Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin; Brodifacoum

EC number: 259-980-5
CAS number: 56073-10-0

CLH-O-0000003395-72-02/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 March 2014

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name:

BRODIFACOUM

EC Number: **259-980-5** (EINECS)
CAS Number: **56073-10-0**
Index Number: **607-172-00-1**

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Version number: 2 **Date: February 2013**

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Part A.

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Brodifacoum***
EC number:	***259-980-5 (EINECS)***
CAS number:	***56073-10-0***
Annex VI Index number:	
Degree of purity:	≥ 95.0% w/w (including both *cis* and *trans* isomers)
Impurities:	Confidential information (please refer to the separate confidential Annex to this CLH report). Based on the available information, none are of toxicological or environmental concern.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox. 1; H310 Acute Tox. 2*; H300 STOT RE.1; H372 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	T+; R27/28 T; R48/24/25 N; R50/53
Current proposal for consideration by RAC	Repr. 1B; H360D Acute Tox. 1; H300Acute Tox. 1; H330 Skin Sens 1; H317	Repr. Cat. 2; R61 T+; R26 T; R48/23 Xi; R 43

	Aquatic Acute 1; H400 Aquatic Chronic 1; H410 *M-factor Acute =10* *M-factor Chronic =10* *Suggested specific conc.limits:* C ≥0,25%; **Acute Tox1 H300** 0.025%≤C<0.25%; Acute Tox 3 H301 0.0025%≤C<0.025% Acute Tox 4 H302 C ≥0,25% **Acute Tox 1H310** 0.025%≤C<0.25%; Acute Tox 3 H311 0.0025%≤C<0.025% Acute Tox 4 H312 C ≥0,25% **Acute Tox 2 H330** 0.025%≤C<0.25%; Acute Tox 3 H331 0.0025%≤C<0.025% Acute Tox 4 H332 C ≥ 0.25 % **STOT RE 1** H372 0,025 %≤ C <0.25 % **STOT RE 2** H373	*Suggested specific conc. limits:* C≥2.5%: T+, N; R26/27/28-48/23/24/25-50/53 0.25%≤C<2.5%: T+, N; R26/27/28-48/23/24/25-51/53 0.025%≤C<0.25%: T; R23/24/25-48/20/21/22-52/53 0.0025%≤C<0.025%: Xn; 20/21/22
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B; H360D Acute Tox. 1; H300 Acute Tox. 1; H310 Acute Tox. 1 H330 STOT RE.1; H372 Skin Sens 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 *M-factor Acute =10* *M-factor chronic =10* *Suggested specific conc.limits:* C ≥0,25%; **Acute Tox1 H300** 0.025%≤C<0.25%; Acute Tox 3 H301 0.0025%≤C<0.025% Acute Tox 4 H302 C ≥0,25% **Acute Tox 1H310** 0.025%≤C<0.25%; Acute Tox 3 H311 0.0025%≤C<0.025% Acute Tox 4 H312 C ≥0,25% **Acute Tox 2 H330** 0.025%≤C<0.25%; Acute Tox 3 H331 0.0025%≤C<0.025% Acute Tox 4 H332 C ≥ 0.25 % **STOT RE 1** H372 0,025 %≤ C <0.25 % **STOT RE 2** H373	Repr. Cat. 2; R61 T+; R26/27/28 T; R48/23/24/25 R43 N; R50/53 *Specific conc. limits:* C≥2.5%: T+, N; R26/27/28-48/23/24/25-50/53 0.25%≤C<2.5%: T+, N; R26/27/28-48/23/24/25-51/53 0.025%≤C<0.25%: T; R23/24/25-48/20/21/22-52/53 0.0025%≤C<0.025%: Xn; 20/21/22

* Minimum classification for a category is indicated by the reference * in the column 'Classification' in Table 3.1, CLP.

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

The present proposal for harmonized Classification and Labelling applies to the substance *Brodifacoum* as proposed for inclusion in Annex I to Directive 98/8/EC, following evaluation of data from two different Applicants (Syngenta and Activa/PelGar, hereafter A and B, respectively) by RMS Italy.
Evaluation of technical equivalence of *Brodifacoum* produced by A and B has been also accomplished, in compliance with the TNsG on the assessment of technical equivalence of substances regulated under Directive 98/8/EC (adopted at the 29th CA Meeting, 28-30 May 2008). Both Tier I evaluation and Tier II evaluation have been carried out in order to assess the technical equivalence of the two *Brodifacoum* sources, which proved to be technically equivalent.
Confidential information on isomeric composition and impurity profile is available separately for either Applicant in the confidential Annex to this CLH report based on the Confidential Annex to the Competent Authority Reports prepared by RMS Italy for the purpose of *Brodifacoum* inclusion in Annex I to Directive 98/8/EC.
Proposed classification based on Regulation EC 1272/2008 (CLP):
Physical/chemical properties: None.

Health hazards:	Acute Tox. 1 H300; Acute Tox. 1 H310; Acute Tox. 1 H330; STOT RE 1 H372 Repr. 1B H360D* Skin Sens 1 H317
Environment:	Aquatic acute 1 H400; Aquatic chronic 1 H410

Proposed classification based on Directive 67/548/EEC:
Physical/chemical properties: None.

Health hazards:	Repr. Cat. 2; R61* T+; R26/27/28 T; R48/23/24/25 Xi; R 43
Environment:	N; R50/53

*Based on the classification for developmental effect by read across to *Warfarin*

Proposed labelling based on Directive 67/548/EEC:

Symbol:	T+; N
Risk phrases:	R26/27/28, R43, R48/23/24/25, R61,R50/53
Safety phrases:	S1/2, S36/37, S45, S60, S61

Proposed labelling based on Regulation EC 1272/2008:

Signal word:	Danger
Symbol:	GHS06, GHS08, GHS07, GHS09
Hazard statement codes:	H300: Fatal if swallowed H310: Fatal in contact with skin H317: May cause an allergic skin reaction H330: Fatal if inhaled H372: Causes damage to organs through prolonged or repeated

exposure
H360D: May damage the unborn child
H400: Very toxic to aquatic life
H410: Very toxic to aquatic life with long lasting effects

The table 3 indicates the current harmonised classification in Annex VI CLP Regulation and the proposed classification.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids				
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral	H 300 Acute Tox 1	C ≥0,25%; Acute Tox1 H300 0.025%≤C<0.25%; Acute Tox 3 H301 0.0025%≤C<0.025% Acute Tox 4 H302	H 300	
	Acute toxicity - dermal	H 310 Acute Tox 1	C ≥0,25% Acute Tox 1H310 0.025%≤C<0.25%; Acute Tox 3 H311 0.0025%≤C<0.025% Acute Tox 4 H312	H 310	
	Acute toxicity – inhalation (Podwer)	H 330 Acute Tox 2	C ≥0,25% Acute Tox 2 H330 0.025%≤C<0.25%; Acute Tox 3 H331 0.0025%≤C<0.025% Acute Tox 4 H332		
3.2.	Skin corrosion / irritation				
3.3.	Serious eye damage / eye irritation				
3.4.	Respiratory sensitisation				
3.4.	Skin sensitisation	H 317			
3.5.	Germ cell mutagenicity				
3.6.	Carcinogenicity				
3.7.	Reproductive toxicity	H 360			

3.8.	Specific target organ toxicity –single exposure				
3.9.	Specific target organ toxicity – repeated exposure	STOT RE H 372	C ≥ 0.25 % STOT RE 1 H372 0,025 %≤ C <0.25 % STOT RE 2 H373	STOT RE H 372	
3.10.	Aspiration hazard				
4.1.	Hazardous to the aquatic environment	H 400 H410	*M-factor Acute =10* *M-factor Chronic =10*	H 400 H 410	
5.1.	Hazardous to the ozone layer		//		

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Symbol: GHS06, GHS08, GHS07, GHS09

Hazard statements: H300: Fatal if swallowed
H310: Fatal in contact with skin
H317: May cause an allergic skin reaction
H330: Fatal if inhaled
H372: Causes damage to organs through prolonged or repeated exposure
H360D: May damage the unborn child
H400: Very toxic to aquatic life
H410: Very toxic to aquatic life with long lasting effects

Precautionary statements: As precautionary statements are not included in Annex VI of Regulation EC 1272/2008, no proposal is made.
Proposed notes assigned to an entry: On the basis of study results, classification of *Brodifacoum* is proposed according to principles detailed in Annex VI of Council Directive 67/548/EEC (with amendments and adaptations) and Regulation EC 1272/2008.
The currently proposed classification according to the DSD criteria and CLP criteria, except Acute Tox. 2 H 300 (CLP); R 26 (DSD) Acute Tox. 1 H330 (CLP) R 43 (DSD) Skin Sens 1 H 317 (CLP), R 48/23 (DSD) and Repr. Cat.2 R61 (DSD); H 360D (CLP).
The proposed have been discussed and agreed by the EU Technical Committee of Classification and Labelling (TC C&L) of Dangerous Substances at their meeting in May 2007.
The proposed specific concentration limits according to Directive 67/548/EEC have been discussed under DSD in the biocide program under directive 98/8/EC.
For skin sensitisation and for toxicity to reproduction, the general concentration limit was proposed to be applied.
Specific concentration limits (SCL_s) for acute and repeated dose toxicity were not agreed, although the method to be used to set SCL_s for acute toxicity (DSD) of any of the 2nd generation anticoagulants under discussion was agreed at the TC C&L May 2007 meeting.
Newly SCL_s calculated according to regulation EC 1272/2008 using the formulae presented in the guidance on CLP.
The proposed classification for environment was agreed in April 2006 by the Technical Committee on Classification and Labelling (TC C&L) of Dangerous Substances.
The classification for human health effects is still under discussion (since May 2007). A provisional classification with R61 was decided in November 2006 by the TC C&L, without a final decision on the category to be used (Repr.Cat 1 or Repr.Cat 2). The proposed classification for *Brodifacoum* for

acute and repeated dose toxicity was agreed upon. In May 2007 the provisionally classification for reprotoxicity was not confirmed as the TC C&L decided to await further results from studies on anticoagulant rodenticides, before finalising the discussion on reprotoxicity. Specific concentration limits for *Brodifacoum* were agreed upon as proposed.
Note: Specific concentration limits (SCL's) for acute and repeated dose toxicity and for environment were agreed by TC C&L (Technical Committee on Classification and Labelling) in May 2007. For toxicity to reproduction, the general concentration limit (Dir 1999/45/EC) of 0.5% was proposed to be applied; the general concentration limit is included here in order to maintain a common manner of expression with documents produced under biocides legislation.

Proposed specific concentration limits based on Regulation EC 1272/2008:

$C \geq 0.1\%$ STOT RE 1

$0.01\% \leq C < 0.1\%$ STOT RE 2

As setting specific concentration limits for acute toxicity is not applicable according to CLP regulation, no values are set.

As regards classification concerning environmental endpoints, R phrases will not be used any longer under CLP but the corresponding information is to be communicated using M factors.

Rationale for specific concentration limits according to Regulation EC 1272/2008:

$$SCLCat1 = \frac{ED}{GV1} \cdot 100\% = \frac{0.01\,mg/kg\,bw/day}{10\,mg/kg\,bw/day} \cdot 100\% = 0.1\%$$

$$SCLCat2 = \frac{ED}{GV2} \cdot 100\% = \frac{0.01\,mg/kg\,bw/day}{100\,mg/kg\,bw/day} \cdot 100\% = 0.01\%$$

ED - Effective Dose: LOAEL 0.01 mg/kg bw/day based on threefold-fourfold increase in prothrombin time after oral application (dog, 42-day range finding study)
GV1 - Guidance Value 1: 10 mg/kg bw/day
GV2 - Guidance Value 2: 100 mg/kg bw/day

A consensus between limit values
1) originating from discussions under Directive 67/548/EEC and
2) calculated according to CLP formulae has not been found. Getting the numerical values in harmony does not seem feasible.
The conclusions from the Directive and the Regulation also do not match.

- According to SCL's ageed on under Directive 67/548/EEC, a typical product containing 50 ppm or 75 ppm (0.0050% or 0.0075%) of difenacoum will be classified as Xn; R20/21/22 and labelled with the "Harmful" symbol as the concentration falls in the concentration range $0.0025\% \leq C < 0.025\%$.
- According to the limit values calculated according to Regulation EC 1272/2008, the respective concentrations do not trigger any labelling requirement since the concentrations are lower than the potential SCL of 0.01% for category 2 classification of STOT, repeated exposure.

From a hazard communication point of view, the existence of a warning label on the packagings of products containing *brodifacoum* is extremely important. The preparations containing *brodifacoum* are intended to kill rodents and are potentially fatal to humans. *Brodifacoum* and the other second generation anticoagulants are intended to kill after a single dosing and it can be expected that the ingestion of even only one bait can cause a casualty in the human population. This hazard should not be overlooked.

