Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at Community level of

Flocoumafen (ISO); reaction mass of: cis-4-hydroxy- 3-(1,2,3,4-tetrahydro-3-(4-(4- trifluoromethylbenzyloxy) phenyl)-1- naphthyl)coumarin; trans-4-hydroxy-3-(1,2,3,4- tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin

EC Number: 421-960-0
CAS Number: 90035-08-8

CLH-O-0000003398-66-03/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

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Flocoumafen

EC Number: 421-960-0

CAS Number: 90035-08-8

Submitted by: Bureau REACH, RIVM, The Netherlands, bureau-reach@rivm.nl

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name:	**Flocoumafen**
EC Number:	**421-960-0**
CAS number:	90035-08-8
Registration number (s):	-
Purity:	≥95.5
Impurities:	Confidential information

Proposed classification based on Directive 67/548/EEC:

Physical/chemical properties: None

Health hazards:	Repro Cat. 3; R63 T+; R26/27/28 T; R48/23/24/25
Environment:	N; R50/53

Proposed classification based on Regulation EC 1272/2008:

Physical/chemical properties: None

Health hazards:	Acute Tox. 1 H300
	Acute Tox. 1 H310
	Acute Tox. 1 H330
	STOT RE 1 H372
	Repr. 2 H361d
Environment:	Aquatic acute 1 H400
	Aquatic chronic 1 H410

Proposed labelling based on Directive 67/548/EEC:

Symbol:	T+; N
Risk phrases:	63-26/27/28-48/23/24/25-50/53
Safety phrases:	(1/2)-28-36/37/39-45-60-61

Proposed labelling based on Regulation EC 1272/2008:

Signal word:	Danger
Symbol:	GHS06, GHS08, GHS09
Hazard statement codes:	H300: Fatal if swallowed H310: Fatal in contact with skin H330: Fatal if inhaled H372: Causes damage to organs through prolonged or repeated exposure H361d: Suspected of damaging the unborn child. H410: Very toxic to aquatic life with long lasting effects

As precautionary statements are not included in Annex VI of Regulation EC 1272/2008, no proposal is made.

Proposed specific concentration limits (if any):

Proposed specific concentration limits based on Directive 67/548/EEC:

Human health

$C \geq 0.8\%$:	T+; R26/27/28 – 48/23/24/25 – R63
$0.4\% \leq C < 0.8\%$:	T+; R26-24/25 – 48/23/24/25 – R63
$0.1\% \leq C < 0.4\%$:	T; R23/24/25 – 48/23/24/25 – R63
$0.01\% \leq C < 0.1\%$:	Xn; R20/21/22 – 48/20/21/22 – R63
$0.003 \leq C < 0.01\%$:	Xn ; R63

Environment

$C \geq 2.5\%$	N; R50/53
$0.25\% \leq C < 2.5\%$	N; R51/53
$0.025\% \leq C < 0.25\%$	R52/53

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

STOT RE 1; H372: C ≥ 0.05% and

STOT RE 2; H373: 0.005% ≤ C < 0.05%

Repr. 2; H361d: C ≥ 0.003%

M-factor according to Regulation EC 1272/2008, including the 2nd ATP (Regulation 286/2011):

The acute M-factor is 10, based on an LC_{50} value of 0.07 mg/L obtained for the fresh water fish *Oncorhynchus mykiss* in a 96-h semi-static study.

The chronic M-factor is 10, based on surrogate chronic data in fish: not rapid degradability and an LC_{50} value of 0.07 mg/L obtained for *Oncorhynchus mykiss* in a 96-h semi-static study.

Proposed notes (if any):

None.

Table 1: 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	None		None	conclusive but not sufficient for classification
2.2.	Flammable gases	None		None	conclusive but not sufficient for classification
2.3.	Flammable aerosols	None		None	conclusive but not sufficient for classification
2.4.	Oxidising gases	None		None	conclusive but not sufficient for classification
2.5.	Gases under pressure	None		None	conclusive but not sufficient for classification
2.6.	Flammable liquids	None		None	conclusive but not sufficient for classification
2.7.	Flammable solids	None		None	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None		None	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	None		None	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	None		None	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	None		None	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	conclusive but not sufficient for classification
2.13.	Oxidising liquids	None		None	conclusive but not sufficient for classification
2.14.	Oxidising solids	None		None	conclusive but not sufficient for classification

2.15.	Organic peroxides	None		None	conclusive but not sufficient for classification
2.16.	Substances and mixtures corrosive to metals	None		None	conclusive but not sufficient for classification
3.1.	Acute toxicity – oral	Acute tox. 1 H300	None	Acute tox 2 * H300	
	Acute toxicity – dermal	Acute tox. 1 H310	None	Acute tox 1 H310	
	Acute toxicity – inhalation	Acute tox. 1 H330	None	Acute tox 2 * H330	
3.2.	Skin corrosion / irritation	None		None	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None		None	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None		None	Data lacking
3.5.	Germ cell mutagenicity	None		None	conclusive but not sufficient for classification
3.6.	Carcinogenicity	None		None	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Repr. 2 H361d	C ≥ 0.003%	None	
3.8.	Specific target organ toxicity –single exposure	None		None	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity –repeated exposure	STOT RE 1 H372	STOT RE 1: C≥0.05% STOT RE 2: 0.005%≤C<0.05%	STOT RE 1	
3.10.	Aspiration hazard	None		None	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 H400 Aquatic Chronic 1 H410	10 10	Aquatic Acute 1 H400 Aquatic Chronic 1 H410	
5.1.	Hazardous to the ozone layer	None		None	conclusive but not sufficient for classification

