Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Rodenticide PT 14 *Reference*: **A5.1/01:** Anonymous (1987) Storm rodenticide – a users's guide, The Shell Guide to Rodent Control (published).	
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)	Primarily commensal rodents: Norway rat (*Rattus norvegicus*) Roof rat (*Rattus rattus*) House mouse (*Mus musculus*)	
5.2.2	Products, organisms or objects to be protected (IIA5.2)	Farm buildings, e.g. barns, animal housings Warehouses Domestic premises Industry buildings Use for public hygiene	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**		X
5.3.1	Effects on target organisms (IIA5.3)	Flocoumafen is a second-generation anticoagulant rodenticide, designed to cause lethal internal haemorrhages following single consumption/ administration.	
5.3.2	Likely concentrations at which the a.s. will be used (IIA5.3)	Concentration of active substance in ready-to-use baits is 0.005 % (m/m) ≡ 50 mg/kg ≡ 50 ppm.	

Section A5 **Subsection (Annex Point)**		**Effectiveness against target organisms and intended uses**	
5.4	**Mode of action (including time delay) (IIA5.4)**	*References*: **A5.4/01:** Vxxxx Cxxxx, Sxxxx Bxxxx (1992): Comparison of rodenticide anticoagulant action in vitro and in vivo. Uxxxx oxxxx Lxxxx, Unpublished Report. **A5.4/02:** vxxxx Sxxxx Nxxxx (1990) PIVKA II and prothrombin in the serum or plasma of rabbits after dosing with WL 108366 (Storm) and vitamin K1 therapy. Sxxxx Pxxxx Bxxxx Lxxxx, Unpublished Report. **A5.4/03:** IPCS (1995) Anticoagulant rodenticides. Environmental health criteria 175, WHO, Geneva (published).	
5.4.1	Mode of action	Flocoumafen, just like other coumarin derivatives, acts as a vitamin K antagonist. In the eukaryotic cell, Vitamin K functions as a coenzyme in the gamma-carboxylation of glutamic acid (Glu) residues, a process involved in the posttranslational stage of protein biosynthesis. The active form of vitamin K is the hydroquinone (KH_2), whose oxidation to vitamin K epoxide (KO) provides the energy for the carboxylation of glutamate. KH_2 is recycled from KO in a two-step reaction by dithiol-dependent reductases. Coumarin-like anticoagulants inhibit the dithiol-dependent reductases, resulting in exhaustion of KH_2 stores. Consequently, carboxylation of glutamate residues in the protein biosynthesis is prevented. Among the proteins which undergo Glu-carboxylation are the blood coagulation factors II (prothrombin), VII, IX and X, which are synthesized in the vertebrate liver. Through the mechanism described above, coumarin derivatives like flocoumafen prevent the formation of blood coagulation factors. Individuals intoxicated by a sufficient dose die of internal haemorrhages within a few days.	
5.4.2	Time delay	A time delay is an intrinsic property of all coumarin-like anticoagulants as a consequence of their the mode of action: As the hepatic vitamin K cycle is disrupted, adverse effects occur after vitamin K deposits are depleted and, as a consequence, internal haemorrhages occur. Following acute toxicity studies, consumption of a lethal dose of flocoumafen typically leads to death within 3–8 days (see Table A5-1 and Section A6.1.1).	x
5.5	**Field of use envisaged (IIA5.5)**	MG03: Pest control	