The administrator should strive for a consistent set of specific concentration limits and one trigger for the labelling obligation. However, determining consistent limit values seems difficult.
On these grounds, setting specific consentration limits seems not to be the most effective way of protecting the public in this case. Instead, a novel 'Special rule for labelling and packaging of certain substances and mixtures' stating "Rodenticide for pest control. Keep out of reach of children." is hereby proposed. This should be introduced in Annex II to Regulation EC 1272/2008 for use in rodenticide packagings where applicable.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness				
Oxidising properties				
Flammability				
Other physico-chemical properties *[Add rows when relevant]*				
Thermal stability				
Acute toxicity	T+R26/27/28	C ≥ 0.25% T+ R26/27/28 0.025% ≤ C< 0.25% T R23/24/25 0.0025% ≤ C <0.025% Xn R20/21/22	T+ R 27/28	
Acute toxicity – irreversible damage after single exposure				
Repeated dose toxicity	T+R 48/23/24/25	C ≥ 0.25% T 48/23/24/25 0.025% ≤ C< 0.25% Xn 48/20/21/22	T 48/24/25	
Irritation / Corrosion				
Sensitisation	Xi R 43			
Carcinogenicity				
Mutagenicity – Genetic toxicity				
Toxicity to reproduction – fertility	T R 61			
Toxicity to reproduction – development				
Toxicity to reproduction – breastfed babies. Effects on or via lactation				
Environment	N R 50-53	C ≥ 2.5% N R50/53 0.25 ≤ C < 2.5% N R51/53 0.025%≤C<0.25% R52/53	N R 50-53	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Very Toxic; Dangerous for the environmental
R-phrases: R 26/27/28-48/23/24/25-43-61-50-53
S-phrases: S 1 /2-36/37-45-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

On the basis of study results, classification of *Brodifacoum* is proposed according to principles detailed in Annex VI of Council Directive 67/548/EEC (with amendments and adaptations) and Regulation EC 1272/2008.
The currently proposed classification according to the DSD criteria and CLP criteria, except Acute Tox. 2 H 300 (CLP); R 26 (DSD) Acute Tox. 1 H330 (CLP) R 43 (DSD) Skin Sens 1 H 317 (CLP), R 48/23 (DSD) and Repr. Cat.2 R61 (DSD); H 360D (CLP).
The proposed have been discussed and agreed by the EU Technical Committee of Classification and Labelling (TC C&L) of Dangerous Substances at their meeting in May 2007.
The proposed specific concentration limits according to Directive 67/548/EEC have been discussed under DSD in the biocide program under directive 98/8/EC.
For skin sensitisation and for toxicity to reproduction, the general concentration limit was proposed to be applied.
Specific concentration limits (SCL_s) for acute and repeated dose toxicity were not agreed, although the method to be used to set SCL_s for acute toxicity (DSD) of any of the 2^{nd} generation anticoagulants under discussion was agreed at the TC C&L May 2007 meeting.
Newly SCL_s calculated according to regulation EC 1272/2008 using the formulae presented in the guidance on CLP.
The proposed classification for environment was agreed in April 2006 by the Technical Committee on Classification and Labelling (TC C&L) of Dangerous Substances.
The classification for human health effects is still under discussion (since May 2007). A provisional classification with R61 was decided in November 2006 by the TC C&L, without a final decision on the category to be used (Repr.Cat 1 or Repr.Cat 2). The proposed classification for *Brodifacoum* for acute and repeated dose toxicity was agreed upon. In May 2007 the provisionally classification for reprotoxicity was not confirmed as the TC C&L decided to await further results from studies on anticoagulant rodenticides, before finalising the discussion on reprotoxicity. Specific concentration limits for *Brodifacoum* were agreed upon as proposed.

2.3 Current harmonised classification and labelling

Repr. Cat. 2; R61;T+; R26; T; R48/23; R43; Repr. Cat. 2; R61; N; R50/53

Suggested specific conc.limits:

C≥2.5%: T+, N; R26/27/28-48/23/24/25-50/53
0.25%≤C<2.5%: T+, N; R26/27/28-48/23/24/25-51/53
0.025%≤C<0.25%: T; R23/24/25-48/20/21/22-52/53
0.0025%≤C<0.025%: Xn; 20/21/22

Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation
T+; R27/28T; R48/24/25; N; R50/53

2.3.1 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

2.4 Current self-classification and labelling

UnKnown

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

UnKnown.

2.4.2 Current self-classification and labelling based on DSD criteria

No

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

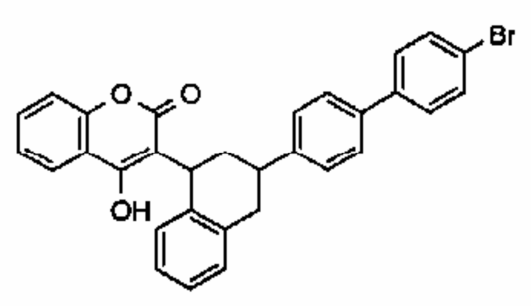
Table 5: Substance identity

EC number:	
EC name:	4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin (EINECS)
CAS number (EC inventory):	
CAS number:	56073-10-0
CAS name:	2H-1-Benzopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-
IUPAC name:	3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin
CLP Annex VI Index number:	607-172-00-1
Molecular formula:	$C_{31}H_{23}BrO_3$
Molecular weight range:	523.4 g/mol

Isomeric Composition: *cis* isomer (CA Index name: *2H-1-Benzopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-, cis-*, CAS-No. 72654-66-1) is a racemic mixture of (1R,3S) and (1S,3R);
trans isomer (CA Index name: *2H-1-Benzopyran-2-one, 3-[3-(4'-*

bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-, trans-, CAS-No. 72654-67-2) is a racemic mixture of (1R,3R) and (1S,3S)

Structural formula:



Brodifacoum consists of a mixture of *cis*/*trans*-isomers. Full details on the isomeric composition are confidential information and have been made available in a separate confidential Annex to this CLH report (based on the Confidential Annex to either Competent Authority Report prepared by RMS Italy for the purpose of *Brodifacoum* inclusion in Annex I to Directive 98/8/EC).

According to the isomeric composition of *Brodifacoum* as reported therein, following the terminology under REACH and CLP *Brodifacoum* should be considered as a multi-constituent substance, since both the *cis*-isomer and the *trans*-isomer fall in the range 10 up to 80% w/w. Consequently, *Brodifacoum* should be named as a reaction mass of the two main constituents as described under isomeric composition below. Nevertheless, part 1.1.1.4 of Annex VI of EC 1272/2008 (CLP) states that whenever possible plant protection products and biocides are designated by their ISO names. As a result, in this proposal preference is given to the use of the ISO name *Brodifacoum*, along with *4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin* as the International Chemical Identifier for inclusion in Annex VI to EC 1272/2008.

1.2 Composition of the substance

The present proposal for harmonized Classification and Labelling applies to Brodifacoum as proposed for inclusion in Annex I to Directive 98/8/EC, following evaluation of data from two different Applicants (Syngenta and Activa/PelGar, hereafter A and B, respectively) by RMS Italy.

Evaluation of technical equivalence of Brodifacoum produced by A and B has been also accomplished, in compliance with the TNsG on the assessment of technical equivalence of substances regulated under Directive 98/8/EC (adopted at the 29th CA Meeting, 28-30 May 2008). Both Tier I evaluation and Tier II evaluation have been carried out in order to assess the technical equivalence of the two Brodifacoum sources, which proved to be technically equivalent.

Confidential information on isomeric composition and impurity profile is available separately for either Applicant in the confidential Annex to this CLH report based on the Confidential Annex to either Competent Authority Report prepared by RMS Italy for the purpose of Brodifacoum inclusion in Annex I to Directive 98/8/EC.

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Brodifacoum EC n°259-980-5	>= 95% (w/w)		

Current Annex VI entry:

Purity/Impurities/Additives

The minimum purity of 95% w/w is supported by the analytical data (5-batch analysis) and has been used in most toxicity and ecotoxicity tests presented by Applicant A for the purpose of *Brodifacoum* inclusion in Annex I to Directive 98/8/EC. A higher minimum purity of 99.2% w/w is supported by the analytical data (5-batch analysis) and has been used in most toxicity and ecotoxicity studies available in the *Brodifacoum* Dossier of Applicant B for the same purpose. Both specifications have been accepted by RMS Italy and, therefore, the minimum purity of 95.0% w/w shall apply for *Brodifacoum*.
No upper limit has been specified by either Applicant, but when results from the 5-batch analyses are treated statistically and expressed as mean±3xSD, a maximum purity of 100% shall apply for *Brodifacoum*.

Brodifacoum does not contain impurities that would be of toxicological or environmental concern. *Brodifacoum* does not contain additives, either. Full details on impurities and their content are regarded as confidential and can be found in the confidential Annex to this CLH report based on the Confidential Annex of either Competent Authority Report prepared by RMS Italy for the purpose of *Brodifacoum* inclusion in Annex I to Directive 98/8/EC.

Table 7: Impurities (non-confidential information)

Not relevant for the classification.

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

The data summarized below are obtained from the Competent Authority Reports prepared by RMS Italy for the purpose of Brodifacoum inclusion in Annex I to Directive 98/8/EC, following evaluation of the physico-chemical studies submitted from both Applicant A and Applicant B. Values in many endpoints are highly or reasonably similar and the reasons for deviations can be usually regarded as experimental.

Brodifacoum does not exhibit hazardous physico-chemical properties. Brodifacoum is thermally stable. Brodifacoum is not highly flammable and it shows no self-ignition below the melting point. Brodifacoum has not oxidizing or explosive properties, either. Brodifacoum does not show signs of reaction with container materials.

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa (Purity)	**A**: Fine powdery solid; colour: cream (92.5% w/w) **B:** White to off-white fine powder (99.7% w/w)	IUCLID 5 section: 4.1	
Melting/freezing point (purity) [method] (purity) [method]	**A:** 232 °C with decomposition (98.7% w/w) [capillary method] **B:** Brodifacoum was observed to darken and decompose at 235.8 °C (100% w/w) [capillary method	IUCLID 5 section: 4.2	
Boiling point	A:Not applicable B: Not determinable	IUCLID 5 section: 4.3	
Relative density (purity) [method] (purity) [method]	A: 1.42 g/cm3 (density) at 25 °C (92.5% w/w) [pycnometer method] B: D204 = 1.530 (>99% w/w) [pycnometer method]	IUCLID 5 section: 4.4 density	
Vapour pressure (purity) [method] (purity)	A: << 10E-6 Pa (20 °C) (98.7% w/w) [gas saturation method] B: 2.6E-22 Pa at 20°C 1.9E-21 Pa at 25°C (99.7% w/w) [estimated by the VP	IUCLID 5 section: 4.6	

[method]	curve – experimental data by VP balance method]		
Surface tension	A: Not applicable (solubility < 1 mg/l) B: Not applicable (solubility < 1 mg/l)	IUCLID 5 section: 4.10	
Water solubility (purity) [method]	A: pH 5.2: 3.83E-3 mg/l at 20 °C pH 7.4: 0.24 mg/l at 20 °C pH 9.3: 10 mg/l at 20 °C (98.7% w/w) [generator column method] B: pH 5: ≤ 3.17E-6 g/l at 20 °C pH 7: 5.80E-5 g/l at 20 °C pH 9: 1.86E-3 g/l at 20 °C (99.7% w/w) [column elution method with re-circulating pump]	IUCLID 5 section: 4.8	
Partition coefficient n-octanol/water	A: 8.5 [calculated by clogp Algorithm of Hansch and Leo] 6.12 [estimated from measured Koc] B: 6.16–6.27 (at pH 5, 10°C) 5.99–6.13 (at pH 5, 20°C) 5.80–5.98 (at pH 5, 30°C) 5.09 (at pH 7, 10°C) 4.92 (at pH 7, 20°C) 4.78 (at pH 7, 30°C) 4.91 (at pH 9, 10°C) 4.78 (at pH 9, 20°C) 4.58 (at pH 9, 30°C)	IUCLID 5 section: 4.7 partition coefficient	

(purity) [method]	(99.7% w/w) [HPLC method]		
Flash point	A: Not applicable (solid) B: Not applicable (solid)	IUCLID 5 section: 4.11	
Flammability (purity) [method] (purity) [method]	A: Not highly flammable (>99%) [EC A.10 - preliminary test] B: Not highly flammable (>99%) [EC A.10 - preliminary test]	IUCLID 5 section: 4.13	
Explosive properties	A: Not explosive on the basis of the structural formula and oxygen balance B: Not explosive based on structure and experience in use	IUCLID 5 section: 4.14	
Self-ignition temperature (purity) [method]	A: No data B: No auto-ignition was observed below the melting temperature (99.7%) [EC A.16]	IUCLID 5 section: 4.12	
Oxidising properties	A: Not oxidising on the basis of the structural formula B: Not oxidising based on structure and experience in use	IUCLID 5 section: 4.15	
Granulometry	A: No data	IUCLID 5 section: 4.5	

(purity) [method]	B: Proportion of test material having an inhalable particle size: less than 100 µm = 14.8% (sieve); less than 10.0 µm = 0.998% (cascade impactor); less than 5.5 µm = 8.14E-02 % (cascade impactor) (*) (99.7%) [OECD 110]		
Stability in organic solvents and identity of relevant degradation products	A: Not required B: --	IUCLID 5 section: 4.17	
Dissociation constant	A: 4.5 [PETE database calculation/ estimation] B: 4.50 [QSAR estimation by ACD/I-Lab Web service]	IUCLID 5 section: 4.21	
Viscosity	A: Not applicable (solid) B: Not applicable (solid)	IUCLID 5 section: 4.22	

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for the classification.

2.2 Identified uses

Not relevant for the classification.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
//			
//			
//			
//			

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of :

- *Explosivity*: based on structural formula and experience in use, *Brodifacoum* does not show explosive properties. No classification is required;

-*Flammability: Brodifacoum* has proved to be not highly flammable. No auto-ignition was observed below the melting temperature. No classification is required.

-*Oxidising potential*: based on structural formula and experience in use, Brodifacoum does not show explosive properties. No classification is required.

3.1.2 Comparison with criteria

No data.

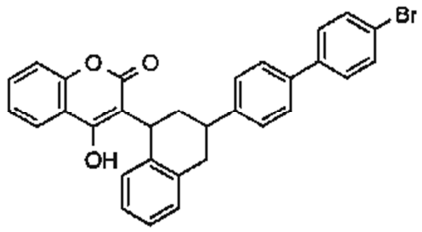
3.1.3 Conclusions on classification and labelling

Brodifacoum does not exhibit hazardous physico-chemical properties. Brodifacoum is thermally stable. Brodifacoum is not highly flammable and it shows no self-ignition below the melting point. Brodifacoum has not oxidizing or explosive properties, either. Brodifacoum shows no signs of reaction with container materials.

4 HUMAN HEALTH HAZARD ASSESSMENT

Brodifacoum, whose structure is shown in Fig. 1, is a so-called second generation anticoagulant rodenticide, which like other coumarin derivatives, is a vitamin K antagonist. They function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver. Death of target organisms is due to massive internal haemorrhages after several days of ingestion of a lethal dose.

Figure 1. The structure of Brodifacoum



Briefly, blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin to the insoluble fibrous protein fibrin, which binds platelets and blood cells to form a solid mass referred to as a blood clot, sealing the site of the haemorrhage and preventing further blood loss. Thrombin is not present in the blood, and is formed at the site of injury from prothrombin. Conversion of prothrombin to thrombin occurs via the coagulation cascade, in which the blood clotting factors are employed. Without these blood factors clotting cannot take place, and the haemorrhage will not be controlled by clot formation. The synthesis of a number of blood coagulation factors is dependent upon vitamin K hydroquinone, which acts as a co-enzyme.

The anticoagulant rodenticides such as Brodifacoum work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. Since, the amount of vitamin K in the body is finite, the progressive block of the regeneration of vitamin K will lead to an increasing probability of a fatal haemorrhage.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Oral Absorption

Brodifacoum (0.21 mg/kg bw) administered orally to rats was rapidly absorbed (T_{max} = 8h; C_{max} 16.1 ng/ml whole blood). The levels declined slowly and about 10% (1.3 ng/ml) was still present at 10 days after dosing. Almost all (82.5 %) the radioactivity in whole blood was found to be associated with the plasma. Based on the radioactivity still associated to the animal tissues, 10 days after the treatment, the oral absorption was > 75%. After a single oral dose of 10 mg/kg of *Brodifacoum* about 64.0% was absorbed and could be accounted for in the liver, carcass and bile 48h after dosing. The rest was recovered in the faeces, as unabsorbed material.