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

Table 2: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	None		None	conclusive but not sufficient for classification
Oxidising properties	None		None	conclusive but not sufficient for classification
Flammability	None		None	conclusive but not sufficient for classification
Other physico-chemical properties	None		None	conclusive but not sufficient for classification
Thermal stability	None		None	conclusive but not sufficient for classification
Acute toxicity - oral	T+; R28	R28: C≥0.8% R25: 0.1%≤ C <0.8% R22: 0.01%≤ C <0.1%	T+; R28 No SCL	
Acute toxicity - dermal	T+; R27	R27: C≥0.8% R24: 0.1%≤ C <0.8% R21: 0.01%≤ C <0.1%	T+; R27 No SCL	
Acute toxicity - inhalation	T+; R26	R26: C≥0.4% R23: 0.1%≤ C <0.4% R20: 0.01%≤ C <0.1%	T+; R26 No SCL	
Acute toxicity – irreversible damage after single exposure	None		None	conclusive but not sufficient for classification
Repeated dose toxicity	T; R48/23/24/25	R48/25:C≥0.1% R48/22: 0.01%≤ C <0.1% R48/24:C≥0.1% R48/21: 0.01%≤C <0.1% R48/23:C≥0.1% R48/20: 0.01%≤ C <0.1%	T; R48/23/24/25 No SCL	
Irritation / Corrosion	None		None	conclusive but not sufficient for classification

Sensitisation	None		None	conclusive but not sufficient for classification
Carcinogenicity	None		None	conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	None		None	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	None		None	Conclusive, but not sufficient for classification
Toxicity to reproduction – development	Repro Cat. 3; R63	C ≥ 0.003%	None	
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	conclusive but not sufficient for classification
Environment	N; R50/53	C ≥ 2.5%, N;R50/53 0.25% ≤ C < 2.5%, N;R51/53 0.025 ≤ C < 0.25%, R52/53	N; R50/53	

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

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

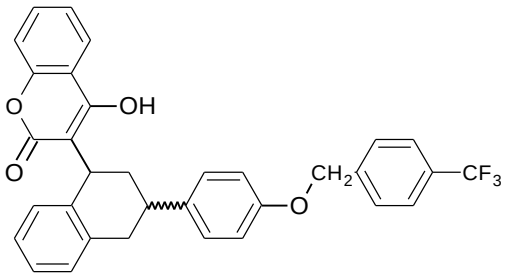
1.1 Name and other identifiers of the substance

Chemical Name:	Flocoumafen
EC Name:	Reaction mass of: cis-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin and trans-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin (IUPAC name according to ELINCS and Annex VI CLP) 4-hydroxy-3-[(1*RS*,3*RS*;1*RS*,3*RS*)-1,2,3,4-tetrahydro-3-[4-(4-trifluoromethylbenzyloxy)phenyl]-1-naphthyl]coumarin (IUPAC name according to Commission Directive 2009/150/EC)
CAS Number:	90035-08-8
IUPAC Name:	Reaction mass of: cis-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin and trans-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin (IUPAC name according to ELINCS and Annex VI CLP) 4-hydroxy-3-[(1*RS*,3*RS*;1*RS*,3*RS*)-1,2,3,4-tetrahydro-3-[4-(4-trifluoromethylbenzyloxy)phenyl]-1-naphthyl]coumarin (IUPAC name according to Commission Directive 2009/150/EC)

The International chemical identifier currently used in Annex VI of CLP and in ELINCS differs from the IUPAC name used in Commission Directive 2009/150/EC although it is only one substance. We propose to bring these two regulations in line. Further, the ISO name "flocoumafen" could be added to the Annex VI entry as is done for many other pesticides and biocides.

1.2 Composition of the substance

Active substance (ISO Common Name):	Flocoumafen*
EC Number:	421-960-0 (ELINCS)
CAS Number:	90035-08-8
Other substance No.	CIPAC No.: 453
IUPAC Name:	Reaction mass of: cis-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin and trans-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin (IUPAC name according to ELINCS and Annex VI of CLP) 4-hydroxy-3-[(1*RS*,3*RS*;1*RS*,3*RS*)-1,2,3,4-tetrahydro-3-[4-(4-trifluoromethylbenzyloxy)phenyl]-1-naphthyl]coumarin (IUPAC name according to Commission Directive 2009/150/EC)

CAS Name:	2H-1-Benzopyran-2-one, 4-hydroxy-3-[1,2,3,4-tetrahydro-3-[4-[[4-(trifluoromethyl)phenyl]methoxy]phenyl]-1-naphthalenyl]-
Molecular Formula:	$C_{33}H_{25}F_3O_4$
Structural Formula:	
Molecular Weight:	542.6 g/mole
Minimum purity of the active substance as manufactured (g/kg or g/l)	Minimum purity 95.5% w/w (50% to 80% cis- and 20% to 50% trans- isomers)
Typical concentration (% w/w):	See below
Concentration range (% w/w):	See below

** the ISO published common name of flocoumafen will be amended to 50%-80%/20%-50% cis/trans-isomers because the name flocoumafen is currently restricted to 40%-60%/60%-40% cis/trans-isomer mixtures.*

Constituents	
IUPAC Name:	cis-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin
CAS Number:	104563-61-3 (cis-isomer)
Concentration	50 – 80%
IUPAC Name:	trans-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin
CAS Number:	104563-60-2 (trans-isomer)
Concentration	20 – 50%

Purity/Impurities/Additives

Technical flocoumafen consists of a mixture of cis/trans-isomers (50% to 80% cis- and 20% to 50% trans-isomers). The minimum purity of flocoumafen is 95.5% w/w. Full details on the impurities and their concentrations in technical flocoumafen are considered to be CONFIDENTIAL. The impurities are considered not relevant for classification and labelling.

