Syngenta	Brodifacoum	February 2004

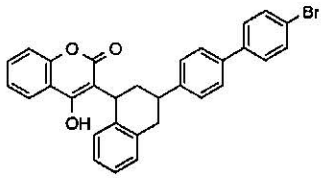
Doc IIIA/Section 1 Applicant

BPD Data Set IIA/Annex Point I

			Official use only
1.1	**Applicant**	**Name:** Syngenta Limited **Address:** European Regional Centre, Priestley Road, Surrey Research Park, Guildford, Surrey, GU2 7YH, United Kingdom **Telephone:** [redacted] **Fax number:** [redacted] **Contact name:** [redacted]	
1.2	**Manufacturer of Active Substance** (if different)	[redacted] **Name:** [redacted] **Address:** [redacted] **Telephone:** [redacted] **Location of manufacturing plant:** [redacted]	
1.3	**Manufacturer of Product(s) (if different)**	[redacted] **Name:** [redacted] **Address:** [redacted]	

Doc IIIA, Section 2 Identity of Active Substance

BPD Annex Point II

Subsection (Annex Point)			Official use only
2.1	**Common name (IIA2.1)**	Brodifacoum	
2.2	**Chemical name (IUPAC) (IIA2.2)**	3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-napthyl]-4-hydroxycoumarin	X
2.3	**Manufacturer´s development code number(s) (IIA2.3)**	PP 581, WBA8117	
2.4	**CAS No and EC numbers (IIA2.4)**		
2.4.1	**CAS-No**	[56073-10-0]	
2.4.2	**EC-No**	EC No: 259-980-5, EINECS No: 289-980-5	X
2.4.3	**Other**		
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**		
2.5.3	**Molecular mass**	523.4	
2.6	**Method of manufacture of the active substance (IIA2.1)**	***Confidential Data***	
2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**	>/= 950 g/kg (>/= 95 % w/w)	X

Doc IIIA, Section 2 Identity of Active Substance

BPD Annex Point II

2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	Please refer to separate documents: IIIA2_8-1B, IIIA2_8-2B, IIIA2_8-3B, IIIA2_8-4B, IIIA2_8-5B ('Identity of impurities and additives').	
2.8.1	**Isomeric composition**	Not relevant.	X
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA2.9)**	Not relevant.	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A2.10

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

Production of active substance

[redacted]

Formulation of the product

[redacted]

ii) Workplace description

Production of active substance

[redacted]

Formulation of the product

- [redacted]

[redacted]

iii) Inhalation exposure

Production of active substance

[redacted]

Formulation of the product

[redacted]

iv) Dermal exposure

Production of active substance

[redacted]

Formulation of the product

[redacted]

2.10.1.2 Intended use(s)

1. Professional Users

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

i) Description of application process

The following tasks are undertaken when using rodenticidal baits:

Decanting of bait from bulk containers may occur;

Loading of bait points with bait;

Topping-up bait points when bait has been consumed; and

Clean-up and disposal of spent baits at the end of the treatment.

'Loading of bait points with bait' and 'Topping-up bait points when bait has been consumed' are essentially identical tasks.

ii) Workplace description

Not relevant for a rodenticide as the 'workplace' is each treatment site. Although the nature of the treatment sites will vary, the tasks undertaken when using a rodenticidal bait are the same.

iii) Inhalation exposure

Potential exposure to professional and non-professional users during application of anticoagulant rodenticide products was simulated by measurement of dermal (hands) and inhalable (air concentration in the breathing zone) residues during a series of representative tasks. (*Chambers JG and Snowdon PJ, 2004. Study to determine potential exposure to operators during simulated use of anticoagulant rodenticide baits. Synergy Laboratories Limited, Study Number SYN/1302, January 2003 (unpublished) [BR-959-0151]*). The tasks elaborated at i) Description of application process, above, were investigated

The study showed inhalational exposures to be significant only when decanting with a mean residue in the breathing zone of <22.8 mg/m^3 product, when using a representative worst case grain bait.

iv) Dermal exposure

Potential exposure to professional and non-professional users during application of anticoagulant rodenticide products was simulated by measurement of dermal (hands) and inhalable (air concentration in the breathing zone) residues during a series of representative tasks. (*Chambers JG and Snowdon PJ, 2004. Study to determine potential exposure to operators during simulated use of anticoagulant rodenticide baits. Synergy Laboratories Limited, Study Number SYN/1302, January 2003 (unpublished) [BR-959-0151]*). The tasks elaborated at i) Description of application process, above, were investigated

Using a representative worst case grain bait, the study showed the following dermal residues:

Decanting product	<221.8 mg product per manipulation
Loading bait into bait points and placing bait points	<10.06 mg product per manipulation
Clean-up and bait disposal	<12.59 mg product per manipulation

2. Non-professional Users including the general public

(i) via inhalational contact	The study referred to above showed inhalational exposures to be significant only when decanting. However, it is not general practice for non-professional users to decant bait. Secondary exposure via the inhalation route is expected to be negligible as the formulation is not volatile and difenacoum has a vapour pressure of 6.7×10^{-9} to 5.4×10^{-14} Pa.
(ii) via skin contact	Using a representative worst case grain bait, the study referred to above showed the following dermal residues: Loading bait into bait points and placing bait points: <10.06 mg product per manipulation Clean-up and bait disposal: <12.59 mg product per manipulation Secondary exposure via the dermal route is expected to be negligible due to the requirement to place baits in inaccessible locations.
(iii) via drinking water	The manner of use of rodenticides precludes contamination of ground and surface water.
(iv) via food	The manner of use of rodenticides precludes contamination of food and feedingstuffs.
(v) indirect via environment	The manner of use of rodenticides at discrete bait points precludes indirect exposure *via* the environment.

2.10.2 Environmental exposure towards active substance

2.10.2.1 Production

(i) Releases into water	[redacted]
(ii) Releases into air	[redacted]
(iii) Waste disposal	[redacted]

2.10.2.2 Intended use(s)

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**
Affected compartment(s): water sediment air soil Predicted concentration in the affected compartment(s)	[redacted]

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

	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 applicants 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*
Remarks	

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	Dermal Exposure concentration
Application MG3/PT14 Professional use and non-professional use	Decanting bait; Placing bait; Clean-up and disposal of bait.	None required, but the use of gloves is recommended for protection against rodent-borne diseases			Simulated Operator Exposure study	<221.8 mg product per manipulation <10.06 mg product per manipulation <12.59 mg product per manipulation

Table A2.10_2: PEC in surface water, sewage treatment plant, ground water and sediment

Compartment/Scenario	Worst case[1]	Realistic worst case (refined)[2]	Normal use (refined)[3]
SEWER			
Surface water			
Sewer – direct release of sewage water	1.59×10^{-6} mg/l (0.0016 µg/l)	1.32×10^{-6} mg/l (0.0013 µg/l)	Realistic worst case reflects normal use
Sewer – during emission from STP	2.38×10^{-7} mg/l (0.00024 µg/l)	1.98×10^{-7} mg/l (0.0002 µg/l)	
Sediment			
Sediment - direct release of sewage water	3.17×10^{-4} mg/kg	2.64×10^{-4} mg/kg	Realistic worst case reflects normal use
Sediment - during emission from STP	4.75×10^{-5} mg/kg	3.96×10^{-5} mg/kg	
Sewage treatment plant			
PECstp (Clocal effluent)	1.61×10^{-5} mg/l	1.34×10^{-5} mg/l	Realistic worst case reflects normal use
Groundwater/soil porewater			
Through application of sewage sludge & aerial deposition	4.84×10^{-10} mg/l (4.8×10^{-7} µg/l)	4.03×10^{-10} mg/l (4×10^{-7} µg/l)	Realistic worst case reflects normal use
WASTE DUMP			
Groundwater/porewater	4.59×10^{-5} mg/l	2.29×10^{-5} mg/l	3.15×10^{-6} mg/l
IN AND AROUND BUILDINGS			
Groundwater/porewater	5.79×10^{-5} mg/l	5.42×10^{-5} mg/l	5.22×10^{-5} mg/l
OPEN AREAS[4]			
Groundwater/porewater	5.35×10^{-4} mg/l	No refinement (for excretion) possible.	4.50×10^{-4} mg/l[4]

1 Worst case, using defaults for usage in ESD. Koc derived from adsorption/desorption study (9155) at pH 7. No refinement for metabolism possible, as brodifacoum is metabolized very slowly.

2 Realistic worst case. Koc 9155 at pH 7. Refinements: 45% of dose is released via urine/faeces.

3 Normal use, using label recommendations for usage. Koc 9155 at pH 7. Refinements: 45% of dose is released via urine/faeces.

4 Realistic refinement to worst case ('normal use') is that bait is only used in containers, therefore 1% direct release during application has been assumed, as for the 'in and around building' scenario.

Table A2.10_3: PEC in soil

Compartment/Scenario	Worst case[1]	Realistic worst case (refined)[2]	Normal use (refined)[3]
SEWER			
Soil			
Sewer - application of sewage sludge & aerial deposition.	7.83 x 10^{-8} mg/kg	6.5 x 10^{-8} mg/kg	Realistic worst case reflects normal use
WASTE DUMP			
Soil	7.41 x 10^{-3} mg/kg	3.71 x 10^{-3} mg/kg	5.10 x 10^{-4} mg/kg
IN AND AROUND BUILDINGS			
Soil	9.36 x 10^{-3} mg/kg	8.77 x 10^{-3} mg/kg	8.44 x 10^{-3} mg/kg
OPEN AREAS[4]			
Soil	8.65 x 10^{-2} mg/kg	No refinement (for excretion) possible.	7.27 x 10^{-2} mg/kg[4]

1 Worst case, using defaults for usage in ESD. No refinement for metabolism possible, as brodifacoum is metabolized very slowly.

2 Worst case, using defaults for usage in ESD. Refinement: 45% of dose is released via urine/faeces. No refinement for metabolism possible as brodifacoum is metabolized very slowly.

3 Normal use, using label recommendations for usage. Refinement: 45% of dose is released via urine/faeces.

4 Realistic refinement to worst case for normal use is that bait is only used in containers, therefore 1% direct release during application has been assumed, as for the 'in and around buildings' scenario. Fraction of product released to soil during use - as default in ESD.

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum

Doc IIIA Subsection No.	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. 1	The capillary method, in accordance with OECD Guideline 102.	[redacted]	**Result:** 232°C with decomposition. **Pressure:** Not applicable.	The temperature curves produced during the melting point determination were atypical for pure material and it was suspected that slight decomposition was occurring. This was confirmed by comparison of melted and unmelted test substance chromatograms produced by HPLC, which showed the presence of decomposition products in the melted sample.	[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. [redacted]	
Melting pt. 2	The capillary method, in accordance with OECD Guideline 102.	[redacted]	**Result:** 201-205°C with decomposition. **Pressure:** Not applicable.	The melting point obtained here for the technical grade active ingredient (TGAI) is lower, as expected, than that obtained for the pure material (see above results). The melting point curves for the TGAI obtained here were somewhat atypical and it was suspected that slight decomposition was occurring. This was confirmed by comparison of melted and unmelted test substance chromatograms produced by HPLC.	[redacted]	[redacted]	Wollerton C, Husband R (1991). Brodifacoum TGAI: Physico-Chemical Data File. [redacted]	
3.1.2 Boiling point								
Boiling pt. 1			**Result:** **Pressure:**	Not applicable. Brodifacoum is a solid at room temperature and a melting point measurement is provided.				X

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum							
Doc IIIA Subsection No.	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
Boiling pt. 2								
3.1.3 Bulk density/ relative density								
Rel. density 1	Pycnometer method, in accordance with OECD Guideline 109.	[redacted]	**Result:** 1.42 g/cm^3 **Test temp:** 25°C		[redacted]	[redacted]	Wollerton C, Husband R (1991). Brodifacoum TGAI: Physico-Chemical Data File. [redacted]	X
3.2 Vapour pressure (IIA3.2)								
Vapour pressure 1	Gas saturation method, in accordance with OECD Guideline 104.	[redacted]	**Temperature:** 20°C **Result:** $<<10^{-9}$ kPa ($<<10^{-8}$ mmHg) ($<<10^{-11}$ atm)	The calibration level was taken to be the limit of determination (LOD) and was such that given the collection time of 18600 minutes, a vapour pressure of $<10^{-9}$ kPa could be determined at the test temperature. No attempt was made to obtain the actual limit of determination of the method, as clearly the above values fall below the cut off point of $<10^{-8}$ kPa at 20°C beyond which vapour pressure ceases to be of environmental significance.	[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. [redacted]	
Vapour pressure 2								

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum

Doc IIIA Subsection No.		Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Official use only
3.2.1	Henry's Law Constant (Pt. I-A3.2)	Calculated according to: $H = P_{vp} / S$, where H = Henry's Law constant (Pa m^3 mol^{-1}) P_{vp} = vapour pressure (Pa at 25°C) S = water solubility (mol m^{-3})	Not applicable.	**Result:** $<<2.18 \times 10^{-3}$ Pa m^3 mol^{-1} at pH 7, and $<<5.23 \times 10^{-5}$ Pa m^3 mol^{-1} at pH 9. Calculated using the following values for brodifacoum: **Molar mass** (Relative Molecular Mass) = 523.4 g mol^{-1} $\mathbf{P_{vp}} = <<10^{-6}$ Pa **S** = 0.24 mg/l **at pH 7** and 20°C $= 4.585 \times 10^{-4}$ mol m^{-3} **S** = 10 mg/l **at pH 9** and 20°C $= 1.911 \times 10^{-2}$ mol m^{-3} **Therefore, at pH 7, H** = 10^{-6} Pa / 4.585×10^{-4} mol m^{-3} $= <<2.18 \times 10^{-3}$ Pa m^3 mol^{-1} **and, at pH 9, H** = 10^{-6} Pa / 1.911×10^{-2} mol m^{-3} $= <<5.23 \times 10^{-5}$ Pa m^3 mol^{-1}	The vapour pressure figure used was 10^{-6} Pa which was actually reported as $<< 10^{-6}$ Pa due to the limit of determination of the method (see above).	[redacted]	[redacted]	Not applicable (calculation given).	
3.3	Appearance (IIA3.3)								
3.3.1	Physical state	Determined by visual inspection at ambient temperature.	[redacted]	Fine powdery solid.		Y	1	Wollerton C, Husband R (1991). Brodifacoum TGAI: Physico-Chemical Data File. [redacted]	

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum

Doc IIIA Subsection No.	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.2 Colour	Determined by visual inspection of the test substance illuminated by standard laboratory fluorescent lighting.	[redacted]	Cream.		[redacted]	[redacted]	Wollerton C, Husband R (1991). Brodifacoum TGAI: Physico-Chemical Data File. [redacted]	
3.3.3 Odour				Not assessed: toxic inhalation hazard (Please refer to Section 6.1.3 of Doc IIIA).				
3.4 Absorption spectra (IIA3.4)								
UV/VIS	Spectrophotometric method in accordance with OECD Guideline 101. A Perkin-Elmer 552 UV spectrophotometer was used. A solution of the test substance having a nominal concentration of 1 x 10^{-5} M was prepared in methanol and scanned between 400 nm and 190 nm using methanol as a reference.	[redacted]	The spectrum was consistent with the accepted structure of brodifacoum (see attached UV spectrum).		[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File [redacted]	
IR	An infrared (IR) spectrum was produced using a Perkin-Elmer 1720 Fourier transform IR spectrophotometer. A KBr disc containing 0.6% w/w brodifacoum was prepared and scanned between 4000 and 450 cm^{-1} using air as a reference.	[redacted]	The spectrum was consistent with the accepted structure of brodifacoum (see attached IR spectrum).		[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File [redacted]	