To support the experimental data on *Brodifacoum* itself, read across to data from some related 2nd generation anticoagulants (i.e. *Difenacoum, Flocoumafen*) can be also applied, based on bridging studies demonstrating the similarity in physico-chemical and toxicological properties of these class

of substances sharing the same mode of action. Anticoagulant rodenticides including *Brodifacoum* are rapidly absorbed via the gastro-intestinal tract and oral absorption is assumed to be 100%, on the basis of amount of radioactivity recovered in the excreta and retained in the tissues.

Inhalation Absorption

For the inhalation route no data are available. Based on the physico-chemical characteristic of the compound, a 100% absorption is considered.

Dermal Absorption

As long as dermal absorption is concerned, *Brodifacoum* is expected to be slowly absorbed through the skin, due to the lipophylicity of the molecule, allowing passive transport through the membrane. *Brodifacoum* dermal absorption was assessed by using a formulation (ready-for-use pellet bait) containing 0.0048% *Brodifacoum* w/w tested *in vitro* on human skin samples In the study over the entire 24 h exposure *Brodifacoum* (determined by LC-MS-MS) was found below the LOQ in the receptor fluid (<3.53% of the applied dose) and in the epidermis (<1.64%), after tape stripping. The applied dose was readily removed by mild skin washing and recovered (108 ±6.25%) in the washing fluid. A 'surrogate value' of 5% dermal absorption was calculated by summing up the amount corresponding to the LOQ in the receptor fluid and in the epidermis after tape stripping, which can be considered as systemically available material. This value can be considered as a worst case, also taking into account that the exposure period exceeds the usual time (*i.e.* 8 hours) of professional handling.

To support these data and to cover the risk characterization depending on the type of formulation (including ready-for-use pellet bait or grains and wax block bait or paste), the read across principle can be applied, based on the close structural relationship, the similar physico-chemical properties and the same mode of action displayed by *Brodifacoum* towards other 2nd generation anticoagulants, such as *Difethialone* and *Difenacoum*. A dermal absorption value = 4% has been adopted for *Difethialone*, whereas in the case of *Difenacoum* two different values have been used for risk characterization depending on the type of formulation, that is 3% (pellets and grains) or 0.047% (wax block bait).

On the basis of the available study and reading across from data on other 2nd generation anticoagulant rodenticides, two different values could be used for risk characterization, depending on the type of formulation, that is 5% (pellets and grains) or 0.047% (wax block bait).

Distribution

After oral absorption *Brodifacoum* is widely distributed and bioaccumulates in the liver with minor concentrations in the kidney. Indeed, 10 days after dosing the proportion of the retained dose was highest in the liver (22.8%), followed by the pancreas (2.3%), and then the kidney (0.8%), heart (0.1%) and spleen (0.2%). The remainder of the dose (≅ 50%) was in the carcass and skin.

Metabolism

Brodifacoum was only partially metabolized: 31.3% and 19.6% of the residues in the carcass and liver, respectively, was unchanged *Brodifacoum*. Two metabolites more polar than the parent compound were detected in the bile, the major one being identified as the glucuronide. The toxicologically relevant chemical species is the parent compound.

Excretion

A small amount (11–14%) of the radioactivity was slowly eliminated in urine and faeces over 10 days following a single oral dose of 0.25 mg/kg. Biliary and renal routes are of equal significance in the elimination of *Brodifacoum*. The rate of elimination as given by the biological half-life, was calculated to be 150–200 days.

The elimination from the liver was biphasic at higher doses. There was a rapid phase (days 1–4) which also corresponded to a reduction in clotting factor synthesis, followed by a slower terminal phase (days 28–84) during which blood clotting function was normal. The half-life of elimination from the liver during the rapid and the slow phase was ≅ 4 and 128 days, respectively. At low dose levels, clotting factor synthesis was unaffected indicating that probably only the slow elimination phase was present in the liver. The half-life of *Brodifacoum* in the liver was calculated in the range of 282–350 days.

Potential for accumulation

Brodifacoum shows a high potential for bioaccumulation: in all studies undertaken and at all dose levels tested, the liver retained the largest % of the dose (half-life in the liver was calculated in the range of 282–350 days).

Analyses of the rat livers from the 90 day feeding study, indicate a non-linear accumulation of *Brodifacoum vs.* dose and time.

4.1.2 Human information

Not evaluated in this dossier.

4.1.3 Summary and discussion on toxicokinetics

Summary of toxicokinetic parameters of Brodifacoum in rats

Absorption	Oral: Almost complete oral absorption (>75-100%) Inhalation: No data are available. 100% assumed as default value Dermal: 5% pellets and grains – 0.05% wax block bait.
Distribution	Widely distributed. High potential for bioaccumulation in the liver
Metabolism	Limited (parent compound as toxicologically relevant compound)
Excretion	Very slow (11 – 14% equally distributed urine and faeces in 10 days)

An almost complete oral absorption (range >75-100%) can be considered, on the basis of the amount of radioactivity recovered in the excreta and retained in the tissues and in comparison with structurally and toxicologically similar 2nd generation anticoagulants. Brodifacoum is widely distributed and bioaccumulates mainly in the liver, with lower concentrations in the kidney. Hepatic bioaccumulation of Brodifacoum is a non-linear vs. dose and time. The elimination kinetic from the liver was biphasic, with an half-life in the range of 282-350 days. The excretion after oral administration is very slow (11–14% in 10 days), occurring via the urine and the bile, both as polar

metabolites (glucuronide) and parent compound. The metabolism of Brodifacoum is limited and the toxicologically relevant chemical species is the parent compound.

As long as dermal absorption is concerned, on the basis of the available study and reading across from data on other 2nd generation anticoagulant rodenticides, two different values could be used for risk characterization depending on the type of formulation, that is 5% (pellets and grains) or 0.047% (wax block bait).

4.2 ACUTE TOXICITY

Table 11 Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral **OECD TG 423**	**< 5 mg/kg bw (female)**		**Szakonyi (2004)** **Doc IIA Activa Pelgar**
Oral **No guideline reported Similar to TG401**	**0.40 mg/kg bw (male)**		**Hadler (1974)** **Doc III A** **Section 6.1.1a** **Syngenta**
Inhalation **OECD TG 403**	**3.05 mg/m^3 (female)**		**Parr-Dobrzanski (1993)** **Doc III A Section 6.1.3 Syngenta**
Dermal **OECD 402**	**7.48 mg/kg bw (female)**		**Szakonyi (2004)** **Doc III A Section 6.1.2a Activa Pelgar**
Dermal **OECD 402**	**3.16 mg/kg bw (female)**		**McCall and Leah (1991)** **Doc III A** **Section 6.1.2 Syngenta**

4.2.1 *Non-human information*

4.2.1.1 Acute toxicity: oral

Acute oral toxicity studies, considered as key studies for classification purposes, are summarized in table 4.2.1.1 and 11.

In the rat study (Szakonyi, 2004) the acute oral LD_{50} value of *Brodifacoum* (technical grade) proved to be below 5 mg/kg body weight, with clinical signs such as decreased activity, lateral position, decreased righting reflex, decreased grip and limb tone, paleness, piloerection, dyspnoea and bleeding from the nose observed starting from five days after treatment. One rat died immediately after onset of symptoms; one died two days after the first symptoms. The third one died without any clinical signs. At necropsy effects were consistent with the well-known anticoagulant effect.
In the mouse study (Hadler, 1974) all the animals dosed with 0.2 mg/kg or less survived until the end of the study. In the top three dose groups (0.5, 1.0 and 2.0 mg/kg) deaths occurred between days 4 and 12. Necropsy of mice found dead during the study showed massive internal hemorrhage, in line with the anticoagulant action of *Brodifacoum*. The oral LD_{50} in the male mouse was determined to be 0.4 mg/kg.

Table 4.2.1-1: Oral acute toxicity

Route	Method Guideline	Species	Dose levels duration of exposure	Value LD_{50}	Reference
Oral	OECD TG 423	Rat	5 mg/kg	< 5 mg/kg bw (female)	Szakonyi (2004) Doc III Section 6.1.1a A Activa Pelgar
Oral	No guideline reported Similar to TG401	Mice	0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg	0.40 mg/kg bw (male)	Hadler (1974) Doc IIIA Section 6.1.1 Syngenta

4.2.1.2 Acute toxicity: inhalation

One acute inhalation toxicity study with rats is available and is listed in table 4.2.1.2 and 11.

In the Parr-Dobrzanski study (1993) 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. Symptoms of toxicity included subcutaneous hemorrhage 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 delayed clinical effects, post-mortem findings and late deaths are all indicative of hemorrhage 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.On other end-points the principle of read-across has been applied. For this reason, data obtained with structurally related compounds with the same mechanism of action (*i.e.* 2nd generation anticoagulants) were considered. On this basis it is expected that the substance is highly toxic after inhalation.

Table 4.2.1-2: Inhalation acute toxicity

Route	Method/ Guideline	Species	Exposure Concentrations (mg/m^3)	Duration of Exposure	Value LC_{50}	Reference
Inhalation	OECD TG 403	Rat	0.82, 1.88, 4.96	4 hours (nose only)	3.05 mg/m^3 (female)	Parr-Dobrzanski (1993) Doc IIIA Section 6.1.3 Syngenta

4.2.1.3 Acute toxicity: dermal

Two acute dermal toxicity studies with rats are available and are summarized in table 4.2.1.3 and 11.

In the rat study by Szakonyi (2004) the dermal LD_{50} is 7.5 mg/kg bw. No dermal changes were found after 24 hours exposure. Clinical symptoms (decreased activity, tremor, lateral position, squatting position, paleness, dyspnoea, piloerection, sanguineous fur around the eyes) appeared 5 and 6 days after treatment in one animal treated at 6 mg/kg group and in all animals of 18 mg/kg group. Mortality occurred between days 5 and 9. In animals found dead, bleeding and hematoma in various organs and tissues, clay colored liver were observed.

In a second study (McCall and Leah, 1991) the dermal LD_{50} is determined to be 3.16 mg/kg bw. 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. All mortalities in higher dosage groups occurred between days 5–11. The animals found dead or sacrificed during the study showed signs of extreme toxicity (pallor, bleeding/bruising, breathing abnormalities) immediately prior to death. There were no signs of skin irritation in any of the animals and no significant signs of toxicity in the surviving male. Post-mortem examination revealed internal hemorrhaging in the animals which died or were killed *in extremis*.

Table 4.2.1-3 Dermal acute toxicity

Route	Method Guideline	Species	Dose levels duration of exposure (mg/kg)	Value LD_{50}	Reference
Dermal	OECD 402	Rat	2, 6, 18	7.48 mg/kg bw (female)	Szakonyi (2004) Doc III A Section 6.1.2a Activa Pelgar
Dermal	OECD 402	Rat	1, 10, 500	3.16 mg/kg bw (female)	McCall and Leah (1991) Doc IIIA Section 6.1.4

					Syngenta

4.2.1.4 Acute toxicity: other routes

Neurotoxicity

None of the acute or subchronic performed tests gave any indication for a potential neurotoxic effect of *Brodifacoum.*

4.2.4 Human information

4.2.3 Summary and discussion of acute toxicity

On the basis of the available information *Brodifacoum* is very toxic after oral administration and also via the dermal and inhalation routes. Death was the result of internal haemorrhage.
Brodifacoum was already evaluated at the written procedure for Plant Protection Products in September 2004. It was the agreed to classify Brodifacoum with T+; R 26/27/28. This classification was confirmed at the May 2007 Meeting.

4.2.4 Comparison with criteria

4.2.5 Conclusions on classification and labelling

Classification proposals according to Directive 67/548/EEC: *Brodifacoum* is very toxic after oral administration (0.4 mg/kg bw < $LD_{50} \leq 5$ mg/kg bw) and also via the dermal (3.16 mg/kg bw < $LD_{50} \leq 7.5$ mg/kg bw) and inhalation routes (LC_{50} = 3.05 mg/m^3). Death was the result of internal haemorrhage. Classification with T+; R26/27/28; "Very toxic by inhalation, in contact with skin and if swallowed" is warranted. (Indication of danger: Very Toxic; T+: R-phrases: R 26/27/28).
Classification proposals according to Regulation EC 1272/2008: Acute Tox. 1 H330; Acute Tox. 1 H310;Acute Tox. 1 H300.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal Brodifacoum presently has a harmonised classification according to CLP for acute toxicity via the dermal route in category 1 and a minimum classification for the oral route in category 2. The dossier submitter (DS) proposed to modify the acute toxicity classification via the oral route to category 1 and to add a classification for acute toxicity via inhalation in category 1. The proposal for classification via the oral route was based on data from one rat study and one mouse study, where the LD_{50}s were <5 mg/kg/day and 0.40 mg/kg/day, respectively. Classification for acute toxicity via the inhalation route is supported by one study in rats, giving a LC_{50} of 3.0 mg/m^3. **Comments received during public consultation** One Member State supported the proposal, and there were no objections. **Assessment and comparison with the classification criteria** The RAC supported the proposal from DS to classify Brodifacoum as Acute Tox. 1 for all

three exposure routes. Indeed, the oral LD_{50} of 0.4 and <5 mg/kg in mice and rats, respectively, are below the CLP trigger value of 5 mg/kg for category 1. The inhalation LC_{50} of 3.0 mg/m^3 in rats is below the CLP trigger value of 50 mg/m^3 for category 1. The category 1 classification for the dermal route is confirmed by two dermal rat studies giving LD_{50}-values of 3.2 and 7.5 mg/kg, which are both below the CLP trigger value of 50 mg/kg for category 1.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

4.3.2 Comparison with criteria

4.3.3 Conclusions on classification and labelling

4.4 Irritation

4.4.1 Skin irritation

Table12: Skin Irritation

Species	Method	Average score 24, 48, 72 h		Reversibility yes/no	Result	Reference Doc III
		Erythema	Oedema			
Rabbit	EPA GL 5 13 77	0.41 (0.5)	0.17 (0.33)	yes	Non irritant according to the score	Parkinson (1978) Doc IIIA Section 6.1.4 Syngenta
Rabbit	OECD 404	0	0	Not relevant	Not irritant	Stahl (2004) Doc IIIA Section 6.1.4 (1) Activa Pelgar

4.4.1.1 Non-human information

4.4.1.2 Human information

4.4.1.3 Summary and discussion of skin irritation

Two skin irritation studies with rats are available and are reported in table 12.