Section A5 **Subsection (Annex Point)**		**Effectiveness against target organisms and intended uses**	
5.6	**User (IIA5.6)**	a) Professionals, including urban pest control operators and farmers. b) Consumers, including home and garden business and homeowners. The product is exposed as ready-to-use bait (wax block). Handling includes fixing the blocks under suitable cover and in tamper-resistant baiting stations.	
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	Flocoumafen is a member of the so-called second-generation anticoagulants that were developed as a response to the advent of Warfarin resistant rats. During product development, there was no evidence of resistance to Flocoumafen (see references in Table A5-2). In order to retrieve more recent information, a literature search was performed on 12 June 2003, covering the databases – among other – "Agricola", "BIOSIS Previews", "CAB Abstracts", "Life Sciences Collection", "National Technical Information Service". The search profile included the terms "flocoumafen", "anticoagulant", "anticoagulants", "rodenticide", "manage", "management", "resistance". However, as a result, no information on resistance to Flocoumafen could be retrieved. Available information on potential cross-resistance against Flocoumafen in Warfarin resistant European pest rodents is presented in Table A5–2. There is no evidence for the occurrence of cross-resistance in *Rattus norvegicus*. Although at least one strain of Warfarin resistant *Mus musculus* (Cambridge cream) showed reduced susceptibility to Flocoumafen, the observed resistance factors of 4.5 and 4.8 (m/f) are not considered as an indication of resistance (Table A5–2, cf. reference A5.7.1/07).	x

Section A5	**Effectiveness against target organisms and intended uses**	
Subsection (Annex Point)		
5.7.2	Management strategies	*References*: **A5.7.2/01:** Anonymous (2003) Anticoagulant Resistance Management Strategy For Pest Management Professionals, Central and Local Government and Other Competent Users Of Rodenticides. CropLife International, Technical Monograph, Brussels, Belgium (published). **A5.7.2/02:** Greaves JH (1995) Managing resistance to anticoagulant rodenticides: an appraisal. Pesticide Science 43: 79-82 (published). **A5.7.2/03:** Prescott CV (2003) A reappraisal of blood clotting response tests for anticoagulant resistance and a proposal for a standardised BCR test methodology. CropLife International, Technical Monograph, Brussels, Belgium (published). According to Greaves (1995) (A5.7.2/02), the objective of resistance management is "to preserve the useful properties of the anticoagulant to which resistance has developed". Since to date no incidents of resistance to Flocoumafen in European rodents are known, immediate action is not indicated. However, development of resistance may be prevented by alternating use of different rodenticides, thus removing selective pressure. As an extract from the cited references, adherence to "good baiting practice", complete eradication of rodent infestations, and better coordination on the level of rodenticide manufacturers, are recommended to avoid resistance problems. The establishment of the "Rodenticide Resistance Action Committee" is assessed as an attempt to face the latter issue. Since resistance is usually a problem regarding Warfarin, Flocoumafen can be seen as a means for effective control of rodent populations resistant against Warfarin. Resistant populations should be quickly eradicated by application of either a more potent anticoagulant (e.g. Flocoumafen) or non-anticoagulant compounds (Greaves 1985, Table A5–2). As a means to detect resistance in rodent populations, the conduct of blood clotting response test (BCR) is proposed (A5.7.2/03).
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	approx. xxxx kg of a.s. contained in rodenticide products are placed onto the market per annum

Table A5-1: Summary table of experimental data on the effectiveness of flocoumafen against target organisms (rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
1) Results adopted from Section A6.1.1 estimating LD_{50} (cross-references to acute oral toxicity)					
Various batches of flocoumafen, > 96 % purity	*Rattus norvegicus*, Fischer 344 strain, male and female; initial body mass: 183–218 g.	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Dosing: 0.20, 0.25, 0.32, 0.4, and 0.50 mg/kg; single administration; observation time: 14 d	LD_{50} = 0.37 mg/kg (95% CI = 0.33–0.42) Time to death (range) = 3–8 d 100 % mortality at doses > 0.50 mg/kg Necropsy findings indicated death from haemorrhages.	Cross-reference: **A6.1.1/01**
As supplied by the manufacturer	*Rattus norvegicus*, Wistar strain, males; initial body mass: 200–250 g *Mus musculus*, C57BL/10 strain, males of 20–25 g body mass.	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Rat: Dosing: 0.100, 0.215, 0.464, and 1.0 mg/kg; Mouse: Dosing: 0.215, 0.464, 1.00, and 2.15 mg/kg; Both: single administration; observation time: 21 d	Rat: LD_{50} = 0.46 mg/kg (95% CI = 0.30–0.72) Time to death (range) = 5–8 d 100 % mortality at doses ≥ 1.0 mg/kg Necropsy findings indicated death from haemorrhages. Mouse: LD_{50} = 0.79 mg/kg (95% CI = 0.58–1.08) Time to death (range) = 5–7 d 100 % mortality at doses ≥ 0.464 mg/kg Necropsy findings indicated death from haemorrhages.	Cross-reference: **A6.1.1/06**
As supplied by the manufacturer	*Mus musculus*, C57BL/10 strain, males and females; 20–25 g body mass.	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Males: Dosing: 0.215, 0.464, 1.00, 2.15, and 4.46 mg/kg Females: Dosing: 0.464, 1.00, 2.15, 4.46, and 10.0 mg/kg Both: single administration; observation time: 21 d	Males: LD_{50} = 0.79 mg/kg (95% CI = 0.58–1.08) Time to death (range) = 4–11 d 100 % mortality at doses ≥ 2.15 mg/kg Necropsy findings indicated death from haemorrhages. Females: LD_{50} = 1.47 mg/kg (no confidence interval reported) Time to death (range) = 6–10 d 100 % mortality at doses ≥ 2.15 mg/kg Necropsy findings indicated death from haemorrhages.	Cross-reference: **A6.1.1/05**