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum							
Doc IIIA Subsection No.	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Relia bility**	**Reference**	**Official use only**
							[redacted]	
NMR	A proton nuclear magnetic resonance (NMR) spectrum was produced using a Jeol GSX270 spectrometer. The sample was run as a 3% w/v solution in deuterated chloroform.	[redacted]	The spectrum was consistent with the accepted structure of brodifacoum (see attached NMR spectrum).		[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File [redacted]	
MS	An electron impact (EI) mass spectrum (MS) was produced by direct insertion using a VG TRIO-1 mass spectrometer.	[redacted]	The spectrum was consistent with the accepted structure of brodifacoum (see attached MS spectrum).		[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File [redacted]	

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum

Doc IIIA Subsection No.	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.5 Solubility in water (IIA3.5)								
Water Solubility 1	Generator column method, in accordance with EPA Guideline CG-1510.	[redacted]	**Result** / **pH** / **Temp (°C)** 0.0038 mg/l / 5.2 / 20 0.24 mg/l / 7.4 / 20 10 mg/l / 9.3 / 20	No breakthrough of brodifacoum occurred *ie* no test substance passed through the extractor column thereby confirming the validity of the results.	[redacted]	[redacted]	Wollerton C, Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File [redacted]	X
Water Solubility 2	Flask method, in accordance with OECD Guideline 105.	[redacted]	**Result** / **pH** / **Temp (°C)** <0.005 mg/l / 4 / 20 <0.005 mg/l / 7 / 20 <40 mg/l / 9 / 20 <0.005 mg/l / Milli-RP Water / 20		[redacted]	[redacted]	Craig WB (2000). Brodifacoum - Physico-Chemical Testing with Brodifacoum: Water Solubility. [redacted]	
3.6 Dissociation constant (pKa)	PETE database estimation/calculation		pKa value of 4.5	Not determined experimentally as brodifacoum is very poorly soluble in water.			Nicholls, P. Physico-chemical Evalution. The Environmental (PETE). Version 3, January 1997. (www.rothamsted.bbsrc.ac.uk/bch/pcgroup/pete.html)	
3.7 Solubility in	Flask method, in accordance with OECD Guideline 105.	[redacted]	**Solvent** / **Brodifacoum solubility** Test temperature was approximately 20°C.		[redacted]	[redacted]	Wollerton C, Husband R (1991).	

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum

Doc IIIA Subsection No.		Method	Purity/ Specification	Results		Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Official use only
	organic solvents, including the effect of temperature on solubility (IIIA3.1)		[redacted]	Hexane	0.088 g/l				Brodifacoum TGAI: Physico-Chemical Data File. [redacted]	
				Toluene	7.2 g/l					
				Dichloromethane	50 g/l					
				Acetone	23 g/l					
				Ethyl acetate	12 g/l					
				Acetonitrile	3.2 g/l					
				Methanol	2.7 g/l					
3.8	Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)					Not applicable. Solvents not used in the brodfiacoum containing rodenticidal products, which are ready-to-use cereal based baits.				

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum							
Doc IIIA Subsection No.	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Relia bility**	**Reference**	**Official use only**
3.9 Partition coefficient n-octanol/water (IIA3.6)								
Partition coefficient 1	The partition coefficient was obtained by calculation using the CLOGP algorithm of Hansch and Leo. The structure of brodifacoum was entered in SMILES notation and the computer program calculated a value using the principlel of fragment and factor contributions.	Not applicable.	**Result**: 8.5 **Temperature:** Not applicable **pH:** Not applicable	An initial estimation based on the structure of brodifacoum indicated that the Log P_{ow} was likely to be greater than 8. This extreme value is outside the range of both the Shake Flask and HPLC Methods, neither of which were therefore attempted. Attempts to determine a value for Log P_{ow} using the Generator Column Method were unsuccessful as, although the experimental work showed that the partition coefficient was >7, it was not possible to determine an actual value. Therefore, a calculation method was used to establish the reported value.	[redacted]	[redacted]	Wollerton C, Husband R (1990). Brodifacoum: Octanol-Water Partition Coefficient [redacted]	
Partition coefficient 2	An estimation of the log Kow species present at pH 7 was made using a well known and frequently used log-log relationship between Kow and Koc: [log Koc=0.5 log Kow + 0.9].	Not applicable	**Result**: 6.12 **Temperature:** Not applicable **pH:** Not applicable	The log Kow of the undissociated species has been estimated using chemical structure (SMILES notation) to be 8.5 (see above, this table). Since reliable Koc data are available from studies conducted at environmentally-relevant pH, an estimation of the log Kow of the species present at pH 7 can be made using a well known and frequently used log-log relationship between	[redacted]	[redacted]	Briggs, G.G. (1981). Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors and the	

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum							
Doc IIIA Subsection No.	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Relia bility**	**Reference**	**Official use only**
				Kow and Koc [log Koc = 0.5 log Kow + 0.9[1]]. Using this relationship, the Kow at pH 7 is estimated as 6.12.			parachor. *J. Agric. Food Chem.*, **29**. pp 1050-1059.	
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	CIPAC MT 46: accelerated storage test of the technical grade active ingredient (TGAI). This included storage of TGAI samples for 14 days in a freezer at -20°C or for 14 days in a temperature controlled oven at +54°C.	[redacted]	The results showed that brodifacoum is stable for at least 14 days at 54°C.	It is concluded from the results of the accelerated storage test that brodifacoum active substance is stable to heat and air. This test result confirms the 30 years in-use experience of brodifacoum and it's formulated products. The additional results of the melting point determination of the active substance indicates that decomposition only occurs at or above the melting point temperature (232°C).	[redacted]	[redacted]	Wollerton C, Husband R (1991). Brodifacoum TGAI: Physico-Chemical Data File. [redacted]	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)				Not applicable as 30 years in-use experience with brodifacoum has shown that it is not flammable and does not evolve any flammable gases.				X
	Flammability of solids – EC A.10.	[redacted]	Flammability: Not a flammable solid.	Syngenta initially stated: Not applicable as 30 years in-use experience with brodifacoum has shown that it is not flammable. However, the RMS required	[redacted]	[redacted]	Garofani, S. (2001). ChemService S.r.l Milan, Italy.- Brodifacoum detremination of the flammability.	X

[1] 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.

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum							
Doc IIIA Subsection No.	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Relia bility**	**Reference**	**Official use only**
				Syngenta to undertake a study, the result of which confirmed this statement.			[redacted]	
3.12 Flash-point (IIA3.9)				Not applicable as brodifacoum is a solid and is not flammable.				
3.13 Surface tension (IIA3.10)				Not determined as brodifacoum is very sparingly soluble in water (see Section 3.5 above) and therefore is not expected to exhibit any surface activity.				
3.14 Viscosity (-)			**result: temperature:**	Not applicable. Brodifacoum is a solid at normal temperature and pressure.				
3.15 Explosive properties (IIA3.11)				The absence of certain reactive groups in the structural formula *[cf Ref: Brethrick, Handbook of Reactive Chemical Hazards, Butterworths, London 1979]*, and its oxygen balance, establish beyond reasonable doubt that brodifacoum is incapable of decomposing, forming gases, or releasing heat very rapidly.				
3.16 Oxidizing properties (IIA3.12)				Examination of the structural formula establish beyond reasonable doubt that brodifacoum is incapable of reacting exothermically with a combustible material (see Section 3.15 above).				
3.17 Reactivity towards container material				Experience in use indicates no reactivity of brodifacoum towards container materials, including polyethylene, high density				

Doc IIIA / Section 3 BPD Data Set IIA / Annex Point III	Physical and Chemical Properties of Active Substance Brodifacoum							
Doc IIIA Subsection No.	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
(IIA3.13)				polyethylene, polypropylene, steel and stainless steel, as required by UN modal transport regulations.				

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/03/2007
Materials and methods	[redacted]

3.2 [redacted]

3.3 [redacted]

3.4 [redacted]

3.8 [redacted]

[redacted]

3.9

[redacted]

3.10

[redacted]

3.11

[redacted]

3.12

[redacted]

3.13

[redacted]

3.14

[redacted]

3.15

[redacted]

3.16

[redacted]

Conclusion

[redacted]

3.2

[redacted]

3.2.1

[redacted]

3.3

[redacted]

[redacted]

3.8 [redacted]

[redacted]

3.11 [redacted]

3.12 [redacted]

3.13 [redacted]

3.14 [redacted]

3.15 [redacted]

3.16 [redacted]

Reliability [redacted]

Acceptability [redacted]

[redacted]

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	

Doc IIIA / Section 4.1
BDP Data Set IIA / Annex Point IV.4.1

Analytical Methods for Detection and Identification

Analytical method for the detection of the active substance brodifacoum in technical grade material

Official use only

1 REFERENCE

1.1 Reference

Analytical Method No. 3455A, which is described in the following report:

Davidson AJ (2000). Brodifacoum - Product Chemistry of Brodifacoum: Analytical Profile of 5 Batches. [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No guideline available, but the method was developed to fulfil the requirements of the Biocidal Products Directive (98/8/EC) and is in accordance with principles given in the SANCO/3030/99 rev 4 guidance document.

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

3.1.2 Cleanup

3.2 Detection

3.2.1 Separation method [redacted]

3.2.2 Detector [redacted]

3.2.3 Standard(s) [redacted]

3.2.4 Interfering substance(s) [redacted]

Doc IIIA / Section 4.1
BDP Data Set IIA / Annex Point IV.4.1

Analytical Methods for Detection and Identification

Analytical method for the detection of the active substance brodifacoum in technical grade material

[redacted]

3.3 Linearity

3.3.1 Calibration range

[redacted]

3.3.2 Number of measurements

[redacted]

3.3.3 Linearity

[redacted]

3.4 Specifity: interfering substances

[redacted]

3.5 Recovery rates at different levels

Nominal Weight Assayed (mg)	Found Weight of total brodifacoum (mg)	[redacted]	[redacted]	Coefficient of Variation (%)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

3.5.1 Relative standard deviation

[redacted]

3.6 Limit of determination

[redacted]

3.7 Precision

Doc IIIA / Section 4.1
BDP Data Set IIA / Annex Point IV.4.1

Analytical Methods for Detection and Identification

Analytical method for the detection of the active substance brodifacoum in technical grade material

3.7.1 Repeatability

Brodifacoum isomer	Concentration of Sample (µg/ml)	[redacted]	Found mean peak area ratio (brodifacoum isomer to internal standard)	Coefficient of variation (%)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

3.7.2 Independent laboratory validation

Not available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

[redacted] The technical grade brodifacoum samples are then quantified using HPLC with u.v. detection at 254 nm.

[redacted]

4.2 Conclusion

4.2.1 Reliability

[redacted]

4.2.2 Deficiencies

[redacted]

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

Doc IIIA / Section 4.1	**Analytical Methods for Detection and Identification**
BDP Data Set IIA / Annex Point IV.4.1	Analytical method for the detection of the active substance brodifacoum in technical grade material

Date	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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	

[redacted]

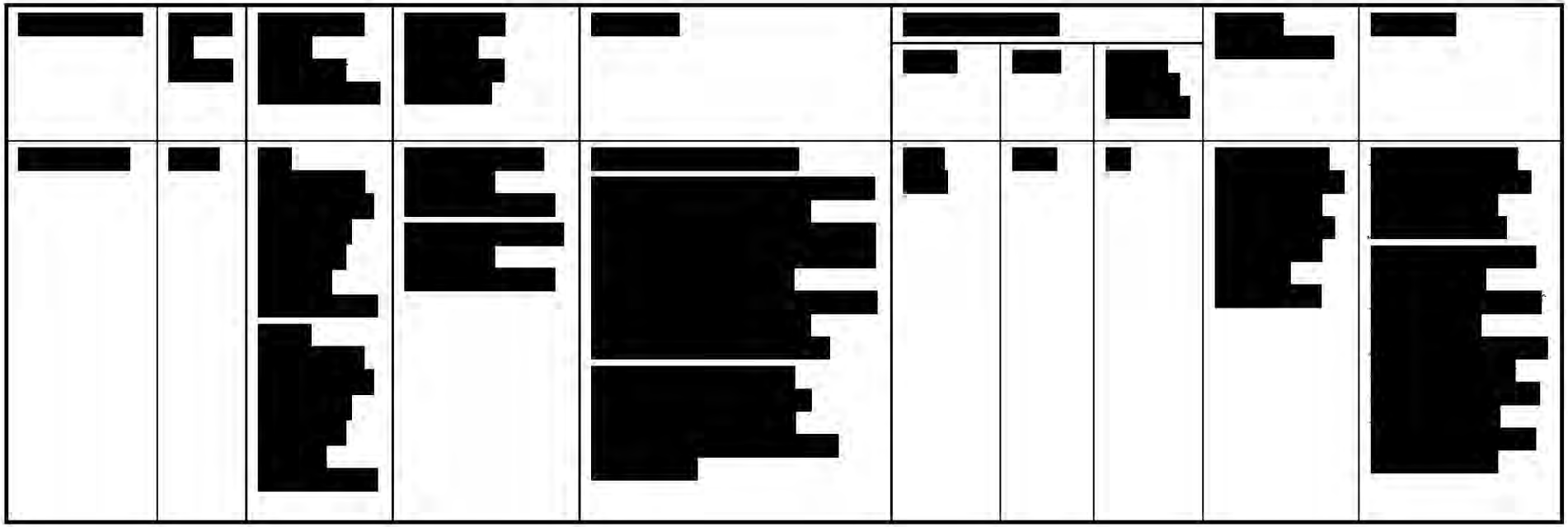


Table 2: Summary of impurity screening results on an area percent basis

Peak retention time (min)[1]	Peak number[2]	% Area for each peak detected in samples of a technical grade brodifacoum (from 5 batches of production)
[redacted]		

Notes:

[1] *cis*-brodifacoum retention time was *ca* 8.5 mins, and *trans*-brodifacoum retention time was *ca* 9.75 mins.

[2] The following impurities were confirmed by matching peaks of reference standards of authentic materials:

[redacted]

Doc IIIA / Section 4.2 Analytical Methods for Detection and Identification

BDP Data Set IIA / Annex Point IV.4.2

Analytical method for the detection of the active substance brodifacoum in water

Official use only

1 REFERENCE

1.1	**Reference**	Craig WB (2000). Brodifacoum - Physico-Chemical Testing with Brodifacoum: Water Solubility. Inveresk Research Report No: 18799. GLP (unpublished) [BR-959-0079]. Experimental work carried out between April 2000 and May 2000.
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No guideline available, but the method is in accordance with the principles given in the SANCO/3029/99 rev 4 guidance document.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	For neutral and low pH water: aliquots of test water samples (4 ml) are added to a volumetric flask (10 ml) and adjusted to volume using acetonitrile. For high pH water: aliquots of test water samples (1 ml) are added to a volumetric flask (20 ml) and adjusted volume using 60:40 v/v acetonitrile:Milli RO water.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	HPLC using a Waters LC1 Liquid Chromatograph coupled to a Waters 474 Fluorescence detector. Column was an Inertsil ODS-2, 150 x 4.6 mm, with a flow rate of 1.5 ml/min and a mobile phase of 62:3:35 v/v/v acetonitrile:propan-2-ol:0.2 M ammonium acetate buffer, pH 4.1 using acetic acid.
3.2.2	Detector	Waters Model 474 Fluorescence detector with excitation wavelength 248 nm and detection wavelength 385 nm.
3.2.3	Standard(s)	Analyte (brodifacoum) as an external standard.