In the Parkinson study (1978) slight signs of irritation were observed at all but one of the application sites at the end of the 24 hour exposure period. All the sites affected showed slight erythema, while two of the intact areas had slight oedema and three of the abraded areas had slight or moderate oedema. The mean skin irritation scores (24 and 72 hours) after application of the test substance *Brodifacoum*, were 0.41 and 0.50 for erythema (intact and abraded skin respectively), and 0.17 and 0.33 for oedema (intact and abraded skin respectively). *Brodifacoum* was concluded to be a slight irritant to rabbit skin, but no classification is required according to the score.
In the rabbit study by Sthal (2004) no primary irritation signs, such as erythema and oedema, occurred during the observation period: therefore an average score of zero was given at each time period. During the study the general state and behaviour of animals were normal. According to EEC directive 2001/59/EEC, the test item has not been classified as irritating for the skin.

4.4.1.4 Comparison with criteria

4.4.1.5 Conclusions on classification and labelling

No classification.

4.4.2 Eye irritation

Table 13 Summary table of relevant eye irritation studies

Species	Method	Average Score				Reversibility yes/no	Result	Reference Doc III
		Cornea	Iris	Conjunctiva				
				Redness	Chemosis			
Rabbit	EPA GL 5 13 77	0/0		0/0	0.67/0	Yes	Non irritant according to the score	Parkinson (1978) Doc IIIA Section 6.1.4 Syngenta
Rabbit	OECD 405	0/0	0/0	0/0	0/0	Yes	Not irritant	Hirka (2004) Doc III A Section 6.1.4 (2) Activa Pelgar

4.4.2.1 Non-human information

4.4.2.2 Human information

4.4.2.3 Summary and discussion of eye irritation

Two eye irritation toxicity studies with rats are available and are reported in table 13.

In the Parkinson study (1978) the mean total score according to the EPA guideline (max 110) for unwashed eyes was 8 (1 – 2 h), 2 (24 h), 1 (48 h) and 1 (72 h) and the mean total score for washed eyes was 6 (1 – 2 h), 2 (24 h), 1 (48 h) and 1 (72 h). All eyes (both washed and unwashed) appeared normal at the end of the seven day observation period. *Brodifacoum* was concluded to be a very mild irritant to both washed and unwashed rabbit eyes, but no classification is required according to the score.
In the study by Hirka (2004), after a single application of the test item into the eyes of the rabbit slight redness and slight to moderate increase discharge excretion were observed in the animals. Chemosis, corneal and iris alteration were not found during the study. 24 hours after treatment every animal was symptom-free. 72 hours after the treatment the study was terminated, since no primary irritation symptoms occurred. The observed symptoms can be evaluated as fully reversible alteration and the test item was concluded to be not irritating to the rabbit eyes.

4.4.2.4 Comparison with criteria

4.4.2.5 Conclusions on classification and labelling

Brodifacoum does not fulfil the EU criteria for classification as a skin or eye irritant. No classification.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

4.5.1 Non-human information

4.5.2 Human information

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

4.5.5 Conclusions on classification and labelling

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 4.6-1: Skin sensitisation

Species	Method	Number of animals sensitized/total number of animals or Stimulation Index	Result	Reference Doc III
Guinea pig	OECD 406 (Test of Ritz and Buehler)	20/30	Skin sensitizer	Robinson (1996) Doc IIIA Section 6.1.5 Syngenta
Guinea pig	OECD 406 (Maximisation test, Magnusson & Kligman)	1/20 Historical positive control: 2,4-DNCB	Not sensitizer (test item : 0.25% of the active substance)	Manciaux(1996) Doc III A Section 6.1.5 (1) Activa Pelgar
Mouse	OECD 429 Local	Stimulation Index less	Not a skin sensitizer	Sanders (2006)

	Lymph Node Assay	than 3 in all 4 groups		Doc III A Section 6.1.5 (2) ActivaPelgar

Table 15: Summary table of relevant skin sensitisation studies

Method	**Results**	Remarks	**Reference**
Guinea pig OECD 406 (Test of Ritz and Buehler)	**Skin sensitizer**		**Robinson (1996)** Doc IIIA Section 6.1.5 Syngenta
Guinea pig OECD 406 (Maximisation test, Magnusson & Kligman)	**Not sensitizer (test item : 0.25% of the active substance)**		**Manciaux (1996)** Doc III A Section 6.1.5 (1) Activa Pelgar
Mouse OECD 429 Local Lymph Node Assay	**Not a skin sensitizer**		**Sanders (2006)** Doc III A Section 6.1.5 (2) Activa Pelgar

4.6.1.1 Non-human information

4.6.1.2 Human information

4.6.1.3 Summary and discussion of skin sensitisation

In the Robinson study (1978) during the induction phase with the test substance, one test animal showed signs of severe toxicity and extensive bruising following the second induction and was humanely killed. The dose level for the third induction was therefore reduced to 0.1% w/v. There were no signs of irritation in any of the test or control animals during the induction phase. Following the challenge with a 0.1% w/v preparation of *Brodifacoum*, scattered mild redness or moderate and diffuse redness was seen in 8 of the 19 test animals. Scattered mild redness was seen in 3 of the 8 control animals (one doubtful reading excluded). The net percentage response was calculated to be 4%. Following challenge with a 0.05% w/v preparation of *Brodifacoum*, scattered mild redness was seen in 7 of the 18 test animals (one doubtful reading excluded). There was no erythematous response in any of the control animals. The net percentage response was calculated to be 39%. *Brodifacoum* was considered to be a moderate skin sensitizer to the guinea pig under the conditions of the test.

In the Manciaux study (1996) the test item was a dilution (0.25%) of the active substance. During the pilot study, 2 mortalities by intradermal route were registered and the substance was well tolerated by cutaneous route. For induction 5% of *Brodifacoum* 0.25% in sterile isotonic saline was used; 50% of the test item was used for challenge. Positive control (75%) with 2,4-DCNB were run separately (historical control from the facility). During the test, 24h after challenge 1/20 animal was sensitized and 48h after challenge 1/20 animal was sensitized. The tested substance is not a skin

sensitizer, but considering that the test item was a substantial dilution (0.25%) of the active substance, results cannot be extrapolated to *Brodifacoum.*
In the study conducted on mice (LLNA), the Stimulation Index (expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group) was less than 3 in all 4 groups, and therefore the result is considered negative. The test material was a non-sensitizer under the conditions of the test.

4.6.1.4 Comparison with criteria

4.6.1.5 Conclusions on classification and labelling

Although *Brodifacoum* showed no sensitizing potential in a LLNA study in mice, it was able to cause skin sensitization in a high number of guinea pig. Therefore, the overall results indicate for *Brodifacoum* a potential for skin sensitization, fulfilling the EU criteria for classification as a skin sensitizer.

Classification proposals according to Directive 67/548/EEC: *Brodifacoum* is considered skin sensitizer Xi R43, according to the criteria of Annex VI of Directive 67/548/EC.

Classification proposals according to Regulation EC 1272/2008: Skin Sens.1 H 317.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

Although Brodifacoum showed no sensitising potential in a Local Lymph Node Assay (LLNA) study in mice, it was able to cause skin sensitisation in a high proportion of guinea pigs in a Buehler test. Therefore, overall the results indicate that Brodifacoum has skin sensitization potential, fulfilling the criteria in the CLP Regulation for classification as a skin sensitiser. The classification proposal according to CLP is Skin Sens.1 (H317).

Comments received during public consultation

Two Member States supported the proposal for classification, whereas one of the MS suggested further sub-categorisation into Skin Sens 1B. No dissenting comments were received.

Assessment and comparison with the classification criteria

This endpoint was very briefly described in the CLH report, so additional information was taken from the Competent Authority Report (CAR). It is noted that the high toxicity of Brodifacoum makes it difficult to study its sensitisation potential.

In a mouse LLNA study, the highest topical concentration not causing general toxicity was 0.001%, and Brodifacoum was not a sensitiser at that concentration. In a Magnusson and Kligman assay in guinea pigs, 0.01% Brodifacoum was used for the first intra dermal induction, 0.25% for the two subsequent topical applications, and 0.12% for the challenge, leading to a conclusion of no sensitisation potential as an allergic reaction was only observed in 1 out of 20 animals.

However, a Buehler test in guinea pigs was positive. The induction was intended to be performed using three weekly topical administrations of 1% Brodifacoum in corn oil, but the concentration had to be reduced to 0.1% at the last induction treatment due to signs of toxicity in one animal. The challenge was performed 2 weeks after the last induction using topical administration of either 0.05 or 0.1% Brodifacoum. With both challenge

concentrations, roughly 40% of the animals showed allergic reactions. The symptoms were described as scattered mild redness at the concentration of 0.05% (no signs in controls), and a mix of scattered mild redness and moderate diffuse redness at 0.1%. However, as 3 out of 8 control animals in the 0.1% group showed scattered mild redness, the difference in incidence between the group exposed to 0.1% and its control group is only 4%, and the effects at 0.1% did not fulfil the criteria for classification. The finding of redness in the controls indicates that there is some source of skin irritation which interfered with the assay. In contrast, the incidence of 39% at the challenge concentration of 0.05% and induction concentration of 1% does in principle fulfil criteria for classification (the incidence is between 15 and 60% at a topical induction dose of 0.2-20%). However, the reaction was very modest, and since irritation was noted at 0.1%, it is difficult to rule out a contribution of irritation to this reaction. Although the negative LLNA and Magnusson & Kligman assays were performed at much lower concentrations of Brodifacoum, these assays are generally more sensitive than the Buehler assay, and the absence of positive reactions in these two assays argue against a sensitisation potential. Although Brodifacoum was weakly positive at (only) one concentration in the Buehler test, a weight of evidence assessment does not support classification for sensitisation.

In conclusion, the RAC considered that there was not sufficient evidence to support classification of Brodifacoum for sensitisation.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

4.6.2.2 Human information

4.6.2.3 Summary and discussion of respiratory sensitisation

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral **OECD TG 408**	**Critical effect: increased blood coagulation time.** NOAEL **1µg/kg bw/d**		**Batten (1984)** Doc IIIA Section 6.4.1(09) Syngenta
Oral. Not reported. Similar to **OECD 409**	**Critical effect: increased blood coagulation time.** NOAEL **3µg/kg bw/d**		**Horner, 1997** Doc IIIA Section 6.4.1(03) Syngenta
Oral **OECD TG 408**	**Critical effect: slight increase in clotting times indices.** NOAEL **40 µg /kg/day**		**Morris, 1995** Doc III A Section 6.4 Activa Pelgar

Table 4.7

Route/ Method	Duration of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Oral Not reported Similar to OECD TG 408	90 days	Rat Wistar Male 10/group	0.02 and 0.08 ppm (corresponding to 1 and 4 µg/kg bw/day)	Critical effect: increased blood coagulation time	4 µg /kg bw/d	1µg/kg bw/d	Batten (1984) Doc IIIA Section 6.4.1(09) Syngenta
Oral Not reported, Similar to OECD TG 409	6 weeks	Dog Beagle Male (1) and female (1)	0.0001, 0.0003, 0.001, 0.003 or 0.01 mg/kgbw/day	Critical effect: increased blood coagulation time	10 µg /kg bw/day	3 µg /kg bw/day	Horner, 1997 Doc IIIA Section 6.4.1(03) Syngenta

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Repeated dose oral studies show that in the rat and in the dog, the clinical signs, haematological and post mortem data were consistent with the known pharmacological action of *Brodifacoum*: impairment of the clotting cascade and increased prevalence of haemorrhage leading to death. There were no indications of other secondary toxicities: any of the other parameters including histopathological analysis revealed no treatment related alterations.
The subchronic 90-day oral toxicity allowed the derivation of the lowest repeated toxicity NOEL= 0.001 mg/kg bw/day. In this study, no treatment related effects on haematological parameters were evidenced at any dose, after 45 days, but statistically significant increases in both the kaolin-cephalin time (KCT) and the prothrombin time (PT) were measured at the highest dose level, 0.004 mg/kg bw/day after 90 days. Based upon this effect on prothrombin times and based on haemorrhagic changes seen at necropsy, the NOEL was set at the next lowest dose, 0.001 mg/kg bw/day.
The study by Morris (1995) shows a higher NOAEL: at 80 µg/kg bw /day in male rats resulted in a slight increased incidence of haemorrhage in two animals and slight increase in clotting times indices. The clinical signs and toxicity are consistent with the mode of action of the rodenticide and its properties of anti-coagulant agent. Females showed no effects in the range of concentrations used. The NOAEL is established in 0.04 mg/kg/day.
There is no inconsistencies among the two studies, although the derived NOELs are different: indeed, the mode of action is the same, as well as the critical effect (altered blood coagulation mechanisms): the study by Batten addressed the measurement of very sensitive parameters related with the mode of action (i.e. increases in both the kaolin-cephalin time and the prothrombin time), and therefore were taken as earlier sings of *Brodifacoum* toxicity. The lowest NOEL is considered appropriate for risk characterization.
Repeated-dose oral studies in the dog show that at doses as low as 3 µg/kg/day in the dog, hemorrhagic effects begin to be seen after 6 weeks administration. The clinical signs, haematological and post mortem data were consistent with the mode of action of *Brodifacoum* and similar to what found in the rat, supporting the NOEL derived from the rat study, although the number of animal tested was quite limited.

4.7.1.2 Repeated dose toxicity: inhalation

No data on repeated inhalation toxicity have been submitted. However, the acute inhalation study (Parr-Dobrzanski, 1993) shows that *Brodifacoum* is acutely toxic by inhalation (LD_{50} = 3.05 mg/m^3). Based on the mode of action shown by *Brodifacoum* independently on the route of exposure, considering the inhalation absorption (100%) and the bioaccumulative nature of *Brodifacoum*, it can be expected that potential repeated exposure by inhalation will probably result in death by induction of a haemorrhagic syndrome. Therefore specific repeated dose inhalation studies would not provide any additional important information.
However, as indicated by data on the low vapour pressure ($2.6x10^{-22}$ Pa at 20°C $1.9x10^{-21}$ Pa at 25°C) of *Brodifacoum*, on dustiness and particle size, the potential for inhalation is low and a repeated dose inhalation toxicity study to be carried out is also considered not justified.

