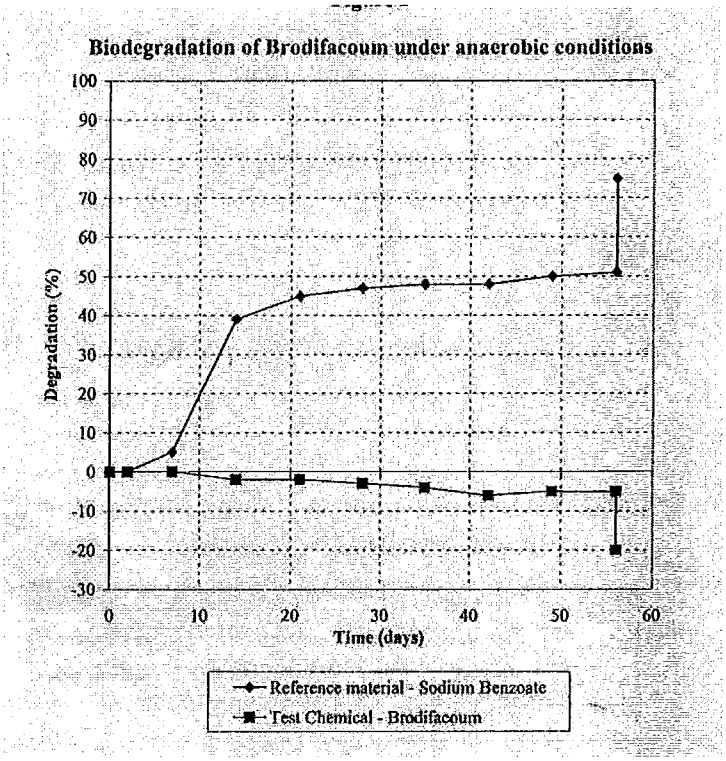
24.3.1	Inoculum / test species	(see table A7_1_2_1_2-1)
24.3.2	Test system	(see table A7_1_2_1_2-2)
24.3.3	Test conditions	(see table A7_1_2_1_2-3)
24.3.4	Method of preparation of test solution	N/A
24.3.5	Initial TS concentration	9.0, 9.3, 9.5 mg
24.3.6	Duration of test	56 days
24.3.7	Analytical parameter	CH_4 and CO_2 evolution
24.3.8	Sampling	2, 7, 14, 21, 28, 35, 42, 49 and 56 days
24.3.9	Intermediates/ degradation products	Not identified
24.3.10	Controls	6 blanks were set up which had no test substance added to it but contained the same amount of wet sludge.
24.3.11	Statistics	N/A

25 RESULTS

25.1 Degradation of test substance

25.1.1	Degradation of TS in abiotic control	N/A
25.1.2	Degradation	Less than 60% biodegradation based on biogas production with a maximum value of 0%.
25.1.3	Graph	See graph for degradation of reference substance
25.1.4	Other observations	The final degradation value recorded (-20% at day 56 suggest that Brodifacoum was inhibitory to the micro-organism population.
25.1.5	Degradation of reference substance	

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1



25.1.6	Intermediates/ degradation products	N/A

26 APPLICANT'S SUMMARY AND CONCLUSION

26.1	**Materials and methods**	The method followed was method 3 of ECETOC Report 28 and ISO DIS 11734
26.2	**Results and discussion**	Brodifacoum gave a negative result (less than 60% biodegradation based on biogas production) with a maximum value of 0%. The final degradation value recorded (-20% at day 56) suggests that Brodifacoum was inhibitory to the micro-organism population.
26.3	**Conclusion**	A reference material, sodium benzoate , was concurrently tested (although this was not a requirement of the test) and showed biodegradation of 75% (by day 56) suggesting that the inoculum was viable.
26.3.1	Reliability	1
26.3.2	Deficiencies	No

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*08/11/06*
Materials and Methods	
Results and discussion	
Conclusion	*Brodifacoum was not degradated in anaerobic condition*
Reliability	*1*
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*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_2_1_2-1: Inoculum / Test organism

Criteria	Details
Nature	Primary anaerobic digestor sludge
Species	N/A
Strain	N/A
Source	Cambridge Sewage Treatment Works
Sampling site	N/A
Laboratory culture	N/A
Method of cultivation	N/A
Preparation of inoculum for exposure	Sludge was passed through a sieve and centrifuged. Then sludge was placed in a water bath at 35°C and a stream of nitrogen bubbled through it and left for two weeks. On the day of the test the sludge was centifuged.
Pretreatment	N/A
Initial cell concentration	Dry sludge solids in test: 5.0g/litre.

Table A7_1_2_1_2-2: Test system

Criteria	Details
Culturing apparatus	
Number of replicates/concentration	3 repeats with test substance and reference material, and 6 repeats for the blank.
Measuring equipment	pressure transducer
Oxidation reduction indicator	No

Table A7_1_2_1_2-3: Test conditions

Criteria	Details
Composition of medium	mineral medium
Additional substrate	No
Solvent	No
Preparation of medium	N/A
Test temperature	Constantly at 35.0°C
pH	between 6.5-6.7
Suspended solids concentration	5.0g/l
Other relevant citeria	None

Section A7.1.2.2.1 Annex Point *IIIA XII.2.1*	**Aerobic aquatic degradation study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Compound is of low water solubility and shown to be negligible biodegradability in ready biodegradation study. Normal rodenticide use practice is to remove product residues in order to minimise possibility of ingestion by non-target organisms. As the active substance is considered to be of low significance for the aquatic environment due to very low water solubility and limited, localised and intermittent uses, an aerobic aquatic degradation study is believed to be unnecessary. The product is also a solid bait which is not applied by spraying or other dispersion over a large area. Furthermore, the product used primarily in indoor or closed environments, and outdoors is subject to rapid photolysis.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.2.2.2 Annex Point *IIIA XII.2.1*	Water/sediment degradation study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. The risk assessment (doc IIB/C) has produced a worse-case sediment PEC/PNEC of 0.2 for release to surface water from an STP during treatment of rat infested city (ESD, 2003) and a worse-case sediment PEC/PNEC of 0.97 for treatment of rat infested city but with bypass of STP, equivalent to direct release of influent to surface water rather than to STP. The PNEC was derived from the equilibrium partition method (TGD, part 2). The PECs were derived from the EUSES 2.03 model, and were based on using a local emission to waste water input value of 8.57E-05kg a.i. /day, derived from the worse case scenario of a campaign to eradicate rats in a heavily infested city, using 30kg of bait blocks over 21 days (as per ESD, 2003). Please see doc IIB/C. On the basis of a sediment PEC/PNEC <1 for both possible scenarios (worse case release to sewers with use of STP and worse case release to sewers with by-pass of STP), a derogation to perform a water/sediment study is requested.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification	*The risk assessment has produced a worse-case sediment PEC/PNEC of 0.2 for release to surface water from an STP during treatment of rat infested city (ESD, 2003) and a worse-case sediment PEC/PNEC of 2.27 for treatment of rat infested city but with bypass of STP, equivalent to direct release of influent to surface water rather than to STP. (see doc IIC 2.1.1)* *The PNEC was derived from the equilibrium partition method (TGD, part 2). The PECs were derived from the EUSES 2.03 model, and were based on using a local emission to waste water input value of 1.93E-04kg a.i. /day, derived from the worse case scenario of a campaign to eradicate rats in a heavily infested city, using 30kg of bait blocks over 21 days (as per ESD, 2003). Please see doc IIB/C.*	

Section A7.1.2.2.2 Annex Point *IIIA XII.2.1*	**Water/sediment degradation study**
Conclusion	*PEC/PNEC >1 for the worse case release to sewers with by-pass of STP*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.3 (1) Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

Official use only

27 REFERENCE

27.1	**Reference**	Drake, R,M (2005) The Estimation of the Adsorption Coefficient (K_{oc}) of Brodifacoum. Chemex International. Ref: ENV7008/120140
27.2	**Data protection**	Yes
27.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
27.2.2	Companies with access to data	PelGar International Ltd. Activa srl
27.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

28 GUIDELINES AND QUALITY ASSURANCE

28.1	**Guideline study**	OECD 121
28.2	**GLP**	Yes
28.3	**Deviations**	

29 MATERIALS AND METHODS

29.1	**Test material**	As given in section 2
29.1.1	Lot/Batch number	7909101
29.1.2	Specification	As given in section 2
29.1.3	Purity	99% (w/w) minimum
29.1.4	Further relevant properties	Must be kept at room temperature in the dark.
29.1.5	Method of analysis	n/a
29.2	**Degradation products**	n/a
29.2.1	Method of analysis for degradation products	n/a
29.3	**Reference substance**	Phenol, Atrazine, Napthalene, 1,2,3-Trichlorobenzene, Phenanthrene, DDT
29.3.1	Method of analysis for reference substance	HPLC was used to give the log K value of the reference substances. A literature value for the log K_{OC} was also used.
29.4	**Soil types**	n/a

Section A7.1.3 (1) Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

29.5 Testing procedure

29.5.1	Test system	HPLC
29.5.2	Test solution and Test conditions	The test material was disperesed in mobile phase (0.002g in 10ml) which was diluted 1 in 5 and this was injected. The quantities injected were 0.8µg.

29.6 Test performance *Non-entry field*

29.6.1	Preliminary test	n/a
29.6.2	Screening test: Adsorption	n/a
29.6.3	Screening test: Desorption	n/a
29.6.4	HPLC-method	Esitmation of the adsorption coefficient (Koc) on soil and sewage sludge using High Performance Liquid Chromatography (HPLC). This experimental method uses HPLC for the estimation of the adsorption coefficient Koc in soil and sewage sludge.
29.6.5	Other test	n/a

30 RESULTS

30.1	**Preliminary test**	n/a
30.2	**Screening test: Adsorption**	Log K_{oc} was between 2.71 and 2.73
30.3	**Screening test: Desorption**	n/a
30.4	**Calculations**	
30.4.1	Ka , Kd	See above
30.4.2	Ka_{oc} , Kd_{oc}	See above
30.5	**Degradation product(s)**	n/a`

31 APPLICANT'S SUMMARY AND CONCLUSION

31.1 Materials and methods

High Performance Liquid Chromatography (HPLC) was used to calculate the Koc of Brodifacoum.

Sample Preparation

The test material was dispersed in mobile phase (0.002g in 10ml) which was diluted 1 in 5 and this was injected. The quantities injected were 0.8µg.

Conditions

Mobile phase: 55:45 methanol:citrate buffer (pH 7.0)

Flow rate: 1ml/min

Section A7.1.3 (1) Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

		Column heater: Not used
		Injection volume: 20µl (standard) and 20µl (sample and blank)
31.2	**Results and discussion**	One peak was detected (in duplicate) with UV detection with log K_{oc} values of 2.73 and 2.71 respectively.
31.2.1	Adsorbed a.s. [%]	n/a
31.2.2	K_a	See above
31.2.3	K_d	See above
31.2.4	Ka_{oc}	
31.2.5	Ka/Kd	
31.2.6	Degradation products (% of a.s.)	
31.3	**Conclusion**	Validity criteria satisfied
31.3.1	Reliability	1
31.3.2	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	*08/11/06*
Materials and Methods	
Results and discussion	
Conclusion	*The HPLC method gives A Koc value much lower than literature values (Koc of 50000 The pesticide manual 13th edition)*
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*

Section A7.1.3 (1) Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

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

Section A7.1.3 (2) Annex Point IIA, VII7.7	**Adsorption/Desorption screening test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	It is believed that the result from the HPLC method gives a Koc which is much lower than it should be. This is based on other values in the literature. Specifically, in the Pesticide Manual (Tomlin, 13th edition) it gives an average Koc of 50000, indicative of non-mobile in soil. Other supportive data is found in the EHC 175 (WHO,1995) where it states that the results of a study by Jackson and Hall (1992) showed that brodifacoum was effectively immobile in four soil types. This conclusion was based on no detectable levels of 14C residues in the leachates. In fact most of the radioactivity applied to the soil was recovered in the top segment. Furthermore, an experimental logP is expected at SafePharm by Sept/Oct 2006 which will be used in the well known equation logKoc = 0.5log P +(0.41 to 0.98, for various soils) (Briggs, G.G. (1981). Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors and the parachor. *J. Agric. Food Chem.*, **29**.1050-1059.) It is expected to provide further supportive evidence that the Koc will indicate that Brodifacoum is non-mobile in soil. If a value of 8.5 (logP in pesticide manual, Tomlin) is used then the following is obtained: Log Koc = 0.5*8.5 + 0.41 = 4.66. Therefore Koc = 45709 Log Koc = 0.5*8.5 +0.98 = 5.23. Therefore Koc = 169824 Under the SSLRC mobility classification, a Koc of >4000 is classed as non-mobile. Both of the estimations, albeit using literature logP, are so far above 4000 that it is very likely that Brodifacoum is non-mobile in various types of soil.	