Doc IIIA / Section 4.2 Analytical Methods for Detection and Identification

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in water

3.2.4	Interfering substance(s)	The system suitability parameters as calculated by the Labsystems Multichrom 2 Version 2.3 were determined as follows: Column Efficiency (theoretical plates): 3378 (*cis*-brodifacoum) and 3787 (*trans*-brodifacoum); Tailing Factor: 1.13 (*cis*-brodifacoum) and 1.10 (*trans*-brodifacoum); Resolution Ratio: 2.04 *cis* to *trans*-brodifacoum.
3.3	**Linearity**	
3.3.1	Calibration range	0, 1.04, 2.08, 4.16, 5.71, 6.24, 8.32 and 10.4 ng total brodifacoum/ml (µg/l).
3.3.2	Number of measurements	2 per concentration level.
3.3.3	Linearity	Correlation coefficients: 0.9967 (*cis*-brodifacoum) and 0.9954 (*trans*-brodifacoum). Therefore, results indicate acceptable linearity of response over the concentration range examined.
3.4	**Specifity: interfering substances**	None. *Cis* and *trans* isomers resolved into separate chromatographic peaks with a resolution ration of 2.04 (*cis* to *trans* brodifacoum).
3.5	**Recovery rates at different levels**	

Isomer	Fortification level (µg/l)	Mean % Recovery (Mean % Assay Accuracy)
Cis-brodifacoum	1.50	73.4
Trans-brodifacoum	0.51	83.7
Cis-brodifacoum	7.49	73.7
Trans-brodifacoum	2.58	84.8

Overall Assay Accuracy (n=6) = 73.5% for *cis*-brodifacoum and 84.25% for *trans*-brodifacoum. The assay accuracy values were outside normal acceptance levels of 85-110% recovery, but were considered acceptable at the level of validation investigated.

3.5.1	Relative standard deviation	See section 3.7.1 below.
3.6	**Limit of determination**	1.04 µg/l total brodifacoum (0.77 µg/l *cis*-brodifacoum + 0.27 µg/l *trans*-brodifacoum).
3.7	**Precision**	
3.7.1	Repeatability	

Isomer	Fortification level (µg/l)	Mean Found Peak Area*	% Coefficient of Variation (Relative Standard Deviation)
Cis-brodifacoum	4.65	884580	2.3

Doc IIIA / Section 4.2 **Analytical Methods for Detection and Identification**

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in water

	Trans-brodifacoum	1.59	266309	3.1

* The system precision was determined by injecting the standard solution of brodifacoum at the given concentration level 10 times.

3.7.2 Independent laboratory validation: Not available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Aqueous samples containing brodifacoum are assayed using HPLC with fluorescence detection. External standardisation is used for quantification purposes.

The limit of determination for brodifacoum in water using this method is 1.04 µg/l.

See Table 1 below for a summary of the method validation data.

4.2 Conclusion

4.2.1 Reliability: 1

4.2.2 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	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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*

Syngenta	Brodifacoum	Dec/2003

Doc IIIA / Section 4.2	**Analytical Methods for Detection and Identification**
BDP Data Set IIA / Annex Point IV.4.2	Analytical method for the detection of the active substance brodifacoum in water

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 1: Summary of method validation data

Test substance	Analytical method	Fortification range/ number of measurements	Linearity of response (correlation coefficient)	Specificity	Recovery rate (%)			Limit of determination	Reference
					Range	Mean	St. dev. (coeff. of variation)		
Brodifacoum	HPLC	0, 1.04, 2.08, 4.16, 5.71, 6.24, 8.32 and 10.4 µg/), with 2 measurements per concentration level.	0.9967 (*cis*-brodifacoum) and 0.9954 (*trans*-brodifacoum).	Method specific to brodifacoum. *Cis* and *trans* isomers resolved into separate chromatographic peaks with a resolution ration of 2.04 (*cis* to *trans*-brodifacoum).	70.7-76.3% for *cis*-brodifacoum and 79.4-91.25 for *trans*-brodifacoum	73.5% for *cis*-brodifacoum and 84.25% for *trans*-brodifacoum	System precision Standard Deviation: 2.3% for *cis*-brodifacoum and 3.1% for *trans*-brodifaocuom	1.04 µg/l	*Craig WB (2000). Brodifacoum - Physico-Chemical Testing with Brodifacoum: Water Solubility. Inveresk Research Report No: 18799. GLP (unpublished) [BR-959-0079].*

Doc IIIA / Section 4.2 Analytical Methods for Detection and Identification

BDP Data Set IIA / Annex Point IV.4.2

Analytical method for the detection of the active substance brodifacoum in rat liver and plasma

Official use only

1 REFERENCE

1.1 Reference

Hall MG (1997). Brodifacoum: Validation of the Methods for the Determination of Brodifacoum in Rat Liver and Plasma. Central Toxicology Laboratory Report No: CTL/M/258 (unpublished) [Supp Series].

The experimental work was carried out between March 1997 and June 1997.

1.2 Data protection

1.2.1 Data owner

1.2.2 Companies with letter of access

1.2.3 Criteria for data protection

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No guideline available, but the method is in accordance with the principles given in the SANCO/3029/99 guidance document.

2.2 GLP

2.3 Deviations

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

Liver analysis:
Liver samples were homogenised and extracted into chloroform:acetone (1:1 v/v), and then the procedure repeated but with acetonitrile:diethyl ether (9:1 v/v). The combined extracts were blown to dryness under a stream of nitrogen and the residues redissolved in 2 ml of hexane.

Plasma analysis:
Plasma samples were acidified using 50% HCL and then extracted into into chloroform:acetone (1:1 v/v), followed by agitation and centrifugation. This extraction procedure was repeated and the extracts combined and then blown to dryness under a stream of nitrogen, and the residues redissolved in 2 ml of hexane.

3.1.2 Cleanup

Both the extracted liver and plasma samples are cleaned up using a solid-phase extraction procedure prior to quantification by liquid chromatography (LC) analysis.

3.2 Detection

3.2.1 Separation method

Liquid chromatography (LC) using an Alltech, Altime C18, 5 µm, 250 x 4.6 mm column, with a flow rate of 2 ml/min and a mobile phase of acetonitrile:water:acetic acid (800:200:4 v/v/v).

Doc IIIA / Section 4.2	**Analytical Methods for Detection and Identification**
BDP Data Set IIA / Annex Point IV.4.2	Analytical method for the detection of the active substance brodifacoum in rat liver and plasma

3.2.2	Detector	Fluorescence detector at excitation and emission wavelengths of 248 nm and 385 nm respectively.
3.2.3	Standard(s)	Analyte (brodifacoum) as external standard.
3.2.4	Interfering substance(s)	None. The extent of matrix interference was investigated by analysing control liver and plasma from 6 male rats, which showed that there was no matrix interference at the retention time of brodifacoum.
3.3	**Linearity**	
3.3.1	Calibration range	Linearity was assess using extracts of control liver or plasma which were spiked with a range of concentrations of brodifacoum (0-2 µg/g for liver, and 0-8 µg/ml for plasma). These spiked extracts were prepared for analysis by LC.
3.3.2	Number of measurements	11 for each of liver and plasma
3.3.3	Linearity	Correlation coefficient = 0.999 for liver analysis Correlation coefficient = 0.996 for plasma analysis
3.4	**Specifity: interfering substances**	The extent of matrix interference was investigated by analysing control liver and plasma from 6 male rats, which showed that there was no matrix interference at the retention time of brodifacoum.

3.5 Recovery rates at different levels

Liver recovery and precision: 7 replicate samples were prepared from control liver spiked at three concentrations: 0.05 µg/g, 0.6 µg/g and 1.8 µg/g and analysed on three separate days.
Plasma recovery and precision: 7 replicate samples were prepared from control plasma spiked at three concentrations: 0.2 µg/ml, 2.4 µg/ml and 7.2 µg/g and analysed on three separate days.

Intra-run analyses accuracy and precision were calculated from concentrations determined from calibration curves prepared from standards extracted on the same day.

Inter-run analyses accuracy and precision were calculated from concentrations determined from 7 replicates at each of the above concentrations on three separate days.

3.5.1 Recovery results for plasma

Fortification level (µg/ml)	Analysis Day	Mean determined conc^n (µg/ml)	Standard Deviation of the mean (µg/ml)	Intra-run Recovery (%)	Inter-run Recovery (%)
0.20	1	0.18	0.00	91.9	88.0
	2	0.17	0.01	86.0	
	3	0.17	0.00	86.2	
2.40	1	2.23	0.30	92.9	95.1
	2	2.28	0.33	95.1	
	3	2.34	0.33	97.3	
7.20	1	8.02	0.82	111	109
	2	7.55	0.71	105	
	3	7.93	0.67	110	

3.5.2 Recovery results for liver tissue

Fortification level (µg/g)	Analysis Day	Mean determined conc^n (µg/g)	Standard Deviation of the mean (µg/g)	Intra-run Recovery (%)	Inter-run Recovery (%)
0.05	1	0.057	0.002	114	118
	2	0.063	0.002	126	
	3	0.057	0.001	115	
0.60	1	0.48	0.02	80.7	84.2
	2	0.52	0.02	86.7	
	3	0.51	0.02	85.2	
1.80	1	1.49	0.25	83.0	91.4
	2	1.81	0.27	100	
	3	1.64	0.29	90.9	

3.5.3 Relative standard deviation

% RSD for plasma = 2.08-14.3

% RSD for liver tissue = 2.00-17.4

3.6 Limit of determination

0.055 µg/ml for plasma;

0.023 µg/g for liver.

3.7	**Precision**	
3.7.1	Repeatability for plasma samples	Intra-run precision: 2.08-14.3 % Inter-run precision: 2.31-3.84%
3.7.2	Repeatability for liver samples	Intra-run precision: 2.00-17.4% Inter-run precision: 3.73-9.84%
3.7.3	Independent laboratory validation	Not available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Liver samples were homogenised and then extracted into organic solvent. The resulting extracts were then cleaned up using solid-phase extraction procedure prior to liquid chromatography (LC). Plasma samples were acidified and then extracted into organic solvent. The resulting extracts were then cleaned up using a solid-phase extraction procedure prior to liquid chromatography (LC). The analytical limits of the method are as follows: ❑ Limit of quantification (determination) for plasma = 0.055 µg/ml (55 µg/l); ❑ Limit of quantification (determination) for liver tissue = 0.023 µg/g (23 ng/g). See Table 1 below for a summary of the method validation data.
4.2	**Conclusion**	The results obtained in this validation study demonstrated that the linearity, reproducibility, specificity and extraction efficiency were acceptable. Accuracy and precision, both intr- and inter-run, were acceptable.
4.2.1	Reliability	1
4.2.2	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	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...

Syngenta	Brodifacoum	Dec/2003

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	

Table 1: Summary of method validation data

Test substance	Analytical method	Fortification range/ number of measurements	Linearity of response (correlation coefficient)	Specificity	Recovery rate (%)			Limit of determination	Reference
					Range	Mean	St. dev. (coeff. of variation)		
Brodifacoum	LC	0-2 µg/g for liver, and 0-8 µg/ml for plasma. 11 measurements per matrix.	Correlation coefficient = 0.999 for liver analysis Correlation coefficient = 0.996 for plasma analysis	The extent of matrix interference was investigated by analysing control liver and plasma from 6 male rats, which showed that there was no matrix interference at the retention time of brodifacoum.	86.0-111% for plasma; 80.7-126% for liver.	97.4% for plasma; 97.9% for liver.	2.08-14.3% for plasma; 2.00-17.4% for liver.	0.055 µg/ml for plasma; 0.023 µg/g for liver.	*Hall MG (1997). Brodifacoum: Validation of the Methods for the Determination of Brodifacoum in Rat Liver and Plasma. Central Toxicology Laboratory Report No: CTL/M/258 (unpublished) [Supp Series].*

Section A4.2 (c)
Annex Point IIA4.2

Analytical Methods for Detection and Identification

of Brodifacoum in water

			Official use only
		1 REFERENCE	
1.1	**Reference**	Martinez, M. P., 2005, Brodifacoum technical: Validation of the analytical method for the determination of the residues in drinking, ground and surface waters, ChemService SpA, Study Nº CH-289/2005	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.1.1	Companies with letter of access	[redacted]	
1.1.2	Criteria for data protection	[redacted]	
		2 GIUDELINE AND QUALITY ASSURANCE	
2.1	**Guidelines**	EEC guideline SANCO/3030/99 rev. 4 Directive 91/414/EEC	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**	Non-entry field	
3.1.1	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	
3.1.2	Cleanup	The residue is redissolved in with 0.5ml of methanol	
3.2	**Detection**	Non-entry field	
3.2.1	Separation method	Separation by HPLC/MS/DAD	X
3.2.2	Detector	DAD detector with an LCQ advantage ionic trap mass detector	X
3.2.3	Standard(s)	Brodifacoum standards: 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml	X
3.2.4	Interfering substance(s)	None identified.	
3.3	**Linearity**		
3.3.1	Calibration range	Brodifacoum standard range: 0.1 – 0.5 µg/ml	
3.3.2	Number of measurements	4 measurements of each standard	
3.3.3	Linearity	The range tested was from 0.1 to 0.5 $\mu g l^{-1}$, corresponding to concentrations from 0.05 to 0.25 $\mu g l^{-1}$ and was found to be linear. r >0.99	X
3.4	**Specifity: interfering**	None specified	

[1]

substances

3.5 Recovery rates at different levels

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	24804390	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	24530660	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)

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Annex Point IIA4.2 *of Brodifacoum in water*

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	33633760	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)

3.5.1 Relative standard deviation — See tables above (3.5)

3.6 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.

3.7 Precision — Non-entry field

3.7.1 Repeatability — Drinking water: Repeatability and recovery tests.

Linear calibration with working standard solutions

Brodifacoum (BDF) (m/z 521)	Standard 1 0.1 µgml^{-1} (peak area)	Standard 2 0.3 µgml^{-1} (peak area)	Standard 3 0.5µgml^{-1} (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 0.1µgml^{-1} (peak area)	Standard 2 0.3 µgml^{-1} (peak area)	Standard 3 0.5µgml^{-1} (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 0.1µgml^{-1} (peak area)	Standard 2 0.3 µgml^{-1} (peak area)	Standard 3 0.5µgml^{-1} (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

3.7.2 Independent laboratory validation

None.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Directive 91/414/EEC
EEC guideline SANCO/3030/99 rev. 4

X

4.2 Conclusion

The analytical method was shown to be specific for brodifacoum residues in each type of water sample.