4.7.1.3 Repeated dose toxicity: dermal

No data have been submitted on dermal repeated toxicity. On the basis of both physico-chemical properties and mode of action of *Brodifacoum* and the results of the acute dermal toxicity

study (McCall J C and Leah A M, 199; rat LD_{50} = 3.16 mg/kg bw), it can be anticipated that subchronic effect due to prolonged skin contact should not be disregarded. Although the dermal absorption is limited, the bioaccumulative nature of *Brodifacoum* is such that repeated dermal administration is likely to cause severe toxic effects at doses lower than those resulting in death following a single dose. Death would be caused by the pharmacological action of the molecule, inducing fatal haemorrhage, the mode of action being similar independently on the route of administration. Therefore specific repeat dose dermal studies would not provide any additional important information to that obtained in repeated dose studies by the oral route.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

Repeated oral exposure to *Brodifacoum* resulted in clinical signs and toxicity consistent with the mode of action of the rodenticide and its properties of anti-coagulant agent (lethal haemorrhages). The NOEL for subchronic oral toxicity both in rats and dog is in the range 1–40 µg/kg/day (the lowest values identified with sensitive end-points, such as increases in both the kaolin-cephalin time and the prothrombin time, related to the mode of action, thus considered as early diagnostic signs). Based on results from the acute dermal and inhalation toxicity studies, route-to-route extrapolation, consistently with the decision adopted for other second generation anticoagulants, it is justified to assume serious damages associated to prolonged exposure through dermal and inhalation routes also.

Brodifacoum was already evaluated at the written procedure for Plant Protection Products in September 2004. It was the agreed to classify *Brodifacoum* with T; R 48/23/24/25. This classification was confirmed at the May 2007 Meeting.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Classification proposal according to Directive 67/548/EEC: *Brodifacoum* is classified with T; R48/23/24/25 “Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed”:

C≥0.25%: T 48/23/24/25
0.025%≤C<0.25%: Xn 48/20/21/22

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification proposal according to Regulation EC 1272/2008: STOT RE 1 H372:

C ≥ 0.25 % STOT RE 1 H372;
0,025 %≤ C <0.25 % STOT RE 2 H373

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

Repeated oral exposure to Brodifacoum resulted in clinical signs and toxicity consistent with the mode of action of the rodenticide and its properties as an anti-coagulant (lethal haemorrhages). The NOEL for subchronic oral toxicity both in rats and dogs was in the range of 0.001–0.040 mg/kg/day (the lowest values identified with sensitive end-points, such as increases in both the kaolin-cephalin clotting time and the prothrombin clotting time, related to the mode of action, thus considered as early diagnostic signs). Based on results from the acute dermal and inhalation toxicity studies, route-to-route extrapolation and consistently with the decision adopted for other second generation anticoagulants, it is justified to assume serious damages associated to prolonged exposure through dermal and inhalation routes also. The classification proposal from the DS according to CLP was STOT RE 1 (H372 (Blood), which is also the current classification in Annex VI of the CLP Regulation.

Comments received during public consultation

One comment was received, from a Member State, supporting the proposal and adding that the classification should apply to all routes of exposure.

Assessment and comparison with the classification criteria

There are three repeated dose toxicity studies available, all of them poorly reported in the CLH report. Information from the CAR shows that increased blood clotting times were found at the top doses in the two 90 day studies in rats (0.004 and 0.080 mg/kg/day, respectively), in the absence of other findings. In the 6 weeks dog study, the 2 dogs in the highest exposure group (0.01 mg/kg/day) had to be killed on day 36 when their blood clotting time reached termination criteria. There were adverse effects in dogs at 0.01 mg/kg/day, which is clearly below 10 mg/kg/day, the guidance value for STOT RE1 in a 90 days study. Whereas the extent to which the finding in the rat studies is adverse is difficult to assess at the doses used in those studies, it is clear that truly adverse effects in rats also will appear at dose levels below the guidance value for STOT RE1 in a 90 day study (10 mg/kg/day).

Regarding the routes of exposure, repeated dose toxicity studies were only available for the oral route. However, the acute toxicity studies indicated that toxicity via the

inhalation and dermal routes was also significant. The RAC therefore supported not specifying the exposure routes in the hazard statement.

The effect levels were well below the guidance value of 10 mg/kg/day for a 90 day study, warranting classification with STOT RE 1; H372 (Causes damage to the blood through prolonged or repeated exposure).

An indicative effect level of 0.01 mg/kg/day from the dog study indicated that a Specific Concentration Limit (SCL) should be set for Brodifacoum, since this is more than one order of magnitude lower than the guidance value (GV). Using Haber's law, the effect level at day 36 was recalculated to give an equivalent 90 day effect level of 0.004 mg/kg/day (0.01 mg/kg/day x 36 days / 90 days). RAC considered, based on the guidance for setting SCLs for repeated dose toxicity, that an effect level of 0.004 mg/kg/day would result in a SCL of 0.04% for STOT RE 1. The SCL value should, according to the guidance, be rounded down to the nearest preferred value of 1, 2 or 5, resulting in a SCL of 0.02% for STOT RE1, and 0.002% for STOT RE 2.

4.9 Germ cell mutagenicity (Mutagenicity):

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies:

Method	Results	Remarks	Reference
Ames test	***Vitro –*** ***- (+S9)*** ***- (-S9)***	Non toxic and non mutagenic	**Thompson P W, 2002** Doc III A Section 6.6.1 Activa Pelgar
Maron and Ames (1983)	***Vitro:*** ***- ve (+S9)*** ***- ve (-S9)***	Cytotoxic for strain TA1538 (-S9) at concentrations above 40 µg/plate; and TA100 (-S9 and +S9) at concentrations above 200µg/plate	Doc III A Section 6.6.1 Syngenta
Gene mutation	***Vitro*** ***- (+S9)*** ***- (-S9)***	Non toxic and non clastogenic	**Durward R, 2004** Doc III A Section 6.6.3 Activa Pelgar
Mammalian Cell Gene Mutation Tests	***Vitro:*** ***- ve (+S9)*** ***- ve (-S9)***	Cytotoxic at concentrations greater than 112.5µg/ml. The addition of auxiliary metabolic activation (S9 mix) appeared to slightly decrease the toxicity	Doc. III A Section 6.6.3 Syngenta
Chromosomal aberration	***Vitro*** ***- (+S9)*** ***- (-S9)***	Non toxic and Non Mutagenic	**Wright N P, 2003** Doc IIIA Section 6.6.2 Activa Pelgar
OECD 473 (1983)	***Vitro:*** ***- ve (+S9)*** ***- ve (-S9)***	Cytotxic at 500 and 1000 µg/ml and precipitation of the test substance	Doc III A Section Syngenta
Mammalian cell transformation assay of Styles (1977): Styles J A (1977), Brit J Cancer, 36, 58	***Vitro*** ***Not tested (+S)*** ***- ve (-S9)***	Increasing cell mortality with increasing dose, with LC_{50} determined to be 20 µg/ml	Doc III A Section 6.6.3 Syngenta
J E Cleaver (1977) - Handbook of Mutagenicity Test Procedures, B. Kilbey *et al* eds., Elsevier, Amsterdam 19-4B; **Abbondandolo *et al* (Roma 1979) - Mutagenesi ambientale, Metodiche di analisi ed. CNR 223-236;** **Benigni *et al*., Mutation Res. 103 (1982) 385-390;** **Ames B N, McCann J, Yamasaki E, Mut. Res. 31 (1975) 347-364;** **Snedecor C W, Statistical Methods, Iowa State College Press, Ames, 5th Edition, 1956.**	***Vitro:*** ***- ve (+S9)*** ***- ve (-S9)***	No statistically significant increases in the incorporation of tritiated thymidine in cultured human Hela cells, either in the presence of absence of metabolic activation up to a dosage concentration of 1000µg/ml. Cytotoxicity was indicated at the higher dose levels (100 and 1000µg/ml) by an inhibition of S-phase	Doc III A Section 6.6.3 Syngenta
OECD 474 Sheldon et All. (1984)	***Vivo*** No statistically significant increase in incidence of micronuclei was seen with		Doc III A Section 6.6.4

	Brodifacoum at any dose level or sampling time, even though the dose levels were equivalent to 80% and 50% of the MLD/7.		Syngenta

Table 4.9: ***In vitro***

Test system Method Guideline	**organism/ strain(s)**	**Concentra_ tions tested**	**Result**		**Remark**	**Reference**
			+ S9	**- S9**		
			+/-/+	+/-/+		
Ames test	*S. Typhimurium* TA 98,100,102, 1535, 1537	0,0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000	—	—	Non toxic and non mutagenic	Thompson P W, 2002 Doc IIIA Section 6.6.1 Activa Pelgar
Maron and Ames (1983)	*Salmonella Typhimurium*: TA 1535, TA 1537,TA 98, TA 100, TA 1538	0.064 – 5000 µg/plate	-ve	-ve	Cytotoxic for strain TA1538 (-S9) at concentrations above 40 µg/plate; and TA100 (-S9 and +S9) at concentrations above 200µg/plate	Doc.III A Section 6.6.1 Syngenta
Gene mutation	Mouse lymphoma	0, 3.13, 6.25, 12.5, 25, 37.5 and 50 µg/ml	—	—	Non toxic and non clastogenic	Durward R, 2004 Doc III A Section 6.6.3 Activa Pelgar
Section 4 Genetic Toxicology, 476. *In Vitro* Mammalian Cell Gene Mutation Tests	Mouse lymphoma cell line L5178Y (-3.7.2C) (TK +/-)	3.9 - 150 µg/ml in cell suspension	-ve	-ve	Cytotoxic at concentrations greater than 112.5µg/ml. The addition of auxiliary metabolic activation (S9 mix) appeared to slightly decrease the toxicity	Doc. III A Section 6.6.3 Syngenta
Chromosomal aberration	human lymphocytes *in vitro*	0, 18.75, 37.5, 75, 150, 225 and 300 µg/ml	—	—	Non toxic and Non Mutagenic	Wright N P, 2003 Doc III A Section 6.6.2 Activa Pelgar
OECD 473 (1983)	Human lymphocytes	5 – 1000	-ve	-ve	Cytotxic at 500 and 1000 µg/ml and precipitation of	Doc.III A

	(male and female)	µg/ml			the test substance	Section 6.6.2 Syngenta
Mammalian cell transformation assay of Styles (1977): Styles J A (1977), Brit J Cancer, 36, 558	Baby Hamster Kidney Fibroblasts (BHK21/C13)	0.12 - 1200 µg/ml	Not tested	-ve	Increasing cell mortality with increasing dose, with LC_{50} determined to be 20 µg/ml	Doc. III A Section 6.6.3 Syngenta
J E Cleaver (1977) - Handbook of Mutagenicity Test Procedures, B. Kilbey *et al* eds., Elsevier, Amsterdam 19-4B; Abbondandolo *et al* (Roma 1979) - Mutagenesi ambientale, Metodiche di analisi ed. CNR 223-236; Benigni *et al*., Mutation Res. 103 (1982) 385-390; Ames B N, McCann J, Yamasaki E, Mut. Res. 31 (1975) 347-364; *Snedecor C W, Statistical Methods, Iowa State College Press, Ames, 5th Edition, 1956*.	Hela cells (human)	1, 10, 100 and 1000 µg/ml	-ve	-ve	No statistically significant increases in the incorporation of tritiated thymidine in cultured human Hela cells, either in the presence of absence of metabolic activation up to a dosage concentration of 1000µg/ml. Cytotoxicity was indicated at the higher dose levels (100 and 1000µg/ml) by an inhibition of S-phase	Doc. III A Section 6.6.3 Syngenta

Table 4.9.1: ***In vivo***

Type of test Method/ Guideline Reference	**Species Strain Sex no/group**	**frequency of application**	**sampling times**	**dose levels**	**Results**	**Remarks**	**Reference**
OECD 474 Sheldon et All. (1984)	*Mus domesticus* (mouse) C57BL/6J male and female 5 + 5	Once	24, 48, 72 hours after treatment	0.187 and 0.30 mg/kg	No statistically significant increase in incidence of micronuclei was seen with *Brodifacoum* at any dose level or sampling time, even though the dose levels were equivalent to 80% and 50% of the MLD/7.		Doc. III A Section 6.6.4 Syngenta

4.9.1 Non-human information

4.9.1.1 In vitro data

4.9.1.2 In vivo data

4.9.2 Human information

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

Brodifacoum was tested in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538, with and without S9-mix, up to 5000 mg/plate, with negative results in all bacterial strain. No clastogenic activity was observed in the *in-vitro* cytogenetic assay in human lymphocytes, performed with and without metabolic activation, up to cytotoxic doses. The *in vitro* mammalian cell mutation assay in mouse lymphoma L5178Y cells also resulted negative, with and without S9-mix, while cytotoxic effects was observed at the highest doses. The substance resulted negative up to cytotoxic concentration in the *in vitro* mammalian chromosome aberration test in human lymphocytes (50% mitotic inhibition at the maximum dosage tested). An *in vivo* mouse micronucleus test gave negative results. Therefore a genotoxic potential of *Brodifacoum* can be ruled out.

4.9.5 Comparison with criteria

4.9.6 Conclusions on classification and labelling

None.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

4.10.1.2 Carcinogenicity: inhalation

4.10.1.3 Carcinogenicity: dermal

4.10.2 Human information

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

As for the chronic toxicity studies, carcinogenicity studies were not considered to be technically feasible and necessary due to the specific action of *Brodifacoum* on the test/target species. The anticoagulant action is the sole pharmacological action of the materials. Short-term studies where vitamin K has been co-administered have not shown any other toxic effects at doses that would have otherwise been lethal. However, administration of vitamin K is not practical for long-term studies in rodents. The absence of carcinogenic potential is supported by the fact that mutagenicity studies on *Brodifacoum* are negative. The likely mechanisms of carcinogenicity are limited to those resulting from effects such as hepatic hypertrophy, or irritancy, and short-term studies show that there are no responses of that nature. It is reasonable to conclude that *Brodifacoum* has no carcinogenic potential. Repeated toxicity studies with second generation anticoagulants cannot be carried out for more than a few weeks due to the accumulative nature and high toxicity of *Brodifacoum*.

Brodifacoum displayed no mutagenic activity in a standard range of genotoxicity tests. No long-term carcinogenicity study was submitted by the two Applicants. In fact, chronic toxicity studies were not considered to be technically feasible due to the specific action of *Brodifacoum* on the test/target species. However, the anticoagulant action is apparently the only pharmacological action of *Brodifacoum*. *Brodifacoum* has no structural alerts for carcinogenicity and no concern about possible non-genotoxic carcinogenic potential can be derived from the toxicological studies. Therefore, the justifications of the Applicants for not-submission of carcinogenicity data was considered acceptable.