Section A7.1.3 (2) Annex Point IIA, VII7.7	Adsorption/Desorption screening test
	Also the bait will be used in very localised areas in and around buildings and in sewers. Direct contact to soil will be limited due to use of bait stations. Based on the above weight-of-evidence it is believed that the risk of brodifacoum reaching groundwater is very low. Also, in the Emission Scenario Document (2003, PT14) it states on page 25 for the scenario for ' in and around buildings' that " a detailed groundwater scenario is not considered necessary due to the limited quantities of active substance , the limited frequency and the limited contamination area". On the basis of all the above arguments, a derogation to perform an adsoption/desorption study (OECD 106) is requested.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*
Evaluation of applicant's justification	
Conclusion	*Brodifacoum is immobile in soil (WHO 1995; Koc = 50000 The Pesticide Manual 13th ed.).*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.4.1 Annex Point *IIIA XII.2.1*	**Field study on accumulation in the sediment**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [X]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.1 Annex Point *IIIA VII.4, XII.1.1*	Aerobic degradation in soil, initial study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Also, in the Emission Scenario Document (2003, PT14) it states on page 25 for the scenario for ' in and around buildings' that " a detailed groundwater scenario is not considered necessary due to the limited quantities of active substance, the limited frequency and the limited contamination area". In addition to this, supportive data in the literature (EHC 175 , WHO 1995) showed that a study by Hall and Priestley (1992) indicated that the half-life was 157 days with 35.8% of applied radioactivity recovered as $^{14}CO_2$ within the test period of 52 weeks. It also stated that Brodifacoum was the major component in the soil extracts throughout the 52 week study. It is accepted that given its fairly long half life, Brodifacoum has a persistant nature in soil, although it does appear to eventually degrade to some degree with harmless CO2 being produced. Also it is vulnerable to rapid photolysis when on the surface of the soil. On the basis of the above a derogation to perform an aerobic degradation in soil is requested.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Brodifacoum half life in soil is 157 days (The Pesticide Manual 13th ed)*	
Remarks		

Section A7.2.1 Annex Point *IIIA VII.4, XII.1.1*	Aerobic degradation in soil, initial study
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.2.1 Annex Point *IIIA VII.4, XII.1.1, XII.1.4*	**The rate and route of degradation including identification of the processes involved and identification of any metabolites and degradation products in at least three soil types under appropriate conditions**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.2.2 Annex Point *IIIA XII.1.1*	Field soil dissipation and accumulation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [x]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.2.3 Annex Point *IIIA XII.1.4*	Extent and nature of bound residues	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [x]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.2.4 Annex Point *IIIA XII.1.1*	**Other soil degradation studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [x]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*	
Evaluation of applicant's justification		
Conclusion	*Other soil degradation studies are not necessary*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.3.1 Annex Point *IIIA XII.1.2*	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant, adsorption and desorption of metabolites and degradation products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [x] Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	It is believed that the result from the HPLC method gives a Koc which is much lower than it should be. This is based on other values in the literature. Specifically, in the Pesticide Manual (Tomlin, 13th edition) it gives an average Koc of 50000, indicative of non-mobile in soil. Other supportive data is found in the EHC 175 (WHO,1995) where it states that the results of a study by Jackson and Hall (1992) showed that brodifacoum was effectively immobile in four soil types. This conclusion was based on no detectable levels of 14C residues in the leachates. In fact most of the radioactivity applied to the soil was recovered in the top segment. Furthermore, an experimental logP is expected at SafePharm by Sept/Oct 2006 which will be used in the well known equation logKoc = 0.5log P +(0.41 to 0.98, for various soils) (Briggs, G.G. (1981). Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors and the parachor. *J. Agric. Food Chem.*, **29**.1050-1059.) It is expected to provide further supportive evidence that the Koc will indicate that Brodifacoum is non-mobile in soil. If a value of 8.5 (logP in pesticide manual, Tomlin) is used then the following is obtained: Log Koc = 0.5*8.5 + 0.41 = 4.66. Therefore Koc = 45709 Log Koc = 0.5*8.5 +0.98 = 5.23. Therefore Koc = 169824 Under the SSLRC mobility classification, a Koc of >4000 is classed as non-mobile. Both of the estimations, albeit using literature logP, are so far above 4000 that it is very likely that Brodifacoum is non-mobile in various types of soil. Also the bait will be used in very localised areas in and around buildings and in sewers. Direct contact to soil will be limited due to use of bait stations. Based on the above weight-of-evidence it is believed that the risk of brodifacoum reaching groundwater is very low. Also, in the Emission Scenario Document (2003, PT14) it states on page 25 for the scenario for ' in and around buildings' that " a detailed groundwater scenario is not considered necessary due to the limited quantities of active substance , the limited frequency and the limited contamination area". On the basis of all the above arguments, a derogation to perform an adsoption/desorption study (OECD 106) is requested.	

Section A7.2.3.1 **Annex Point *IIIA XII.1.2***	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant, adsorption and desorption of metabolites and degradation products**
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*08/11/06*
Evaluation of applicant's justification	
Conclusion	*On the basis of literature data brodifacoum can be considered immobile in soil.*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.3.2 Annex Point *IIIA XII.1.3*	**Mobility in at least three soil types and where relevant mobility of metabolites and degradation products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [x] Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	It is believed that the result from the HPLC method gives a Koc which is much lower than it should be. This is based on other values in the literature. Specifically, in the Pesticide Manual (Tomlin, 13th edition) it gives an average Koc of 50000, indicative of non-mobile in soil. Other supportive data is found in the EHC 175 (WHO,1995) where it states that the results of a study by Jackson and Hall (1992) showed that brodifacoum was effectively immobile in four soil types. This conclusion was based on no detectable levels of 14C residues in the leachates. In fact most of the radioactivity applied to the soil was recovered in the top segment. Furthermore, an experimental logP is expected at SafePharm by Sept/Oct 2006 which will be used in the well known equation logKoc = 0.5log P +(0.41 to 0.98, for various soils) (Briggs, G.G. (1981). Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors and the parachor. *J. Agric. Food Chem.*, **29**.1050-1059.) It is expected to provide further supportive evidence that the Koc will indicate that Brodifacoum is non-mobile in soil. If a value of 8.5 (logP in pesticide manual, Tomlin) is used then the following is obtained: Log Koc = 0.5*8.5 + 0.41 = 4.66. Therefore Koc = 45709 Log Koc = 0.5*8.5 +0.98 = 5.23. Therefore Koc = 169824 Under the SSLRC mobility classification, a Koc of >4000 is classed as non-mobile. Both of the estimations, albeit using literature logP, are so far above 4000 that it is very likely that Brodifacoum is non-mobile in various types of soil. Also the bait will be used in very localised areas in and around buildings and in sewers. Direct contact to soil will be limited due to use of bait stations. Based on the above weight-of-evidence it is believed that the risk of brodifacoum reaching groundwater is very low. Also, in the Emission Scenario Document (2003, PT14) it states on page 25 for the scenario for ' in and around buildings' that " a detailed groundwater scenario is not considered necessary due to the limited quantities of active substance , the limited frequency and the limited contamination area". On the basis of all the above arguments, a derogation to perform an adsoption/desorption study (OECD 106) is requested.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has*	

Section A7.2.3.2 Annex Point *IIIA XII.1.3*	**Mobility in at least three soil types and where relevant mobility of metabolites and degradation products**
	agreed on the delayed 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	*30/11/06*
Evaluation of applicant's justification	
Conclusion	*On the basis of literature data brodifacoum can be considered immobile in soil.*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.3.1 Annex Point *IIIA VII.5*	**Phototransformation in air (estimation method), including identification of breakdown products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	**Variation:** Photodegradation characteristics of the active substance have been estimated using the EPIWIN v 3.12 programme. The indirect photolysis half-life of of Brodifacoum with OH radicals is 2.205 hours (rate const. = 7.8831 x 10^{-12}cm^3/molecule/sec) and 2.015 hours (rate const. = 13.650000 x 10^{-17} cm^3/molecule/sec) with ozone. ***Atmospheric risk:*** Brodifacoum has a low volatility and emissions to the air compartment are expected to be low ***Global warming:*** Brodifacoum shows no absorption in the so-called atmospheric window (800-1200 nm) and therefore, according to the TGD on risk assessment (Part II, Section 3.7.2) is not a potential greenhouse gas. ***Stratospheric ozone:*** According to the TGD on risk assessment (Part II, Section 3.7.2) ozone depletion potential values approach zero for molecules with atmospheric halftimes less than one year. Brodifacoum has an estimated half-life of approximately 2 hours, therefore is predicted to have no effect on stratospheric ozone. ***Tropospheric ozone:*** According to the TGD on risk assessment (Part II, Section 3.7.2) there is at present no procedure available to estimate the effect on tropospheric ozone if only the basic characteristics of a substance are known. (Brodifacoum has a tropospheric half-life of approximately 2 hours). ***Acidification:*** Oxidation of Brodifacoum does not cause the formation of nitrogen containing oxides, and due to the low expected emissions to the air compartment, it is not expected that Brodifacoum will have an effect on acidification of the receiving soil or surface water. Calculation for this study: see 'references'. EPIWIN v 3.12 programme calculation of BCF factor.	X
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*30/11/06*	
Evaluation of applicant's justification		

Section A7.3.1 Annex Point *IIIA VII.5*	**Phototransformation in air (estimation method), including identification of breakdown products**
Conclusion	*Agreed with notifier*
Remarks	According to TGD the cOH should be 0.5 x 10^6 molec/cm3 and the time 24 h; the new value is t1/2 = 6.61 h.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.3.2 Annex Point *IIIA XII.3*	**Fate and behaviour in air, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [x]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound , rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Compound is stable in air and has low v.p. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*30/11/06*	
Evaluation of applicant's justification		
Conclusion	*Agreed with notifier*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.1.1 Acute toxicity to fish

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		32 REFERENCE
32.1	**Reference**	Report: The Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) of BRODIFACOUM Technical. XXXXX - March 2003. XXXXX report ENV5803/120140 Acute toxicity study of test substance brodifacoum technical.
32.2	**Data protection**	Yes
32.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
32.2.2	Companies with Access to data	PelGar International Ltd. Activa srl
32.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		33 GUIDELINES AND QUALITY ASSURANCE
33.1	**Guideline study**	OECD 203 (1992 version)
33.2	**GLP**	Yes
33.3	**Deviations**	Yes; DMF control at higher concentration than guideline value; test substance concentration not maintained at 80% or higher. Both of these deviations are not considered to reduce the reliability or accuracy of the results. Please see comments later.
		34 MATERIALS AND METHODS
34.1	**Test material**	As given in section 2
34.1.1	Lot/Batch number	ECO120140
34.1.2	Specification	As given in section 2
34.1.3	Purity	Minimum 99 % brodifacoum
34.1.4	Composition of Product	Not applicable
34.1.5	Further relevant properties	Not Applicable
34.1.6	Method of analysis	HPLC
34.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_1-1
34.3	**Reference substance**	Yes
34.3.1	Method of	Acute toxicity: 96 hour LC_{50} of Potassium dichromate on rainbow trout

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

	analysis for reference substance	with test concentrations of, 0 (control), 32, 56, 100, 180 and 320mg/l.
34.4	**Testing procedure**	
34.4.1	Dilution water	See table A7_4_1_1-2
34.4.2	Test organisms	See table A7_4_1_1-3
34.4.3	Test system	See table A7_4_1_1-4
34.4.4	Test conditions	See table A7_4_1_1-5
34.4.5	Duration of the test	96 hours
34.4.6	Test parameter	Mortality
34.4.7	Sampling	Test substance analysis of each concentration carried out as soon as possible after sampling at 0, 24, 48, 72 and 96 hours with samples being frozen until analysis
34.4.8	Monitoring of TS concentration	Yes Start of study, before and after renewal of solutions at 24, 48, 72 hours, and at the end of the 96 hour exposure period.
34.4.9	Statistics	LC_{50} determined by the Spearman-Karber method NOEC and LOEC determined by Fisher's Exact test
	35 RESULTS	
35.1	**Limit Test**	Not performed
35.1.1	Concentration	Not Applicable
35.1.2	Number/ percentage of animals showing adverse effects	Not Applicable
35.1.3	Nature of adverse effects	Not Applicable
35.2	**Results test substance**	
35.2.1	Initial concentrations of test substance	0 (control), DMF(control), 0.06, 0.13, 0.25, 0.5 and 1.0 mg/l

35.2.2 Actual concentrations of test substance	**mg/l Nom.**	**0**	**0.06**	**0.13**	**0.25**	**0.5**	**1.0**
	0 hrs fresh	0	0.03	0.06	0.16	0.28	0.58
	24 hrs aged	0	0.03	0.06	0.08	0.18	0.48

24 hrs renewed	0	0.04	0.08	0.15	-	-
48 hrs aged	0	0.03	0.06	0.05	-	-
48 hrs renewed	0	0.04	0.08	-	-	-
72 hrs aged	0	0.03	0.05	-	-	-
72 hrs renewed	0	0.04	0.08	-	-	-
96 hrs aged	0	0.02	0.04	-	-	-
Mean measured conc		*0.03*	*0.06*	*0.11*	*0.23*	*0.53*

-No replacement solutions required

35.2.3 Effect data (Mortality): Mortality: see table A7_4_1_1-6

LC_{50} plus 95% confidence limits: see table A7_4_1_1-7

35.2.4 Concentration / response curve

Exposure	Nominal concentration (mg/l)					
period(hours)	**Control**	**0.06**	**0.13**	**0.25**	**0.50**	**1.0**
0	0	0	0	0	0	0
24	0	0	0	0	100	100
48	0	0	0	100	100	100
72	0	0	42.9	100	100	100
96	0	0	57.1	100	100	100

35.2.5 Other effects: Not stated

35.3 Results of controls

35.3.1 Number/ percentage of animals showing adverse effects: No effects

35.3.2 Nature of adverse effects: No effects

35.4 Test with reference substance: Performed

35.4.1 Concentrations: Potassium dichromate at nominal concentrations 0(control), 32,56, 100, 180 and 320 mg/l

35.4.2 Results: 24 hour LC_{50} = 240 mg/l (206 – 279 mg/l; 95% confidence limits)

48 hour LC_{50} = 240 mg/l (206 – 279 mg/l; 95% confidence limits)

72 hour LC_{50} = 133 mg/l (91 – 201 mg/l; 95% confidence limits)

96 hour LC_{50} = 133 mg/l (91 – 201 mg/l; 95% confidence limits)