Section A4.2 (c)
Annex Point IIA4.2

Analytical Methods for Detection and Identification

of Brodifacoum in water

		The range tested was from 0.1 to 0.5 $\mu g ml^{-1}$, corresponding to concentrations from 0.05 to 0.25 $\mu g l^{-1}$ 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.	
4.2.1	Reliability	1	X
4.2.2	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	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

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	

Doc IIIA / Section 4.2 **Analytical Methods for Detection and Identification**

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in plasma and liver tissue

Official use only

		1 REFERENCE
1.1	**Reference**	O'Bryan SM and Constable DJC (1991). Quantification of Brodifacoum in Plasma and Liver Tissue by HPLC. Journal of Analytical Toxicology, May/June 1991, Volume 15, Number 3, Pages 144-147 (published) [C5.1/03].
1.2	**Data protection**	[redacted]
1.2.1	Data owner	Syngenta
1.2.2	Companies with letter of access	*Give name of company/companies which have the right to use these data on behalf of the data owner (see TNsG in support of AnnexVI)*
1.2.3	Criteria for data protection	[redacted]I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No guideline available, but the method is in accordance with the principles given in the SANCO/3029/99 guidance document.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Preliminary treatment**	
3.1.1	Enrichment	The internal standard (difenacoum) is added quantitatively to the plasma (10 ml) and liver tissue (2 g) samples. The brodifacoum and internal standard are then extracted from the plasma with two sequential 10 ml volumes of acetonitrile:ether (9:1), and from the liver tissue by grinding with 10 ml acetonitrile. The extracts are evaporated to dryness under nitrogen, and then acetonitrile (2 ml) added to reconstitute the residues, and the resulting solution analysed by HPLC.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	HPLC using a Hypersil (5 µm) ODS C_{18} 100 mm x 4.6 mm I.D. column, with a flow rate of 1 ml/min and a mobile phase of acetonitrile: *N*-propanol: 0.2M ammonium acetate buffer, pH4 (62:3:35).
3.2.2	Detector	Hewlett-Packard fluorescence detector, Model 1046A, at excitation and emission wavelengths of 248 nm and 285 nm respectively.
3.2.3	Standard(s)	Difenacoum as an internal standard.
3.2.4	Interfering substance(s)	No interferences were observed with any of the following related compounds: Bromadiolone, coumarin, difenacoum, diphacinone, warfarin, and

		vitamin K_1.
3.3	**Linearity**	
3.3.1	Calibration range	A calibration curve was prepared by analysis of standard solutions of brodifacoum in acetonitrile at concentrations of 5, 10, 20, 50 and 100 µg/l. Each of the standards contained 10 µg/l difenacoum as the internal standard.
3.3.2	Number of measurements	Not given in method report.
3.3.3	Linearity	Correlation coefficient = 0.999
3.4	**Specifity: interfering substances**	No interferences were observed between brodifacoum and any of the 4-hydroxycoumarin related compounds (bromadiolone, coumarin, difenacoum, diphacinone, warfarin) and vitamin K_1. Coumarin (1 mg/ml) was not detected by this method. Both the *cis*- and *trans*-isomers of brodifacoum and difenacoum are detected by this method. The predominant *cis*-isomer was chosen to quantify brodifacoum for the following two reasons: ➢ Earlier metabolism studies utilising thin-layer chromatography demonstrated that the *cis:trans*-isomer ratio (60:40) is not altered during disposition of brodifacoum Therefore, no error will result when one isomer is consistently used for both standardisation and quantification. ➢ A study of 20 human plasma samples collected from a normal population showed that naturally occurring plasma constituents occasionally co-elute with the *trans*-isomer, which would positively bias quantification of brodifacoum. However, the *trans*-isomer, if separated from plasma constituents, provides additional qualitative evidence for the presence of brodifacoum in plasma and liver tissue samples. Similarly, the *cis*-isomer of difenacoum was used for the internal standardisation.

3.5 Recovery rates at different levels

- Plasma recovery samples were prepared by spiking 2 ml of plasma with brodifacoum.
- Liver tissue recovery samples were prepared by spiking 2 g of liver with brodifacoum immediately before mincing to facilitate binding with the tissue.

3.5.1 Recovery results for plasma

Target spiked (nominal) conc^n of brodifacoum in plasma (µg/l)	Recovered conc^n of brodifacoum in plasma (µg/l)	Mean % recovery	Standard deviation (SD)	Coefficient of variation (CV) ie % Relative standard deviation (%RSD)
10	9.4	94	9	10
20	21	105	28	26
50	56	111	1	1
100	115	115	7	7
500	498	100	2	2

Overall recovery range: 94 - 115
n = 10 (2 at each conc^n)
Mean recovery = 105
SD = 13
CV = 12

NB Brodifacoum was also spiked into 3 additional samples of whole blood which were frozen and then thawed to haemolyse the samples. The mean recovery was 89%, with a CV of 9%, which demonstrated that gross haemolysis does not interfere with the brodifacoum assay.

3.5.2 Recovery results for liver tissue

Target spiked (nominal) conc^n of brodifacoum in liver tissue (ng/g)	Recovered conc^n of brodifacoum in liver tissue (ng/g)	Mean % recovery	Standard deviation (SD)	Coefficient of variation (CV) ie % Relative standard deviation (%RSD)
10	11	110	18	17
50	54	108	11	9
100	92	92	8	9
200	170	85	12	13
400	370	93	4	4
600	580	97	4	4
800	750	94	8	9
1000	920	92	7	8

Overall recovery range: 85 - 110
n = 40 (5 at each conc^n)
Mean recovery = 96
SD = 8
CV = 9

3.5.3 Relative standard deviation

% RSD for plasma = 12

		% RSD for liver tissue = 9
3.6	**Limit of determination**	Limit of quantification (determination) for plasma = 5 µg/l Limit of quantification (determination) for liver tissue = 10 ng/g Limit of detection for plasma = 2 µg/l Limit of detection for liver tissue = 5 ng/g
3.7	**Precision**	
3.7.1.	Repeatability for plasma samples	**Within assay precision:** To determine within assay precision, 30 plasma samples were spiked with brodifacoum (10 samples each spiked with 20, 100 and 500 µg/l). The overall mean (*n*=30) recovery was 92%, with a CV of 16%. **Between assay precision:** To determine between assay precision, 3 plasma samples were spiked with 20, 100 and 500 µg/l brodifacoum and were analysed in each of 5 different assays. The overall mean (*n*=15) recovery was 109%, with a CV of 10%.
3.7.2	Repeatability for liver samples	**Within assay precision:** To determine within assay precision, 20 liver samples were spiked with brodifacoum (10 samples each spiked with 75 and 300 ng/g). The overall mean (*n*=20) recovery was 96%, with a CV of 14%. **Between assay precision:** To determine between assay precision, 5 liver samples were spiked with 10, 100, 400, 600 and 1000 ng/g brodifacoum and were analysed in each of 5 different assays. The overall mean (*n*=25) recovery was 96%, with a CV of 11%.
3.7.3	Independent laboratory validation	Not available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

After addition of difenacoum as the internal standard, brodifacoum is extracted from plasma samples with two sequential volumes of acetonitrile:ethyl ether (9:1), and from liver samples by grinding the tissue with acetonitrile. The extracts are evaporated to dryness under nitrogen and then acetonitrile added to reconstitute the residues, and the resulting solution analysed using reverse-phase chromatography and fluorescence detection.

The analytical limits of the method are as follows:

- Limit of quantification (determination) for plasma = 5 µg/l
- Limit of quantification (determination) for liver tissue = 10 ng/g
- Limit of detection for plasma = 2 µg/l
- Limit of detection for liver tissue = 5 ng/g

See Table 1 below for a summary of the method validation data.

4.2 Conclusion

The recovery results (as given in section 3.5 above) illustrate that recoveries from the human plasma and rat liver tissue used in the validation of the method are accurate and precise.

Additional comparisons were made at the test laboratory with bovine serum and horse serum spiked with brodifacoum in the range 10 - 500

µg/l, and gave a recovery of 98% with a CV of 14% for bovine serum. Analyses of the horse serum however, showed that it contained interfering constituents which allowed only a qualitative determination of brodifacoum. The analyses results for the bovine serum samples were not as accurate or precise as rat plasma recoveries. These comparative studies suggest that normal control and recovery samples should be prepared from the same species of animal and analysed with the sample in question whenever brodifacoum quantification is required.

During the method validation at the test laboratory for this method of analysis, a frozen canine liver sample was received from a veterinarian requesting confirmation of a suspected brodifacoum poisoning. This canine plasma and liver was analysed and found to contain 81 µg/l and 1,800 ng/g brodifacoum respectively.

Therefore, the precise and accurate recovery data for spiked human plasma and rat liver samples demonstrate the efficacy of this method in assaying plasma and liver for brodifacoum. The sensitivity, the simple extraction procedure, and the adaptability to a wide range of animal species, make this HPLC method appropriate for use in clinical chemical or toxicology laboratories.

4.2.1 Reliability 1

4.2.2 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	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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	

Table 1: Summary of method validation data

Test substance	Analytical method	Fortification range/ number of measurements	Linearity of response (correlation coefficient)	Specificity	Recovery rate (%)			Limit of determination	Reference
					Range	Mean	St. dev. (coeff. of variation)		
Brodifacoum	HPLC	A calibration curve was prepared by analysis of standard solutions of brodifacoum in acetonitrile at concentrations of 5, 10, 20, 50 and 100 µg/l. Each of the standards contained 10 µg/l difenacoum as the internal standard.	0.999	No interferences were observed between brodifacoum and any of the 4-hydroxycoumarin related compounds (bromadiolone, coumarin, difenacoum, diphacinone, warfarin) and vitamin K_1. Coumarin (1 mg/ml) was not detected by this method.	94-115% for plasma; 85-110% for liver.	105% for plasma; 96% for liver.	12% for plasma; 9% for liver.	5 µg/l for plasma; 10 ng/g for liver tissue.	*O'Bryan SM and Constable DJC (1991). Quantification of Brodifacoum in Plasma and Liver Tissue by HPLC. Journal of Analytical Toxicology, May/June 1991, Volume 15, Number 3, Pages 144-147 (published) [C5.1/03].*

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

Annex Point IIA, IV.4.2(e) Brodifacoum residues in treated food and feedingstuffs

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1 REFERENCE

1.1	**Reference**	Turnbull, G. (2005). Validation of Analytical Methodology to Determine Rodenticides in Food Matrices. Central Science Laboratory unpublished report number PGD-180, 16 June 2005.
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	SANCO/825/00 rev. 6.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Extraction	Brodifacoum is extracted from cucumber, wheat grain and lemon by blending with ethyl acetate. Extraction from oilseed rape is by blending with acetone. Meat is extracted by grinding with anhydrous sodium sulphate and shaking with a mixture of dichloromethane and acetone.
3.1.2	Cleanup	Cucumber, oilseed rape and lemon: SPE catridge (Oasis Max). Wheat grain and meat: Gel permeation chromatography (S-X3 biobeads, mobile phase: cyclohexane + ethyl acetate).
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. Mobile phase: 10 mM ammonium acetate and methanol gradient.
3.2.2	Detector	MS-MS: transitions 521 to 79 m/z, 523 to 81 m/z.
3.2.3	Standard(s)	Internal standard (coumatetralyl).
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	Four (in duplicate).
3.3.3	Linearity	R^2 = 0.9095 to 0.9936.

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

Annex Point IIA, IV.4.2(e) Brodifacoum residues in treated food and feedingstuffs

3.4 Specifity: interfering substances

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

3.5 Recovery rates at different levels

Recovery from fortified representative matrices was as follows:

Matrix	Fortification level (mg/kg)	Recovery (%)		
		range	mean	n
Cucumber	0.01	82 - 103	91	5
	0.10	86 - 106	94	5
	overall	82 - 106	92	10
Wheat	0.01	88 - 126	107	5
	0.10	71 - 90	84	5
	overall	71 - 126	95	10
Meat	0.01	62 - 86	73	5
	0.10	45 - 87	61	5
	overall	45 - 87	67	10
Oilseed rape	0.01	75 - 99	86	5
	0.10	110 - 134	119	5
	overall	75 - 134	103	10
Lemon	0.01	74 - 93	84	5
	0.10	62 - 89	76	5
	overall	62 - 93	80	10

Overall recovery was within the acceptable range 70 to 110% for each matrix except meat (67%).

3.5.1 Relative standard deviation

RSD values based on the recovery determinations were as follows:

Matrix	Fortification level (mg/kg)	RSD (%)	Overall RSD (%)
Cucumber	0.01	8.5	8.4
	0.10	8.9	
Wheat	0.01	13.1	17.2
	0.10	9.0	
Meat	0.01	13.0	22.0
	0.10	28.8	
Oilseed rape	0.01	9.7	18.5
	0.10	7.7	
Lemon	0.01	9.7	12.0
	0.10	13.2	

3.6	**Limit of determination**	The limit of determination is 0.01 mg/kg (defined as the lowest concentration at which acceptable recovery has been demonstrated).
3.7	**Precision**	
3.7.1	Repeatability	RSD values are presented above under 3.5.1. Overall RSD for each matrix are below 20% and are within acceptable limits defined in SANCO/825/00 except for meat. For meat, the RSD was 22% which is slightly above the acceptance criterion.
3.7.2	Independent laboratory validation	Not applicable.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Brodifacoum is extracted from cucumber, wheat grain and lemon by blending with ethyl acetate. Extraction from oilseed rape is by blending with acetone. Meat is extracted by grinding with anhydrous sodium sulphate and shaking with a mixture of dichloromethane and acetone. Extracts are purified by SPE catridge (cucumber, oilseed rape and lemon) or gel permeation chromatography (wheat grain and meat) prior to determination by LC/MS/MS.
4.2	**Conclusion**	The methods for determination of residues of brodifacoum in representative food and feedingstuffs (cucumber, wheat grain, meat, oilseed rape and lemon) 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 the meat method, accuracy and precision were slightly outside the acceptance criteria, but it is concluded that the method will be appropriate for monitoring purposes. The methods require equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the methods are suitable for enforcement purposes.
4.2.1	Reliability	1
4.2.2	Deficiencies	None

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

Doc IIIA / Section 4.2 Analytical Methods for Detection and Identification

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in soil

Official use only

1 REFERENCE

1.1 Reference

Koubek KG and Ussary JP (1979). An HPLC Method for the Determination of Brodifacoum in Soil. ICI Americas Inc Report Method No: TMU0423/A (unpublished) [F2.1/04].

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letter of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No guidelines available, but the method is in accordance with the principles given in the SANCO/3029/99 guidance document.

2.2 GLP

[redacted]

2.3 Deviations

[redacted]

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

Samples are extracted with 30% methanol in chloroform and the insolubles removed by filtration. The solvent is removed with roto-evaporation and the residue is then redissolved in 15% methylene chloride in cylcohexane.

3.1.2 Cleanup

Interfering substances are removed by gel permeation chromatography. The solvent is again removed by roto-evaporation, and the residue redissolved in HPLC mobile phase and analysed by adsorption chromatography using a u.v. detector.

3.2 Detection

3.2.1 Separation method

HPLC using µPorasil stainless steel 30 cm x 3.9 mm i.d. column, with a flow rate of 1.5 ml/min and a mobile phase of 75 parts cyclohexane: 25 parts methylene chloride: 0.6 parts glacial acetic acid.

3.2.2 Detector

u.v. detector at 254 nm and 0.05 AU full scale.

3.2.3 Standard(s)

Analyte (brodifacoum) as an external standard.

3.2.4 Interfering substance(s)

None - these are removed by the gel permeation step.

3.3 Linearity

Doc IIIA / Section 4.2 Analytical Methods for Detection and Identification

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in soil

3.3.1	Calibration range	0.04, 0.50, 1.00, 2.00, 5.00 and 10.00 ppm brodifacoum in soil samples.
3.3.2	Number of measurements	2, 1, 6, 2, 1 and 5 for fortification levels of 0.04, 0.50, 1.00, 2.00, 5.00 and 10.00 ppm brodifacoum respectively in soil samples.
3.3.3	Linearity	Correlation coefficient not given in method report.
3.4	**Specifity: interfering substances**	None - these are removed by the gel permeation step.

3.5 Recovery rates at different levels

Fortification level (ppm)	Range of recovery	Mean recovery	% Recovery
0.04	0.04 - 0.04	0.04	100
0.50	0.49	0.49	98
1.00	0.87 - 1.06	0.96	96
2.00	1.89 - 1.94	1.92	96
5.00	4.79	4.79	96
10.00	9.21 - 10.30	9.62	96

3.5.1	Relative standard deviation	Not given in method report.
3.6	**Limit of determination**	0.04 ppm brodifacoum in soil.
3.7	**Precision**	
3.7.1	Repeatability	Not given in method report.
3.7.2	Independent laboratory validation	Not available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Samples are extracted with 30% methanol in chloroform and the insolubles removed by filtration. The solvent is removed with roto-evaporation and the residue is then redissolved in 15% methylene chloride in cylcohexane.