1.3 Physico-chemical properties

Table 1.1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference number
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	Fine crystalline solid (purified active ingredient, 99.4%)	A3.1.1/01-02
VII, 7.2	Melting/freezing point	3.2	166.1-168.2°C (purified active ingredient, 99.4%) 185-188°C (cis-isomer, >99%) 158-163°C (trans-isomer, ~95%)	A3.1.1/01 A3.1.1/03
VII, 7.3	Boiling point	3.3	No boiling point exists at atmospheric pressure	A3.1.1/01
VII, 7.4	Relative density	3.4 density	D_4^{20}= 1.40 (purified active ingredient, 99.4%)	
VII, 7.5	Vapour pressure	3.6	$<1x10^{-3}$ Pa at 20°C $<1x10^{-3}$ Pa at 25°C $<1x10^{-3}$ Pa at 50°C (purified active ingredient, 99.4%)	A3.2/01
VII, 7.6	Surface tension	3.10	Not considered to be required due to low solubility (< 1 mg/L)	
VII, 7.7	Water solubility	3.8	Solubility (20°C) (mg/l ± SD) pH 4 0.0024 ± 0.00003 pH 7 0.114 ± 0.005 pH 9 14.0 ± 0.39 (purified active ingredient, 99.4%) Solubility in deionised water = 0.14 ± 0.045 mg/l	A3.5/01
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	pH 4 > 6.12 at 20°C pH 7 = 6.12 at 20°C pH 9 = 5.11 at 20°C (purified active ingredient, 99.4%)	A3.9/01
VII, 7.9	Flash point	3.11	Not relevant (substance is not a liquid)	-
VII, 7.10	Flammability	3.13	Not "highly flammable" according to the guideline criteria (EC A.10)	A3.11/01
VII, 7.11	Explosive properties	3.14	Based on the structure of flocoumafen, no explosive properties are expected.	A3.15/01
VII, 7.12	Self-ignition temperature		No self-ignition of the	

			test substance was observed up to 400 °C.	
VII, 7.13	Oxidising properties	3.15	Based on the structure of flocoumafen, no oxidising properties are expected.	A3.16/01
VII, 7.14	Granulometry	3.5	No information available	
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	Solvent Solubility (g/l) Methanol 14.1 (20°C) Toluene 31.3 (20°C) n-Octanol 17.4 (20°C) (TGAI, 98.6%)	A3.7/01
XI, 7.16	Dissociation constant	3.21	4.5 ± 0.4 (based on water solubility) 4.5 ± 1.0 (based on molecular structure)	A3.6/01 A3.6/02
XI, 7.17	Viscosity	3.22	Not relevant for solid substances	-
	Auto flammability	3.12	No induction of self-ignition	A3.11/02
	Reactivity towards container material	3.18	Lupolen (BASF trademark for polythene) is used as packaging material. Corrosiveness towards packaging material, containers, or apparatus has not been observed.	A3.17/01
	Thermal stability	3.19	Stable up to 250 °C under air and nitrogen	A3.1.1/01

2 MANUFACTURE AND USES

Flocoumafen-containing products already marketed are known to contain flocoumafen in concentrations ranging from 0.005% to 0.5%.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex VI of Regulation EC 1272/2008

Flocoumafen is currently classified in Annex VI of Regulation EC 1272/2008 (Index number: 607-375-00-5) under the International Chemical Identification of:

reaction mass of: *cis*-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin; trans-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin.

According to Table 3.1:

	Classification		Labelling	
Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)
Acute Tox. 2 * Acute Tox. 1 Acute Tox. 2 * STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H330 H310 H300 H372 ** H400 H410	GHS06 GHS08 GHS09 Dgr	H330 H310 H300 H372 ** H410	

According to Table 3.2:

Classification	Labelling
T+; R26/27/28 T; R48/23/24/25 N; R50-53	T+; N R: 26/27/28-48/23/24/25-50/53 S: (1/2-)28-36/37/39-45-60-61

3.2 Self classification(s)

The CLP self-classification according to the ECHA inventory as of March 2012 for CAS 90035-08-8 is:

Classification		Labelling			Specific Concentration limits, M- Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms Signal Word Code(s)			

Acute Tox. 2	H300	H300		GHS06 GHS09 GHS08 Dgr			23
Acute Tox. 1	H310	H310					
Acute Tox. 2	H330	H330					
STOT RE 1	H372	H372					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					

4 ENVIRONMENTAL FATE PROPERTIES

The environmental fate properties assessment for flocoumafen is based on the revised Competent Authority Report (CAR) and the draft final CAR, document IIA, prepared in the context of the possible inclusion of flocoumafen in Annex I of Council Directive 98/8/EC (May 2009 + January 2009, RMS The Netherlands) concerning the placing biocidal products on the market. The assessment report is publicly available at:
http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/assessment_cleanpdf/_EN_1.0_&a=d

No registration dossiers were available for this substance on 15/06/2012.

All tables in the present assessment are copied from the draft final CAR. The tables are renumbered in accordance with the paragraph numbers.

4.1 Degradation

4.1.1 Stability

Hydrolysis.

The results of a preliminary hydrolysis test (Table 4.1) indicated that flocoumafen is hydrolytically stable at pH 4, 7 and 9. Thus, it was concluded that the hydrolytic half-life (DT50) is above one year at environmentally relevant pH. Abiotic degradation by this pathway is considered negligible.

Table 4.1: Half-live times for the hydrolysis of flocoumafen at 50 °C.

Guideline/ Test method	pH	Temperature [°C]	Initial Test concentration, [mg/l]	DT50	Reference
EC C.7, OECD 111	4, 7, 9	50 ± 0.5	0.001	> 1 yr	A7.1.1.1.1/01

Photolysis in water.

A study has been performed assessing the photolysis of flocoumafen in water (Table 4.2). The results show that flocoumafen is susceptible to photo-transformation in water with a DT_{50} of 1.67 days. Four major transformation products were detected in the study. However, only two could be identified as 4-hydroxy-3-[3-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-1-naphthyl]coumarin (42-49% of parent compound) and 4-(trifluoromethyl)-benzoic acid (13-37% of parent compound). The unidentified transformation products amounted to 28-31% and 12-30% of the parent compound.