X

36 APPLICANT'S SUMMARY AND CONCLUSION

36.1 Materials and methods: OECD 203

Section A7.4.1.1 Acute toxicity to fish

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36.2	**Results and discussion**	Test substance is extremely insoluble in water(c.0.03 ppm), of very low vapour pressure, and is subject to rapid photolysis (half-life c.7.5 hrs)	
36.2.1	LC_0	48 hour LC_0 = not provided	
		96 hour LC_0 = not provided	
36.2.2	LC_{50}	24 hour LC_{50} = 0.159 mg/l (95% confidence limits not possible to determine)	
		48 hour LC_{50} = 0.081 mg/l (95% confidence limits not possible to determine)	
		72 hour LC_{50} = 0.062 mg/l (0.048 – 0.078 mg/l; 95% confidence limits)	
		96 hour LC_{50} = 0.042 mg/l (95% confidence limits not possible to determine)	
36.2.3	LC_{100}	48 hour LC_{100} = not provided 96 hour LC_{100} = not provided	
36.3	**Conclusion**	R50/R53- Very toxic to aquatic organisms applies. All validity criteria can be considered as fulfilled except one(see deviations in 2.3). Clear dose-response relationship shown (see table A7_4_1_1-8)	
36.3.1	Other Conclusions	LC50 is greater than the water solubility.	
36.3.2	Reliability	1	X
36.3.3	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	*November 2006*
Materials and Methods	*Accepted*
Results and discussion	*A NOEC can be set = 0.03 mg/l (measured).*
Conclusion	*Accepted*
Reliability	*2*
Acceptability	*acceptable* *A major deficiency observed in the test report is the lack of clear information on the concentrations of solvent used and of a justification for using a DMF concentration 10 fold higher than that recommended by OECD 23.* *The applicant has provided acceptable clarifications on this issue (see note to Table A7_4_1_1-1). Therefore results are considered reliable despite of the major deficiency, due to the lack of effects in the solvent control.*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

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

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

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

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

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	De-chlorinated mains tap water
Alkalinity	214 mg/l
Hardness	*286 mg/l calcium carbonate
PH	7.0 – 8.0
Oxygen content	99-101% ASV
Conductance	766µS/cm
Holding water different from dilution water	No

*The value (286 mg/l CaCO3) given in the report represents an average figure for hardness derived from historic analyses. The guideline states the range 10-250 mg/l CaCO3 is preferable, not mandatory, therefore it is considered that the slightly higher level does not adversely affect the data obtained.

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow Trout (*Oncorhynchus mykiss*)
Source	Bilbury Trout Farm, Cirencester
Wild caught	No
Age/size	Mean length: 49.3 mm Weight: 1.56 g
Kind of food	Keystart Fingerling 25
Amount of food	Feed at 1% of body weight per day
Feeding frequency	Daily
Pretreatment	No

Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	*Semi-static
Renewal of test solution	Test solutions replaced every 24 hours
Volume of test vessels	10 litre volume plastic aquaria
Volume/animal	1.43 litres/fish
Number of animals/vessel	7
Number of vessels/ concentration	7 vessels: 0 (control), DMF (control),0.06, 0.13, 0.25, 0.5 and 1.0 mg/l
Test performed in closed vessels due to significant volatility of TS	No

*It was considered that a flow through study was not required because the semi-static guideline gave the option of determining the toxicity based on measured concentrations. At the time the semi-static study was commissioned , the test laboratory did not have any data available that indicated that it was not an appropriate or acceptable study

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	14.0 – 15.0 °C
Dissolved oxygen	95 – 100%
PH	8.2 – 8.5
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	
Photoperiod	16 hours light and 8 hours dark

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	Mortality							
	Total Number				Cumulative Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
*0 (Control DMF)	0	0	0	0	0	0	0	0
0.06	0	0	0	0	0	0	0	0
0.13	0	0	3	4	0	0	42.9	57.1
0.25	0	7	7	7	0	100	100	100
0.50	7	7	7	7	100	100	100	100
1.0	7	7	7	7	100	100	100	100
Temperature [°C]	14.5	15.0	14.0	14.0				
pH	8.4	8.3	8.4	8.3				
Oxygen [%ASV]	95-100	96-99	97-100	96-97				

*In accordance with UK Home Office recommendations concerning animal welfare, a single control containing DMF was set up for the Acute fish study. The preliminary range finding test had shown that DMF at the

required concentration did not adversely affect trout, therefore it was considered acceptable to reduce the total number of fish used by eliminating the extra control.

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	-	-	-	-
LC_{50}	0.081(m)	Not possible to determine	0.042(m)	Not possible to determine
LC_{100}	-	-	-	-

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

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

	fulfilled	Not fulfilled
Mortality of control animals <10%	*YES	
Concentration of dissolved oxygen in all test vessels > 60% saturation	YES	
Concentration of test substance ≥80% of initial concentration during test		**NO
Criteria for poorly soluble test substances	YES	

* Please see comment under table A7_4_1_1_6 above. No mortalities were recorded in the Control plus DMF throughout the full test period. As this is a more stringent control than dilution water alone it is thought that the test control validity criteria can be considered to be fulfilled.

** It is accepted that this criterion was not met. However the LC50 values have been recalculated using mean measured values which have now given lower LC50 values than originally reported. Therefore it is considered that the recalculated LC50 values are now sufficiently accurate for use in any risk assessment.

Section A7.4.1.2 Acute toxicity to invertebrates

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

			Official use only
		37 REFERENCE	
37.1	**Reference**	Report: The Toxicity to *Daphnia magna* of BRODIFACOUM Technical. XXXXX - March 2003. XXXXX report - ENV5802/120140 Acute toxicity study of test substance brodifacoum technical.	
37.2	**Data protection**	Yes	
37.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
37.2.2	Companies with Access to data	PelGar International Ltd. Activa srl	
37.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		38 GUIDELINES AND QUALITY ASSURANCE	
38.1	**Guideline study**	OECD 202	
38.2	**GLP**	Yes	
38.3	**Deviations**	No	X
		39 MATERIALS AND METHODS	
39.1	**Test material**	As given in section 2	X
39.1.1	Lot/Batch number	ECO120140	
39.1.2	Specification	As given in section 2	
39.1.3	Purity	Minimum 99 % brodifacoum	
39.1.4	Composition of Product	Not applicable	
39.1.5	Further relevant properties	Not applicable	
39.1.6	Method of analysis	HPLC	
39.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1)	
39.3	**Reference substance**	Yes	
39.3.1	Method of analysis for reference substance	Aquatic toxicity: 48 hour EC_{50} of potassium dichromate on *Daphnia magna*. Concentrations: 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/	

Section A7.4.1.2 Acute toxicity to invertebrates

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

39.4 Testing procedure

39.4.1	Dilution water	See table A7_4_1_2-2	
39.4.2	Test organisms	See table A7_4_1_2-3	X
39.4.3	Test system	See table A7_4_1_2-4	
39.4.4	Test conditions	See table A7_4_1_2-5	
39.4.5	Duration of the test	48 hours	
39.4.6	Test parameter	Immobility	
39.4.7	Sampling	Test substance analysis of each concentration carried out as soon as possible after sampling at 0, 24 and 48 hours with samples being frozen until analysis	
39.4.8	Monitoring of TS concentration	Yes Start of study, before and after renewal of solutions at 24 hours, and at the end of the 48 hour exposure period.	
39.4.9	Statistics	EC_{50} determined graphically with 95% confidence limits according to the method of ToxCalc™ Version 5.0 NOEC and LOEC determined by Fisher's Exact test	X

40 RESULTS

40.1 Limit Test Not performed

40.1.1	Concentration	Not applicable
40.1.2	Number/ percentage of animals showing adverse effects	Not applicable
40.1.3	Nature of adverse effects	Not applicable

40.2 Results test substance

40.2.1 Initial concentrations of test substance: 0 (control), DMF(control),0.13, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/l

40.2.2 Actual concentrations of test substance

mg/l Nom	**0**	**0.13**	**0.25**	**0.5**	**1.0**	**2.0**	**4.0**
0 hrs fresh	0	0.10	0.12	0.33	0.73	0.73	1.80
24 hrs aged	0	0.04	0.10	0.26	0.51	0.82	2.70
24 hrs renewed	0	0.09	0.17	0.30	0.83	1.34	-

Section A7.4.1.2 Acute toxicity to invertebrates

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

		48 hrs aged	0	0.06	0.10	0.22	0.43	0.79	-
		Mean measured	*0*	*0.07*	*0.12*	*0.28*	*0.63*	*0.92*	

40.2.3	Effect data (Immobilisation)	Immobility: See table A7_4_1_2-6; EC_{50} and 95% confidence limits: See table A7_4_1_2-7	X
40.2.4	Concentration / response curve	Slope: 2.9 with 95% confidence limits 2.0 – 3.8.	
40.2.5	Other effects	None stated	
40.3	**Results of controls**	No effects	
40.4	**Test with reference substance**	Performed	
40.4.1	Concentrations	Potassium dichromate at nominal concentrations 0(control), 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg /l	
40.4.2	Results	24 hour EC_{50} = 1.3 mg/l (1.1 – 1.6 mg/l; 95% confidence limits) 48 hour EC_{50} = 0.9 mg/l (0.8 – 1.1 mg/l; 95% confidence limits) 48 hour NOEC = 0.56 mg/l 48 hour 100% mortality = 3.2 mg/l	

41 APPLICANT'S SUMMARY AND CONCLUSION

41.1	**Materials and methods**	OECD 202	
41.2	**Results and discussion**	Test substance is extremely insoluble in water (c.0.03 ppm), of very low vapour pressure, and is subject to rapid photolysis (half-life c.7.5 hrs)	
41.2.1	EC_0	-	
41.2.2	EC_{50}	24 hour EC_{50} = 0.78 mg/l (0.71 – 0.85 mg/l; 95% confidence limits) 48 hour EC_{50} = 0.25 mg/l (0.20 – 0.31 mg/l; 95% confidence limits)	
41.2.3	EC_{100}	-	
41.3	**Conclusion**	R51 /R53 Toxic to aquatic organisms applies. All validity criteria can be considered as fulfilled. Clear dose-response relationship shown (see table A7_4_1_2-8) LC50 is greater than the water solubility.	
41.3.1	Reliability	1	X
41.3.2	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

Section A7.4.1.2 Acute toxicity to invertebrates

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

Date	*November 2006- September 2009*
Materials and Methods	3.1 Test material: Brodifacoum technical
Results and discussion	4.2.3 Effect data: In the original study report the EC50 are based on nominal concentrations. They have been recalculated by the study owner based on measured concentrations, upon request by RMS. In table A7_4_1_2-7, wrong confidence limits are reported. The reported EC_0 and EC_{100} concentration are nominal values (not measured values as indicated). The EC_0 is reported as 0.25 mg/l (nominal). At this concentration (NOEC, based on statistical analysis), 10% of animals was immobilize, therefore the RMS proposes to set the EC_0 at 0.07 mg/l (measured). The EC_{100} expressed as measured concentration is 0.92 mg/l.
Conclusion	48 hour EC_{50} = 0.25 mg/l (0.20 – 0.31) (measured). Very toxic to Daphnia. 48h NOEC = 0.12 mg/l (measured) Accepted
Reliability	2
Acceptability	acceptable A major deficiency observed in the test report is the lack of clear information on the concentrations of solvent used and of a justification for using a DMF concentration 10 fold higher than that recommended by OECD 23. The applicant has provided acceptable clarifications on the issue above (see note to Table A7_4_1_2-1). Therefore results are considered reliable despite of the major deficiency, due to the lack of effects in the solvent control.
Remarks	2.3 deviations: DMF concentration 10 fold higher than that recommended by OECD 23.

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

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

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

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

Table A7_4_1_2-2: Dilution water

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

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

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Shell Research Laboratories
Age	Less than 24 hours
Breeding method	Cultured under semi-static conditions. Isolation of gravid animals.
Kind of food	A suspension of *Chlorella vulgaris*
Amount of food	1 mg organic carbon per litre of culture water

Feeding frequency	Daily
Pretreatment	None
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

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

Table A7_4_1_2-5: Test conditions

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

Table A7_4_1_2-6: Immobilisation data

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

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

Table A7_4_1_2-7: Effect data

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

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

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

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

*The test laboratory have stated that the concentration of dissolved oxygen fulfilled the OECD 202 requirement of being ≥ 60% of the air saturation value at the end of the test, at the temperature used. It is assumed by the applicant that this is equivalent to >3mg/l stated in the validity criteria of the guideline.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		42 REFERENCE	
42.1	**Reference**	Report: The Growth Inhibition of the alga *Selenastrum capricornutum* by BRODIFACOUM Technical. XXXXX - March 2003. XXXXX. Report -ENV5801/120140 Toxicity study of test substance brodifacoum technical.	
42.2	**Data protection**	Yes	
42.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
42.2.2	Companies with Access to data	PelGar International Ltd. Activa srl	
42.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		43 GUIDELINES AND QUALITY ASSURANCE	
43.1	**Guideline study**	OECD 201	X
43.2	**GLP**	Yes	
43.3	**Deviations**	No	X
		44 MATERIALS AND METHODS	
44.1	**Test material**	As given in section 2	
44.1.1	Lot/Batch number	ECO120140	
44.1.2	Specification	As given in section 2	
44.1.3	Purity	Minimum 99 % brodifacoum	
44.1.4	Composition of Product	Not applicable	
44.1.5	Further relevant properties	Not applicable	
44.1.6	Method of analysis	HPLC	
44.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_3-1)	
44.3	**Reference substance**	Yes	
44.3.1	Method of analysis for reference substance	Aquatic toxicity: 72 hour EC_{50} of Potassium dichromate on *Selenastrum capricornutum* with test concentrations of, 0 (control), 0.18, 0.32, 0.56, 1.0 and 1.8 mg/l.	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

44.4 Testing procedure

44.4.1 Culture medium

Nutrient	*Final concentration in culture medium (mg/l)*
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.2H_2O$	18
$MgSO_4.7H_2O$	15
KH_2PO_4	1.6
$FeCl_3.6H_2O$	0.08
$Na_2EDTA.2H_2O$	0.1
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$ZnCl_2$	0.003
$CoCl_2.6H_2O$	0.0015
$CuCl_2.2H_2O$	*0.00001
$Na_2MoO_4.2H_2O$	0.007
$NaHCO_3$	50

**The original figure of 0.0001 was a typographical error, examination by the test laboratory of the raw data for the preparation of the stock solutions for the algal nutrient growth medium showed that the concentration of CuCl2.2H2O used for the study was correct, 0.01µg/l as stated in the guideline. The test laboratory acknowledges that this should have been noted during the preparation of the report and audit.*

44.4.2	Test organisms	see table A7_4_1_3-2
44.4.3	Test system	see table A7_4_1_3-3
44.4.4	Test conditions	see table A7_4_1_3-4
44.4.5	Duration of the test	72 hours
44.4.6	Test parameter	Cell multiplication inhibition
44.4.7	Sampling	Sampling at 0, 24, 48 and 72 hrs
44.4.8	Monitoring of TS concentration	Yes Start and end of test period
44.4.9	Statistics	EC_{50} values estimated using a logarithm-linear or logarithm-probit plot of concentration and percent growth inhibition.