Interfering substances are removed by gel permeation chromatography. The solvent is again removed by roto-evaporation, and the residue redissolved in HPLC mobile phase and analysed by adsorption chromatography using a u.v. detector.

The limit of determination for brodifacoum in soil using this method is 0.04 ppm.

See Table 1 below for a summary of the method validation data.

Doc IIIA / Section 4.2 **Analytical Methods for Detection and Identification**

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in soil

4.2 Conclusion

4.2.1 Reliability 2

4.2.2 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	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

Table 1: Summary of method validation data

Test substance	Analytical method	Fortification range/ number of measurements	Linearity of response (correlation coefficient)	Specificity	Recovery rate (%)			Limit of determination	Reference
					Range	Mean	St. dev. (coeff. of variation)		
Brodifacoum	HPLC	2, 1, 6, 2, 1 and 5 measurements respectively for fortification levels of 0.04, 0.50, 1.00, 2.00, 5.00 and 10.00 ppm brodifacoum respectively in soil samples.	Not given in method report.	Method is specific for brodifacoum: interfering substances are removed by the gel permeation step.	96 - 100%	97%	Not given in method report.	0.04 ppm brodifacoum in soil.	*Koubek KG and Ussary JP (1979). An HPLC Method for the Determination of Brodifacoum in Soil. ICI Americas Inc Report Method No: TMU0423/A (unpublished) [F2.1/04].*

Doc IIIA / Section 4.2 Analytical Methods for Detection and Identification

BDP Data Set IIA / Annex Point IV.4.2 Analytical method for the detection of the active substance brodifacoum in water

Official use only

1 REFERENCE

1.1	**Reference**	Sanderson DJ and Austin DJ (1990). The Determination of Residues of Brodifacoum (ICIA0581) and Difenacoum (ICIA0580) in Potable Water. ICI Agrochemicals Report Method No: 173 (unpublished) [F2.2/03].
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No guideline available, but the method is in accordance with the principles given in the SANCO/3029/99 guidance document.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Samples are extracted by liquid-liquid partitioning with dichloromethane. After rotary and blown-air evaporation, the dried extracts are resuspended in the mobile phase used for final quantitative determination by HPLC.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	HPLC using a Spherisorb S5C8 25 cm x 4.6 mm I.D column, with a flow rate of 1.0 ml/min and a mobile phase of 77:23 Methanol:Water (with 0.16 g/l ammonium acetate + 0.12 g/l glacial acetic acid).
3.2.2	Detector	Perkin-Elmer LS-4 Fluorescence Spectrometer, with fluorimetric detection at 270 nm (excitation) and 390 nm (emission).
3.2.3	Standard(s)	Analyte (brodifacoum) as an external standard.
3.2.4	Interfering substance(s)	Method validation using controls showed that co-extractives were observed to interfere with brodifacoum determination during the final chromatographic step. When the water extractives were least diluted, *ie* at the 0.1 µg/l fortification level, the co-extractives were equivalent only to <0.003 µg/l of brodifacoum. The mean values of the co-extractive

		concentrations were subtracted from the corresponding brodifacoum concentrations observed in the fortified samples prior to calculation of the recovery values.
3.3	**Linearity**	
3.3.1	Calibration range	0, 0.1, 0.2, 0.5 and 1.0 µg/l.
3.3.2	Number of measurements	4, 2, 2, and 2 measurements, for fortification levels of 0.1, 0.2, 0.5 and 1.0 µg/l respectively.
3.3.3	Linearity	Correlation coefficient not given in method report.
3.4	**Specifity: interfering substances**	Method validation using controls showed that co-extractives were observed to interfere with brodifacoum determination during the final chromatographic step. When the water extractives were least diluted, *ie* at the 0.1 µg/l fortification, the co-extractives were equivalent only to <0.003 µg/l of brodifacoum. The mean values of the co-extractive concentrations were subtracted from the corresponding brodifacoum concentrations observed in the fortified samples prior to calculation of the recovery values. The co-extractive levels (expressed as brodifacoum equivalents) were determined as follows: 0.002, 0.002, 0.002 and 0.003 µg/l (mean: 0.002 µg/l).
3.5	**Recovery rates at different levels**	**Fortification level (µg/l)** → **% Recovery** 0.1 → 85, 81, 84, 85 0.2 → 98, 96 0.5 → 108, 114 1.0 → 85, 81 Mean = 92%
3.5.1	Relative standard deviation	Not given in method report.
3.6	**Limit of determination**	0.1 µg/l
3.7	**Precision**	
3.7.1	Repeatability	> +/- 5%
3.7.2	Independent laboratory validation	Not available.
		4 APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Materials and methods**	Samples are extracted by liquid-liquid partition with dichloromethane. After rotary and blown-air evaporation, the dried extracts are resuspended in the mobile phase used for final quantitative determination by HPLC using fluorometric detection. Recovery samples

are accurately fortified with the analyte as the external standard.

The limit of determination for brodifacoum in potable water using this method is 0.1 µg/l.

See Table 1 below for a summary of the method validation data.

4.2 Conclusion

4.2.1 Reliability 1

4.2.2 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	[redacted]
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

Table 1: Summary of method validation data

Test substance	Analytical method	Fortification range/ number of measurements	Linearity of response (correlation coefficient)	Specificity	Recovery rate (%)			Limit of determination	Reference
					Range	Mean	St. dev. (coeff. of variation)		
Brodifacoum	HPLC	4, 2, 2, and 2 measurements, for fortification levels of 0.1, 0.2, 0.5 and 1.0 µg/l respectively.	Not given in method report.	Method validation using controls showed that co-extractives were observed to interfere with brodifacoum determination during the final chromatographic step. When the water extractives were least diluted, ie at the 0.1 µg/l fortification, the co-extractives were equivalent only to <0.003 µg/l of brodifacoum. The mean values of the co-extractive concentrations were subtracted from the corresponding brodifacoum concentrations observed in the fortified samples prior to calculation of the recovery values.	81-114%	92%	Not given in method report.	0.1 µg/l	*Sanderson DJ and Austin DJ (1990). The Determination of Residues of Brodifacoum (ICIA0581) and Difenacoum (ICIA0580) in Potable Water. ICI Agrochemicals Report Method No: 173 (unpublished) [F2.2/03].*

Doc IIIA/Section 4.3 **BPD Data Set IIIA/Annex Point 4.3**	***Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant***	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [] **Limited exposure** []	**Technically not feasible** [] **Scientifically unjustified** [] **Other justification** [✓]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [[✓]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	

Doc IIIA/Section 4.3 **BPD Data Set IIIA/Annex Point 4.3**	***Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant***
Remarks	

Official use only

5.1	**Function (IIA5.1)**	PT14 – Rodenticide The codes and terms are not currently available.
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	*Rodents:* *Rattus norvegicus, Rattus rattus, Mus musculus*
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	For the purpose of the protection of public health, including: • Prevention of transmission of disease; • Prevention of the contamination of food and feedingstuffs and other materials, with urine, faeces and rodent hairs, at all stages of their production, storage and use; • Protection of buildings and structures including pipes, cables and overall integrity; • Protection of livestock, wild and domestic; • Social abhorrence and stigma; • Legal requirement, for example, UK Prevention of Damage by Pests Act 1954. The borderline document, 'Guidance document agreed between the Commission services and the competent authorities of Member States for the biocidal products Directive 98/8/EC and for the plant protection products Directive 91/414/EEC on: Borderline between Directive 98/8/EC concerning the placing on the market of Biocidal product and Directive 91/414/EEC concerning the placing on the market of plant protection products states, "... rats and mice can contaminate with their excrements a much greater quantity of plant products (with the consequent danger of transmission of diseases) compared with the quantity directly devoured. Considering that there could be a need to control the population of rodents in plant growing areas not because they devour crops but because they multiply and can subsequently spread to human settlements, it is agreed that products used for this specific purpose are biocidal product". The codes and terms are not currently available.

Doc IIIA/Section 5 **Effectiveness against target organisms and intended uses**
BPD Data Set IIA/Annex Point V.

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)

Anticoagulant rodenticides disrupt the normal blood-clotting mechanisms, resulting in increased bleeding tendency and, eventually, profuse haemorrhage.

Signs of anticoagulant poisoning in rats and mice include lethargy, hunched posture and vein clearing in the ears. Blood around the eyes, mouth and anus, indicating internal haemorrhaging, appears prior to death (*extract from WHO, 1995. Environmental Health Criteria 175 – Anticoagulant Rodenticides. International Programme on Chemical Safety, pages 22 and 55*).

For the control of rats and mice by professional and non-professional users.

For rat control: use up to 50g of bait, in appropriate dry locations, at protected bait points, approximately 10 metres apart, until no bait has been consumed for three days.

For mouse control: use up to 15g of bait, in appropriate dry locations, at protected bait points, 2-5 metres apart, until no bait has been consumed for three days.

In general, rodenticide treatments with anticoagulant rodenticides would be expected to achieve control within 35 days.

All efficacy studies have been summarised using the standard format IIIB5_10.

Please refer to table A5-1 Summary table of experimental data on the effectiveness of the active substance, brodifacoum [CAS 56073-10-0], when formulated into the representative biocidal product against target organisms.

5.3.2 Likely concentra-tions at which the A.S. will be used (IIA5.3)

PT14

The concentration of the active substance present in the biocidal products is 50 ppm (0.005% w/w) brodifacoum.

5.4 Mode of action (including time delay) (IIA5.4)

5.4.1 Mode of action

5.4.2 Time delay

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-dependant post-translation processing before they are converted into the respective procoagulant zxymogens. The point of action appears to be the inhibition of K1 epoxide reductase (*extract from WHO, 1995. Environmental Health Criteria 175 – Anticoagulant Rodenticides. International Programme on Chemical Safety, page 20*).

Clinical signs are progressive and occur 12 to 18 hours after ingestion of a toxic dose, ultimately leading to death 3 to 10 days later. Effects are reversible by administration of the antidote vitamin K1 which stimulates the regeneration of the clotting factors.

5.5 Field of use envisaged (IIA5.5)

MG03: Pest control

Product type (PT) 14 – Rodenticide.

Doc IIIA/Section 5 BPD Data Set IIA/Annex Point V.	Effectiveness against target organisms and intended uses
Further specification	Rodenticidal baits (containing 50ppm brodifacoum, as the active substance) may be used indoors, around buildings, away from buildings, around waste sites and in sewers. The product is used in the same manner in all of these situations; the bait is placed in discrete locations within the infested area, it is not dispersed or broadcast within the environment. The products are primarily used to treat existing infestations. For rat control, protected bait points containing up to 50g of product are used, at intervals of up to 10 metres apart. For mouse control, protected bait points containing up to 15g of product are used, at intervals of 2-5 metres apart. An adequate number of baits points are placed in dry locations, protected from the weather and in appropriate positions to help prevent access by non-target animals. The number of bait points employed and the amount of product used is dependant on: the treatment site; the size and severity of the infestation; the user; and the users requirements and needs. A large number of bait points would be used on a site where the existing infestation is heavy, maximum control is required, immigration pressure is high and the user is professionally competent. Conversely, a low number of bait points would be used in a domestic premise where the householder had sightings of a rodent pest and considered it necessary to take some action. The common strategy for best rat control, given that rats generally live outdoors, is to place protected baits between where rats live and feed so that they encounter the bait before encountering alternative foods. Bait points are thus best placed around harbourages and living areas, along runs where rats habitually travel, at entry points into buildings and around areas where rats are known to feed. As mice are sporadic feeders, and generally live indoors within inaccessible spaces and voids, the strategy for best mouse control is to place many bait points throughout the area where mice are known to feed. Bait points are inspected frequently and the bait point is replenished when bait take is observed. When no further take is observed for more than three days it is considered that control has been achieved and bait and bait points are removed from the site. It is normally expected that a typical baiting treatment of an infestation will not exceed a 35 day duration. At the conclusion of a rodent control treatment all remains of bait and bait containers are removed from the site and disposed of safely. Some Member States have specific disposal requirements; for example, in the UK non-professional users can dispose of their waste direct to landfill sites (*via* domestic refuse) but professional users have to dispose of waste as controlled waste under EU waste legislation. Rodent bodies must be disposed of using the same routes.
5.6 User (IIA5.6)	

Doc IIIA/Section 5 BPD Data Set IIA/Annex Point V.	Effectiveness against target organisms and intended uses
Industrial	
Professional	This user group is not exposed to the active substance, except when formulated in a rodenticidal product at the concentration of 50ppm. The following tasks are undertaken when using rodenticidal baits: • Decanting of bait from bulk containers may occur; • Loading of bait points with bait; • Topping-up bait points when bait has been consumed; and • Clean-up and disposal of spent baits at the end of the treatment. 'Loading of bait points with bait' and 'Topping-up bait points when bait has been consumed' are essentially identical tasks. Although gloves are not necessary when handling the product they are recommended for protection against exposure to rodent-borne diseases. It is expected that a professional user would undertake a risk assessment to the standards required by the Chemical Agents Directive, 98/24/EC in order to determine if any exposure controls are required for any specific task on specific treatment sites.
General public	This user group is not exposed to the active substance, except when formulated in a rodenticidal product at the concentration of 50ppm. The following tasks are undertaken when using rodenticidal baits: • Decanting of bait from bulk containers may occur; • Loading of bait points with bait; • Topping-up bait points when bait has been consumed; and • Clean-up and disposal of spent baits at the end of the treatment. 'Loading of bait points with bait' and 'Topping-up bait points when bait has been consumed' are essentially identical tasks. Although gloves are not necessary when handling the product they are recommended for protection against exposure to rodent-borne diseases. Exposure is indirectly limited by controls on pack sizes available to this user group.
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	There are two main forms of resistance to anticoagulants. The first, *technical* resistance, is a small change in the susceptibility of a rodent strain to an anticoagulant which is detectable but which has no obvious effect on control efficacy. The second, *practical* resistance, is a level of resistance which may have a detrimental effect on the outcome of control treatments. Practical resistance to the first-generation anticoagulants is widespread in Europe in both Norway rats and House mice. There are also foci of practical resistance to difenacoum and bromadiolone in these two species in some countries of the EU (e.g. UK and Denmark). There is no incidence of any practical resistance to brodifacoum in either rats or mice in the EU, although a focus of technical resistance in Norway rats exists at a site in the UK.

Various methods are available for the identification of resistant rodents and resistant populations, each has its advantages and disadvantages. The Blood Clotting Response (BCR) Test is the method advocated by the Rodenticide Resistance Action Committee of CropLife International and susceptibility BCR baselines for Norway rats and House mice are published for brodifacoum, and other anticoagulants, which permit resistance in these species to be identified. Resistance detection methods based on gene probes are under development.

Brodifacoum is fully effective against all anticoagulant resistant rat and mouse populations to be found in the EU. The correct use of products based on brodifacoum both help prevent the development of resistance and provide effective control of anticoagulant resistant rodents.

(Reference: Buckle A, 2004. Brodifacoum, Resistance to the Anticoagulants and Resistance Management. Syngenta Limited, (unpublished), [BR-959-0149].)