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Table A5-1: Summary table of experimental data on the effectiveness of flocoumafen against target organisms (rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
As supplied by the manufacturer	*Rattus rattus*, 28 males, 20 females; 112–278 g body mass	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Males: Dosing: 0.10, 0.215, 0.464, 1.0, 2.15, and 4.46 mg/kg Females: Dosing: 0.464, 1.00, 2.15, and 4.46 mg/kg Both: single administration; observation time: 21 d	Males: LD_{50} = 1.78 mg/kg (95% CI = 1.21–2.61) Time to death (range) = 6–11 d 100 % mortality at doses ≥ 4.64 mg/kg Necropsy findings indicated death from haemorrhages. Females: LD_{50} = 1.0 mg/kg (95% CI = 0.64–1.56) Time to death (range) = 4–13 d 100 % mortality at doses ≥ 2.15 mg/kg Necropsy findings indicated death from haemorrhages	Cross-reference: **A6.1.1/08**
As supplied by the manufacturer	*Rattus norvegicus*, Wistar strain, females	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Dosing: 0.215, 0.464, 1.0, and 2.15 mg/kg; single administration; observation time: 14 d; no control group	LD_{50} = 0.56 mg/kg (95% CI = 0.38–0.82) Time to death (range) = 4–8 d 100 % mortality at doses ≥ 1.00 mg/kg Necropsy findings indicated death from haemorrhages	Cross-reference: **A6.1.1/07**
Purity > 99 %	*Mus musculus*, CF1 strain, males and females; 17–28 g body mass	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Dosing: 0.75, 1.5, 3.0, 6.0, and 12.0 mg/kg; single administration; observation time: 42 d; no control group	Males: LD_{50} = 2.9 mg/kg (95% CI = 1.7–5.0) Time to death (range) = 2–9 d 100 % mortality at doses ≥ 12.0 mg/kg Necropsy findings indicated death from haemorrhages. Females: LD_{50} = 2.0 mg/kg (95% CI = 1.0–3.7) Time to death (range) = 3–10 d 100 % mortality at doses ≥ 6.0 mg/kg Necropsy findings indicated death from haemorrhages	Cross-reference: **A6.1.1/10**