45 RESULTS

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

45.1 Limit Test Not performed

45.1.1 Concentration: Not applicable

45.1.2 Number/ percentage of animals showing adverse effects: Not applicable

45.2 Results test substance

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

45.2.2 Actual concentrations of test substance

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

45.2.3 Growth curves

45.2.4 Concentration / response curve

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

45.2.5 Cell concentration data: see table A7_4_1_3-5

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

45.2.7 Other observed effects: None stated

45.3 Results of controls No effects

45.4 Test with reference substance Performed

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

45.4.1	Concentrations	Potassium dichromate at nominal concentrations 018, 0.32, 0.56, 1.0 and 1.8 mg/l	
45.4.2	Results	E_bC_{50} 0 – 48 hrs 0.59 mg/l	
		E_rC_{50} 0 - 48 hrs 0.86 mg/l	
		E_bC_{50} 0 - 72 hrs 0.58 mg/l	
		E_rC_{50} 0 - 72 hrs 0.88 mg/l	
		46 APPLICANT'S SUMMARY AND CONCLUSION	
46.1	**Materials and methods**	OECD 201.	
46.2	**Results and discussion**	Test substance is extremely insoluble in water (c.0.03 ppm), of very low vapour pressure, and is subject to rapid photolysis (half-life c.7.5 hrs)	
46.2.1	NER_CO	0 – 48 hrs NER_CO < 0.032 mg/l	X
46.2.2	E_rC_{50}	0 – 48 hrs = 0.74 mg/l; 0 – 72 hrs = 0.27 mg/l	X
46.2.3	E_bC_{50}	0 – 48 hrs = 0.15 mg/l; 0 – 72 hrs = 0.06 mg/l	X
46.3	**Conclusion**	R50 /R53 Very toxic to aquatic organisms applies. All validity criteria can be considered as fulfilled. Clear dose-response relationship shown	
		EC50 is greater than the water solubility of the compound.	
46.3.1	Reliability	1	X
46.3.2	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	*November 2006*
Materials and Methods	2.1 Guideline study: OECD 201, version 1984

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Results and discussion	5.2.1-5.2.3: the endpoints reported here are those retrieved from the study report based on nominal concentration. The EC50 have been recalculated by the study owner based on average measured concentrations, upon request by RMS. 5.2.1: NOErC < 0.032 mg/l, to be corrected into NOErC = 0.014 mg/l (comparison made with solvent control). 5.2.2: 72h E_rC_{50} = 0.12 mg/l (extrapolated value), as reported in 4.2.6 5.2.3: 72h E_bC_{50} = 0.04 mg/l, as reported in 4.2.6. Following the discussion at TMV07 on the same study (included in a different CAR), the algae endpoints were recalculated on the basis of the geometric mean concentrations, hence: **EbC_{50} 0.016 mg/l** **ErC_{50} 0.04 mg/l.** **These last endpoints have been used in the risk assessment**
Conclusion	*Accepted*
Reliability	*2*
Acceptability	acceptable *Remarks made on a earlier version of the Doc. IIIA have been presented to the applicant which has provided adequate clarifications.*
Remarks	2.3 Deviations: surface/volume ratio is not in line with OECD 201 guideline as the optimal ratio for 250 ml conical flasks is obtained with 100 ml of test solution (the experiment reported 200 ml of test solution in 250 ml conical flasks). DMF concentration 10 fold higher than that recommended by OECD 23. The deviations are considered to not affect the results of the study. The "Concentration of test substance ≥80% of initial concentration during test"as reported in the table for the validity criteria, is not exact. Nevertheless such a requirement is not foreseen as validity condition by the OECD 201(1984).
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

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

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

Table A7_4_1_3-2: Test organisms

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

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	*200 ml in 250 ml conical flask
Culturing apparatus	Haemocytometer and microscope
Light quality	White light at 6000 – 10000 lux.
Procedure for suspending algae	Shaking at 200 rpm
Number of vessels/ concentration	6 replicates for 0 (control), 3 replicates per test

	concentration, 3 replicates for DMF (Control)
Test performed in closed vessels due to significant volatility of TS	No

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

Table A7_4_1_3-4: Test conditions

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

Table A7_4_1_3-5: Cell concentration data

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

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

3. Tables for Applicant's Summary and Conclusion

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

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

Section A7.4.1.4 Inhibition to microbial activity

Annex Point IIA7.4 Inhibition of activated sludge respiration by Brodifacoum

Official use only

47 REFERENCE

47.1	**Reference**	Staniland, J. (2004) An evaluation of the effect of Brodifacoum on the inhibition of activated suldge respiration according to OECD 209. Chemex Environmental International Ltd. Ref: ENV7009/120140
47.2	**Data protection**	Yes
47.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
47.2.2		PelGar International Ltd. Activa srl
47.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

48 GUIDELINES AND QUALITY ASSURANCE

48.1	**Guideline study**	OECD 209
48.2	**GLP**	Yes
48.3	**Deviations**	None

49 MATERIALS AND METHODS

49.1	**Test material**	As given in section 2
49.1.1	Lot/Batch number	7909101
49.1.2	Specification	As given in section 2
49.1.3	Purity	100% (w/w)
49.1.4	Composition of Product	n/a
49.1.5	Further relevant properties	Store in cool, dry place
49.1.6	Method of analysis	n/a
49.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The OECD 23 guideline "Guidance Document On Aquatic Toxicology Testing Of Difficult Substances and Mixtures" lists relevant OECD ecotoxicology guidelines (see page 14, Table 1) and the OECD 209 guideline "Activated Sludge, Respiration Inhibition Test" is not included. The OECD 209 guideline does not reference OECD 23, nor is there any reference to "protocol adjustment" for materials with low water solubility. The OECD 209 test guideline was therefore followed and direct addition of the test material (at the stated concentrations) to the biological system was considered appropriate

X

Section A7.4.1.4 Inhibition to microbial activity

Annex Point IIA7.4 Inhibition of activated sludge respiration by Brodifacoum

		The study plan generated for this study states in section 9.1 "It may not be possible to determine an EC50 value for substances with low water solubility." This reflected Chemex's concern that an EC50 based on nominal concentrations would not represent the EC50 based on the actual dissolved concentrations (if it were possible to determine these for a material of low solubility in this test system). The OECD 23 guideline defines a poorly (or sparingly) water-soluble substance as a substance with a limit of water solubility of up to100mg/l. The solubility of Brodifacoum (<0.1mg/l) falls much lower than this OECD 23 definition. Chemex acknowledges the difficulty in assessing the relevance of the OECD 209 test for use with Brodifacoum, however Chemex was required to perform the study as commissioned.
49.3	**Reference substance**	3,5-dichlorophenol (3,5-DCP) 97% purity
49.3.1	Method of analysis for reference substance	n/a
49.4	**Testing procedure**	*Non-entry field*
49.4.1	Culture medium	Batches of synthetic medium were freshly prepared for each test as described in OECD test guidelines 209.
49.4.2	Inoculum / test organism	(see table A7_4_1_4-2)
49.4.3	Test system	(see table A7_4_1_4-3)
49.4.4	Test conditions	(see table A7_4_1_4-4)
49.4.5	Duration of the test	3 hours
49.4.6	Test parameter	Respiration inhibition
49.4.7	Analytical parameter	Oxygen measurement
49.4.8	Sampling	Dissolved oxygen (mg/l) was measured every minute in each vessel for 10 minutes.
49.4.9	Monitoring of TS concentration	No.
49.4.10	Controls	3 vessels with reference substance at 5, 15 and 30 mg/l. 2 vessels which were blanks. 1 abiotic control.
49.4.11	Statistics	The results obtained from each vessel were plotted as graphs of dissolved oxygen concentrations (mg/litre) against time (minutes). The respiration rates (R) in each vessel were obtained from the linear parts of each graph using the equation: $R = (Q1-Q2)/st) \times 60$

Section A7.4.1.4 Inhibition to microbial activity

Annex Point IIA7.4 Inhibition of activated sludge respiration by Brodifacoum

		Where: R = Respiration rate in mg O_2 per litre per hour Q1= The first measurement on the linear part of the graph of the dissolved oxygen concentration (mg/l) v time Q2= The last measurement on the linear part of the graph of the dissolved oxygen concentration (mg/l) v time st = The time interval between Q1 and Q2 in minutes.
		50 RESULTS
50.1	**Preliminary test**	Not performed
50.1.1	Concentration	
50.1.2	Effect data	
50.2	**Results test substance**	*Non-entry field*
50.2.1	Initial concentrations of test substance	0, 63.0, 124.3, 251.0, 501.3, 850.7, 1003 mg/l
50.2.2	Actual concentrations of test substance	n/a
50.2.3	Growth curves	n/a
50.2.4	Cell concentration data	120.0 ml of activated sludge n each vessel except the abitoic control.
50.2.5	Concentration/ response curve	*Plot of the percent inhibition vs. concentration of test substance*
50.2.6	Effect data	Could not be calculated as no vessels showed inhibition of activated sludge.
50.2.7	Other observed effects	None
50.3	**Results of controls**	The results of the abiotic control showed that there was no oxygen consumption in vessels without the addition of the inocula. The control vessels which had no test substance in showed no inhibition to the consumption of oxygen by the activated sludge.
50.4	**Test with reference substance**	Performed
50.4.1	Concentrations	5, 15, 30 mg/l

Section A7.4.1.4 Inhibition to microbial activity

Annex Point IIA7.4 Inhibition of activated sludge respiration by Brodifacoum

50.4.2	Results	The EC_{50} for the reference substance was estimated graphically at 5.7mg/l.	
		51 APPLICANT'S SUMMARY AND CONCLUSION	
51.1	**Materials and methods**	OECD 209. A range of test substance concentration were added to a synthetic sewage medium containing an activated sludge inoculum. Each treatment was placed into a conical flask and vigorously aerated for 3 hours. After 3 hours, the test solution was transferred to a 250ml BOD bottle and the oxygen electrode inserted in such a way as to exclude oxygen. The rate of oxygen consumption was measured in each bottle over a 10 minute period. Percentage inhibition of respiration rate was then estimated by comparison with unenexposed blanks and a dose-response curve was obtained by plotting percent inhibition of respiration against exposure concentration of Brodifacoum. The sensitivity of the activated sludge micro-organsims was assessed by determining the EC_{50} of the reference compound 3,4-dichlorphenol (3,5-DCP) during the definitive test.	
51.2	**Results and discussion**	Brodifacoum did not cause any significant effects on activated sludge respiration inhibition at tge concentrations tested, up to and including 1003mg/l. Therefore the EC_{50} value of Brodifacoum could not be calculated but is greater than 1003mg/l	X
51.2.1	EC_{20}		
51.2.2	EC_{50}	>*1003mg/l	X
51.2.3	EC_{80}		
51.3	**Conclusion**	The reference compound 3,5-DCP indicated the sensitivity of the activated sludge was within the correct range of 5 to 30mg/l, with an EC_{50} of 5.7mg/l (estimated graphically). The respiration rates of two blank treatments were within 15% of the mean value.	
51.3.1	Reliability	1 * The applicant believes that the study was carried out according to the guideline. Therefore the study itself should receive a high reliability for how it was performed. However it is accepted that given the low solubility of brodifacoum, it is extremely unlikely that 1003mg/l would have been achieved in solution without the use of any co-solvent. It is proposed therefore that the water solubility limit of 0.1mg/l is used for this study and taken forward to the risk assessment, until further refinement using the water solubility figure from a new SPL study (due Aug/Sept 2006) is available.	X
51.3.2	Deficiencies	No	