4.10.5 Comparison with criteria

4.10.6 Conclusions on classification and labelling

None.

4.11 Toxicity for reproduction

Table 20: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Fertility **Oral OECD 416**	**NO(A)EL Parental (mg/kg/day): m 0.003, f 0.001;** **NO(A)EL F1 (mg/kg/day):): m 0.003, f 0.003;** **NO(A)EL F2 (mg/kg/day):): m 0.003, f 0.003;**	Critical effect: High dose Parental, F1 and F2,, mid-dose parental females: haemorrhagic diathesis.	Szakonyi, 2004 Doc IIIA Section 6.8.2 Activa Pelgar
Developmental **Oral gavage** **OECD 414**	**NO(A)EL maternal toxicity: 0.002 mg/kg bw/day** **NO(A)EL developmental toxicity: equal or higher than 0.005 mg/kg bw/day**	Critical effect: Deaths with internal haemorrhages. No developmental effects (maternal toxicity)	**Doc III A** **Section 6.8.1** Syngenta
Developmental **Oral gavage** **OECD 414**	**NO(A)EL maternal toxicity: 0.002 mg/kg bw/day** **NO(A)EL developmental toxicity: Equal or higher than 0.004 mg/kg/day**	Critical effect: Dams: Increase in Kaolin-cephalin and prothrombin time at 0.004 mg/kg/day. No developmental effects	Morris, 1995 Doc IIIA Section 6.8.1 (2) Activa Pelgar
Developmental **Oral gavage** **OECD 414**	**NO(A)EL maternal toxicity: 0.001 mg/kg bw/day** **NO(A)EL developmental toxicity: Equal or higher than 0.020 mg/kg bw/day**	Critical effect: Deaths with internal haemorrhages. No developmental effects (maternal toxicity)	Doc III A Section 6.8.1 Syngenta
Developmental **Oral gavage** **OECD 414**	**NO(A)EL maternal toxicity: Equal or higher than 0.040 mg/kg bw /day** **NO(A)EL developmental toxicity: Equal or higher than 0.040 mg/kg bw /day**	No maternal or developmental effects	Morris, 1995 Doc IIIA Section 6.8.1 (1) Activa Pelgar

4.11.1 Effects on fertility

No specific effects on fertility or reproductive performance effects were observed at doses eliciting general toxicity. Dose related induction of haemorrhagic diathesis was consistent with the anti-coagulant properties of the active substance. Female animals were more sensitive than the male animals in the Parental generation. The NOEL and LOEL were 0.001 and 0.003 mg/kg bw/day, respectively, based on parental toxicity associated to anticoagulant effect. Overall, parental animals were more sensitive than F1 and F2 animals.

4.11.1.1 Non-human information

4.11.1.2 Human information

No specific information on reproductive or developmental effects of *Brodifacoum*.
Warfarin is an established human teratogen, sharing the same chemically active group as *Brodifacoum*.

4.11.2 Developmental toxicity

In a series of studies compliant with OECD 414 in rats and rabbits, there was no evidence of developmental effects up to the dose levels tested of 0.020 and 0.040 mg/kg bw for rat and rabbit, respectively. The specific anticoagulant effects of the compound were clearly shown in rats and rabbits, the latter appearing more sensitive.

4.11.2.1 Non-human information

4.11.2.2 Human information

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

The available studies provided no hint that *Brodifacoum* may elicit reproductive or developmental effects at dose levels at which the specific anticoagulant effects are not induced.
However, the issue of human developmental hazard remains an open one. (tables 4.11 and 4.11.1)
It is widely recognized that the conventional OECD Guideline 414 may have limitations in the detection of possible teratogenic effects of coumarin-related compounds. In particular, *Brodifacoum* has the same chemically active group as the recognized human teratogen *Warfarin* (classified as Repr. category 1). Taking into account the limitations of the current protocol, the potential species-specificity of effects and the structure-activity similarity with *Warfarin*, the EU approach towards anticoagulant rodenticides is a precautionary one. Specific areas of uncertainties as regards the comparison with Warfarin of 2nd generation anticoagulants concern the placental transfer, as well mode of action in developing tissues (extrahepatic vitamin K deficiency). Such areas of uncertainties make it difficult to rule out the developmental toxicity of 2nd generation anticoagulants and support a conservative read-across with Warfarin.
Accordingly, *Brodifacoum* should be classified for developmental toxicity with Repr. Cat. 2; R61.

Table 4.11 Effects on fertility

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses**	**critical effect**	**NO(A)EL Parental (mg/kg/day)**		**NO(A)EL F1 (mg/kg/day)**		**NO(A)EL F2 (mg/kg/day)**		**Reference**
						m	f	m	f	m	f	
Oral	OECD 416	Rat Wistar 25 /sex/group	2-generation	0,0.001,0.003 and 0.006 mg/kg bw/day	High dose Parental, F_1 and F_2, mid- dose parental females: haemorrhagic diathesis	0.003	0.001	0.003	0.003	0.003	0.003	Szakonyi, 2004 Doc IIA Activa Pelgar

4.11.1Developmental toxicity

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses**	**Critical effects dams fetuses**	**NO(A)EL maternal toxicity**	**NO(A)EL developmental toxicity**	**Reference**
Oral - gavage	OECD 414	Dutch Rabbit 15/group	Gestation days 6-18	0, 0.001, 0.002, 0.005 mg/kg bw/d	(maternal toxicity): deaths with internal haemorrhages. No developmental effects	0.002 mg/kg bw/day	Equal or higher than 0.005 mg/kg bw/day	Doc. IIIA 6.8.1 (Syngenta)
Oral - gavage	OECD 414	Rabbit, New Zealand White, 20/group	Gestation days 6-18	0, 0.001, 0.002, 0.004 mg/kg bw/day	Dams: Increase in Kaolin-cephalin and prothrombin time at 0.004 mg/kg/day. No developmental effects	0.002 mg/kg bw/day	Equal or higher than 0.004 mg/kg/day	Morris, 1995 Doc III A Section 6.8.1 (2) Activa Pelgar
Oral - gavage	OECD 414	Rat 20/group	Gestation day 6-15	0, 0.001, 0.010, 0.020 mg/kg bw/day	(maternal toxicity): deaths with internal haemorrhages. No developmental effects	0.001 mg/kg bw/day.	Equal or higher than 0.020 mg/kg bw/day.	Doc. IIIA 6.8.1 Syngenta
Oral	OECD 414	Pregnant Rat	Gestation day 6-15	0, 0.01,	No maternal or developmental	Equal or higher	Equal or higher than	Morris, 1995

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Doses	Critical effects dams fetuses	NO(A)EL maternal toxicity	NO(A)EL developmental toxicity	Reference
		20/group		0.020, 0.040 mg/kg bw/day	effects	than 0.040 mg/kg bw /day	0.040 mg/kg bw /day	Doc III A Section 6.8.1 (1) Activa Pelgar

4.11.5 Comparison with criteria

4.11.6 Conclusions on classification and labelling

Classification proposal according to Directive 67/548/EEC: Toxic Repr. Cat. 2; R61*
(*Based on the classification for developmental effect by read across to *Warfarin*).

Classification proposal according to Regulation EC 1272/2008: Toxic Repr. 1B H360D*
(*Based on the classification for developmental effect by read across to *Warfarin*).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

According to the DS, the available studies provided no hint that Brodifacoum may elicit reproductive or developmental effects at dose levels at which the specific anticoagulant effects are not induced.

However, it is recognised that the conventional OECD Guideline 414 may have limitations in the detection of possible teratogenic effects of coumarin-related compounds. In particular, Brodifacoum contains the same chemically active 4-hydroxycoumarin group as the recognized human teratogen Warfarin (classified in Annex VI of CLP as Repr. 1A). Taking into account the limitations of the current test design, the potential species-specificity of effects and the structure-activity similarity with Warfarin, the EU approach towards anticoagulant rodenticides should according to the DS be a precautionary one. Specific areas of uncertainty regarding the comparison of 2nd generation anticoagulants with Warfarin concern their placental transfer, as well as their mode of action in developing tissues (extra-hepatic vitamin K deficiency). Such areas of uncertainty make it difficult to rule out the developmental toxicity of 2nd generation anticoagulants and support a conservative read-across from Warfarin. Accordingly, Brodifacoum should be classified according to CLP for developmental toxicity with Toxic Repr. 1B; H360D.

Comments received during public consultation

Five member states disagreed with the proposal and instead proposed classification as Repr. 1A; H360D based on the human evidence of developmental toxicity of Warfarin. Six industry organisations disagreed with the proposal, mainly based on the observation that reliable animal studies showed that there was no developmental toxicity in rats or rabbits, and that therefore there should be no classification for Brodifacoum.

It is noted that the DS has changed their position after the public consultation, and in the RCOM expressed that classification with Repr. 1A; H360D was supported.

Assessment and comparison with the classification criteria

Brodifacoum and Warfarin share the same AVK MoA, i.e., they inhibit vitamin K epoxide reductase, an enzyme involved with blood coagulation and bone formation. Several other AVK rodenticides have also been developed with the same MoA but which are more effective rodenticides. They have similar functional groups and all inhibit both vitamin K epoxide reductase and vitamin K reductase. Vitamin K is necessary for proper functioning of carboxylases needed for both blood coagulation and bone development.

In humans, Warfarin is known to cause death of embryos and foetuses and malformations, mainly nasal hypoplasia. Since deformation of the naso-maxial part of the face is very specific, it is also referred to as human "Warfarin embryopathy", and Warfarin is consequently classified as a known human developmental toxicant in category Repr. 1A (H360D).

Two other coumarins, i.e., Acenocoumarol and Phenprocoumon, are also used in medicine because of their anticoagulant properties. They are also known human teratogens, with five and eight cases of congenital anomalies (85% involving the nose) reported until 2002 for Acenocoumarol and Phenprocoumon, respectively (van Driel, 2002). It has been argued that the second generation rodenticides have different half-lives to Warfarin and are therefore less likely to be teratogens. Therefore, it is noteworthy that Acenocoumarol and Phenprocoumon exhibit teratogenicity despite having different half-lives to Warfarin. Thus, half-lives of 2-8 hours are reported for Acenocoumarol, 30-45 hours for Warfarin, and 156-172 hours for phenprocoumon (Rane and Lindh, 2010). It seems that the MoA is more important than half-life as a determinant for developmental toxicity expressed as a specific deformation of the face.

Although there are only 3 human cases described for Brodifacoum, two of them indicate similar effects of Brodifacoum and Warfarin in humans, and more severe effects in the fetus than in the mother. Thus, they support the position that Brodifacoum may exert similar developmental toxicity as Warfarin in humans.

Although the experimental animal studies on Brodifacoum do not indicate any developmental toxicity, there are uncertainties as to the predictability of these studies for humans, and there is also some theoretical basis for assuming that humans and experimental animals may respond differently to the AVK rodenticides, including Brodifacoum.

Overall, the RAC is of the opinion that Brodifacoum should be classified similarly to Warfarin when it comes to developmental toxicity, i.e., in category Repr. 1A (H360D). The reasons are the similar MOAs, some supporting human evidence of developmental toxicity of Brodifacoum and the other therapeutically used AVK coumarins, and the likelihood that experimental animal data derived from standard test protocols is not predictive for effects in humans.

Regarding a specific concentration limit (SCL) for Brodifacoum, it is acknowledged that the specific data on developmental toxicity of Brodifacoum is too scarce to guide the setting of a SLC.

However, for Warfarin there is sufficient data to set a SCL for developmental toxicity. Thus, based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg/day) may cause developmental toxicity and could perhaps be regarded as an ED10 level. This human ED10 value would, using the CLP guidance for setting SCLs based on animal data, belong to the high potency group (<4 mg/kg/day). The CLP guidance states that for an ED10 <4 mg/kg/day, the SCL is 0.03%, and for an ED10 below 0.4 mg/kg/day the SCL becomes 0.003%. If the starting point was an ED10 value obtained from animal studies (0.125 mg/kg/day; Kubaszky *et al.* 2009), it would qualify Warfarin for the high potency group and also result in a SCL of 0.003%. Thus, the RAC has

concluded on a SCL of 0.003% for developmental toxicity for Warfarin.

As the other AVK rodenticides are equally or more toxic than Warfarin, it is not considered appropriate to apply the generic concentration limit for these substances (0.3%), but instead to base the SCLs on that proposed for Warfarin. Thus, the RAC is of the opinion that the SCL for Warfarin can be used as a surrogate SCL for the other AVK rodenticides, resulting in a SCL of 0.003% for Brodifacoum.

Detailed discussion by RAC

Brodifacoum is a second generation AVK rodenticide, having the same MoA as Warfarin (EHC, 1995). Warfarin is known to cause death of embryos or foetuses and malformations, mainly nasal hypoplasia in humans. Since the deformation of the naso-maxial part of the face is very specific, it is also referred to as human "Warfarin embryopathy", and Warfarin is consequently classified as a known human developmental toxicant in category Repr. 1A (H360D).

In addition to skeletal malformations, Warfarin may cause spontaneous abortion, stillbirth, neonatal death, premature delivery, and ocular atrophy, of which spontaneous abortion and stillbirth appear to be the most frequent (affecting ca. 27% of pregnancies), and naso-maxial hypoplasia the most frequent among live births (ca. 5% of pregnancies). Substitution of Warfarin by Heparin during the first trimester of pregnancy removes the risk of naso-maxial hypoplasia. Differences in human sensitivity to AVK agents mainly relate to metabolic polymorphisms in the enzymes CYP2C9 and VKORC1 (Verhoef *et al.*, 2013), but may also depend on e.g. vitamin K intake via the food, and differences in parameters related to hepatic accumulation, protein binding and placental transfer.

Brodifacoum and warfarin share the same MoA, i.e., they inhibit vitamin K epoxide reductase, an enzyme involved with blood coagulation and bone formation. Several other AVK rodenticides have also been developed with the same mode of action (MoA) but are more efficient rodenticides. They have similar functional groups: 4-hydroxycoumarin, 1,3-indanedione (Chlorofacinone) and 2-hydroxy-4-thiochromenone (Difethialone) and all inhibit vitamin K epoxide reductase and vitamin K reductase. Vitamin K is necessary for proper functioning of carboxylases needed for both blood coagulation and bone development. Effects on blood coagulation are shared between all AVKs, and as vitamin K also is involved in bone formation, effects on bone formation are expected but not proven for other AVK rodenticides. Effects on the foetal bone formation can theoretically either be direct via inhibited enzymes in the foetus or indirect via inhibition in the dam resulting in low circulating concentrations of vitamin K.