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Table A5-1: Summary table of experimental data on the effectiveness of flocoumafen against target organisms (rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
2) Studies allocated specifically to Section A5					
As supplied by the manufacturer	*Mus musculus*, CF57BL/10 strain, males of 20–25 g body mass; the tested strain is less susceptible to warfarin than standard laboratory mice	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Dosing: 0.316, 1.00, 3.16, and 10.0 mg/kg; single administration; observation time: 21 d; no control group	LD_{50} = 1.78 mg/kg (no confidence interval reported) Time to death (range) = 2–16 d 100 % mortality at doses ≥ 3.16 mg/kg Necropsy findings indicated death from haemorrhages; inter-strain differences in anticoagulant susceptibility are stated.	**A5.3/01:** Sxxxx (1984b)
Cis- and trans-flocoumafen, considered to be pure, respectively	*Rattus norvegicus*, males of Wistar strain and Welsh strain (homozygous for warfarin resistance), respectively; 200–250 g body mass	Prothrombin time determination: Plasma from blood samples of treated rats was mixed with thromboplastin and the time until first appearance of a gel clot stopped.	Dosing: 0.1 to 0.6 mg/kg, separated by steps of 0.1 mg/kg; the lower or upper end of the scale omitted in certain tests; single administration via the peritoneal route; prothrombin time determination 72 h after administration	Prothrombin time ED_{50} <u>Wistar rats:</u> cis-isomer: ED_{50} = 0.39 mg/kg trans-isomer: ED_{50} = 0.37 mg/kg racemic mixture: ED_{50} = 0.32 mg/kg <u>Welsh strain rats (warfarin resistant):</u> cis-isomer: ED_{50} = 0.32 mg/kg trans-isomer: ED_{50} = 0.48 mg/kg racemic mixture: ED_{50} = 0.37 mg/kg	**A5.3/02:** Sxxxx (1984c)

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Table A5-1: Summary table of experimental data on the effectiveness of flocoumafen against target organisms (rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
As supplied by the manufacturer	*Mus musculus*, wild type; mice used in the pen trials were known to be warfarin resistant	Pen trials: Toxicity testing of test substance mixed with feed. Field trials: Pre- and post-treatment survey of populations in farm buildings by live-capture and tracking-boards.	Pen trials: Feed mixed with 0.005 % flocoumafen offered to four family groups in a choice-feeding test. Field trials: 0.005 % flocoumafen bait supplied in ten buildings; baiting was ceased when no signs of feeding occurred for three days (after 3–7 weeks).	Pen trials: 100 % mortality in all four trials time to death: 3–10 d (mean = 5.1 d) Field trials: Population reduction was 100 % in seven trials, and ranged between 87 % and 96 % in the other three. Survivors were susceptible to flocoumafen in laboratory tests, but survived longer (mean = 8.9 d).	**A5.3/03:** Rowe, Bradfield, Swinney (1985)
As supplied by the manufacturer	*Rattus norvegicus*, Wistar strain, males of 187–225 g body mass; Welsh strain (homozygous for warfarin resistance), females of 137–170 g body mass; *Mus musculus*, CF57BL/10 strain, males of 15–20 g body mass.	Acute toxicity tests by feeding of bait containing defined concentrations of active substance, in order to determine effective concentrations (LC_{50}) of the a.s. in bait.	No-choice feeding of bait prepared with test substance for 24 h; estimation of food consumption; observation period: 21 d; rats were singly caged, mice caged in groups of five individuals.	Wistar rats: Bait LC_{50} > 5.0 ppm food intake as 50 ppm bait to deliver LD_{50} dose = 9.2 % Welsh strain rats: Bait LC_{50} = 5.0–10.0 ppm food intake as 50 ppm bait to deliver LD_{50} dose: not reported Mice: Bait $LC_{50} \approx 5.0$ ppm food intake as 50 ppm bait to deliver LD50 dose = 7.9 % Remark: LC_{50} values were merely roughly assessed instead of estimated using valid statistical procedures.	**A5.3/04:** Sxxxx (1983b)

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Table A5-1: Summary table of experimental data on the effectiveness of flocoumafen against target organisms (rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
As supplied by the manufacturer	*Rattus norvegicus*, wild warfarin-susceptible, homozygous Welsh and Scottish (both warfarin-resistant), and homozygous Hampshire (difenacoum-resistant) strains, wild *R. rattus*, wild warfarin-resistant *Mus domesticus*, body masses in a normal range, respectively (for more details please refer to the original document). Further three tropical rodent species tested are not referred to for the purpose of this dossier.	Laboratory choice and no-choice feeding tests of 50 ppm Flocoumafen bait vs. blank bait, following EPPO guidelines; bait offered for 1 day	Housing in plastic or wire cages at 20 ± 2 °C and natural daylight regime; 3 weeks observation period	Total mortality in wild susceptible *R. norvegicus*, difenacoum-resistant *R. norvegicus*, and Scottish *R. norvegicus* (both sexes, respectively), female Welsh *R. norvegicus*, and female *R. rattus*. 90 % mortality in male *R. rattus* and male Welsh *R. norvegicus*, respectively. 80 % and 70 % mortality in male and female *Mus domesticus*, respectively.	**A5.3/05:** Gill (1992)