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

Section A7.4.1.4 Inhibition to microbial activity

Annex Point IIA7.4 Inhibition of activated sludge respiration by Brodifacoum

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2006- August 2009*
Materials and Methods	*3.2 Preparation of TS solution for poorly soluble or volatile test substances: it is agreed that OECD guideline 23 (page 14, Table 1) does not applies to OECD 209, directly; however the OECD guideline 23 "may also be relevant to other tests" as reported at page 13 "Scope". Furthermore, in testing Brodifacoum following OECD 209, it should be considered that the guideline "is most readily applied to substances which, due to their water solubility and low volatility, are likely to remain in water". For this reason, the known water solubility of the substance is one of the prerequisites of OECD 209.* *As a consequence, the need and soundness of using solvents are evident.* *Since no solvents were used, it is expected that the actual concentration was far less than 1003mg/l.*
Results and discussion	*5.2 and 5.2.2: taking into account the statement of the applicant at 5.3.1 (given in response to the RMS comments on a earlier version of the Doc. IIIA), the results should be expressed as EC_{50} > Brodifacoum water solubility, to be used for risk assessment.* *The water solubility indicated by the applicant (0.1 mg/l, pH 5.8-6.9, OECD 105) has to be corrected into 5.8E-5 g/l (pH 7, 20°C) as resulted from the new study provided to the RMS in December 2006.* *For the needs of the risk characterisation, the water solubility of 0.058 mg/l can be used as the $NOEC_{microorganisms}$.*
Conclusion	*No inhibitory effect on microorganisms was observed at 1003 mg/l in the respiration inhibition test. However, the results of the study cannot be accepted due to an evident underestimation of the toxicity. As the test was performed at concentrations far above the water solubility of brodifacoum it is deemed as a conservative approach to assume that NOEC is equal or greater the water solubility limit for brodifacoum of 0.058 mg/l.*
Reliability	*3*
Acceptability	*Acceptable.* *Although the results of the study (EC_{50} >1003mg/l) are not reliable, the study can be used to derive the $NOEC_{microorganisms}$ on the basis of the brodifacoum water solubility (EC_{50} >* 0.058 mg/l).
Remarks	*Table A7_4_1_4-1 to be fulfilled according to the original study report.*
	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*

Section A7.4.1.4	**Inhibition to microbial activity**
Annex Point IIA7.4	Inhibition of activated sludge respiration by Brodifacoum

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

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

Criteria	Details
Dispersion	Yes/No
Vehicle	Yes/No *(If yes, specify: e.g. organic solvents, emulsifiers or dispersants)*
Concentration of vehicle	*Give the concentration (% v/v)*
Vehicle control performed	Yes/No *(If yes, specify)*
Other procedures	*e.g. test in completely filled closed vessels for testing volatile test substance*

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	n/a
Strain	n/a
Source	sewage treatment plant treating predominantly domestic sewage
Sampling site	Cambridge Sewage Treatment Works, Milton road, Cambridge
Laboratory culture	no
Method of cultivation	n/a
Preparation of inoculum for exposure	Sludge was centrifuged and the pellet resuspended in dechlorinated tap water.
Pretreatment	n/a
Initial cell concentration	1.51g/l dry weight in each test vessel.

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	250ml (nominal) BOD bottles with ground glass stoppers.
Number of culture flasks/concentration	12 vessels, with 0, 63.0, 124.3, 251.0 501.3, 850.7, 1003, 0 mg/l of the test material concentration. The remainaing 4 vessels contained an abitoic contronl and 5, 15, 30 mg/l of tne reference material.
Aeration device	Aquarium type air pump.
Measuring equipment	Dissolved oxygen meter.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	21 ±2°C
pH	n/a
Aeration of dilution water	Yes, air flow
Suspended solids concentration	9.6 g of synthetic sewage in each vessel

Section A7.4.2 Annex Point *IIIA XIII.2.3*	**Bioconcentration**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	The bioconcentration factor can be estimated using the following equation from the TGD, part II: Log BCFfish = -0.20 * logKow^2 + 2.74 * logKow - 4.72 (eq. 75) Log BCFfish = -0.20 * 8.5^2 + 2.74 * 8.5 – 4.72 = 4.12 BCF fish = 13183 Log Kow = 8.5 is taken from the Pesticide Manual (13th edition). The above equation is for use on substances with log Kow >6. The value for logKow >4 found elsewhere in the dossier is not entirely trustworthy and a further study to determine the log Kow is to be carried out at SafePharm Laboratories in August 2006, with the report expected in September 2006. When this value is obtained the calculated BCF can be refined. The above BCF of 13183 indicates that Brodifacoum is expected to be very bioaccumulative (TGD, part II, page 169). A derogation to perform a bioconcentration on fish is requested for the following reasons: i) very high value obtained indicating a very high biocumulative potential, therefore it is accepted that brodifacoum is likely to bioconcentrate in fish, without the need to prove it experimentally ii)animal welfare grounds as it would be a waste of experimental fish iii) predicted low exposure (IIB) in the aquatic compartment	X
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*December 2006*	

Section A7.4.2 Annex Point *IIIA XIII.2.3*	**Bioconcentration**
Evaluation of applicant's justification	*The new study on Kow mentioned by the applicant has been made available and accepted by the RMS.* *As a result, the experimental Log Pow of brodifacoum is 4.92.* *The BCF_{fish} is therefore calculated using the equation 74 (TGD, part II) as:* *Log BCF_{fish} = 0.85 * logKow – 0.70 = 3.482, leading to a BCF_{fish} = 3034*
Conclusion	*BCF_{fish} is 3034, estimated from the experimentally derived Log Pow of 4.92.*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.1 Annex Point *IIIA XIII.2.1*	**Prolonged toxicity to an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	A derogation for a study on the prolonged toxicity to an appropriate species of fish is requested on the following grounds: a) Expected exposure to the aquatic compartment is very low. The EUSES 2.03 model (see IIB) indicates a local PEC in water of only 8.28E-07mg/l, from use of wax blocks in sewers. Using the LC50 for fish (A7.4.1) of 0.042mg/lt and an assessment factor of 1000 (TGD, partII) we get a PNEC of 4.2E-05. This give a PEC/PNEC ratio of 0.02. This is well below the limit value of 1.0. A generic value of at least 80% for sewer connection to an STP in the EU (TGD, part II, appendix 12) shows that there is a low probability risk from untreated sewer water. . In untreated sewer water we have a PEC of 4.03E-06mg/l (EUSES 2.03 _bypass STP). So the PEC/PNEC is 0.1.This latter scenario is for a realistic worse-case in a city with a severe rat infestation and would also require that the city was not connected to a STP, a highly unlikely combination. b) The use pattern for the wax bait block is in sewers and in and around buildings. The sewer case is discussed above. In and around buildings it is highly unlikely that the active would end up in surface water because it is in the form of a solid block and this is not going to be deposited into water. Any of the active that does go onto soil, due to for example excretion from the rat is not going to reach groundwater since the active has a very high Koc (average 50000, pesticide manual, 13th edition) indicative of being non-mobile in soil. c)Brodifacoum has very low solubility in water (<0.1mg/l, doc IIIA, also new study is to be conducted at SafePharm Ltd in August /Sept 2006 to obtain a more accurate value) and will strongly adhere to sediment (high Koc) making it non-bioavailable to aquatic organisms such as fish. This assumes it dissolves in the first place which is unlikely. d)Brodifacoum undergoes rapid direct photolysis (see Doc IIIA) with half-lives ranging between 23 and 366 minutes depending on the season. Also EPIWIN (Doc IIIA)predicts that the indirect photolysis half-life is 2.205 hours. So, on the above basis, a derogation for a study on prolonged toxicity in fish is requested on grounds of: limited exposure; use pattern; very low water solubility; high Koc; rapid photolysis of the active. Furthermore it would be a waste of experimental animals and is considered an unnecessary animal test under the BPD directive.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

Section A7.4.3.1 Annex Point *IIIA XIII.2.1*	**Prolonged toxicity to an appropriate species of fish**
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2006*
Evaluation of applicant's justification	*The applicant justification is acknowledged in principle on regard of the absence of risk for water compartment and partition of the substance into sediments.* *Furthermore, according to TNsG (ch. 3, part A), this test is usually not required, as it does not add information useful to the risk assessment.*
Conclusion	*There is no need of the above study.*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.2 Annex Point *IIIA XIII.2.2*	**Effects on reproduction and growth rate on an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	A derogation for a study of the effects on reproduction and growth rate on an appropriate species of fish is requested on the following grounds: a) Expected exposure to the aquatic compartment is very low. The EUSES 2.03 model (see IIB) indicates a local PEC in water of only 8.28E-07mg/l, from use of wax blocks in sewers. Using the LC50 for fish (A7.4.1) of 0.042mg/lt and an assessment factor of 1000 (TGD, partII) we get a PNEC of 4.2E-05. This give a PEC/PNEC ratio of 0.02. This is well below the limit value of 1.0. A generic value of at least 80% for sewer connection to an STP in the EU (TGD, part II, appendix 12) shows that there is a low probability risk from untreated sewer water. . In untreated sewer water we have a PEC of 4.03E-06mg/l (EUSES 2.03 _bypass STP). So the PEC/PNEC is 0.1.This latter scenario is for a realistic worse-case in a city with a severe rat infestation and would also require that the city was not connected to a STP, a highly unlikely combination. b) The use pattern for the wax bait block is in sewers and in and around buildings. The sewer case is discussed above. In and around buildings it is highly unlikely that the active would end up in surface water because it is in the form of a solid block and this is not going to be deposited into water. Any of the active that does go onto soil, due to for example excretion from the rat is not going to reach groundwater since the active has a very high Koc (average 50000, pesticide manual, 13th edition) indicative of being non-mobile in soil. c)Brodifacoum has very low solubility in water (<0.1mg/l, doc IIIA, also new study is to be conducted at SafePharm Ltd in August /Sept 2006 to obtain a more accurate value) and will strongly adhere to sediment (high Koc) making it non-bioavailable to aquatic organisms such as fish. This assumes it dissolves in the first place which is unlikely. d)Brodifacoum undergoes rapid direct photolysis (see Doc IIIA) with half-lives ranging between 23 and 366 minutes depending on the season. Also EPIWIN (Doc IIIA) predicts that the indirect photolysis half-life is 2.205 hours. So, on the above basis, a derogation for a study on prolonged toxicity in fish is requested on grounds of: limited exposure; use pattern; very low water solubility; high Koc; rapid photolysis of the active. Furthermore it would be a waste of experimental animals and is considered an unnecessary animal test under the BPD directive.	X
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has*	

Section A7.4.3.2 Annex Point *IIIA XIII.2.2*	**Effects on reproduction and growth rate on an appropriate species of fish**
	agreed on the delayed 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	*November 2006*
Evaluation of applicant's justification	The applicant justification is acknowledged in principle, on regard of the absence of risk to the water compartment, based on acute toxicity data. The other elements provided by the applicant such as limited exposure, use pattern, water solubility, Koc and photolysis are of limited relevance as justification for the non submission of the study. Besides, taking into account that STPs normally have a screening system placed upstream the active sludge tank, it is likely that the actual exposure of surface waters would be even lower than the calculated one, as carcasses of poisoned rodents and fragments of baits are removed. It is therefore concluded that there is sufficient margin of safety to exclude long-term risk for the aquatic compartment. Further, it has to be taken into account that a study on fish reproduction and growth rate would probably not contribute to the risk assessment as it is likely to fail, considering the high toxicity of the substance and also the technical difficulties to maintain, extract and measure brodifacoum at low concentrations upon long term.
Conclusion	Risk to the aquatic compartment is not expected therefore there is not need of a chronic study with fish
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.3.1
Annex Point IIIA XIII.2.3

Bioaccumulation in an appropriate species of fish-
Effect in Rainbow Trout -

Official use only

52 REFERENCE

52.1 Reference	XXXXX (2004) The Bioconcentration potential of Brodifacoum in Rainbow Trout under flow-through conditions XXXXX Report ENV6597/120140
52.2 Data protection	Yes
52.2.1 Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
52.2.2 Companies with letter of access	PelGar International Ltd. *Activa srl*
52.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

53 GUIDELINES AND QUALITY ASSURANCE

53.1 Guideline study	OECD Guideline 305
53.2 GLP	Yes
53.3 Deviations	No

54 MATERIALS AND METHODS

54.1 Test material	As given in section 2
54.1.1 Lot/Batch number	ECO120140
54.1.2 Specification	As given in section 2
54.1.3 Description	Off white powder
54.1.4 Purity	100%
54.1.5 Stability	Not stated
54.1.6 Method of analysis	HPLC analysis
54.2 Reference substance	Yes – Dimethylformamide (DMF)
54.2.1 Method of analysis for reference substance	HPLC analysis
54.3 Testing/estimation procedure	
3.3.1 Test system/performance	Test vessel – 300 litre volume TITAN medium density polyethylene water storage tanks. Dilution water – Dechlorinated mains tap water. Test organisms – Eighty trout were placed in each of the control and test vessels. The average weight of the fish at the start of the test was 5.17g.

Section A7.4.3.3.1
Annex Point IIIA XIII.2.3

Bioaccumulation in an appropriate species of fish-

Effect in Rainbow Trout -

Loading – At the beginning of the test the loading ratio of Rainbow Trout to water was estimated to be:

Control 0.2992g fish tissue per litre test solution day^{-1}

0.04µg/l 0.3009g fish tissue per litre test solution day^{-1}

0.04µg/l 0.3002g fish tissue per litre test solution day^{-1}

Test concentrations – A preliminary stock solution of 0.04g/l Brodifacoum in Dimethylformamide (DMF) was prepared. Secondary stock solutions were prepared daily at concentrations of 0.04 and 0.004mg/l in distilled water. A control solution of 1ml DMF per litre distilled water was also prepared.

Test procedure – Flow-through. Peristaltic pumps were used to dose both the dilution water and test stock solutions.

Test conditions – The test vessels were maintained at10°C.

Observation frequency – Observations and records of mortalities were made every 24 hours.

Sampling frequency – Water samples were taken 24 hours before and immediately before addition of the trout on day 0, and (then) on days 1,3,6,10 and 14.

Feeding rate – The trout were fed on a daily basis on Trouw (UJ) Ltd Nutra Trout Fry 02 Crumb fish feed at a rate of 1% body weight day^{-1}.