Table 4.2: Photolysis of flocoumafen in water

Guideline/ Test method	Initial molar Test concentration	Total recovery of test substance [% of appl. a.s.]	Photolysis rate constant [kcp]	Direct photolysis sunlight rate constant [kpE]	Reaction quantum yield [φcE]	Half-life [t1/2E]	Reference
Draft OECD	Label "289":	58–108 %	3.97 ×	0.005–	8.90 ×	1.67d	A7.1.1.1.2/01

guideline "Phototransformation of chemicals in water, direct and indirect photolysis", August 2000; SETAC procedures	0.62, 0.51 and 1.79 mg/l Label "290": 0.74, 0.64 and 2.10 mg/l		10-5 s–1	0.957 d–1 (calculated by program ABIWAS for normal climatic conditions at a latitude of 52 °N)	10-4 (± 3.69 × 10-4)	(normal) month: April	

Photooxidation in air

Table 4.3: Photooxidation of flocoumafen in air

The photooxidation of flocoumafen in air has been estimated using AOPWIN. According to these calculations, flocoumafen has a potential for rapid photo-oxidative degradation in air with a half-life of 0.123 days and 0.085 days in reaction with OH-radicals and ozone, respectively.

Guideline/ Test method	**Initial molar TS concentration**	**Total recovery of TS [% of appl. a.s.]**	**OH radical rate constant (kOH)**	**Ozone rate constant (kOzone)**	**Half-life, reaction with OH-radicals,** t½ (•OH)	**Half-life, reaction with ozone,** t½ (Ozone)	**Reference**
SAR-based estimation (AOPWIN)	Not applicable	Not applicable	86.76 × 10–12 cm3/molec. × s	13.65 × 10–17 cm3/molec. × s	0.123 d (≡ 1.479 h)	0.085 d (≡ 2.015 h)	A7.3.1/01

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

No data.

4.1.2.2 Screening tests

Readily biodegradability

The results of a ready biodegradability test are summarised in Table 4.4. In an OECD 301 B test (Modified Sturm test), only 6% degradation was observed after 29 days. The test concentration was above the expected water solubility. The observed degradation rate in the test might have been affected by the poor bioavailability of the test compound. However, it can be assumed that flocoumafen was dissolved in the test media up to its water solubility and that this part was available for biodegradation. Based on the test results, flocoumafen should be considered as not readily biodegradable.

Table 4.4: Biodegradability of flocoumafen.

Guideline/ Test method	Inoculum			Additional substrate	Test conc.	Degradation		Reference
	Type	Conc.	Adaptation			Incubation period	Degree	
OECD 301 B	Activated sludge	Not reported	No	No	c. 14 mg/l	29 d	6 %	A7.1.1.2.1/02

Anaerobic biodegradation.

Results from a study following test method ISO11734 on the anaerobic degradation of flocoumafen are summarised in Table 4.5. No degradation was observed after 60 days of incubation. The test concentration was above the expected water solubility. Although the observed degradation rate in the test might have been affected by the poor bioavailability of the test compound, it can be assumed that flocoumafen was dissolved in the test media up to its water solubility and that this part was available for biodegradation. These test results indicated that flocoumafen is not biodegradable under anaerobic conditions,

Table 4.5: Anaerobic degradation of flocoumafen.

Guideline/ Test method	Test parameter	Inoculum			Additional substrate	Test conc.	Degradation		Reference
		Type	Conc.	Adaptation			Incubation period	Degree	
ISO 11734	Headspace pressure, DIC	Digested sludge	Suspended solids 2 g/L	No	No	68 mg/l	60 d	-9 %	A7.1.2.1.2/01

4.1.2.3 Simulation tests

Biodegradation in soil.

Aerobic degradation.

In a study according to OECD guideline 307 (2002), the degradation of flocoumafen in soil was studied using four soils and two different radiolabels at an application rate of 0.5 mg/kg soil. The distribution of radioactivity among acid volatiles, i.e. CO_2 (basic and organic volatiles were not detected), the non-extractable and extractable fraction indicated that flocoumafen was degraded at a low to moderate rate. Recovery varied from 85.6 – 102.4% (2 outlier excluded). Mineralization ranged between 2.5 - 15.6% after 120 days of incubation. The extractable radioactivity was nearly exclusively due to the parent compound, while non-extractable radioactivity ranged from 8.3 – 47.4%. No significant metabolites were detectable. The mineralisation rate and the extent of the formation of non-extractable radioactivity were strongly dependent on the soil type, particularly on sorption potential and microbial biomass. No reliable mineralization rate can be determined. Due to the fact that extractable metabolites were formed only to minor degree, the results do not reveal any further detail with respect to the degradation pathway of flocoumafen. Accumulation of any metabolites was not observed. Degradation was found to be most appropriately described by first order kinetics. The resulting dissipation times are presented in Table 4.6.

Table 4.6: Single 1st order DT50 values for the biological degradation flocoumafen in soil.

Soil	Temperature (°C)	Coumarin labelled ^{14}C flocoumafen DT50 (days)	Trifluoromethylphenyl labelled ^{14}C-flocoumafen DT50 (days)	Reference
Borstel, loamy sand	20	281	311	A7.2.1/02
Borstel, loamy sand	10	443	1293	
Soest, silt loam	20	219	226	
Marisfeld, silty clay loam	20	71	74	
Osnabrück, loamy sand	20	442	421	

An average half-life of flocoumafen in soil can be estimated by calculating the geometric mean of all experimental DT50 values at 20 °C. Accordingly, the mean dissipation half-life amounts to DT50 = 213 days.

4.2 Summary and discussion of persistence

In summary, flocoumafen is hydrolytically stable. Flocoumafen appears to be susceptible to primary degradation due to photo-transformation in water and photo-oxidation in air. However, flocoumafen is not ready biodegradable and is not degraded under anaerobic conditions. In a simulation test in soil, mineralisation of flocoumafen was only 2.5-15.6% after 120 days. Overall, flocoumafen is considered not readily or rapidly degradable for the purpose of classification and labelling.