Table A5-2: Summary table of experimental data and review articles on possible resistance against flocoumafen in the target organisms (commensal rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
As supplied by the manufacturer	*Mus musculus*, female hybrids from crosses between male C57BL10 and Cambridge Cream, which had previously survived a 250 ppm warfarin diet; 15–20 g body mass	Acute oral toxicity trial; guideline compliance not stated, and not assessable due to poor documentation of the study.	Dosing: 1.00, 3.16, and 10.0 mg/kg; observation period: 21 d	LD_{50} < 1.00 mg/kg (confidence interval indeterminable) Time to death (range): not reported 100 % mortality at doses ≥ 1.00 mg/kg Necropsy findings indicated death from haemorrhages	**A5.7.1/01:** Fxxxx (1985)
As supplied by the manufacturer	*Mus musculus*, Cambridge Cream strain (warfarin resistant), males and females of 13–20 g body mass	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Dosing: 1.0, 3.16, 10.0, and 31.6 mg/kg (the highest dose in females only); observation period: 21 d	<u>Males:</u> LD_{50} = 3.55 mg/kg (95 % CI = 2.02–6.24) Time to death (range): 5–7 d; 100 % mortality at doses ≥ 10.0 mg/kg; 0 % mortality at 1.00 mg/kg; <u>Females:</u> LD_{50} = 7.08 mg/kg (95 % CI = 3.19–15.7) Time to death (range): 3–8 d; 100 % mortality at doses ≥ 31.6 mg/kg; 0 % mortality at 1.00 mg/kg; Resistance factor: 4.5 (males); 4.8 (females).	**A5.7.1/02:** Sxxxx (1985)

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Table A5-2: Summary table of experimental data and review articles on possible resistance against flocoumafen in the target organisms (commensal rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
As supplied by the manufacturer	*Rattus norvegicus*, Welsh strain (warfarin resistant), males and females of 175–250 g body mass.	Acute oral toxicity trial; guideline compliance not stated, but the study was conducted in the style of EC method B.1	Dosing: 0.1, 0.215, 0.464, 1.0, and 3.16 mg/kg (the highest dose in females only); observation period: 21 d; due to increased dietary vitamin K demands in warfarin resistant rats, drinking water containing 20 ppm vitamin K was supplied during housing, which was replaced by tap water three days prior to dosing.	Males: LD_{50} = 0.46 mg/kg (95 % CI = 0.26–0.84) Time to death (range): 2–13 d; 100 % mortality at doses ≥ 1.0 mg/kg; Females: LD_{50} = 0.42 mg/kg (95 % CI = 0.24–0.75) Time to death (range): 4–10 d; 100 % mortality at doses ≥ 1.0 mg/kg; 0 % mortality at 0.1 mg/kg; The LD_{50} values coincide with those of susceptible rats.	**A5.7.1/03:** Sxxxx (1986b)
Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Reference is made primarily to warfarin resistance, with respect to affected species, geographical distribution, genetic aspects, and detection methods. Recommendations are given for management strategies: Development of new rodenticides, and eradication of resistant populations by application of non-anticoagulant agents such as, e.g., calciferol.	**A5.7.1/04:** Greaves (1985)
Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Extensive review of warfarin resistance, with focus on the molecular mechanism. Although no reference to flocoumafen is made, concern is expressed that continued use of second generation 4-hydroxycoumarin anticoagulants in general may favour resistance against the whole class of compounds by selective pressure.	**A5.7.1/05:** MacNicoll (1986)

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Table A5-2: Summary table of experimental data and review articles on possible resistance against flocoumafen in the target organisms (commensal rodents). Generally, the substance functions as a rodenticide (PT 14) and the envisaged field of use is pest control (MG03).

Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	A general review of the mode of action of all coumarin-based anticoagulants is given and mechanisms of Warfarin-resistance (biochemical and pharmacokinetic) are discussed. The long pharmacokinetic half-life of Flocoumafen and its fluorinated side chain which effectively prevents biotransformation (also see Section A6.2) may be seen as a reason why resistance to Flocoumafen (as well as Brodifacoum) has not yet been reported.	**A5.7.1/06:** Thijssen (1995)
Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Not applicable (review article)	Chapter 8 ("The anticouagulants. III. Resistance) of the cited publication is referred to for the purposes of this dossier. The history of Warfarin resistance in rats and mice is reviewed. Resistance is defined as the inheritable (genetically fixed) ability of rodents to survive 50–500 fold doses of anticoagulant, compared to "normal" individuals. Cross-resistance is discussed as a general problem with anticoagulants, but reference to Flocoumafen is not made, since this active was not yet marketed at the time of publication.	**A5.7.1/07:** Meehan (1984)

References (Tables A5-1 and -2):

A5.7.1/01:

Fxxxx Jxxxx (1985) An experimental note on: The acute oral LD_{50} of WL108366 in hybrid mice. Sxxxx Lxxxx, Unpublished Report, February 1, 1985.

A5.3/05:

Gill JE (1992) Laboratory evaluation of the toxicity of Flocoumafen as a single-feed rodenticide to seven rodent species. International Biodeterioration & Biodegradation 30: 65-76 (published).

A5.7.1/04:

Greaves JH (1985) The present status of resistance to anticoagulants. Acta Zoologica Fennica 173: 159-162 (published).

A5.7.1/05:

MacNicoll AD (1986) Resistance to 4-hydroxycoumarin anticoagulants in rodents. In National Research Council (ed.), Pesticide Resistance: Strategies and Tactics for Management: 87-99. Washington D.C., National Academy Press (published).

A5.7.1/07:

Meehan (1984) Rats and mice - their biology and control. The Rentokil Library, Rentokil Ltd., East Grinstead, UK.

A5.3/03:

Rowe FP, Bradfield A, Swinney T (1985) Pen and field trials on a new anticoagulant rodenticide flocoumafen against the house mouse (*Mus musculus* L.). Journal of Hygiene 95: 623-627 (published).

A5.3/04:

Sxxxx Rxxxx (1983b) Acute feeding of bait containing novel anticoagulants to non-resistant and resistant rats, and non-resistant mice – bait LC_{50} determination. Sxxxx Lxxxx, Unpublished Report, July 14, 1983.

A5.3/01:

Sxxxx Rxxxx (1984b) The acute oral toxicity of WL108366 in C3H/He mice. Sxxxx Lxxxx, Unpublished Report, November 14, 1984.

A5.3/02:

Sxxxx Rxxxx (1984c) Prothrombin time determination, in rats, for cis- and trans-WL108366. Sxxxx Lxxxx, Unpublished Report, March 27, 1984.

A5.7.1/02:

Sxxxx, Rxxxx (1985) The acute oral toxicity of WL108366 to Cambridge Cream mice. Sxxxx Lxxxx, Unpublished Report, June 10, 1985.

A5.7.1/03:

Sxxxx Rxxxx (1986b) An experimental note on: The acute oral toxicity of WL108366 in male and female warfarin-resistant *Rattus norvegicus*. Sxxxx Lxxxx, Unpublished Report, July 10, 1986.

A5.7.1/06:

Thijssen HHW (1995) Warfarin-based rodenticides: mode of action and mechanism of resistance. Pesticide Science 43: 73-78 (published).