5.7.2 Management strategies

Guidelines for the management of resistance to anticoagulant rodenticides have been published *(RRAC, 2003. Anticoagulant resistance management strategy for pest management professionals, central and local government and other competent users of rodenticides. CropLife International (Rodenticide Resistance Action Committee) Technical Monograph. Brussels. p 18 and www.croplife.org)* which offer advice to practitioners. The document first provides general guidance on a broad approach to rodent pest management which is based on the principles of Integrated Pest Management (IPM). This approach should be the foundation of all rodent control operations and would be expected to reduce the risk of the development of resistance and to optimise rodent management at resistant foci. The advice is directed in two different scenarios:

1. To avoid the development of resistance in susceptible rodent populations
   - Use anticoagulant rodenticides. Ensure that all baiting points are inspected weekly and old bait is replaced where necessary.
   - Undertake treatment according to the label until the infestation is completely cleared.
   - On completion of the treatment remove all unused baits.
   - Do no use anticoagulant rodenticides as permanent baits routinely. Use permanent baits only where there is a clear and identified risk of immigration or introduction or where protection is afforded to high-risk areas.
   - Monitoring of rodent activity should be undertaken using visual survey, through the use of non-toxic placebo monitors or by other effective means.
   - Record details of treatment.
   - Where rodent activity persists due to problems other than resistance, use alternative baits or baiting strategies, extend the baiting programme or apply alternative control techniques to eliminate the residual infestation (acute or sub-acute rodenticides, gassing or trapping).

- Ensure that complete elimination of the infestation is achieved.
- As appropriate during the rodenticide treatment, apply effective Integrated Pest Management measures (remove alternative food sources, remove water sources, remove harbourage and proof susceptible areas against rodent access).

2. Treatment of rodent infestations containing resistant individuals

- Where rodent infestations containing resistant individuals are identified, immediately use an alternative anticoagulant of higher potency. If in doubt, seek expert advice on the local circumstances. NB. Brodifacoum is fully effective in all resistant foci in Europe and, as the most potent anticoagulant available, it is appropriate for use against any rodent infestation that is resistant to any other anticoagulant rodenticide.
- Alternatively, use an acute or sub-acute but non-anticoagulant rodenticide.
- In both cases, it is essential that complete elimination of the rodent population is achieved. Where residual activity is identified apply intensive trapping to eliminate remaining rodents. Gassing or fumigation may be useful in specific situations.
- Apply thorough Integrated Pest Management procedures (environmental hygiene, proofing and exclusion).
- Do not use anticoagulant rodenticides as permanent baits as routine. Use permanent baits only where there is a clear and identified risk of immigration or introduction or where protection is afforded to high risk areas.
- Record details of treatment.

(Reference: Buckle A, 2004. Brodifacoum, Resistance to the Anticoagulants and Resistance Management. Syngenta Limited, (unpublished), [BR-959-0149].)

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

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

A5-1 Summary table of experimental data on the effectiveness of the active substance, brodifacoum [CAS 56073-10-0], when formulated into the representative biocidal product against target organisms.

Product Type 14 – Rodenticide. For use in all situations, in and around buildings, away from buildings, waste dumps and sewers. (The codes and terms are not currently available.

Test substance	Test organism(s)	Test system	Test conditions	Test results: effects, mode of action, resistance	Reference
Klerat Pellets containing 50ppm brodifacoum	Norway rat (*Rattus norvegicus*)	Field trial, in accordance with EPPO, 1982. Guidelines for the biological evaluation of rodenticides. No. 2. Field tests against synanthropic rodents (*Mus musculus, Rattus norvegicus, R. rattus*). Also, this study complies with the TNsG on Product Evaluation Chapter 7 and its appendices – Product Type 14 – Rodenticides.	Study was carried out in the field following the proposed Directions for Use on the product label.	[redacted]	[redacted]
*Talon Pellets containing 50ppm brodifacoum	Norway rat (*Rattus norvegicus*)	Field trial, carried out using methods which are compliant with current guidelines, for example, TNsG on Product Evaluation Chapter 7 and its appendices – Product Type 14 – Rodenticides; and EPPO, 1999, Guidelines for the	Study was carried out in the field following the Directions for Use on the product label.	[redacted]	[redacted]

Test substance	Test organism(s)	Test system	Test conditions	Test results: effects, mode of action, resistance	Reference
		efficacy evaluation of rodenticides. Field tests against synanthropic rodents (*Mus musculus, Rattus norvegicus, R. rattus*).			
*Talon Pellets containing 50ppm brodifacoum	Norway rat (*Rattus norvegicus*)	Field trial, carried out using methods which are compliant with current guidelines, for example, TNsG on Product Evaluation Chapter 7 and its appendices – Product Type 14 – Rodenticides; and EPPO, 1999, Guidelines for the efficacy evaluation of rodenticides. Field tests against synanthropic rodents (*Mus musculus, Rattus norvegicus, R. rattus*).	Study was carried out in the field following the Directions for Use on the product label.	[redacted]	[redacted]
*Talon Pellets containing 50ppm brodifacoum	Norway rat (*Rattus norvegicus*)	Field trial, carried out using methods which are compliant with current guidelines, for example, TNsG on Product Evaluation Chapter 7 and its appendices – Product	Study was carried out in the field following the Directions for Use on the product label.	[redacted]	[redacted]

Test substance	Test organism(s)	Test system	Test conditions	Test results: effects, mode of action, resistance	Reference
		Type 14 – Rodenticides; and EPPO, 1999, Guidelines for the efficacy evaluation of rodenticides. Field tests against synanthropic rodents (*Mus musculus, Rattus norvegicus, R. rattus*).			
Klerat Pellets containing 50ppm brodifacoum	House mouse (*Mus domesticus*)	Field trial, in accordance with EPPO, 1982. Guidelines for the biological evaluation of rodenticides. No. 2. Field tests against synanthropic rodents (*Mus musculus, Rattus norvegicus, R. rattus)* and MAFF, 1990. Guidelines on efficacy tests for rodenticides. MAFF Working Document 10/2. Also, this study complies with the TNsG on Product Evaluation Chapter 7 and its appendices – Product Type 14 – Rodenticides.	Study was carried out in the field following the proposed Directions for Use on the product label.	[redacted]	[redacted]
*Talon Pellets containing 50ppm	Norway rat (*Rattus norvegicus*) and	Field trial, carried out using methods which	Study was carried out in the field following	[redacted]	[redacted]

Test substance	Test organism(s)	Test system	Test conditions	Test results: effects, mode of action, resistance	Reference
brodifacoum	House mouse (*Mus musculus*)	are compliant with current guidelines, for example, TNsG on Product Evaluation Chapter 7 and its appendices – Product Type 14 – Rodenticides; and EPPO, 1999, Guidelines for the efficacy evaluation of rodenticides. Field tests against synanthropic rodents (*Mus musculus, Rattus norvegicus, R. rattus*).	the Directions for Use on the product label.		

*[redacted]

Doc IIIA / Section 5.4	**Acute Oral Toxicity (Effect of Massive Oral Doses > LD_{95} in Mice)**
BPD Data Set IIA / Annex Point V.5.4	

1 REFERENCE

1.1 Reference

[redacted], 1975, 'Massive Oral Acute Dose of WBA 8119 to the Mouse', [redacted]

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

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

2.2 GLP

No. GLP was not compulsory at the time.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

WBA 8119 (brodifacoum).

3.1.1 LOT/BATCH NUMBER

[redacted]

3.1.2 SPECIFICATION

As given in Section 2 of Doc. IIIA.

3.1.3 DESCRIPTION

Powder.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Mus domesticus (House mouse).

3.2.2 STRAIN

LAC.

3.2.3 SOURCE

OLAC Ltd.

3.2.4 SEX

Male.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

25 - 30g.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

10 (male).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to at least 6 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

50, 100 and 500 mg/kg.
These doses are multiples of 125, 250 and 1250 times the measured LD_{50} in mice of 0.4mg/kg.

3.3.4 VEHICLE

Polyethylene glycol (PEG 300) with distilled water.

3.3.5 CONCENTRATION IN VEHICLE

5, 10 and 50 g/l.

3.3.6 TOTAL VOLUME APPLIED

0.25 to 0.3 ml (10 ml/kg).

3.4 Examinations

The mice were observed for up to 6 days after treatment. Clinical signs and mortalities were noted. Macroscopic examinations were performed at autopsy.

3.5 Method of determination of LD_{50}

Not applicable.

4 RESULTS

4.1 LD_{50}

LD_{50} values were not determined in this study.

The aim of the study was to observe the effect of WBA 8119 in acute dosages greatly in excess of that recorded for the LD_{95} in order to reveal any secondary mode of action, not to determine the LD_{50}.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY (MASSIVE DOSES) OF WBA 8119 (BRODIFACOUM) TO MALE MICE

Dose (mg/kg)	n Dead/ n Investigated	Time of Death (Range)	Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations
50	10/10	Days 3 - 5	All the mice in the three dose groups died between days 1 - 6. The mice which died from day 3 onwards exhibited hunched posture, anaemic appearance, and blood around tail and nose. Internal postmortem examination revealed massive internal haemorrhages and some liver enlargement. No other atypical lesions were noted. The four mice which died on the first day in the top dose group (500 mg/kg) were noted to be subdued and hunched three hours after intubation. Death occurred between 8 and 16 hours, possibly due to maladministration. Convulsions were noted at death in one animal which is consistent with maladministration. Internal examination revealed no haemorrhages and no atypical lesions.
100	10/10	Days 3 - 6	
500	10/10	Days 1- 5	
LD_{50}: Not determined in this study			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material WBA 8119 (brodifacoum); Batch [redacted]; Purity: >[redacted] %. The test material as a solution in PEG 300 with distilled water, was administered to groups of 10 male mice by oral gavage at dose levels of 50, 100 and 500 mg/kg (125, 250 and 1250 times the measured LD_{50} of 0.4 mg/kg. Animals were observed daily for up to 6 days, then sacrificed and autopsied.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

All the mice in the three dose groups died between days 1 - 6. The mice which died from day 3 onwards exhibited hunched posture, anaemic appearance, blood around tail and nose. Internal postmortem examination revealed massive internal haemorrhages and some liver enlargement. No other atypical lesions were noted.

The four mice which died on the first day in the top dose group (500 mg/kg) were noted to be subdued and hunched three hours after intubation. Death occurred between 8 and 16 hours. Convulsions were

noted at death in one animal. Internal examination revealed no haemorrhages and no atypical lesions in any case.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	-	House mouse (*Mus domesticus*)/ LAC/ Male/ 10 per group	50, 100, 500	Single dose	Mouse (male)/ effect of very high acute oral doses)	All the mice in the three dose groups died by day 6	[redacted], 1975, [redacted]

5.4 Conclusion

All the mice in the three dose groups died in this study. The mice which died from day 3 onwards exhibited symptoms typical as a result of ingestion of an indirect anticoagulant.
Four of the mice in the top dose group of 500 mg/kg died on the first day between 8 and 16 hours after intubation. These four animals did not exhibit symptoms typical of an indirect anticoagulant as internal examination revealed no haemorrhages and no atypical lesions. It was considered that there may be an indication of an alternative mode of action of the test material at a dose equivalent to 1250 times the acute oral LD_{50}.

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

Doc IIIA / Section 5.4

BPD Data Set IIA / Annex Point V.5.4

Acute Oral Toxicity (Effect of Massive Oral Dose in Rats and Mice)

1 REFERENCE

1.1 Reference

[redacted], 1978, 'The Effect of Massive Acute Oral Exposure to Pindone, Warfarin and Brodifacoum', [redacted]

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

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

2.2 GLP

No. GLP was not compulsory at the time.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum.

3.1.1 LOT/BATCH NUMBER

Bx. Ref S/T 647.

3.1.2 SPECIFICATION

As given in Section 2.

3.1.3 DESCRIPTION

Solid.

3.1.4 PURITY

>91 %.

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Rattus norvegicus (Rat).
Mus domesticus (House mouse).

3.2.2 STRAIN

Rat: Wistar.
Mice: BKTO.

3.2.3 SOURCE

Not specified.

3.2.4 SEX

Rat: male.
Mice: male.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Rat: 250g
Mice: 25g.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

4 per group (rats and mice).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to at least 4 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

Rat: 100, 200, 500, 1000 and 2000 mg/kg.
Mice: 100, 200, 500, 1000 and 2000 mg/kg.

3.3.4 VEHICLE

Deionised water with 2% Tween 80.

3.3.5 CONCENTRATION IN VEHICLE

Not specified.

3.3.6 TOTAL VOLUME APPLIED

Not specified.

3.4 Examinations

Rats and Mice:
The animals were observed for clinical signs of toxicity and mortality hourly during the first 8 hours after treatment and daily thereafter. Those animals which died within the first 24 hours were examined at death. After 4 days, the surviving animals were sacrificed and examined.

3.5 Method of determination of LD_{50}

Not applicable.

4 RESULTS

4.1 LD_{50}

LD_{50} values at 24 hous after treatment were not estimated in this study.

The aim of the study was to observe and compare the potency and symptoms of the proposed secondary mode of action, through massive acute oral doses of the three anticoagulants brodifacoum, pindone and warfarin.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY (MASSIVE DOSES) OF BRODIFACOUM TO THE RAT			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (Range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
100	0/4	-	There were no mortalities within the first 24 hours after treatment and no mortalities in the 100, 500 and 1000 mg/kg dosage groups. The major symptoms noted were hunched or splayed posture, rapid ventilation movements leading to audible gasping, fluid around the mouth and convulsive movement immediately prior to death. Macroscopic examination of the animals that died and those surviving to the end of the observation period showed a minor degree of internal haemorrhages at the highest dose levels.
200	1/4	Day 4	
500	0/4	-	
1000	0/4	-	
2000	2/4	Days 2 - 4	
LD_{50} not determined.			

ACUTE ORAL TOXICITY (MASSIVE DOSES) OF BRODIFACOUM TO THE MOUSE			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (Range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
100	1/4	Day 4	There were no mortalities within the first 24 hours after treatment but there were mortalities within all the dosage groups during the observation period. The major symptoms noted were hunched or splayed posture, rapid ventilation movements leading to audible gasping, fluid around the mouth and convulsive movement immediately prior to death. Macroscopic examination of the animals that died and those surviving to the end of the observation period showed a minor degree of internal haemorrhages at the highest dose levels.
200	1/4	Day 4	
500	1/4	Day 4	
1000	2/4	Day 4	
2000	4/4	Days 2 - 4	
LD_{50} not determined.			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material brodifacoum; Batch bx ref S/T 647.; Purity >90%. The test material as a wet ground aqueous suspension in deionised water with 2% Tween 80, was administered to groups of 4 male rats or mice by oral gavage at dose levels of 100, 200, 500, 1000 and 2000 mg/kg. Animals were observed daily for up to 4 days, then sacrificed and macroscopically examined.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

Rat

There were no mortalities within the first 24 hours after treatment and no mortalities in the 100, 500 and 1000 mg/kg dosage groups.
The major symptoms noted were hunched or splayed posture, rapid ventilation movements leading to audible gasping, fluid around the mouth and convulsive movement immediately prior to death.
Macroscopic examination of the animals that died and those surviving to the end of the observation period showed a minor degree of internal haemorrhages at the highest dose levels.