4.3 Environmental distribution

4.3.1 Adsorption/desorption

In a screening study according to OECD 121, the Koc value for flocoumafen was estimated to be 68510 L/kg and 134858 L/kg for the cis- and the trans-isomers of flocoumafen, respectively. Flocoumafen can be considered to be immobile in soil.

4.3.2 Volatilisation

The vapour pressure of flocoumafen is $<1x10^{-5}$ hPa at 20°C. Two values for Henry's law constant of flocoumafen are available. The first (H <3.871 Pa·m^3/mol) is calculated from measured values for vapour pressure and water solubility, whereas the second (H = 7.43 × 10^{-8} Pa·m3/mol) is derived from the molecular structure (QSAR estimate). The latter indicates that volatilisation of the substance from water surfaces is expected to be negligible. This is in line with other chemical parameters determined for flocoumafen, such as the low vapour pressure and the high Koc.

4.3.3 Distribution modelling.

No data.

4.4 Bioaccumulation

4.4.1 Aquatic bioaccumulation

4.4.1.1 Bioaccumulation estimation

The log Kow of flocoumafen has been experimentally determined to be higher than 6.12 at pH 4, 6.12 at pH 7 and 5.11 at pH 9. These values exceed 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).

4.4.1.2 Measured bioaccumulation data

An experimental study to determine the BCF in fish has not been submitted.

4.4.2 Terrestrial bioaccumulation

No data.

4.4.3 Summary and discussion of bioaccumulation

The log Kow of flocoumafen has been experimentally determined to be higher than 6.12 at pH 4, 6.12 at pH 7 and 5.11 at pH 9. An experimentally derived BCF value is not available.

The log Kow values for flocoumafen fulfil the criterion for bioaccumulation potential conform Directive 67/548/EEC and Regulation EC 1272/2008 since it exceeds the value of 3 and 4, respectively.

4.5 Secondary poisoning

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

The summaries included in this proposal are partly copied from the Competent Authority Report, Document IIA prepared in the context of the possible inclusion of flocoumafen in Annex I to Council Directive 98/8/EC (January 2009 and older versions, RMS The Netherlands). Summaries are only copied if relevant for the classification and labelling of the substance. References to individual studies (given as study numbers) should be seen as references to the CAR, which is publicly available at
http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/assessment_cleanpdf/_EN_1.0_&a=d

No registration dossiers were available for this substance on 15/06/2012.

Flocoumafen is an active substance for use in rodenticides for control of rats and mice. Flocoumafen is a 4-hydroxycoumarin derivate with an anticoagulant action. Flocoumafen inhibits the vitamin K1-epoxide cycle, thereby interrupting the supply of vitamin K1 necessary for producing blood clotting factor precursors.

Flocoumafen is used as a mixture of cis and trans isomers. The cis:trans isomer ratio for the various test substances used in the toxicological studies are within the range specified by the notifier.

RAC general comment

Flocoumafen belongs to a group of compounds known as the anticoagulant rodenticides, i.e. those with an anti-vitamin K (AVK) mode of action (MoA) which are used mainly as active substances in biocidal products for pest control of rats, mice and other rodents. Some of the substances had an existing harmonised classification. However, at the time of writing, only Warfarin is currently classified for toxicity to reproduction in category 1A.

The eight AVK rodenticides were previously discussed by the Technical Committee on Classification and Labelling of Dangerous Substances (TC C&L) of the European Chemicals Bureau (ECB) (2006 – 2008). However, the work was transeferred to be continued at ECHA and to that end Member State Competent Authorities (MSCAs) were requested to prepare CLH proposals.

CLH proposals for eight AVK rodenticides, Coumatetralyl (Denmark), Difenacoum (Finland), Warfarin (Ireland), Brodifacoum (Italy), Flocoumafen (The Netherlands), Difethialone (Norway) Chlorophacinone (Spain) and Bromodialone (Sweden), these eight AVK rodenticides were submitted by eight different Dossier Submitters (DS). (Ireland, Italy, the Netherlands, Sweden, Norway, Denmark, Spain, Finland). The dossiers were handled as a group and but the Committee for Rrisk Aassessment (RAC) had agreed to proceeded to evaluate the proposals on a substance by substance basis in comparison comparing with the human data available for Warfarin (and other AVKs) and, relying on a weight-of–evidence approach as required by Regulation 1272/2008 (CLP).

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

5.1.1 Oral route

Several oral toxicokinetic studies in rats with flocoumafen were available.

Groups of ten male Fischer rats received approximately 14 mg/kg bw of coumarin-U-^{14}C-labelled or trifluoromethyl-phenyl-U-^{14}C-labelled flocoumafen by gavage (A6.2/01). Identification and quantification of metabolites was performed in samples of urine, faeces and liver. The major part of

radioactivity was excreted via faeces (63.2 % and 71.0 % of the dose). Mainly (56-57% of faeces associated radioactivity) unchanged parent compound was identified in samples of faeces. In total 83-86% of faeces associated radioactivity was identified. In contrast, 0.6 % and 6.1 % of the administered dose were detected in urine. Metabolites detected in urine samples showed to be more polar than the metabolites detected in faeces. After single oral administration of 14 mg/kg bw of coumarin-U-^{14}C-labelled or trifluoromethyl-phenyl-U-^{14}C-labelled flocoumafen, total oral absorption amounted to 17% of the administered dose, based on radio label found in urine, liver and cage wash. Radioactive residues detected in pooled liver samples amounted to 9.3-15.2 % of the dose. The cis and trans isomers of the parent compound formed the major (60-63% of liver associated radioactivity) residue in liver. In total 83-86% of liver associated radioactivity was identified. Based on the metabolite identifications, it is concluded that the main routes of biotransformation were represented by phase I reactions oxidising all ring systems of the test item. Conjugation of the parent was observed with glucuronic acid. Cleavage of the benzyl ether bridge played a minor role in metabolism of flocoumafen, but explained the urine metabolites detected at low quantities.