Considering the likely similar/identical MoA for Brodifacoum and warfarin, a question is whether they would have similar developmental toxicological effects in humans. There are three case reports on effects of Brodifacoum in pregnant women that can be informative.

Zurawski and Kelly (1997) described a case where a (22nd week) pregnant woman suffered from haemorrhagic diathesis after the ingestion of Brodifacoum, and aggressive vitamin K therapy cured her and led to the birth of a healthy infant.

Yan (2013) describes a case where a (37th week) pregnant woman was examined for "gross hematuria" (blood in the urine) in the absence of other clinical signs. Obstetric ultrasound revealed a live fetus with evidence of intracranial hemorrhage. Administration of vitamin K and prothrombin complex normalised the coagulopathy in the mother, but the neonate was delivered stillborn with severe haemorrhagic changes in the brain and lungs. The presence of Brodifacoum was confirmed in the mother's blood (1310 ng/ml), in cord blood (652 ng/ml) and placenta (1033 ng/ml).

Mehlhaff *et al.* (2013) reported a case with bleeding diathesis (spontaneous mucosal

bleeding) in the mother after oral ingestion of Brodifacoum. After correction of the coagulopathy, the patient was taken for urgent cesarean delivery. The 32nd week neonate showed evidence of foetal coagulopathy and died at 4 days of life.

Although there are only 3 cases, two of them indicate severe effects in the foetus, which in contrast to the coagulopathy in the mother was not curable with vitamin K administration, and thus led to more serious effects in the fetus than in the mother. These cases support the position that Brodifacoum may exert similar developmental toxicity to warfarin in humans.

Another question is whether the apparently negative rat developmental studies for Brodifacoum have predictive value for effects in humans, and how much weight the negative data should be given in a weight of evidence analysis which also includes human evidence.

Human warfarin embryopathy may involve foetotoxicity (e.g., spontaneous abortion and stillbirth), ocular atrophy, and skeletal malformations. The animal developmental toxicity studies on Brodifacoum do not show any fetal toxicity, and this could either be because of no such inherent toxicity or that animal studies are not sufficiently predictive for effects in humans. A comparison of the animal and human effects of warfarin was therefore performed.

In some rat studies, warfarin was shown to cause foetotoxicity, fetal haemorrhages, and ocular effects. With very specific design of the studies, bone-related malformations can also appear in rat studies (Howe and Webster, 1992). The rat fetal effects will be discussed further below, in order to assess to what extent rat studies on AVK rodenticides are predictive for effects in humans.

Developmental toxicity - haemorrhage

Increased incidence (without a clear dose-response relationship) of foetal haemorrhages, external or visceral, were observed in a recent, reliable study on rats exposed to warfarin (Kubaszky, 2009; see CLH report on Warfarin). However, it should be noted that small foetal haemorrhages are not easily detected, and in the reporting of the Kubaszky study (2009) it is stated specifically that clinical observations were made "*with special attention to external signs of haemorrhages*". Considering the lack of a dose-response relationship, it can be questioned if the haemorrhages are substance-related. On the other hand, one may not expect a very clear dose-response considering the small dose spacing in this study (0.125-0.25 mg/kg/day).

AVK rodenticides act via inhibiting the formation of vitamin K, which in the next step acts by regulating carboxylases, and the AVK rodenticides therefore have effects on the processes (e.g., coagulation, bone formation) regulated by these carboxylases. It is noted that the expression of carboxylases in the foetal liver, which is responsible for the coagulation system, starts at day 16 (Romero *et al.*, 1998), so it is unexpected that haemorrhages are found at rather similar incidences in foetuses exposed until day 15 as in foetuses exposed until day 19. In both cases foetuses were dissected at day 20.
However, a (poorly reported) study on Warfarin by Mirkova and Antonov (1983; see CLH report on Warfarin) also reported foetal haemorrhages, and James *et al.* (1989; see CLH report and CAR on Flocoumafen) reported a low incidence of haemorrhage in controls that did not increase with increasing exposure to another AVK rodenticide, flocoumafen.

It seems that haemorrhages sometimes can be picked up in an OECD 414 study, but it is not clear how severe they need to be or if special attention is needed to note them.

Brodifacoum data: No foetal haemorrhages were reported in the rat studies on Brodifacoum and there are different opinions regarding how to interpret the absence of fetal haemorraghes in these studies. In contrast, a case report (Munday and Thompson, 2003) describes how an apparently healthy dog gave birth to pups, where 8 out of 13

pups died of haemorrhage within 2 days. The finding of Brodifacoum in the pup livers was taken as evidence of AVK-intoxication.

Developmental toxicity – bone effects

Human Warfarin embryopathy includes effects on bone formation, typically in the nose region. There were equivocal indications of disturbed ossification in skull bones (in foetuses from one mid-dose litter) in the Kubaszky study (2009). The finding of malformed skulls only concerned one single litter from the mid-dose, with malformations in 2 out of 7 pups, indicating that a relationship with treatment is not likely. The critical period for nasal and skeletal development is not the same for humans (during the first trimester) and rats (late foetal/early postnatal period), and it is concluded that this malformation can therefore not be picked up by a standard rat/rabbit OECD 414 study.

Developmental toxicity – ocular effects

In the recent rat study on Warfarin, a low incidence of an extremely rare foetal ocular effect was observed (Kubaszky, 2009), potentially supporting that prenatal animal toxicity studies can pick up this effect of Warfarin. However, the ocular effects were only noted at the high dose and at such a low incidence (in 1 out of 17 test protocol 1-litters and 3 out of 21 test protocol 2-litters at the dose of 0.2 mg/kg/day) that, if they would be caused by other rodenticides, they would only occasionally occur in normal sized studies (n=20). No such effects were noted in other Warfarin studies (e.g., Mirkova and Antonov, 1983).

Developmental toxicity – general foetal toxicity

Foetal toxicity has been indicated in the Warfarin study by Kubaszky (2009), but only in one of the subgroups and in the presence of severe maternal toxicity (mortality). Foetal toxicity was also indicated in a poorly reported study by Mirkova and Antonov (1983).

Brodifacoum data: No foetal toxicity was observed in the developmental toxicity studies with Brodifacoum.

Dose-effect relationship between haemorrhages and nose/bone defects

It is not known from the human AVK data if there are differences in the dose-effect relationship between haemorrhages and nose/bone defects in humans. If, for instance, it would be the case that in humans (and animals) haemorrhages always occur before nose/bone defects (because of marked inhibition of vitamin K epoxide reductase leading to reduced carboxylation of the critical bone proteins), then one could use the absence of haemorrhages in animal studies to conclude that nose/bone defects also will not be induced. But since this information is not available, that conclusion cannot be drawn for the AVKs.

Based on available literature, one may rather speculate that the opposite may be true in humans, i.e., that bone effects may precede haemorrhagic effects of AVKs. Cases of Warfarin-induced teratogenicity with no reported haemorrhagic event are reported. For example, Baillie (1980) described "*a term infant with a hypoplastic nose due to failure of development of the nasal septum. No other abnormality was detected on routine clinical examination. X-rays of pelvis and femora showed stippling in the greater trochanters and left pubis and also abnormal vertebral bodies at S4/5*". A similar case with slightly enlarged head and flattened face with a depressed nasal bridge and small nose, stippling of the vertebrae and femoral epiphyses was noted in a stillborn neonate in the 26th week of gestation where no abnormalities other than mild hydrocephalus, nasal hypoplasia, foetal growth restriction were revealed (by autopsy) (Tongsong *et al.*, 1999). Van Driel et al. (2002) summarised the foetal outcome in cohort studies on use of coumarins during pregnancy and reported two-fold higher prevalence of embryopathy (22 cases of skeletal anomalies seen after in utero exposure to coumarins) than bleeding (11 cases), if

coumarins were given from the beginning and throughout the pregnancy. For the cases reported for Acenocoumarol and Phenprocoumon, 85% involved the nose and only one case mentions bleedings (7%) (van Driel et al., 2002).

A possible explanation for the presence of bone effects with no haemorrhagic effects could be related to the specificity of haemostatic mechanisms in the developing foetus. During in utero development, vitamin K levels are low in the foetus, even close to deficiency levels (Howe and Webster, 1994). Coagulation factors do not cross the placenta, and vitamin K crosses the placenta at a very low rate, with concentrations in the cord plasma at 0.2-0.3% of maternal plasma concentration (Shearer, 1982). Therefore, concentrations of the vitamin K dependent clotting factors (II, VII, IX, and X), as well as of the proteins C and S, are reduced at birth to about 50% of normal adult values. Nevertheless, due to other, non-vitamin K-dependent mechanisms (e.g. higher plasma concentration of von Willebrand factor and higher haematocrit level), healthy neonates have normal haemostasis and are no more prone to bleeding diathesis than adults (Revel-Vilk 2012).

A biochemical basis for a higher sensitivity of the bone system than of the hepatic coagulation system in humans is also suggested in the literature. Thus, the recycling of vitamin K 2,3-epoxide to vitamin K hydroquinone, which is essential for modification of glutamic acid residues to gamma-carboxyglutamate in vitamin K–dependent proteins (including coagulation factors, protein C, S, and Z, Matrix Gla protein – MGP, and osteocalcin), requires two steps. In the first step, the vitamin K 2,3-epoxide is reduced to vitamin K, and in the second step vitamin K is further reduced to the hydroquinone. The first step is catalysed by vitamin K epoxide reductase (VKOR) both in hepatic and extra-hepatic tissues, while in the second step VKOR is essential only in extra-hepatic tissues. In hepatic tissue other enzymes, such as DT diaphorase (NADH-dependent reductase, which is not inhibited by Warfarin), are also involved (Teichert *et al.* 2008). Wallin and co-authors showed, for example, that in vascular smooth muscle cells the activity of DT-diaphorase is 100 times lower compared to liver tissue, whereas VKOR is 3 times higher (Wallin *et al..* 1999).

It could be expected, therefore, that extra-hepatic tissues are more sensitive to vitamin K deficiency or inhibition (such as that induced by Warfarin) than hepatic tissue. Undercarboxylated osteocalcin (ucOC) has been found in healthy adults with normal coagulation (prothrombin time within the normal range), and its level decreased by approximately 50% after one-week vitamin K supplementation (1000 micrograms of vitamin K1 per day) (Binkley *et al.* 2000). Because of the high accumulation of vitamin K in the liver, the liver will take up vitamin K from the blood at the expense of other tissues also needing vitamin K (Vermeer, 2001). The dose of vitamin K that inhibited the effect of Warfarin on blood coagulation could not prevent Warfarin-induced inhibition of gamma-carboxylation of osteocalcin in rats ("liver-bone dichotomy" model) (Price and Kaneda 1987). Similarly, Warfarin induced bone and cartilage changes in the absence of haemorrhages in developing rats treated concomitantly with Warfarin and vitamin K1 during the first 12 weeks of life (Howe and Webster 1992).

Human experiences of vitamin K deficiencies also support the conclusion that the bone system is very sensitive, and even more sensitive than the coagulation system. Thus, facial malformations identical to those caused by Warfarin may be caused in humans by many agents that decrease the concentrations of vitamin K, such as the anticonvulsant phenytoin (Howe *et al.*, 1995), other coumarin drugs such as Acenocuomarol and Phenprocoumoun (Hetzel *et al.*, 2006), liver dysfunction (Xie *et al.* 2013), and genetic vitamin K epoxide reductase deficiency (Keppler-Noreuil and Wenzel, 2012).

Overall, it is concluded that there might be differences between how humans and experimental animals respond to the AVK rodenticides, and also differences between different human beings. It is therefore difficult to exclude human developmental toxicity based on negative animal studies, particularly considering that there are a few cases of

developmental toxicity seen in humans exposed to Brodifacoum and other AVK coumarins used therapeutically.

Toxicokinetics and transplacental transfer

The AVK rodenticides have different physico-chemical characteristics (e.g., a range of 0.7-6.3 for the log Pow and 292-542 for the molecular weight) which lead to differences in kinetics, mainly expressed as different half-lives. This affects the potency, but a comparison of the toxicity profiles shows much smaller differences than indicated by the 5-6 orders of magnitude difference in lipophilicity. Thus, the anticoagulants have LD_{50}-values in rats of 0.25-15 mg/kg. In repeated dose (generally 90 days) studies, the NOAELs and LOAELs in rats varied between 1-30 and 4-100 ug/kg/day, respectively. The NOAELs for maternal toxicity in the developmental toxicity studies varied between 1-125 ug/kg/day in rats and 2-12 ug/kg/day in rabbits. It is concluded that there are quantitative differences in potency but no major qualitative differences are expected. It cannot be ruled out that the ratio between maternal toxicity and fetal toxicity is affected somewhat, but it is noted that the AVK-drugs Acenocoumarol and Phenprocoumon exhibit teratogenicity despite having different pharmacokinetics (half-lives) than Warfarin. Thus, half-lives of 2-8 hours are reported for Acenocoumarol, 30-45 hours for Warfarin, and 156-172 hours for Phenprocoumon (Rane and Lindh, 2010). It seems that the MoA is more important than half-life as determinant for developmental toxicity.

It is not fully clear to what extent teratogenicity is caused by direct effects of the coumarin in the fetus and to what extent decreased maternal levels of vitamin K indirectly affect the fetus.

Due to differences in physicochemical properties and toxicokinetics (metabolism, liver accumulation, etc.) the transplacental transfer might differ between the various AVKs. Only one study has investigated the transplacental transfer of AVKs in rats. Johnson (2009; see CLH report on Flocoumafen) studied the transplacental transfer of Warfarin and Flocoumafen in rats, at a stage when the placenta is fully developed (GD 19). From this study it appears that both Warfarin and Flocoumafen can cross the maternal-foetal placental barrier in rats. However, in the rat there is a lower foetal availability of Flocoumafen than of Warfarin (the normalized Flocoumafen plasma concentration was 7-fold lower than that of Warfarin), but the concentration of Flocoumafen was higher in the foetus than in the dam, whereas the opposite was true for Warfarin. Other AVK anticoagulants have been shown to cross the placenta in humans, e.g., Acenocoumarol and Phenindione (Hoyer 2010).

Brodifacoum data: There are no animal data for Brodifacoum. Like Flocoumafen, Brodifacoum is expected to pass the placenta, although presumably in lower amounts than Warfarin. Yan *et al.* (2013) has shown that Brodifacoum passes over to the human foetus, as the concentration in cord blood (at the 37nd week) was about half the concentration in the mother's blood. Regarding the nose/bone defects, it has been noted that the sensitive stage in humans is the first trimester, when the placenta is not fully developed. Thus, for this malformation in humans, differences in transplacental transfer may be of no relevance.