Mouse

There were no mortalities within the first 24 hours after treatment but there were mortalities within all the dosage groups during the observation period.
The major symptoms noted were hunched or splayed posture, rapid ventilation movements leading to audible gasping, fluid around the mouth and convulsive movement immediately prior to death.
Macroscopic examination of the animals that died and those surviving to the end of the observation period showed a minor degree of internal haemorrhages at the highest dose levels.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Result	Reference
Oral		Rat (*Rattus norvegicus*)/ Wistar/ Male/ 4 per group	100, 200, 500, 1000, 2000	Single dose	Rat (male)/ effect of very high acute oral doses	Mortalities occurred between Days 2 and 4.	M R Hadler, 1978, RIC0576 (C2.1/15)
Oral		House mouse (*Mus domesticus*)/ BKTO / Male/ 4 per group	100, 200, 500, 1000, 2000	Single dose	Mouse (male)/ effect of very high acute oral doses	Mortalities occurred between Days 2 and 4.	M R Hadler, 1978, RIC0576 (C2.1/15)

5.4 Conclusion

The symptoms noted for brodifacoum were far less marked than the other two anticoagulants (warfarin and pindone) also tested in the study.
Although there were no deaths in the animals treated with brodifacoum in the first 24 hours after treatment, there were mortalities in some of the warfarin and pindone dosage groups. Therefore, the study showed that at massive oral doses, death can occur for warfarin and pindone within 24 hours; the symptoms noted were not those expected of an indirect anticoagulant and indicative of respiratory involvement. The potency in order of decreasing potency of this secondary effect in the three anticoagulants tested are pindone>warfarin>brodifacoum.

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

Acceptability Remarks

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

Doc IIIA /
Section 6.1.1

Acute Oral Toxicity
(Oral LD_{50} in Mice)

BPD Data Set IIA /
Annex Point VI.6.1.1

1 REFERENCE

1.1 Reference

[redacted], 'Acute Oral Toxicity of WBA 8119 to Male Mice', Ward Blenkinsop and Company Limited, Agricultural Research, RIC0559 (C2.1/04), 1st November 1974 [C2.1/04].

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

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

2.2 GLP

No. Study pre-dates the requirement for GLP

2.3 Deviations and Deficiencies

Not applicable X

3 MATERIALS AND METHODS

3.1 Test Material

WBA 8119 (brodifacoum).
3.1.1 LOT/BATCH NUMBER

Not available.

3.1.2 SPECIFICATION

As given in Section 2 of Doc. IIIA. X

3.1.3 DESCRIPTION

Powder.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

As given in section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Mus domesticus (House mouse).

3.2.2 STRAIN

LAC.

3.2.3 SOURCE

OLAC Ltd.

3.2.4 SEX

Male.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

30 - 35 g.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

10 (male).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to 21 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg.

3.3.4 VEHICLE

Polyethylene glycol (PEG 300).

3.3.5 CONCENTRATION IN VEHICLE

0.01, 0.02, 0.05, 0.10, and 0.20 g/l.

3.3.6 TOTAL VOLUME APPLIED

0.3 - 0.35 ml (10 ml/kg).

3.4 Examinations

The mice were observed for up to 21 days after treatment. Clinical signs and mortalities were noted. Macroscopic examinations were performed at autopsy.

3.5 Method of Determination of LD_{50}

Acute oral LD_{50} by best line of fit of the data.

4 RESULTS

4.1 LD_{50}

0.40 mg/kg to male mice.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY OF WBA 8119 (BRODIFACOUM) TO MALE MICE			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (Range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
0.1	0/10	-	The animals dosed with 0.2 mg/kg or less all survived the study. All deaths occurred between days 4 - 12. Autopsy of all the mice which died during the study showed massive internal haemorrhage.
0.2	0/10	-	
0.5	10/10	Days 7 - 10	
1.0	10/10	Days 4 - 12	
2.0	10/10	Days 4 - 9	
LD_{50}: 0.4 mg/kg			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

X

Test material WBA 8119 (brodifacoum); Purity: >91 %. The test material as a solution in PEG 300, was administered to groups of 10 male mice by oral gavage at dose levels of 0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg. Animals were observed daily for a period of 21 days, then sacrificed and autopsied.

5.2 Reliability

Reliability indicator: 3).

5.3 Findings

No mortalities were observed in the dose groups of 0.1 and 0.2 mg/kg. The mortalities in the top three dose groups (0.5, 1.0 and 2.0 mg/kg) occurred between days 4 - 12. The post-mortem examination showed massive internal haemorrhages in all the mice which died.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	-	House mouse (*Mus domesticus*)/ LAC/ Male/ 10 per group	0.1, 0.2, 0.5, 1.0, 2.0	Single dose with 21 day observation period	Mouse (male) oral LD_{50}	0.4	M R Hadler, 1974, RIC0559 [C2.1/04]

5.4 Conclusion

The oral LD_{50} of WBA 8119 (brodifacoum) in the male mouse was determined to be 0.4 mg/kg. The results of the post-mortem examinations indicate that the mortalities were due to the anticoagulant action of brodifacoum.

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

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

Doc IIIA /
Section 6.1.1

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

BPD Data Set IIA /
Annex Point VI.6.1.1

1 REFERENCE

1.1 Reference

[redacted], 1993, 'Brodifacoum: Acute Oral Toxicity', ICI Central Toxicology Laboratory, CTL/P/3918 (C2.1/20).

1.2	**Data protection**	Yes.
1.2.1	Data owner	Syngenta Limited.
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 active substance 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 and Deficiencies

None X

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum.

3.1.1 LOT/BATCH NUMBER

[redacted]

3.1.2 SPECIFICATION

As given in Section 2 of Doc. IIIA.

3.1.3 DESCRIPTION

Off-while solid.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

Please refer to section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Rattus norvegicus (Rat).

3.2.2 STRAIN

Specific pathogen free (SPF) Wistar-derived albino rats (Alpk: APfSD strain).

3.2.3 SOURCE

Barriered Animal Breeding Unit, ZENECA Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.

3.2.4 SEX

Male and female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Young adults / 224 - 311 g (male), 186 - 250 g (female).

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

5 (male) and/or 5 (female).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to 15 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

0.25, 0.35, 0.50, 0.75 mg/kg.

3.3.4 VEHICLE

Polyethylene glycol (PEG 300).

3.3.5 CONCENTRATION IN VEHICLE

0.025, 0.035, 0.050, 0.075 g/l.

3.3.6 TOTAL VOLUME APPLIED

Male: 2.24 - 3.11 ml;
Female: 1.86 - 2.50 ml;
(10 ml/kg).

3.4 Examinations

The rats were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made once daily, or twice daily whenever there were significant signs of toxicity, up to day 15. The animals were weighed on the day before dosing (day -1), the day of dosing (day1), day 3, day 5, day 8, and day 15.
Animals *in extremis* and/or showing signs of bleeding and those surviving at the end of the study were humanely killed by inhalation of halothane Ph Eur (Fluothane, Zeneca Pharmaceuticals) vapour followed by exsanguination, and together with those found dead were subjected to a macroscopic post-mortem examination.

3.5 Method of Determination of LD_{50}

Calculated from the mortality data (the mortality data included animals that were killed *in extremis* and/or showing signs of bleeding) by logistic regression using nominal dose values. Confidence limits for females were calculated using a likelihood ratio interval (reference 1). Approximate confidence limits for males are given by the highest dose with no mortalities and the lowest dose with 100% mortality. Slope estimates could not be calculated for either sex due to the mortality patterns.
4 RESULTS

4.1 LD_{50}

0.418 mg/kg to the male rat (approximate 95% confidence limits: 0.350, 0.500)
0.561 mg/kg to the female rat (approximate 95% confidence limits: 0.472, 0.667)**4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations**

ACUTE ORAL TOXICITY OF BRODIFACOUM TO THE RAT

Dose (mg/kg)	**n Dead/ n Investigated**		**Time of Death (range)**		**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
	Male	**Female**	**Male**	**Female**	**Male and Female**
0.25	0/5	0/5	-	-	There were no deaths or significant signs of toxicity at the two lowest dose levels of 0.25 and 0.35 mg/kg. All of the males and one of the females dosed with 0.50 mg/kg were either found dead or were killed *in extremis* by day 9. Signs of toxicity observed prior to death included pallor, subdued behaviour or decreased activity, bruising and bleeding from the nose or rectum. Signs of slight toxicity were observed in the surviving females. At 0.75 mg/kg, all of the females were killed *in extremis* and/or showing signs of bleeding by day 6. Signs of toxicity observed prior to death included pallor, decreased activity, bleeding (eg from the nose, ear markings and paws) and bruising. After the initial weight loss due to fasting, most animals exceeded their day -1 bodyweight by day 3. Most of the animals surviving to termination continued to gain weight throughout the study. Most of the animals that were found dead or were humanely killed showed a reduction in weight prior to death. At *post mortem* examination, free or clotted blood was observed in the thoracic and abdominal cavity, the kidney, oesophagus and subcutis. Discolouration of a variety of organs was also observed.
0.35	0/5	Not dosed	-	-	
0.5	5/5	1/5	Days 5 - 9	Day 9	
0.75	Not dosed	5/5	-	Days 4 - 6	
LD_{50}: 0.418 mg/kg to the male rat 0.561 mg/kg to the female rat					

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material brodifacoum; Batch P3/D7534/53; Purity 96.1%.
The test material as a solution in PEG 300, was administered to groups of 5 male and/or 5 female rats by oral gavage at dose levels of 0.25, 0.35, 0.50, 0.75 mg/kg. The animals were observed daily for signs of systemic toxicity and mortalities, and their body weights recorded at intervals throughout the study.

5.2 Reliability

Reliability indicator: 1).

5.3 Findings

There were no deaths or significant signs of toxicity at the two lowest dose level of 0.25 and 0.35 mg/kg. All of the males and one of the females dosed with 0.50 mg/kg were either found dead or were killed *in extremis* by day 9. Signs of toxicity observed prior to death included pallor, subdued behaviour or decreased activity, bruising and bleeding from the nose or rectum. Signs of slight toxicity were observed in the surviving females. At 0.75 mg/kg, all of the females were killed *in extremis* and/or showing signs of bleeding by day 6. Signs of toxicity observed prior to death included pallor, decreased activity, bleeding (eg from the nose, ear markings and paws) and bruising.

After the initial bodyweight loss due to fasting, most animals exceeded their day -1 bodyweight by day 3. Most of the animals surviving to termination continued to gain weight throughout the study. Most of the animals that were found dead or were humanely killed showed a reduction in weight prior to death.

Macroscopic abnormalities among those animals that died or were humanely killed during the study included haemorrhage in various organs, discolouration or pallor of internal organs and stomach and ileal contents, increased fluidity of rectal contents, renal pelvic dilatation and limb and ear trauma. In the surviving animals, observations included red areas in the thymus, pallor of the kidney and renal pelvic dilatation.
With the exception of the renal pelvic dilatation, which is a common spontaneous finding, all of the above signs are consistent with brodifacoum's mode of toxicity, anticoagulation.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	OECD 401	Rats (*Rattus norvegicus*)/ Alpk: APfSD / Male/ 5 per group	0.25, 0.35, 0.50	Single dose with 15 day observation period	Rat (male) oral LD_{50}	0.418 (approximate 95% confidence limits: 0.350, 0.500)	Duerden L, 1993, CTL/P/3918 (C2.1/20)
Oral	OECD 401	Rats (*Rattus norvegicus*)/ Alpk: APfSD / Female/ 5 per group	0.25, 0.50, 0.75	Single dose with 15 day observation period	Rat (female) oral LD_{50}	0.561 (approximate 95% confidence limits: 0.472, 0.667)	Duerden L, 1993, CTL/P/3918 (C2.1/20)

5.4 Conclusion X

The oral LD_{50} of brodifacoum in rats was determined to be 0.418 mg/kg to the male rat (approximate 95% confidence limits 0.350 - 0.500), and 0.561 mg/kg to the female rat (approximate 95% confidence limits: 0.472, 0.667).

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

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

Doc IIIA /
Section 6.1.2

Acute Dermal Toxicity
(Dermal LD_{50} in the Rat)

BPD Data Set IIA /
Annex Point VI.6.1.2

1 REFERENCE

1.1 Reference

[redacted], 1991, 'Brodifacoum Technical: Acute Dermal Toxicity to the Rat', ICI Central Toxicology Laboratory, CTL/P/3595 [C2.1/19], 20 Dec 1991.

1.2	**Data protection**	Yes.
1.2.1	Data owner	Syngenta Limited.
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 active substance for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

OECD 402.

2.2 GLP

[redacted]

2.3 Deviations and Deficiencies

None . X

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum.

3.1.1 LOT/BATCH NUMBER

[redacted]

3.1.2 SPECIFICATION

As given in Section 2.

3.1.3 DESCRIPTION

Off-white solid.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Rattus norvegicus (rat).

3.2.2 STRAIN

Wistar-derived albino rats / Crl: (WI)BR strain.

3.2.3 SOURCE

Charles River Limited, Manston Road, Margate, Kent, UK.

3.2.4 SEX

Male and female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Young adults / 260 - 358 g (male), 179 - 233 g (female).

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

5 male and 5 female per dose group.

3.3 Administration/Exposure

Dermal.

3.3.1 POSTEXPOSURE PERIOD

Observed to 14 days after treatment.

3.3.2 AREA COVERED

Approximately 4 cm x 6 cm on the dorso-lumbar region.

3.3.3 OCCLUSION

Occlusive dressing of gauze patch , covered by a patch of plastic film and held in position using adhesive bandage, and secured by two pieces of PVC tape wrapped around the animal.

3.3.4 VEHICLE

Polyethylene glycol (PEG 600) for the lowest dose group of 1 mg/kg.
Olive oil for the middle dose group of 10 mg/kg.
Corn oil for the top dose group of 500 mg/kg.

3.3.5 CONCENTRATION IN VEHICLE

Approximately 0.5 g/l for the test substance in PEG 600 (lowest dose group of 1 mg/kg).
Approximately 12.9 g/l for the test substance in olive oil (middle dose group of 10 mg/kg).
Approximately 643.7 for the test substance in corn oil (top dose group of 500 mg/kg).

3.3.6 TOTAL VOLUME APPLIED

Approximately 0.52 ml for the test substance in PEG 600 (lowest dose group of 1 mg/kg).
Approximately 0.2ml for the test substance in olive oil (middle dose group of 10 mg/kg).
Approximately 0.2ml for the test substance in corn oil (top dose group of 500 mg/kg).

3.3.7 DURATION OF EXPOSURE

24 hours.

3.3.8 REMOVAL OF TEST SUBSTANCE

The skins of the animals were cleansed with clean warm water.

3.4 Examinations

The animals were observed for signs of systemic toxicity once between 1 and 4 hours after application and then once daily, for systemic toxicity and skin irritation, up to day 15. Each animal was weighed immediately before treatment (day 1) and on days 3, 4, 8 and 15. Animals *in extremis* and those surviving to the end of the study were humanely killed and subjected to a macroscopic post-mortem examination.

3.5 Method of Determination of LD_{50}

Calculated from the mortality data by logistic regression for the males, and was estimated using linear log-dose interpolation for the females, using nominal dose values. Confidence limits for the males were calculated using a likelihood ratio interval (reference 1), and approximate confidence limits for the females were given by the highest dose with no mortalities and the lowest dose with 100% mortality.

4 RESULTS

4.1 LD_{50}

Male rat: 5.21 mg/kg (95% confidence limits 1.95 - 13.8).
Female rat: 3.16 mg/kg (approximate confidence limits 1.00 - 10.00).