54.3.1	Estimation of bioconcentration	N/A

55 RESULTS

55.1 Experimental data

55.1.1	Mortality/behaviour	Early termination of the uptake phase was necessary due to the number of mortalities recorded.
55.1.2	Lipid content	Please see section 3.3 of report.
55.1.3	Concentrations of test material during test	A preliminary stock solution of 0.04g/l Brodifacoum in Dimethylformamide (DMF) was prepared. Secondary stock solutions were prepared daily at concentrations of 0.04 and 0.004mg/l in distilled water. A control solution of 1ml DMF per litre distilled water was also prepared. The final test concentrations of 0 (Control), 0.004 and 0.04µg/l Brodifacoum were prepared by proportional dilution of the test material to dilution water at a rate of 1 ml per litre dilution water. All records of flow rates can be found in Appendix 2.
55.1.4	Bioconcentation factor	Not determined.
55.1.5	Uptake and depuration rate constants	The uptake phase of the study was scheduled to last 28 days however, this had to be shortened to 14 days after mortalities on the 0.04µg/l test concentration were recorded.
55.1.6	Depuration time	N/A
55.1.7	Metabolites	Not identified.

Section A7.4.3.3.1
Annex Point IIIA XIII.2.3

Bioaccumulation in an appropriate species of fish-

Effect in Rainbow Trout -

55.1.8 Other observations

Significant difficulties were experienced in attempting to extract and measure Brodifacoum in the water samples in this study. This is supported by the published literature on Brodifacoum, which indicates that this material can not be successfully extracted from water at low concentrations. Therefore all results from this study are derived from the nominal concentrations based on flow rates of the dilution water and test material and analysis of the fish tissue.

55.2 Estimation of bioconcentration

X

The following calculation was carried out after the original summary was submitted, in response to comments from the RMS (July 2006). The original text in this section has been deleted.

Approximate log Kow = 8.5 (pesticide manual, 13 edition)

From the TGD (part II, equation 75), for substances with a log Kow >6.0 is used:

$\log BCF_{fish} = -0.20 * \log Kow^2 + 2.74 * \log Kow - 4.72$

$\log BCF_{fish} = -0.20 * 8.5^2 + 2.74 * 8.5 - 4.72$

$\log BCF_{fish} = 4.12$

$BCF_{fish} = 13182$

56 APPLICANT'S SUMMARY AND CONCLUSION

56.1 Materials and methods

OECD Guideline 305

56.2 Results and discussion

This study has demonstrated that at the concentrations tested the test material Brodifacoum could not successfully be identified in extracts of the bodily tissues of Rainbow Trout by HPLC analysis. Analysis of Brodifacoum in water samples was not possible due to problems encountered with the extraction procedure. Early termination of the uptake phase was necessary due to the number of mortalities recorded. Under these circumstances it is not possible to determine the Bioconcentration Factor (BCF_{ss}) of Brodifacoum in Rainbow trout.

Significant difficulties were experienced in attempting to extract and measure Brodifacoum in the water samples in this study. This is supported by the published literature on Brodifacoum, which indicates that this material can not be successfully extracted from water at low concentrations. Therefore all results from this study are derived from the nominal concentrations based on flow rates of the dilution water and test material and analysis of the fish tissue.

56.3 Conclusion

The BCF value was not obtained, hence the validity criteria has not been met.

56.3.1 Reliability

*4 (see below)

56.3.2 Deficiencies

No preliminary trials were run to assess the method of analysis in water and fish. A test should have been performed using radiolabelled test material so that analysis could be conducted. The BCF is a quotient of the fish tissue concentration and the water concentration, which has not been determined in this

Section A7.4.3.3.1
Annex Point IIIA XIII.2.3

Bioaccumulation in an appropriate species of fish-

Effect in Rainbow Trout -
study. The mortality levels at the higher level are unacceptable but indicate that possible accumulation has occurred since the animals have died. This implies that the animals have reached a critical body burden of the compound which has resulted in death.

* A review of this summary by the applicant, after comments from the RMS, agrees with the RMS comments. Contrary to what was originally stated in 4.2 above, a new estimate is now given. Please see this in 4.2. This calculated value indicates a **high bioconcentration factor**, which is expected from the structure and known accumulation in mammals.

Evaluation by Competent Authorities

Date	*November 2006*
Materials and Methods	*Accepted*
Results and discussion	*4.2 Estimation of bioconcentration: Bioconcentration in fish can be calculated from the new experimental Log Pow, leading to a BCF_{fish} of 3034 (see section 7.4.2, RMS comments).*
Conclusion	*Accepted*
Reliability	*3*
Acceptability	*The study did not meet the validity criteria and therefore is not acceptable.*
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	

Section A7.4.3.3.2 Annex Point IIA XIII 2.3	Bioaccumulation in an appropriate invertebrate species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	According to the TNsG on data requirements, this test is required if there is a direct release to marine/brackish waters. There is no release to marine/brackish waters therefore a derogation to carry out this study is requested.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	*Accepted*	
Conclusion	*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		

Section A7.4.3.4 Annex Point IIIA XIII 2.4	Effects on reproduction and growth rate with an invertebrate species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	A derogation for a study of the effects on reproduction and growth rate on an appropriate invertebrate species is requested on the following grounds: a) Expected exposure to the aquatic compartment is very low. The EUSES 2.03 model (see IIB) indicates a local PEC in water of only 8.28E-07mg/l, from use of wax blocks in sewers. Using the LC50 for fish (A7.4.1) of 0.042mg/lt and an assessment factor of 1000 (TGD, partII) we get a PNEC of 4.2E-05. This gives a PEC/PNEC ratio of 0.02. This is well below the limit value of 1.0. A generic value of at least 80% for sewer connection to an STP in the EU (TGD, part II, appendix 12) shows that there is a low probability risk from untreated sewer water. In untreated sewer water we have a PEC of 4.03E-06mg/l (EUSES 2.03 _bypass STP). So the PEC/PNEC is 0.1.This latter scenario is for a realistic worse-case in a city with a severe rat infestation and would also require that the city was not connected to a STP, a highly unlikely combination. b) The use pattern for the wax bait block is in sewers and in and around buildings. The sewer case is discussed above. In and around buildings it is highly unlikely that the active would end up in surface water because it is in the form of a solid block and this is not going to be deposited into water. Any of the active that does go onto soil, due to for example excretion from the rat is not going to reach groundwater since the active has a very high Koc (average 50000, pesticide manual, 13th edition) indicative of being non-mobile in soil. c)Brodifacoum has very low solubility in water (<0.1mg/l, doc IIIA, also new study is to be conducted at SafePharm Ltd in August /Sept 2006 to obtain a more accurate value) and will strongly adhere to sediment (high Koc) making it non-bioavailable to aquatic organisms such as daphnia. This assumes it dissolves in the first place which is unlikely. d) Brodifacoum undergoes rapid direct photolysis (see Doc IIIA) with half-lives ranging between 23 and 366 minutes depending on the season. Also EPIWIN (Doc IIIA) predicts that the indirect photolysis half-life is 2.205 hours. Also note that the above PEC/PNEC is based on that of fish. Daphnia is shown to be less sensitive to fish, at least from acute studies (Daphnia EC50 at 48hr = 0.25mg/l compared to LC50 fish 0.042mg/l on measured concentrations, see updated summaries in Doc IIIA). Therefore there is likely to be even less risk to Daphnia than to fish. So, on the above basis, derogation is requested for a study of the effects on reproduction and growth rate on an appropriate invertebrate species on grounds of: limited exposure; use pattern; very low water solubility;	

Section A7.4.3.4 Annex Point IIIA XIII 2.4	Effects on reproduction and growth rate with an invertebrate species
	high Koc; rapid photolysis of the active. Furthermore it would be a waste of experimental animals and is considered an unnecessary animal test under the BPD directive.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*
Evaluation of applicant's justification	*The applicant justification is acknowledged in principle on regard of the absence of risk for water compartment and partition of the substance into sediments.* *This test is normally not required for PT14. Need of submission arises in the case of risk for the aquatic compartment or long term exposure, provided that invertebrates are the most relevant organisms for further testing (TNsG Ch. 3, part A, Fig. 3.1).* *Due to the absence of risk, based on the acute toxicity data, the Decision Table of Appendix 1 (TNsG) does not apply.* *Based on the results of the acute toxicity tests, invertebrates are considered the less relevant (10 fold less sensitive) of the aquatic organisms tested.*
Conclusion	*The study is not required*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.5.1 Annex Point IIIA XIII 3.4-	Effects on sediment dwelling organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [X] **Scientifically unjustified** [X]	
Limited exposure [X]	**Other justification** [ ]	
Detailed justification:	PNECsediment = 0.045 mg/kgwwt (see Doc II). This is now based on an updated aquatic LC50 for fish (see Doc IIIA), using measured concentration values as requested for A 7.4.1.1. The PEC sediment is predicted by the EUSES 2.03 model as 9E-04mg/kgwwt, obtained from normal use in sewers going to STP. This would give a sediment PEC/PNEC of 0.02. The substance is predicted to bind to soil, and therefore according to the TGD (pt II), the PEC/PNEC ratio is increased by a factor of 10 to give a PEC/PNEC ratio of 0.2. In the worse case scenario (see EUSES 2.03_bypass STP), a predicted value for PEC sediment is 4.38E-03 mg/kg $_{wwt}$. This would give a sediment PEC/PNEC of 0.097. The substance is predicted to bind to soil, and therefore according to the TGD, the PEC/PNEC ratio is increased by a factor of 10 to give a PEC/PNEC ratio of 0.97. Although this value is close to 1, it must be stressed that it represents a very worse case combination of a 21-day treatment using 30kg of rodenticide bait within the sewer system of a large population (10000 PE, from ESD, 2003 for PT14) heavily infested with rats and would require that the population was not connected to an STP. This is a very unlikely scenario in the EU. It is considered therefore that on the grounds of low exposure and no indicated risk form the environmental risk assessment, that derogation for performing this study is requested. There is no concern for the sediment compartment from this use scenario.	X
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	*This test is normally not required for PT14. Need of submission arises in the case of risk for the aquatic compartment or long term exposure, provided that sediment organisms are the most relevant fauna for further testing.*	
Conclusion	*No need of the study.*	

Section A7.4.3.5.1 Annex Point IIIA XIII 3.4-	Effects on sediment dwelling organisms
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.5.2 Annex Point IIIA XIII 3.4	Aquatic plant toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [X]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Compound is of very low water solubility and is not used in situations where aquatic plants are exposed. It is used in highly localised and limited areas such as sewers where aquatic plants do not exist, and it is not applied in a widespread fashion to extensive areas where leaching and run-off which might contaminate aquatic plants is possible.	X
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	*The arguments raised by the applicant are not pertinent as justification.* *Rather, RMS argues that this test is normally not required for PT14. Need of submission arises in the case of risk for the aquatic compartment or long term exposure, provided that aquatic plants are the most relevant organisms for further testing.*	
Conclusion	*No need of the study as there is not evidence that brodifacoum would be toxic to aquatic plants to a greater extent than to other aquatic organisms.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.5.1.1 Annex Point IIA VII7.4	Inhibition to microbial activity (terrestrial)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Although the use pattern does not exclude exposure to soil (via excretion from rat) the water solubility of brodifacoum is very low (<0.1mg/l, see doc IIIA). Therefore it should not affect bacteria in the pore water to any degree. Secondly, data from the ready biodegradation study (A 7.1.1.2.1) provides information on the toxicity of brodifacoum to micro-organisms in sewage sludge. According to the OECD 301B guideline, if the degradation of the reference is greater than 25% after 14 days, then the test substance is not considered to be inhibitory to the mico-organisms. In the study the degradation in the toxicity reference after 14 days was 60 % and therefore significantly more than the guideline required. Also the OECD 301B guideline states that the ready biodegradability study can take inoculum from a variety of places including sewage treatment plants and soil, therefore it seems reasonable that the results from the use of the sewage inoculum can be transferable to the micro-organisms found in the soil (at least in the pore water part of it). The microbial respiration inhibition study (A7.4.1.4) is not believed to be entirely invalid (please see comments from test lab, in the robust summary). A strong argument can be made that if there were no signs of inhibition when 1000mg/l of brodifacoum was present (in whatever form) in the study, it is not likely to be a problem at the predicted level of 0.011mg/kg in soil, in whatever form (see doc IIB). Also, it can be argued that in the above study, the brodifacoum was present in solution at its solubility limit of 0.1mg/l and still did not show any inhibition at that level. It is not likely that this concentration would be found in soil-pore water in the soil due to Brodifacoum's very high Koc (50000, pesticide manual) indicating a strong tendency to adhere to soil. In fact the risk assessment calculates a soil pore water value of 1.13E-04mg/l (ESD(2003) PT14 and TGD, 2003). The risk assessment (doc IIB/C) has also shown that there is no risk to the soil compartment with respect to earthworms. The PEC/PNEC obtained was 0.011. This was based on the calculated PEC (using the ESD, 2003) for soil (0.011mg/kg) as a result of direct and indirect release (via rat) of the active from bait blocks. The PNEC was based on an EC50 earthworm of >994mg/kg soil with an AF of 1000 giving >0.994mg/kg. Finally the area of use is limited to areas such as sewers and in and around buildings and it is not applied in a widespread fashion to extensive areas. For all of the above reasons a derogation to perform a study on inhibition to microbial inhibition (terrestrial) is requested.	
Undertaking of intended data submission [X]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has*	

Section A7.5.1.1 Annex Point IIA VII7.4	Inhibition to microbial activity (terrestrial)
	agreed on the delayed 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	*December 2006*
Evaluation of applicant's justification	*Accepted. This test is normally not required for PT14.*
Conclusion	*No need of the study*
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Official use only

		57 REFERENCE
57.1	**Reference**	XXXXX (2005) The toxitiy to *Eisenia foetida foetida* of Brodifacoum. XXXXX. Ref:ENV7010/120140
57.2	**Data protection**	Yes
57.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
57.2.2	Companies with access to data	PelGar International Ltd. Activa srl
57.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		58 GUIDELINES AND QUALITY ASSURANCE
58.1	**Guideline study**	Static test conditions according to SOP E260 based on OECD 207.
58.2	**GLP**	Yes
58.3	**Deviations**	No
		59 METHOD
59.1	**Test material**	As given in section 2
59.1.1	Lot/Batch number	Chemex reference: ECO120140
59.1.2	Specification	As given in section 2
59.1.3	Purity	100% (w/w)
59.1.4	Composition of Product	N/A
59.1.5	Further relevant properties	Insoluble, must be kept in cool, dry place.
59.1.6	Method of analysis	N/A
59.2	**Reference substance**	2-Chloracetamide (98%)
59.2.1	Method of analysis for reference substance	N/A
59.3	**Testing procedure**	
59.3.1	Preparation of the test substance	(see table A7_5_1_2-1)
59.3.2	Application of the test substance	Test substance added with fine sand and mixed.