It is concluded that all AVK rodenticides are expected to cross the placenta, and although there might be some quantitative differences, the toxicokinetic aspects are not contradictory, but rather support the similarity between the effects of Warfarin and Brodifacoum in humans.

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4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference

EC C.7, OECD 111	DT50: > 1 yr		R. Fabbrini
EC C.7, OECD 111	DT50: > 1 yr		Mathis SNG, Benner JP and Skidmore MW
OPPS 835-2210	Half-life [t1/2E]: < 1 d		R.M. Drake
OECD 301B	Incubation period: *28 days* Degree[%]:*0%*		R.M. Drake
OECD 302	Incubation period: *56 days* Degree[%]:*2%*		R.M. Drake
OECD (1992) 301D	Incubation period: *28 days* Degree[%]:*3.5%*		Kelly C:R.Clayton
ISO 11734 and method 3 of ECETOC report no. 28	Incubation period: *56 days* Degree[%]:*0%*		R.M. Drake
EPA Guidelines, 162-1 (October 1982).	DT50: 157		Hall BE and Priesley

5.1.1 Stability

Hydrolysis

Two hydrolysis studies are available and are reported in table 4.1.1-1. The studies indicated that Brodifacoum is hydrolytically stable at pH 4, 7 and 9; the hydrolytic half-life (DT50) is above one year at environmentally relevant pH.

Photolysis in water

Photolysis of Brodifacoum was fast with 38% removal in the first hour of exposure. Greater than 89% photolysis was noted to have occurred by around three hours. Furthermore, whatever the season, the half-life of Brodifacoum is less than one day. In the laboratory the substance completes photolysis. No degradation products were detected.

Photo-oxidation in air

The photo-oxidation of Brodifacoum in air has been estimated using AOPWIN. According to these calculations, Brodifacoum has a potential for rapid photo-oxidative degradation in air with a half-life of 6.61 h, considering COH 0.5 x 106 molec/cm3 and the time 24 h.

5.1.2 Biodegradation

Anaerobic biodegradation

Results from a study following test method ISO11734 on the anaerobic degradation of Brodifacoum are summarised in Table 4.1.2-2. No degradation was observed after 56 days of incubation. These test results indicated that Brodifacoum is not biodegradable under anaerobic conditions.

Degradation in soil

Brodifacoum is persistent in soil with DT50 value of 157 days. Mineralization rate is about 35.8% and the non-extractable bound residue is maximum 23.6% in one soil after 365 days.

5.1.2.1 Biodegradation estimation

No data.

5.1.2.2 Screening tests

No study on the inherent biodegradability has been submitted by the applicant based on the fact that the substance is poorly soluble and therefore, the test is technically very difficult to perform.

5.1.2.3 Simulation tests

Was not degradated in anaerobic condition.

5.1.3 Summary and discussion of degradation

Abiotic degradation

Brodifacoum showed to be hydrolytically stable under environmentally relevant conditions (DT50 > 1 year). Brodifacoum was found to be susceptible to photo-transformation in water (DT50 < 1 d) and photo-oxidation in air (DT50 = 0.275 d for reaction with OH-radicals).

Biodegradation in water

Brodifacoum is not readily or inherently or anaerobically biodegradable.

Degradation in soil

Brodifacoum is persistent in soil with a DT50 value of 157 days. Mineralization rate is about 35.8% and the non-extractable bound residue is maximum 23.6% in one soil after 365 days.

5.2 Environmental distribution

No data.

5.2.1 Adsorption/Desorption

Brodifacoum is immobile in soil (Koc > 9155 l/kg). Brodifacoum is not expected to contaminate groundwater.

5.2.2 Volatilisation

Brodifacoum has a low vapour pressure (<<1 x 10-6 Pa) and a Henry's Law constant of 2.18 x 10-3 Pa m3 mol-1 (pH 7). Release to air via water is expected to be negligible. This is also supported by calculations using the TGD on risk assessment for percent release to air from a sewage treatment plant where a default of 0 is given (i.e., no release to air). The manufacture of Brodifacoum is in a closed system. There are no releases to air of Brodifacoum from manufacturing, formulating, use or disposal phases.

5.2.3 Distribution modelling

No Data.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log Kow of Brodifacoum has been experimentally determined to be 4.92 at pH 7 and 20°C. This value exceeds the guidance values for bioaccumulation for classification purposes according to Directive 67/548/EEC (log Kow > 3) and Regulation EC 1272/2008 (log Kow > 4).

5.3.1.2 Measured bioaccumulation data

No reliable experimental data are available for the bioaccumulation of Brodifacoum in fish.

5.3.2 Summary and discussion of aquatic bioaccumulation

The log Kow of Brodifacoum has been experimentally determined to be 4.92 at pH 7 and 20°C.

An experimentally derived BCF value is not available.

The log Kow value for Brodifacoum fulfils the criteria for bioaccumulation potential according to Directive 67/548/EEC and Regulation EC 1272/2008 as it exceeds the value of 3 and 4, respectively.

5.4 Aquatic toxicity

Table 23: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
OECD 203 (1992)	LC_{50} 0.042	Species: Rainbow trout	W. J Craig
OECD (1984) Guideline 202 Part 1	Results (mg a.s./l) EC_0 : 0.07 EC_{50}: 0.25 EC_{100}: 0.92	Species: Daphnia magna	W. J Craig
OECD 201 (1984)	Results (mg a.s./l) NOE_r-C: 0.01 E_bC_{50}: 0.016 E_rC_{50}[1]: 0.04	Species: Selenastrum capricornutum (renamed Pseudokirkneriella sub capitata)	W. J Craig

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

One fully reliable GLP study with Rainbow trout, carried out following OECD 203, is available.

Seven fish were exposed under semistatic conditions to nominal concentration of Brodifacoum: 0 (control), DMF (control), 0.06, 0.13, 0.25, 0.5 and 1.0 mg a.s./l. Mean measured concentrations of old and new solutions (4 old and 4 fresh) were: 0.03, 0.06, 0.11, 0.23, 0.53 mg a.s./l and were used to express results. No mortality was recorded at the lowest concentration, while 100% fish died at 0.11 mg a.s./l. The 96h LC50 was calculated equal to 0.042 mg a.s./l.

5.4.1.2 Long-term toxicity to fish

No data are available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Short-term toxicity to aquatic invertebrates

The results of an acute GLP study with Daphnia magna, carried out according to OECD 202, are available. Twenty daphnids (divided in 4 replicates) were exposed to nominal concentrations of Brodifacoum of 0 (control), DMF (control), 0.13, 0.25, 0.5, 1.0, 2.0 and 4.0 mg a.s./l. The measured mean concentrations of new and old test solutions were 0,0.07, 0.12, 0.28 ,0.63, 0.92 mg a.s./l, which were used to express the results. The calculated 48h EC50 was 0.25 mg/l (0.20 – 0.31). No statistically significant effect were observed at 0.12 mg/l.

5.4.2.2 Long-term toxicity to aquatic invertebrates

No data are available.

5.4.3 Algae and aquatic plants

A 72h study with algae, carried out with Selenastrum capricornutum (renamed Pseudokirkneriella subcapitata) according OECD 201 and under GLP provisions, is available. Nominal test concentrations were 0 (control), DMF, 0.032, 0.056, 0.10, 0.18, 0.32 mg/l. The endpoints of the study have been recalculated on the basis of the geometric mean concentrations, hence: NOEC = 0.010 mg/l, EbC50 0.016 mg/l and ErC50 0.04 mg/l. The result for growth rate is that considered for risk assessment.

5.4.4 Other aquatic organisms (including sediment)

No data are available**.**

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

No data are available

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Related to environment Brodifacoum LC50 is below 1 mg/L (the 96h LC50 Rainbow trout equal to 0.042 mg a.s./l; 48h EC50 Daphnia magna equal to 0.25 mg/l; static 72h ErC50 Selenastrum capricornutum equal to 0.04 mg/l) with proposal for specific concentration limits: Cn≥2,5 (DSD); M=10 (CLP) and the substance is not readily biodegradable. Classification proposal according to Directive 67/548/EEC.

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

There is a current entry in Annex VI of CLP Regulation for Brodifacoum with an environmental classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) under CLP. The DS proposed to add M-factors of 10 to both - the Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 classifications according to CLP.

Degradation

Degradation was studied in two hydrolysis tests, one photolysis test in water, three ready biodegradability tests, one inherent biodegradation test and finally one degradation test in soil.

The DS considered Brodifacoum as hydrolytically stable (DT_{50} > 1 yr) and rapidly photodegradable with an experimental half-life < 1 day. It was degraded rapidly in the atmosphere by reaction with OH radicals, although the presence of this compound in air is not expected due to its low vapour pressure.

In the CLH report, table 21 summarised all relevant information on degradation, including data about ready and inherent degradation. However, the DS did not provide a detailed

evaluation of these tests in the report. According to these data, Brodifacoum is not readily or inherently biodegradable under test conditions. In the ready biodegradability tests according to the OECD 301B, OECD 301D, and ISO 11734 guidelines, the level of degradation was between 0-3.5%, being therefore below the ready biodegradability pass levels of 60 or 70%. In the inherent biodegradation test according to the OECD 302D draft guideline, the degradation was 2%.

Brodifacoum showed a very slow degradation under aerobic conditions in soil with a DT_{50} of 157 days and a mineralization rate about 35.8% after 365 days.

Based on the available data Brodifacoum was proposed as not-rapid/ready degradable.

Bioaccumulation

The experimental log K_{ow} of Brodifacoum is 4.92 at pH 7 and 20 ºC. This value is above the cut-off values of log K_{ow} ≥4 (CLP). Experimental bioconcentration tests are not available.

In conclusion, due to its high log K_{ow} value, the DS concluded that Brodifacoum has potential for bioaccumulation.

Aquatic toxicity

Acute toxicity studies in fish (*Oncorhynchus mykiss*), invertebrates (*Daphnia magna*) and algae (*Pseudokirchneriella subcapitata*) were reported by the DS. Long-term tests in fish and invertebrates are not available but the algae test submitted in the CLH report can be considered for acute (E_rC_{50}) and chronic (NOE_rC) hazard assessment.

All the acute endpoints (EC_{50}) reported in the CLH dossier for the three trophic levels are lower than 1 mg/L: fish LC_{50} (96h) = 0.042 mg/L; invertebrate EC_{50} (48h) = 0.25 mg/L and algae ErC_{50} (72h) = 0.04 mg/L, all of them based on mean measured concentrations, meaning the fish and algae are the most sensitive trophic levels for acute toxicity. A NOE_rC value of 0.01 mg/l was reported for algae.

Comments received during public consultation

Four member states supported the environmental classification proposed by the dossier submitter.

In their post-public consultation response to the comments received, the DS confirmed that the proposed M-factor of 10 should apply to both aquatic acute toxicity and aquatic chronic toxicity.

RAC assessment and comparison with criteria

Degradation

RAC agreed that Brodifacoum can be considered hydrolytically stable and rapidly photodegradable based on the information provided in the CLH report.

RAC also agreed that Brodifacoum is not readily or inherently biodegradable under test conditions, with a level of degradation lower than 4% after 28 days. Furthermore, in an aerobic soil study Brodifacoum shows a very slow degradation (DT_{50}=157 days), therefore, based on these data, RAC agreed with the DS that Brodifacoum should be considered as **not rapidly degradable** according to the CLP criteria.

Bioaccumulation

The experimental log K_{ow} for Brodifacoum is 4.92 which is above the cut-off values of log K_{ow}≥4, therefore RAC agrees with the DS that Brodifacoum has **high potential for bioaccumulation**.

Aquatic toxicity

Under CLP, the acute toxicity category should be based on the lowest E(L)C_{50}, in this case two trophic levels show similar toxicity, i.e. fish (*Oncorhynchus mykiss)* and algae *(Pseudokirchneriella subcapitata)* with E(L)C_{50} of 0.042 mg/l and 0.04 mg/l, respectively. These values are ≤ 1 mg/l, therefore Brodifacoum classifies as Aquatic Acute category 1 (H400), with an M-Factor of 10, because both values are between 0.01 and 0.1 mg/l.

Regarding chronic toxicity, no adequate chronic toxicity data is available for all three trophic levels. Only chronic toxicity for algae was included in the CLH report and according to this data, and taking into account that the substance is not rapidly degradable, a classification as Aquatic Chronic category 1 (H410) and an M-factor of 10 is applicable for Brodifacoum based on a NOE_rC of 0.01 mg/L, since 0.001 < NOEC ≤ 0.01.

However, due to the lack of chronic data for fish and invertebrates, the surrogate approach should also be considered. Brodifacoum is not rapidly degradable and the log K_{ow} ≥ 4 and the highest acute toxicity was reported for fish, i.e. LC_{50} (fish) ≤ 0.1mg/L (0.042 mg/L), the resulting classification from the surrogate approach is Aquatic Chronic 1 (H410) with an M- factor of 10 (0.01 < L(E)C_{50} ≤ 0.1). Therefore, the long-term hazard classification based on the chronic algae toxicity and the surrogate approach (fish acute toxicity) is the same.

In conclusion, RAC agreed with the DS's proposal to classify Brodifacoum according to CLP criteria as Aquatic Acute 1 (H400) with an M-factor of 10 and Aquatic Chronic 1 (H410) with M-factor of 10.

6 OTHER INFORMATION

No data.

7 REFERENCES

REFERENCE LIST

The studies considered as confidential information are available separately for either Applicant in the confidential Annex to this CLH report.

Applicant A

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Anon	6.12/02	2004	The treatment of anticoagulant poisoning: Advice to physicians. Issued jointly by Zeneca Public Health, Sorex Limited, Lipha SA, BASF and Bayer. Not GLP, unpublished. [Advice to physicians1]	Y	Syngenta
Anon	8/01	1999	Brodifacoum Technical, EC Safety Data Sheet, Version 7.	Y	Syngenta
Confidential Data	6.4.1/02	1984	Brodifacoum: 90-Day Feeding Study In Rats. ICI Central Toxicology Laboratory, Report No: CTL/P/862. GLP, unpublished. [C2.3/03].	Y	Syngenta
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Berry D	6.18/01	2003	Brodifacoum: Global Evaluation of Toxicological and Metabolism Studies. Central Toxicology Laboratory Report No:	Y	Syngenta

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8 ANNEXES

No data