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE DERMAL TOXICITY OF Brodifacoum TO THE RAT

Dose (mg/kg)	n Dead/ n Investigated		Time of Death (range)		Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations
	Male	Female	Male	Female	Male and Female
1	0/5	0/5	-	-	There were no mortalities or significant signs of toxicity in the lowest dose group of 1 mg/kg. The test substance was non-irritant to males and practically non-irritant to females. In the middle dose group of 10 mg/kg, four males were found dead or killed *in extremis* between days 7 - 11, and all females were killed *in extremis* between days 6 - 8. There were no significant signs of toxicity in the surviving male. In the top dose group of 500 mg/kg all the animals were killed *in extremis* between days 5 - 8. Post-mortem examination revealed widespread internal haemorrhage in the animals which died or were killed *in extremis*.
10	4/5	5/5	Days 7 - 11	Days 6 - 8	
500	5/5	5/5	Days 5 - 6	Days 5 - 8	
LD_{50}: Male rat: 5.21 mg/kg Female rat: 3.16 mg/kg					

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material brodifacoum; Batch Y00052/035 (Bx 0007); Purity 95.6%.
The test material as a solution in PEG 600 (for the 1 mg/kg dose group), as a paste in olive oil (for the 10 mg/kg dose group) or as a paste in corn oil (for the 500 mg/kg dose group), was applied to the shorn backs of groups of 5 male and 5 female rats, and occluded with dressings for an exposure period of 24 hours. Animals were observed for signs of systemic toxicity up to day 15 and bodyweights recorded. Animals *in extremis* and those surviving to the end of the study were humanely killed and subjected to a macroscopic post-mortem examination.

5.2 Reliability

Reliability indicator: 1.

5.3 Findings

Animals treated with a single dermal application of 1 mg/kg showed no significant signs of toxicity or skin irritation considered to be compound related. Following a dermal application of 10 mg/kg, four males were found dead or killed *in extremis* between days 7 - 11, and all the females were killed *in extremis* between days 6 - 8. The animals which died or were killed showed signs of extreme toxicity (pallor, bleeding/ bruising, breathing abnormalities) immediately prior to death, but had previously only shown minor symptoms.

There were practically no signs of skin irritation in any of the animals and no significant signs of toxicity in the surviving male. Following the 500 mg/kg application, all the males were killed *in extremis* on day 5 - 6 and all the females between days 5 - 8. The symptoms were similar to those seen at 10 mg/kg.
All mortalities in all dosage groups occurred between days 5 - 11.
Most animals dosed with 1 mg/kg exceeded their initial bodyweight by day 8 and continued to gain weight for the rest of the study. All the males dosed with 10 mg/kg lost weight early in the study, and one showed further weight loss prior to termination. Most of the females at this dose rate gained weight but one showed a weight loss prior to death. Four males and all the females dosed with 500 mg/kg gained weight initially and some showed further weight gain prior to death.
Post-mortem examination revealed internal haemorrhaging in the animals which died or were killed *in extremis*. There was discolouration of bowel contents and a number of organs. Pelvic dilatation and distention of the bladder were also seen in a number of animals but these are common symptoms in rats of this age and were not considered to be compound related.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Dermal	OECD 402	Rat *(Rattus norvegicus)*/ Wistar-derived albino rats Crl: (WI)BR/ Male/ 5 per group	1, 10, 500	Single dose with 15 day observation period	Rat (male) dermal LD_{50}	5.21 (95% confidence limits 1.95 - 13.8)	McCall J C and Leah A M, 1991 CTL/P/3595 (C2.1/19)
Dermal	OECD 402	Rat (*Rattus norvegicus)*/ Wistar-derived albino rats Crl: (WI)BR/ Male/ 5 per group	1, 10, 500	Single dose with 15 day observation period	Rat (female) dermal LD_{50}	3.16 (approximate confidence limits 1.00 - 10.00)	McCall J C and Leah A M, 1991 CTL/P/3595 (C2.1/19)

5.4 Conclusion X

The dermal LD_{50} of brodifacoum in rat was determined to be:
Male rat: 5.21 mg/kg (95% confidence limits 1.95 - 13.8).
Female rat: 3.16 mg/kg (approximate confidence limits 1.00 - 10.00).
The clinical signs and results of the post-mortem examinations indicate that the mortalities were due to the anticoagulant action of brodifacoum.

Evaluation by Competent Authorities
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EVALUATION BY RAPPORTEUR MEMBER STATE

Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	[redacted]
	COMMENTS FROM ...
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	

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

Doc IIIA /
Section 6.1.3

Acute Inhalation Toxicity
(Inhalation LC_{50} in the Rat)

BPD Data Set /
Annex Point VI.6.1.3

1 REFERENCE

1.1 Reference

[redacted] 1993, 'Brodifacoum: 4-Hour Acute Inhalation Toxicity Study in the Rat', [redacted], /P/4065 [C2.1/21].

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

Yes, study conducted in accordance with OECD guideline 403.

2.2 GLP

Yes.

2.3 Deviations and Deficiencies

None X

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum.

3.1.1 LOT/BATCH NUMBER

P3/D7534/53 (R98581)/(Y00052/038).

3.1.2 SPECIFICATION

As given in Section 2 of Doc IIIA.

3.1.3 DESCRIPTION

Off-while solid.

3.1.4 PURITY

[redacted]% pure.

3.1.5 STABILITY

As given in Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Rattus norvegicus (Norway rat).

3.2.2 STRAIN

Specific pathogen free (SPF) Wistar-derived albino rats (Alpk: APfSD strain).

3.2.3 SOURCE

[redacted].

3.2.4 SEX

Male and female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Young adults / 273 - 332 g (male), 215 - 234 g (female).

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

5 (male) and 5 (female).

3.3 Administration/Exposure

Inhalation.

3.3.1 POST EXPOSURE PERIOD

Observed to 14 days after treatment.

3.3.2 CONCENTRATIONS

Test Group	Nominal Brodifacoum Concentrations (mg/m^3)	Analytical Brodifacoum Concentrations (mg/m^3)
1	10.35	4.40
2	3.50	1.72
3	1.05	0.69

The atmospheres of the test material were controlled on the basis of the total particulate concentration. The concentrations of test atmospheres were as follows:

Test Group	Target Particulate Concentrations (mg/m^3)	Measured Particulate Concentrations (mg/m^3)
1	5.0	4.96
2	1.5	1.88
3	0.5	0.82

It was considered that with a generation system of the type employed in this study, the volatility of acetone (the solvent vehicle) and the fine particle size generated, that all the solvent had evaporated following generation of the test atmosphere and the particulate concentration represented brodifacoum technical material.
Therefore, the particulate concentrations were used to identify the exposure levels.

3.3.3 PARTICLE SIZE

Test Group	Median Size (MMAD/µm)	Geometric Standard Deviation (µm)
1	0.68	2.54
2	0.89	1.91
3	0.80	3.09

3.3.4 TYPE OR PREPARATION OF PARTICLES

The particles were prepared from a solution of brodifacoum in acetone using a glass concentric-jet atomiser.

3.3.5 TYPE OF EXPOSURE

Nose only exposure.

3.3.6 VEHICLE

Acetone.

3.3.7 CONCENTRATION IN VEHICLE

5 g/l of brodifacoum in a solution of acetone.

3.3.8 DURATION OF EXPOSURE

4 hours.

3.4 Examinations

The animals were observed frequently during exposure for clinical signs of toxicity. At the end of the 4 hour exposure period each animal was given a detailed clinical examination. The animals were also given a detailed examination on each day of the 14 day observation period.
The animals were weighed the day before exposure (day -1), prior to exposure on day 1, and then on days 2, 3, 8 and 15.
Post-mortem examinations on all animals were carried out with particular attention being given to abdominal and thoracic viscera. Lungs (with trachea and larynx attached) were excised and trimmed, and the weights recorded (following removal of the larynx).
The animals which were killed *in extremis* were similarly examined as soon after death as possible but organ weights were not recorded.

3.5 Method of Determination of LC_{50}

The LC_{50} was estimated by logistic regression. Confidence limits were calculated using a likelihood ratio interval.

4 RESULTS

4.1 LC_{50}

4.86 mg/m^3 to the male rat (95 % confidence limits 2.97 - 11.1 mg/m^3);
3.05 mg/m^3 to the female rat (95 % confidence limits 1.88 - 4.96 mg/m^3).

4.2 Effects /4.3 Reversibility/4.4 Findings of Histopathological Examinations

ACUTE INHALATION TOXICITY OF BRODIFACOUM TO THE RAT					
Dose (mg/m^3)	**n Dead/ n Investigated**		**Time of Death (range)**		**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
	Male	**Female**	**Male**	**Female**	**Male and Female**
4.96	3/5	5/5	Days 4 - 6	Days 4 - 6	During exposure and immediately after exposure, clinical abnormalities generally associated with restraint (stains around the snout, wet fur, hunched posture and piloerection) were seen in all groups. During the post exposure period, the clinical condition of most of the animals exposed to 0.82 and 1.88 mg/m^3 brodifacoum improved, and in general, only minor changes (piloerection) were evident after day 4. Three males and all the females exposed to 4.96 mg/m^3 were killed *in extremis* or found dead on days 4, 5 and 6. The post-mortem examinations of these animals showed treatment related effects including internal haemorrhages, enlarged and/or discoloured thymuses and staining of the nares. No treatment related effects were identified in the surviving animals that were sacrificed at the end of the study. After a small initial bodyweight loss, attributable to the exposure method, all surviving animals gained weight throughout the remainder of the study.
1.88	0/5	0/5	-	-	
0.82	0/5	0/5	-	-	
LD_{50}: 4.86 mg/m^3 to the male rat; 3.05 mg/m^3 to the female rat.					

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material brodifacoum; Batch P3/D7534/53 (R98581)/(Y00052/038); Purity [redacted]%.
Groups of 5 male and 5 female Alpk: APfSD rats were exposed for 4 hours to atmospheres containing brodifacoum at particulate concentrations of 0.82, 1.88 or 4.96 mg/m^3; the median particle sizes/geometric standard deviations were 0.80/3.09, 0.89/1.91 and 0.68/2.54 µm respectively.
All animals were examined pre-exposure for clinical abnormalities. The animals were observed frequently during the inhalation period and given a detailed clinical examination at the end of the exposure. The animals were observed for signs of systemic toxicity daily for 14 days, and the bodyweight recorded on days -1, 1, 2, 3, 8 and 15.
Those animals surviving the study were humanely killed and, along with any animals killed *in extremis*, were subjected to post-mortem examination. The animals were examined as soon after death as possible.

5.2 Reliability

Reliability indicator: 1).

5.3 Findings

During exposure and immediately after exposure, clinical abnormalities generally associated with restraint (stains around the snout, wet fur, hunched posture and piloerection) were seen in all groups. During the post exposure period, the clinical condition of most of the animals exposed to 0.82 and 1.88 mg/m^3 brodifacoum improved, and in general, only minor changes (piloerection) were evident after day 4.
Three males and all the females exposed to 4.96 mg/m^3 were killed *in extremis* or found dead on days 4, 5 and 6. Symptoms of toxicity included subcutaneous haemorrhage of the head and thorax, signs of bleeding from hind limbs and snout, decreased activity, increased respiratory depth, reduced respiratory rate and shaking.
A small initial bodyweight loss was seen in animals from all exposure groups, probably due to the use of restraint during exposure. All surviving animals gained weight throughout the remainder of the study.
The post-mortem examinations of the animals that were found dead or were killed *in extremis* showed treatment related effects including internal haemorrhages, enlarged and/or discoloured thymuses and staining of the nares.
No treatment related effects were identified in the surviving animals which were sacrificed at the end of the study.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Exposure Concen-trations (mg/m^3)	Duration of Exposure	Endpoint	Value (mg/m^3)/ Remarks	Reference
Inhalation	OECD guideline 403	Norway rat (*Rattus norvegicus*)/ Alpk: APfSD/ Male and Female/ 5 each per group	0.82, 1.88, 4.96	4 hours	Rat (male and female) inhalation LC_{50}	Male: 4.86 (95 % confidence limits 2.97 - 11.1); Female: 3.05 (95 % confidence limits 1.88 - 4.96)	[redacted] J, 1993, [redacted] /P/4065 ([redacted])

5.4 Conclusion X

The 4 hour LC_{50} of brodifacoum in the rat was determined to be:
4.86 mg/m^3 to the male rat (95 % confidence limits 2.97 - 11.1 mg/m^3), and
3.05 mg/m^3 to the female rat (95 % confidence limits 1.88 - 4.96 mg/m^3).

The delayed clinical effects, post-mortem findings and late deaths are all indicative of haemorrhage which are typical of exposure to anticoagulant rodenticides.
The lack of any significant clinical effects and the lack of gross abnormalities at necropsy in those animals surviving to termination, demonstrates a rapid recovery from exposure to non-lethal concentrations of the test material.

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

Remarks

Doc IIIA / Section 6.1.4 — **Acute Eye Irritation (Eye Irritation in the Rabbit)**

BPD Data Set IIA / Annex Point VI.6.1.4

1 REFERENCE

1.1 Reference

[redacted], 1978, 'Brodifacoum: Skin and Eye Irritation', ICI Central Toxicology Laboratory, CTL/P/404 [C2.1/10], 26th July 1978.

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

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

EPA guidelines 5 13 77.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

None stated.

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum (PP581).

3.1.1 LOT/BATCH NUMBER

Not specified.

3.1.2 SPECIFICATION

As given in Section 2.

3.1.3 DESCRIPTION

Buff coloured solid.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Oryctolagus cuniculus (rabbit).

3.2.2 STRAIN

New Zealand White (male).

3.2.3 SOURCE

Not specified.

3.2.4 AGE/WEIGHT AT STUDY INITIATION

2 - 2.5 kg.

3.2.5 NUMBER OF ANIMALS PER GROUP (SEX)

One group of 9 male rabbits (6 unwashed and 3 washed eyes).

3.3 Study Design and Methods

3.3.1 APPLICATION

3.3.1.1 Preparation of test substance

The brodifacoum was used as received for the study.

3.3.1.2 Amount of active substance instilled

100 mg per eye.

3.3.1.3 Exposure period

Single treatment: 3 animals had the treated eye irrigated 30 seconds after instillation of test substance, and 6 were left unwashed.

3.3.1.4 Post treatment observation period

7 days.

3.3.2 EXAMINATIONS

3.3.2.1 Scoring system

The ocular lesions were scored using the Draize scale:

	CORNEA		IRIS	CONJUNCTIVA		
	a: Opacity	b: Area	c	d: Redness	e: Chemosis	f: Discharge
Score	0 to 4	0 to 4	0 to 2	0 to 3	0 to 4	0 to 3
Maximum	a x b x 5 = 80		c x 5 = 10	(d + e + f) x 2 = 20		
Total score	80 + 10 + 20 = 110					

The initial pain reaction was assessed on a 0 - 5 point scale.

A modified form of the system described by Kay and Calandra was used to interpret and classify the numerical scores.

3.3.2.2 Examination time points

Animals observed at 1-2 hours after application, then at 24 hours, 48 hours, 72 hours, day 4, day 7.

3.3.2.3 Other investigations

The effect of irrigating the eye for 1 minute with clean lukewarm water, 30 seconds after instillation of the test substance was investigated in 3 rabbits.

4 RESULTS

4.1 Scores at different time points/
4.2 Reversibility

RESULTS FOR EYE IRRITATION TO RABBIT WITH BRODIFACOUM (Unwashed eyes)				
	Cornea (Opacity)	**Iris**	**Conjunctiva**	
			Redness	**Chemosis**
Score (average of animals investigated)	**0 to 4**	**0 to 2**	**0 to 3**	**0 to 4**
1 - 2 h	Not assessed	0.5	1	0.83
24 h	0	0	0.83	0.33
48 h	0	0	0.67	0
72 h	0	0	0.50	0
Average 24 h, 48 h, 72 h	0	0	0.67	0.11
Area affected	-	Not specified		
Mean total score (max 110)**	1 - 2 h: 8 24 h: 2 48 h: 1 72 h: 1			
Reversibility*	-	c	c	c
Average time for reversion	-	24 h	7 days	48 h

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

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