Elimination and retention of ^{14}C-flocoumafen after a single oral dose of 0.14 mg/kg in corn oil was studied in Fischer 344 rats (A6.2/03). In addition, the effect of flocoumafen on prothrombin time was investigated. Total oral absorption amounted to 69% in males and 75% in females of the administered dose, based on radio label found in urine, tissues (liver, skin and kidney) and cage wash. The major route of elimination was via faeces (23–26% of the administered dose), with urine accounting for less than 0.5% of the administered dose. Upon study termination after 7 days, 74% to 79% of the dose was retained in the animals (including amount retained in intestines) with approx. 50 % of the retained radioactivity located in the liver. Elevated prothrombin times relative to the normal control range of 10–11 seconds were observed at 4 and 8 hours after administration. The maximum prothrombin time was reached after 24 hours (approx. 2-fold of the normal control range) and returned to the control range by 48 hours after administration. Radioactivity in whole blood samples of the rats reached a maximum of 0.025 µg equivalents/mL at 4 hours and rapidly declined thereafter. About 70% of the hepatic radioactivity was found to be unchanged flocoumafen. It was concluded that the concentration of radioactivity in the liver (1.2–1.6 mg equivalents/g) appears to have only a transient effect on the synthesis of blood clotting factors possibly because it was stored in a non-bioavailable form.

The rate of depletion of ^{14}C-flocoumafen from selected tissues after a single oral dose of 0.14 mg/kg was tested in Fischer 344 rats (A6.2/04). Radioactivity was found to be retained for a long period in tissues and the depletion of residues from the selected tissues was very slow. Radioactive residues determined in liver samples remained at a plateau value of 1.2 µg/g during the 7 days after administration and depleted slowly thereafter with a calculated half-life of about 215 days. Depletion of radioactivity from kidney, fat and muscle occurred in a biphasic manner. Initially, elimination from these tissues was rapid (half-life periods of 18.5 to 29.5 days). After 16-30 days, the depletion slowed considerably resulting in half life periods of 191 to 273 days. Depletion from intestines did not show biphasic behaviour and occurred with a half-life of 205 days. Depletion of radioactivity from blood occurred in a bi-phasic manner. Initially, elimination from blood was rapid (half-life period of 3.1 days). After 7 days, the depletion slowed considerably resulting in a half-life of 341 days.

The metabolic fate of ^{14}C-flocoumafen after a single low (0.14 mg/kg) or a single high oral dose (14 mg/kg) in corn oil was studied in Fischer 344 rats (A6.2/05). Further tests were performed with

male rats dosed intravenously and bile duct cannulated rats dosed intra-peritoneally. Flocoumafen was a major component of faeces (37-53%) and liver (81-97%) associated radioactivity. Other components were characterised as polar and non-polar components, but not identified. Bile did not contain flocoumafen. The reports lack detail on important aspects of the study: no TCL chromatograms are presented, only one HLPC chromatogram is given, no integration results are given, several measurements were not reported (e.g. combustion results after extraction). Due to lack of identification of degradation products, the metabolic pathway of flocoumafen in rat cannot be determined from this study (which was the primary purpose of this study).

Male Fischer 344 rats were treated with multiple oral doses of ^{14}C-flocoumafen at a rate of 0.1 mg/kg (high dose) or 0.02 mg/kg (low dose) at weekly intervals for 10 and 14 weeks, respectively (A6.2/06, A6.2/07). The major route of elimination of radioactivity was via faeces (28-42% of the weekly dose per week), principally as the unchanged parent compound (44-74% of faeces associated radioactivity). Two other more polar metabolite fractions and one less polar metabolite of unknown identity were determined. Elimination via the urine was low (0.7-0.8%/week). In all animals, appreciable cellular accumulation of radioactivity occurred in the liver (up to 34-51% of the cumulative dose). The major component detected in liver extracts of both dose groups was unchanged flocoumafen (76-88% of liver associated radioactivity). At the low dose level, hepatic residues increased proportionately with the dose received, whereas at the upper dose level, hepatic residues reached a plateau 4 weeks after commencement of the study. The order of tissue concentrations was liver > kidney > skin > muscle > fat > blood. Progressive increases in tissue radioactivity were seen for all tissues throughout the treatment period. Radioactive residue concentrations in liver, kidney, skin and muscle of the recovery animals decreased (222 days after the last dose) 2- to 2.5-fold.

5.1.2 Inhalatory route

No data.

5.1.3 Dermal route

Dermal absorption, metabolism and elimination of flocoumafen were studied in male rats following a single percutaneous administration of 0.17 mg ^{14}C-flocoumafen/kg (3µg/cm^2) (A6.2/02). Animals were exposure non-occlusively for 7 days. Elimination of radioactivity via excreta was slow following administration, with 10.3% of the administered dose via urine and 30.9% of the administered dose via faeces. Radioactive residues were widely distributed within tissues and organs. In liver 25.4% of the administered dose was located, 4.6% of the administered dose was located in GI tract, spleen, heart, brain, testes, kidneys and lungs, and 5.02% was found in the remaining carcass. Total dermal absorption amounted to approximately 76% of the administered dose. However, it should be noted that the exposure period was 7 days, instead of approximately 6-8 hours. As the MOE and the risk index (based on AEL) are calculated based on a daily dose, the present study is not considered suitable for risk assessment purposes. Furthermore, in the present study flocoumafen was administered in acetone. This vehicle does not represent the normal exposure conditions of flocoumafen, as Storm BB is a cereal based wax-bound bait block.