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

59.3.3	Test organisms	(see table A7_5_1_2-2)
59.3.4	Test system	(see table A7_5_1_2-3)
59.3.5	Test conditions	(see table A7_5_1_2-4)
59.3.6	Test duration	14 days
59.3.7	Test parameter	Mortality
59.3.8	Examination	Number of alive animals was obsereved after 7 days
59.3.9	Monitoring of test substance concentration	No
59.3.10	Statistics	ToxCalc version 5.0 "Comprehensive Toxicity Data Analysis and Database Software" was used to calculate LD_{50} and confidence limits.

60 RESULTS

If appropriate, include tables. Sample tables are given below

60.1 Filter paper test Not performed

60.1.1	Concentration	N/A
60.1.2	Number/ percentage of animals showing adverse effects	N/A
60.1.3	Nature of adverse effects	N/A

60.2 Soil test *Non-entry field*

60.2.1	Initial concentrations of test substance	0, 318, 556 and 994 mg/kg
60.2.2	Effect data (Mortality)	(see table A7_5_1_2-5 and table A7_5_1_2-6)
60.2.3	Concentration / effect curve	N/A
60.2.4	Other effects	N/A

60.3 Results of controls

60.3.1	Mortality	No mortality seen
60.3.2	Number/ percentage of earthworms showing	N/A

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

		adverse effects	
	60.3.3	Nature of adverse effects	N/A
60.4	**Test with reference substance**		Performed
	60.4.1	Concentrations	32, 56, 99, 178, 316 mg/kg
	60.4.2	Results	LC_{50} 194 mg/kg dry weight

61 APPLICANT'S SUMMARY AND CONCLUSION

61.1	**Materials and methods**	Static test conditions according to SOP E260 based on OECD 207.
61.2	**Results and discussion**	No animals were killed by the test substance at any concewntration tested (<994 mg/kg). 0 out of 40 control organisms died during the study and this represents an acceptable level of health of the test organism maintained under test conditions.
61.2.1	LC_0	n/a
61.2.2	LC_{50}	>994 mg/kg* *The OECD 207 guideline "Earthworm Acute Toxicity Tests" specifies concentrations up to 1000mg/kg. Any minor deviations in test sample concentrations are derived from calculations based on actual sample mass weighed in the preparation of the test substrate. This difference between rangefinder and definitive test concentrations (1mg/kg) has little significance at a level of 1000mg/kg
61.2.3	LC_{100}	n/a
61.3	**Conclusion**	(see validity criteria summarized in table A7_5_1_2-7).
61.3.1	Other Conclusions	n/a
61.3.2	Reliability	1
61.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2006*
Materials and Methods	*Accepted*
Results and discussion	*Accepted*
Conclusion	*Accepted*
Reliability	*1*

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

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

Table A7_5_1_2-1: Preparation of TS solution

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

Table A7_5_1_1-2: Test organisms

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

Table A7_5_1_1-3: Test system

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

Table A7_5_1_2-4: Test conditions

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

Table A7_5_1_2-5: Mortality data

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

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

Table A7_5_1_2-6: Effect data

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

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

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

	fulfilled	Not fulfilled
Mortality of control animals < 10%	Yes	

Section A7.5.1.3 Annex Point IIIA XIII 3.4	Terrestrial plant toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum has a very high Koc (50000, pesticide manual, 13th ed.) indicative of very strong adherence to soil particles. It also has a very low water solubility (<0.1mg/l , see Doc IIIA). These two factors combined, mean that the available brodifacoum in solution that can be taken up by the roots of plants will be extremely low. The risk assessment (IIB) shows a calculated value of 0.011mg/kg active in soil from use of wax bait blocks in and around buildings (from ESD, 2003). Most of this will be partitioned to the soil, as explained above (Koc, water solubility), leaving a very low soil pore-water concentration, so providing further evidence of limited exposure. It is used in highly localised and limited areas such as sewers where plants do not exist, and it is not applied in a widespread fashion to extensive areas. It is not applied as a spray or vapour which might contaminate plants. Many years of use in a wide range of situations have shown no effect on plants. There is no evidence in the literature that Brodifacoum is toxic to plants. For the above reasons a derogation to perform a study on terrestrial plant toxicity is requested.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*December 2006*	
Evaluation of applicant's justification	*Accepted. This test is normally not required for PT14.*	
Conclusion	*No need of the study*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	

Section A7.5.1.3 Annex Point IIIA XIII 3.4	Terrestrial plant toxicity
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.2.1 Annex Point IIIA XIII 3.2	Reproduction study with other soil non-target macro-organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The risk assessment (IIB/C) shows that the PEC/PNEC for the soil compartment is 0.011. The PNEC is derived from the earthworm acute LC50 of > 994mg/kg (see Doc IIIA). This indicates no risk to the soil compartment which includes other non-target macro-organism (which is taken into account in the PNEC figure when an AF of 1000 is used) since the ratio is well below 1. The product wax bait block is used in highly localised and limited areas such as sewers where such creatures do not exist, and it is not applied in a widespread fashion to extensive soil areas. For the above reasons a derogation to perform this study is requested.	X
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*December 2006*	
Evaluation of applicant's justification	*This test is normally not required for PT14. Nevertheless, need of submission arises in the case of risk for the terrestrial compartment or direct or long term exposure, provided that earthworms (or other macro-organisms) are the most relevant species for further studies.* *As concern risk, the characterisation was carried out with both the PNEC obtained from the earthworm acute toxicity study and the PNEC derived through the equilibrium partition method, as required by the TGD (part II, 3.6.2). In both cases, no risk was observed.* *With regard to exposure, it is likely that the amount of brodifacoum entering soil when used in and around buildings, results in a long term exposure for soil organisms. However, there is evidence that earthworms (EC_{50} >994 mg/kg) are not the relevant organisms for further studies.*	
Conclusion	*No need of the study*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

Section A7.5.2.1 Annex Point IIIA XIII 3.2	Reproduction study with other soil non-target macro-organisms
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.2.2 Annex Point -	Long-term test with terrestrial plants	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Brodifacoum has a very high Koc (50000, pesticide manual, 13th ed.) indicative of very strong adherence to soil particles. It also has a very low water solubility (<0.1mg/l , see Doc IIIA). These two factors combined, mean that the available brodifacoum in solution that can be taken up by the roots of plants will be extremely low. The risk assessment (IIB) shows a calculated value of 0.011mg/kg active in soil from use of wax bait blocks in and around buildings (from ESD, 2003). Most of this will be partitioned to the soil, as explained above (Koc, water solubility), leaving a very low soil pore-water concentration, so providing further evidence of limited exposure. It is used in highly localised and limited areas such as sewers where plants do not exist, and it is not applied in a widespread fashion to extensive areas. It is not applied as a spray or vapour which might contaminate plants. Many years of use in a wide range of situations have shown no effect on plants. There is no evidence in the literature that Brodifacoum is toxic to plants. For the above reasons a derogation to perform a study on long-term tests with terrestrial plants is requested.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	*November 2006*	
Evaluation of applicant's justification	*This test is normally not required for PT14. Need of submission arises in the case of risk for the terrestrial compartment or long-term exposure, provided that terrestrial plants are the most relevant species for risk assessment.* *As concern risk, the characterisation was carried out with both the PNEC obtained from the earthworm acute toxicity study and the PNEC derived through the equilibrium partition method, as required by the TGD (part II, 3.6.2). In both cases, no risk was observed.* *Data on microbial activity (7.5.1.1) and terrestrial plant toxicity (7.5.1.3) were considered as not due; consequently there is not need to submit a long-term study on terrestial plants*	
Conclusion	*No need to submit a long-term study on terrestial plants.*	

Section A7.5.2.2 Annex Point -	Long-term test with terrestrial plants
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.5.3.1.1 Acute oral toxicity on birds
Annex Point IIIA XIII 1.1

Official use only

		62 REFERENCE
62.1	**Reference**	XXXXX (2005) Acute oral toxicity of Brodifacoum Technical of Japanese Quail. XXXXX. Study code: 04/903-115FU
62.2	**Data protection**	Yes
62.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force
62.2.2		PelGar International Ltd. Activa srl
62.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		63 GUIDELINES AND QUALITY ASSURANCE
63.1	**Guideline study**	OPPTS 850.2100
63.2	**GLP**	Yes
63.3	**Deviations**	*No * On comments from the RMS (during review), the test laboratory made the following statement: At the time of the study it was recognized that the Draft OECD Guideline 223, Avian Acute Oral Toxicity Test was often accepted in principle before being fully issued. It was agreed with the Sponsor that a compromise between the OPPTS and the draft OECD Guideline was a valid approach to demonstrating avian acute toxicity of the test item. At the time of the study, it was almost impossible to obtain bobwhite quail at a suitable quality and timing, so the Japanese quail, which is accepted by all the OECD draft and issued avian toxicity guidelines, was selected for this study.
		64 METHOD
64.1	**Test material**	As given in section 2
64.1.1	Lot/Batch number	04359
64.1.2	Specification	As given in section 2
64.1.3	Purity	100% w/w
64.1.4	Composition of Product	n/a
64.1.5	Further relevant properties	n/a
64.1.6	Method of analysis in the diet	n/a
64.2	**Administration of**	see table A7_5_3_1_1-1

Section 7.5.3.1.1 Acute oral toxicity on birds
Annex Point IIIA XIII 1.1

the test substance		
64.3	**Reference substance**	No
64.3.1	Method of analysis for reference substance	n/a
64.4	**Testing procedure**	*Non-entry field*
64.4.1	Test organisms	see table A7_5_3_1_1-2
64.4.2	Test system	see table A7_5_3_1_1-3
64.4.3	Diet	The birds were offered poultry pullet standard diet ad libitum. The birds were fed with the carrier (corn oil)
64.4.4	Test conditions	(see table A7_5_3_1_1-4)
64.4.5	Duration of the test	Single dose followed by 14 days observation
64.4.6	Test parameter	Mortality
64.4.7	Examination / Observation	Clinical observations, body weight measurements, food consumption and necropsy.
64.4.8	Statistics	LD50 was calculated by SPSS PC+ statistical software using probit analysis and was determined with 95% confidence limits. Statistical analysis was performed on the body weight data with SPSS statistical programme. Variance was determined by F-test.
	65 RESULTS	
65.1	**Limit Test / Range finding test**	Mortality at the control dose was 0/2. Mortality at 0.2 mg/kg-bw was 0/2 Mortality at 2 mg/kg-bw was 0/2 Mortality at 20 mg/kg bw was 1/2 Mortality at 200 mg/kg 2/2 Mortality at 2000 mg/kg 2/2
65.1.1	Concentration	Dosing volume at the time was 5ml/bw.
65.1.2	Number/ percentage of animals showing adverse effects	Not stated in report
65.1.3	Nature of adverse effects	Not stated in report
65.2	**Results test substance**	*Non-entry field*

Section 7.5.3.1.1 Acute oral toxicity on birds
Annex Point IIIA XIII 1.1

65.2.1	Applied concentrations	A constant dose volume of 5 mg/kg-bw was used
65.2.2	Effect data (Mortality)	(see table A7_5_3_1_1-5); report LD_{50} value (including 95 % c.l.) Male LD50 is 20mg/kg-bw Female LD50 18mg/kg-bw Male and Female LD50 19mg/kg-bw
65.2.3	Body weight	No test item effects were observed
65.2.4	Feed consumption	No changes in food consumption observed
65.2.5	Concentration / response curve	*Slope of the concentration-mortality curve*
65.2.6	Other effects	*Describe any other observations differentiating organisms in tests and controls (e.g. any signs of intoxication, abnormal behaviour); give details on macroscopic pathological examination of birds died during the observation period and animals sacrificed after test termination*
65.3	**Results of controls**	
65.3.1	Number/ percentage of animals showing adverse effects	See report
65.3.2	Nature of adverse effects	See report
65.4	**Test with reference substance**	Not applicable
65.4.1	Concentrations	
65.4.2	Results	

66 APPLICANT'S SUMMARY AND CONCLUSION

66.1 Materials and methods	OPPTS 850:2100
66.2 Results and discussion	The mean body weight gain and the food consumption of the birds did not show any toxicological important statistically changes compared to the control. During necropsy of birds that died the macroscopic changes observed were those that would be expected with any acute circulatory failure (as the cause of death)
66.2.1 LD_{50}	19 mg/kg-bw
66.3 Conclusion	The test was considered to have met the validity criteria, because the mortality in the control group was below 10% at the end of the test.