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	14 January 2005
Materials and Methods	**(5.3) Table A5-1, part *1) Results adopted from Section A6.1.1*:** ref. A6.1.1/01: The LD50 was 0.42 mg/kg (males) and 0.31 mg/kg (females). The combined LD50 was 0.37 mg/kg. The time to death was 3-7 days (males) and 5-8 days (females). Mortality was 80% at the highest test dose of 0.50 mg/kg (males) and 100% at test doses ≥0.4 mg/kg (females). Reliability is 1. ref. A6.1.1/06: Per test dose, 5 male mice were used, but only 4 male rats. The test substance purity was not reported ("considered to be pure"). In (male) mice, 100% mortality occurred at doses ≥2.15 mg/kg (not: ≥0.464 mg/kg). Reliability is 2 (only 4 male rats per dose, test substance purity not reported). ref. A6.1.1/05: Each dose was tested in 5 animals of each sex. The test substance purity was not reported ("considered to be pure"), hence reliability is 2. ref. A6.1.1/08: Each dose was tested in only 4 animals of each sex. The test substance purity was not reported. The highest test dose was 4.64 mg/kg (not: 4.46 mg/kg). Reliability is 2 (only 4 rats of each sex per dose, test substance purity not reported). ref. A6.1.1/07: Each dose was tested in only 4 female rats. The test substance purity was not reported. Reliability is 2 (only 4 female rats per dose, test substance purity not reported). ref. A6.1.1/10: Each dose was tested in 5 animals of each sex. The combined LD50 was 2.4 mg/kg (95% CI 1.6-3.4). Reliability is 1. **(5.3) Table A5-1, part *2) Studies allocated specifically to Section A5*):** ref. A5.3/01: (1) The mouse strain was C3H/He instead of CF57BL/10. Per test dose, 5 male mice were used. (2) Results: add that 0% mortality occurred at ≤1 mg/kg. (3) The test substance purity was not reported ("considered to be pure"), hence reliability is 2. ref. A5.3/02: Per test dose, 3 male rats were used. The test substance purity was not reported ("considered to be pure"), hence reliability is 2. ref. A5.3/03: (1) Population reduction in the other three field trials ranged between 87% and 95% (not 96%). (2) Warfarin resistance of the mice was claimed, but not tested. (3) The test substance purity was not reported. (4) Because of (2) and (3), reliability is 3. ref. A5.3/04: (1) Each dose was tested in 5 animals of each sex. (2) The acute oral LD50 for Wistar rats and mice was reported to be 0.46 and 0.79 mg/kg bw, respectively (data from cross-reference A6.1.1/06). (3) The percentages food intake as 50 ppm bait were not experimentally determined, but represent calculated values, based on the aforementioned LD50 values and assuming that a rat and a mouse consumes every day 10% and 20%, respectively, of its own body weight. (4) The test substance purity was not reported, hence reliability is 2. ref. A5.3/05: (1) Each test employed 10 animals of each sex, except for *R. rattus* (10 males and 5 females). (2) Replace "Laboratory choice and no-choice feeding tests" by "Laboratory no-choice feeding tests" (choice feeding tests were also reported but not included in the summary). (3) The test substance purity was not reported, hence reliability is 2. **(5.4.2)** The overall ranges for the time to death were 3-13 days (rat) and 2-16 days (mouse). **(5.7.1)** Table A5-2: ref. 5.7.1/01: (1) Each dose was tested in only 4 female mice. (2) The test substance purity was not reported. (3) Because of (1) and (2), reliability is 2.

	ref. 5.7.1/02: (1) Each dose was tested in 5 animals of each sex. (2) The resistance factor was calculated by dividing the LD50 of the tested strain (Cambridge Cream) by that of the standard susceptible strain C57BL/10. (3) Cambridge Cream mice were claimed to be Warfarin resistant, but experimental data confirming Warfarin resistance were not shown. (4) The test substance purity was not reported ("considered to be pure"). (5) Because of (3) and (4), reliability is 3. ref. 5.7.1/03 (and point 5.7.1): (1) Each dose was tested in only 4 animals of each sex. (2) Dose levels for males were 0.1, 0.215, 0.464 and 1 mg/kg, and for females 0.1, 0.316, 1 and 3.16 mg/kg. (3) The test substance purity was not reported. (4) Because of (1) and (3), reliability is 2.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	