Therefore, as no suitable dermal absorption data are available, an estimate of dermal absorption is made based on the physical and chemical properties of flocoumafen. Based on the molecular weight of 542.6 mg/ml and the log Pow of 6.12 (at pH 7), a dermal absorption of 10% should be considered for risk assessment purposes. The 10% default value can be quantitatively adjusted based on analogy to other second generation anticoagulants. Those second-generation anticoagulants for

which draft or final CA report are already available are comparable with flocoumafen regarding the physiochemical properties generally considered to have the decisive impact on the potential for skin penetration, along with experimentally determined dermal absorption rates. Accordingly, all these compounds have very similar molecular masses and distribution coefficients. Since flocoumafen very well matches the physiochemical properties of the other second-generation coagulants for which measured dermal absorption rates are available, it is very reasonable to assume a comparable skin penetration rate by analogy (see Table 5.1). This is particularly the case since the average composition of bait materials does not vary substantially between products of different formulators, so that matrix influences which may lead to varying absorption rates during handling of bait materials is not to be expected. Similarly, the actual handling may safely be assumed to be similar with all products of one bait type. Therefore, the proposal would be 4%. For now, the 4% and 10% value would be used in the first tier of a tiered approach.

Table 5.1: Comparison of flocoumafen with other second-generation anticoagulants regarding dermal absorption, based on the information published in CA reports in the context of Directive 98/8/EC

Compound	Molecular mass [g/mol]	Log Pow	Dermal absorption [%]
Brodifacoum	523.4	6.12	5%
Difenacoum	444.5	6.13	3%
Difethialone	539.5	6.29	4%
Flocoumafen	542.6	6.12	**4%**

5.1.4 Supportive data

Table 5.2: Supportive data on metabolism

The following studies are considered to contain additional information concerning metabolism in rats and are thus presented in tabular format as supportive data: (all studies were non-GLP studies)			
Study number	**Title**	**System**	**Results**
A6.2/08	Determination of the residues and the half-life of the rodenticides brodifacoum, bromadiolone and flocoumafen in the livers of rats during 200 days after single oral doses of each at a dose level of 0.2 mg/kg	Male Sprague-Dawley rats	Test compounds (0.2 mg/kg) dissolved in PEG 300 were administered to male rats orally by gavage and residues in liver were determined for up to 200 days after dosing. In contrast to reference A.6.2/04, a biphasic depletion of residues in liver was observed. During the initial 28 days the half-life of elimination was approx. 6 days for flocoumafen. Estimates of the terminal half-life from the slope of the fitted bi-exponential curve gave a half-life of 159 days.

The following studies are considered to contain additional information concerning metabolism in rats and are thus presented in tabular format as supportive data: (all studies were non-GLP studies)			
Study number	**Title**	**System**	**Results**
A6.2/09	The percutaneous fate of the rodenticide flocoumafen in the rat: role of non-biliary intestinal excretion	a) Male Fischer 344 rats (dermal or i.v. administration) b) Male Wistar rats (bile duct cannulation, i.p. administration)	The results reported for the dermal exposure are identical to reference A6.2/02. The results reported for the i.v. and i.p. administration were already summarised in reference A6.2/5.
A6.2/10	An experimental note on: The release of body residues of WL108366 with phenylbutazone	Four male New Zealand White rabbits	Phenylbutazone was administered to rabbits from previous body residence studies with flocoumafen, whose prothrombin times had returned to within the normal range, or near to the normal range. Sequential prothrombin time determinations indicated the potential of phenylbutazone to release bound flocoumafen from the body. The results of the study also suggested that elimination of flocoumafen had occurred as a result of phenylbutazone administration.
A6.2/11	The effect of phenobarbitone and Warfarin administration on hepatic ^{14}C-WL108366 residues in Fischer 344 rats	Male Fischer 344 rats (5 animals per group)	Seven days after receiving a single oral dose of ^{14}C-flocoumafen (0.10 mg/kg) male Fischer rats received either a single oral dose of Warfarin (0.12 mg/kg) or single daily, oral doses of phenobarbitone (50 mg/kg) for 5 days. Fourteen days after the administration of flocoumafen no significant differences could be detected in the concentration of radioactivity determined in the livers of either the Warfarin- or phenobarbitone-treated animals when compared to the respective control animals. Both substances caused a 2-fold increase in the amount of urinary elimination of radioactivity, which had a negligible effect on the body burden of flocoumafen.
A6.2/12	The fate of the rodenticide flocoumafen in the rat: retention and elimination of a single oral dose	Fischer 344 rats	This publication is based on the following references: A6.2/03, A6.2/04 and A6.2/11.
A6.2/13	Metabolic and toxicological studies on the anticoagulant rodenticide, flocoumafen	Male and female Beagle dogs (4 per sex)	Dogs received one to two single doses of 0.5 mg flocoumafen/kg in corn oil, followed by 7 days of therapy with vitamin K (2 or 5 mg/kg). This therapeutic regime was effective and suitable for dogs intoxicated with flocoumafen, whereas no difference was observed in antidotal response using either 2 or 5 mg/kg vitamin K per day. Severe intoxication, as observed in gross haemorrhaging was successfully treated by whole blood transfusion. Flocoumafen was retained in the liver at concentrations similar to those in other species.

The following studies are considered to contain additional information concerning metabolism in rats and are thus presented in tabular format as supportive data: (all studies were non-GLP studies)			
Study number	**Title**	**System**	**Results**
A6.2/14	The fate of the anticoagulant rodenticide flocoumafen in the rat and in quail	Male Fischer 344 rats; male quail	Flocoumafen is extremely toxic to rats, it undergoes very limited biotransformation and is eliminated very slowly. By contrast, it is less toxic to quail, being extensively metabolised and also rapidly eliminated. However, persistent hepatic flocoumafen residues were seen in both species.

5.1.5 Summary and conclusion

Absorption

After single low (0.14 mg/kg) oral administration of ^{14}C-flocoumafen, total absorption amount to 69% in males and 75% in females, based on radio label found in urine, tissues (liver, skin and kidneys) and cage wash. After single high (14 mg/kg) oral administration of ^{14}C-flocoumafen, total absorption amount to 17% of the administered dose based on the radio label found in urine, liver and cage wash.