Section 7.5.3.1.1 Acute oral toxicity on birds
Annex Point IIIA XIII 1.1

66.3.1 Reliability 2

66.3.2 Deficiencies Yes

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2006*
Materials and Methods	*Accepted*
Results and discussion	*Accepted*
Conclusion	*Accepted*
Reliability	*2*
Acceptability	Acceptable. Following the clarifications provided by the applicant, minor deficiencies still remain (test species) not affecting the reliability of the study. No further testing for acute toxicity to birds is deemed necessary.
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_3_1_1-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	Yes, corn oil
Concentration of the carrier [% v/v]	5ml/kg bw
Other vehicle	No
Function of the carrier / vehicle	Solvent for test substance, facilitation of uptake and digestion.

Table A7_5_3_1_1-2: Test animals

Criteria	Details
Species/strain	Japanese quail
Source	Dezso Rokolya, Csavoly Hungary
Age (in weeks), sex and initial body weight (bw)	Birds were young adults, at least 16 weeks old at the start of the treatment. Birds were both male and female. Male bw- 145-181g Female bw 173-210g
Breeding population	*n/a*
Amount of food	*Total amount and amount per feeding*
Age at time of first dosing	See above
Health condition / medication	All birds were in apparent good health

Table A7_5_3_1_1-3: Test system

Criteria	Details
Test location	indoor in holding pens
Holding pens	*Type, size and material of pens; any furthter details of importance (use of cleaning detergents)*
Number of animals	30 per sex
Number of animals per pen [cm²/bird]	5
Number of animals per dose	5
Pre-treatment / acclimation	The birds were fasted for 15 hours prior to dosing, but drinking water was continuously supplied.
Diet during test	The birds were offered poultry standard diet ad libitum.
Dosage levels (of test substance)	Control, 3.1, 6.3, 12.5, 25.0 50.0 mg/kg-bw
Replicate/dosage level	*n/a*
Feed dosing method	Gavage
Dosing volume per application	A constant dose volume of 5 ml/kg-bw was used for all groups
Frequency, duration and method of animal monitoring after dosing	Clinical observations All test birds were observed during the first 60 mins after dosing and then at 3h, 4h and 5h after the treatment and then once each day for 14 day thereafter. Records were maintained of all mortality, signs of toxicity or abnormal behaviour. Food consumption Average estimated feed consumption was determined for each dosage group and the control for Days 0-3, 3-7, and 7-14. Feed consumption was determined by measuring the change in the weight of the feed provided to the birds over a given period of time. Necropsy The birds which died during the study were examined as soon as possible after death. All surviving birds were sacrificed and examined for gross pathological changes.
Time and intervals of body weight determination	Individual body weights were measured on Day (-14), (-7), 0, 3, 7 and 14 of the test.

Table A7_5_3_1_1-4: Test conditions (housing)

Criteria	Details
Test temperature	Temperature during the test ranged from 18.6-24.1°C
Shielding of the animals	
Ventilation	
Relative humidity	Ranged from 47-62%

Photoperiod and lighting	8 hours of light per day

Table A7_5_3_1_1-5: Mortality data after test termination

Test substance dosage level [mg/kg bw]	Mortality after test termination (.... days)									
	Total number per dose level					Percentage per dose level				
	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
Control	10					0				
3.1	10					0				
6.3	10					0				
12.5	10					40				
25	10					50				
50	10					100				
Temperature [°C]	18.6 – 24.1									
Relative humidity	47 – 62 %									

Table A7_5_3_1_1-6: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	YES	

Section A7.5.3.1.2 Annex Point IIIA XIII 1.2	Short-term toxicity on birds	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The risk assessment shows that there is already a very high risk of secondary poisoning to birds (based on a NOEC of 0.014mg/kg, 6 week reproduction study). The PEC/PNEC values for secondary poisoning to birds are in some cases many thousands. It is extremely unlikely that the NOEC from a short term study in birds is going to be thousands of times bigger than 0.014mg/kg. Therefore the conclusion is that even if a study was carried out, it would not change the conclusion of the risk assessment and therefore would be a waste of experimental animals and would be contrary to Article 8 of the BPD.	
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	*November 2006*	
Evaluation of applicant's justification	*The applicant justification is acknowledged.* *Furthermore, following the endorsement of the "Addendum relevant to Biocides to the TGD on Risk Assessment (Endorsed at the 23rd CA meeting Nov. 2006), PNECoral derivation for the primary and secondary poisoning assessment of anti-coagulant rodenticides", the need to test the short term toxicity of anticoagulant rodenticides ceases in the case that a* $NOEC_{reproduction,\ bird}$ *is available.*	
Conclusion	*A short term toxicity study on birds is not required, being a bird reproduction study available.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	

Section A7.5.3.1.2 Annex Point IIIA XIII 1.2	Short-term toxicity on birds
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.5.3.1.3 Effects on reproduction of birds
Annex Point IIIA XIII 1.3

			Official use only
		67 REFERENCE	
67.1	**Reference**	XXXXX (2005) Avian reproduction toxicity test of Brodifacoum Technical in Japanese Quails (Coturnix coturnix japonica) XXXXX. Study Code: 03/778-206FU	
67.2	**Data protection**	Yes	
67.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
67.2.2		PelGar International Ltd. Activa srl	
67.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
		68 GUIDELINES AND QUALITY ASSURANCE	
68.1	**Guideline study**	OECD 206 (1984) and draft OECD 206 (1998)* *It was agreed with the Sponsor that in the protocol the exposure period given in the Draft OECD 206 Test guideline (treatment period of 6 weeks) was more appropriate than the 20 weeks given in the old 1984 guideline). The test laboratory followed the time line of the more modern guideline, which they understood is common practice in current EU registrations.	
68.2	**GLP**	Yes	
68.3	**Deviations**	No	X
		69 METHOD	
69.1	**Test material**	As given in section 2	
69.1.1	Lot/Batch number	04359	
69.1.2	Specification	As given in section 2	
69.1.3	Purity	100 % w/w	
69.1.4	Composition of Product	n/a	
69.1.5	Further relevant properties	None	
69.1.6	Method of analysis	n/a	
69.2	**Administration of the test substance**	(see table A7_5_3_1_3-1)	

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Annex Point IIIA XIII 1.3

69.3	**Testing procedure**		*Non-entry field*	
	69.3.1	Test organisms	(see table A7_5_3_1_3-2)	
	69.3.2	Test system	(see table A7_5_3_1_3-3)	X
	69.3.3	Diet	Not stated in the report	X
	69.3.4	Test conditions	(see table A7_5_3_1_3-4)	
	69.3.5	Duration of the test	6 weeks exposure of test material. The test was continued for 14 days after the hatching period.	
	69.3.6	Test parameter	Mortality, clinical signs, feed consumption, gross macroscopic examination, organ weight and conditions and viability of the eggs.	
	69.3.7	Examination / Observation	(ee table A7_5_3_1_3-3	
	69.3.8	Statistics	The heterogeneity of variance between groups was checked by Barlett's homogeneity of variance test. Where no significant heterogeneity was detected, a one-way analysis of variance was carried out. If the obtained result was positive, Duncan's Multiple Range test was used to assess the significance of intergroup differences.	
			70 RESULTS	
			If appropriate, include tables. Sample tables are given below	
70.1	**Limit Test / Range finding test**		Not performed	
	70.1.1	Concentration		
	70.1.2	Number/ percentage of animals showing adverse effects	See report	
	70.1.3	Nature of adverse effects		
70.2	**Results test substance**		*Non-entry field*	
	70.2.1	Applied concentrations	0, 17.5, 35.0 and 70 µg/kg bw	
	70.2.2	Effect data (Mortality and reproductivity)	(see table A7_5_3_1_3-5) The reproductive NOEC is 0.1 µg/ml water concentration (nominal 17.5 µg/kg body weight, measures 14 µg/kg body weight.	X
	70.2.3	Body weight	There was no effect caused by any of the dose levels on body weights of adult birds following exposure. Body weights of the one-day old hatchlings showed significant and biologically important decreases in the high dose group compared to the	

Section 7.5.3.1.3 Effects on reproduction of birds
Annex Point IIIA XIII 1.3

control.

Body weights of the 14-day old survivors in the exposed groups increased in the high dose group compared to the control group but it may have occurred due to the significantly lower number of the hatchlings per surface area of cages.

70.2.4 Food consumption

During the 6 weeks exposure the average food consumption of the exposed animals showed a statistically significant decrease when compared to the control on weeks 3 and 4.

Considering the degree of decrease and the fact that the food consumption measurement is only an estimation in case of the birds, only the reduction in the high dose group is considered to be test item related.

70.2.5 Results of residue analysis

Nominal concentration	Mean of measured concentrations (mg/L)	Mean of measured concentration in percentage of the nominal
0.1	0.07	69%
0.2	0.18	87%
0.4	0.30	91%

70.2.6 Other effects

70.3 Results of controls

70.3.1 Number/ percentage of animals showing adverse effects

No clinical signs or macroscopic alterations could be seen in the control animals.

70.3.2 Nature of adverse effects

No adverse effects seen in control

71 APPLICANT'S SUMMARY AND CONCLUSION

71.1 Materials and methods

The objective of the study was to investigate the effect of six-week exposure of the test item on quails reproduction. Three different dose groups- 17.5, 35.0 and 70.0 (from the second week: 52.5) µg/kg- and one control group were tested in the study. Each dose group consisted of 12 male and 24 female birds, which were 9 weeks old at the beginning of the test. Test birds were housed indoors by dosage groups in pens. Each pen consisted of one male and females. Birds were exposed to drinking water containing the test item for a period of six weeks.

Effects on adult health, body weight gain, food consumption, pathological changes and reproductive parameters were monitored and evaluated (eggs laid, fertility, viability, hatchability, eggs cracked/broken, egg mass, egg shell thickness, embryonic death. Furthermore the 14-day old survivors, their body weights, food consumption and general state of health were observed.

X

Section 7.5.3.1.3 Effects on reproduction of birds
Annex Point IIIA XIII 1.3

		Mode of Treatment: Birds received the drinking water prepared with test item ad libitum. The applied concentration levels in the drink water: 0.1mg/L, 0.2mg/L and 0.4 (from the second week: 0.3) mg/L. These water concentrations are equal to nominal 17.5, 35.0 and 70.0 (52.5) µg/kg body weight (measured: 14, 33, 52 µg/kg body weigh) dose groups. Exposure to the test substance was for 6 weeks.	
71.2	**Results and discussion**		
71.2.1	NOEC	See conclusion	
71.3	**Conclusion**	Under the conditions of the study the reproductive NOEC is 0.1 µg/ml water concentration (nominal 17.5 µg/kg body weight. Measured 14 µg/kg body weight) and the value of the reproductive LOEC is 0.2 µg/ml water concentration (nominal 35 µg/kg body weight, measured 33 µg/kg body weight).	
71.3.1	Reliability	1	X
71.3.2	Deficiencies	None	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	*November 2006*

Section 7.5.3.1.3 Effects on reproduction of birds
Annex Point IIIA XIII 1.3

Materials and Methods	*3.3.2 Test system:* *OECD 206 does not mention ethanol as a suitable carrier. Since the interference of ethanol with anticoagulants is known in human medicine (Chung-Eun et al., Investigations of the Effects of Ethanol on Warfarin Binding to Human Serum Albumin. Journal of Biomedical Science 2000;7:114-121), the applicant was asked to clarify whether ethanol was given to control birds and provide evidence of non interference of ethanol with brodifacoum.* *The applicant acknowledged the non administration of ethanol to control birds and justified the use of ethanol because (see alsoTable A7_5_3_1_3-1):* *- it is one of the few suitable solvents for this class of chemistry,* *- there is an experienced lack of toxicity and it is readily metabolised by birds and mammals at the levels used.* *The applicant furthermore agreed that high levels of ethanol can interfere with warfarin metabolism but evidenced that no metabolites of warfarin are common to Brodifacoum and the metabolic pathways are not the same. Also, the protein binding of warfarin is different to that of modern anti-coagulants so no comparisons can be made regarding effects of ethanol. In the very unlikely event of an interference, such as that observed for warfarin, the test system would be more sensitive and would hence give a more conservative safety evaluation. Only the opposite would be of any regulatory concern.* *As a conclusion, although the non administration of ethanol to control birds is in any case a deficiency of the test, the reasons provided as justification would indicate that results do not underestimate toxicity.* *Applied concentrations: The treatment was made via drinking water. The conversion from mg/l to µg/kg body weight is not scientifically supported.*
Results and discussion	*4.2.2 Effect data:* *NOEC is based on a 6 weeks exposure time instead of the 20 weeks required by OECD 206 (version 1984, adopted); consequently, the applicant was asked to clarify such deviation.* *The applicant agreed that the exposure time is shorter than that indicated by the OECD guideline 206 (version 1984, adopted) but stated on animal welfare grounds, that a 20 week study would inevitably end up killing some of the parent birds before the end of the 20 weeks even at low doses, due to its high toxicity to birds and very bioaccumulative (vB) nature. In this respect, reference has been made also to the Draft OECD Test guideline 206 (1998) which in fact indicates a treatment period of 6 weeks, as appropriate.* *The RMS acknowledges the difficulty of testing a very toxic and bioaccumulative substance on long term.*
Conclusion	*Under the conditions of the study, the reproductive 6 weeks NOEC is 0.1 mgll water concentration and the value of the reproductive LOEC is 0.2 mg/l water concentration .*
Reliability	*3*
Acceptability	*Not acceptable. Exposure was via drinking water instead of food and the duration was shorter than recommended by OECD 206 (6 weeks instead of 20weeks).* *At the TMIII09 it was agreed that the lack of a reliable study would not results in a data gap..*