Based on physical chemical properties of flocoumafen, a dermal absorption percentage of 10% should be considered for risk assessment purposes. Based on comparison of flocoumafen with other second-generation anticoagulants also a dermal absorption percentage of 4% was considered for risk assessment purposes.

Elimination

Within 7 days after administration of a single low dose (0.14 mg/kg), males and females excreted 0.35-0.45% and 26.16-23.07% of the administered dose in urine and faeces, respectively. Male rats that had received a high dose (14 mg/kg) of coumarin-U-14C-labelled or trifluoromethyl-phenyl-U-14C-labelled flocoumafen excreted 0.6-6.1% and 63.2-71.0% of the administered dose within 72 hours.

Distribution

Flocoumafen accumulates in tissues after dermal and oral administration. Flocoumafen is extensively distributed, with highest tissue levels in liver. The order of tissue concentrations was liver > kidneys > skin > muscle > fat > blood. Radio label was found to be retained for a long period in tissues, e.g. the calculated half life of flocoumafen in liver was 215 days. Radioactivity levels in tissues and organs of males were very similar to those in females.

Metabolism

In samples of faeces and liver, mainly the unchanged parent compound was identified. In urine no parent compound was identified, but polar metabolites.

The main routes of biotransformation were represented by phase I reactions oxidising all ring systems of flocoumafen. Conjugation of flocoumafen was observed with glucuronic acid. Cleavage of the benzyl ether bridge played a minor role in metabolism of flocoumafen, but explained the urine metabolites detected at low quantities. The proposed metabolic pathway of flocoumafen in rat is shown in Fig. 5.1.

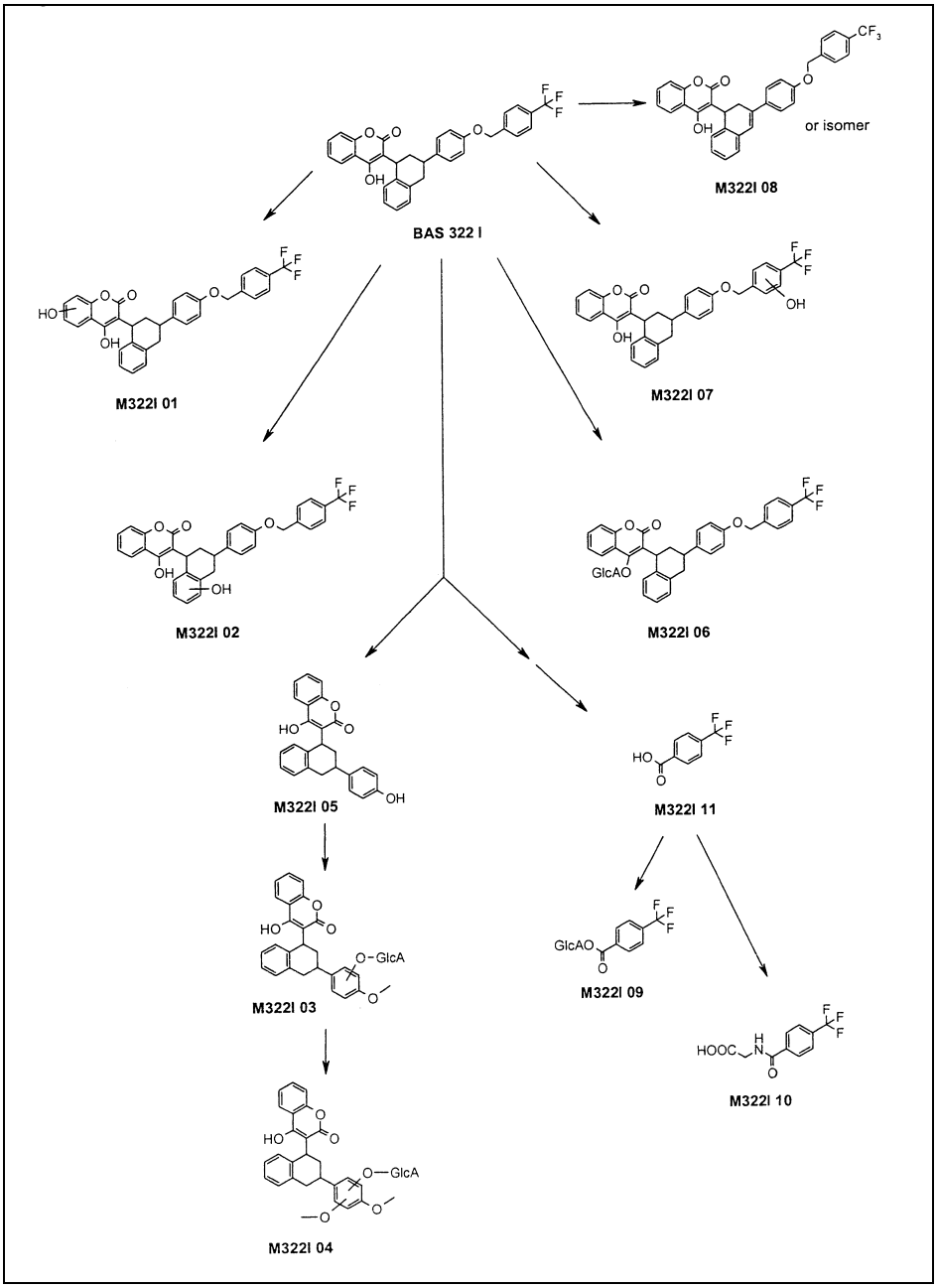


Figure 5.1: Proposed metabolic pathway in the rat (A6.2/01)

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

The acute oral toxicity of flocoumafen (purity: 97.6%; cis-trans isomer ratio=57:43) was tested in Fischer 344 rats (A6.1.1/01) (see Table 5.4). Groups of 5 male and 5 female rats received a single dose of 0.20, 0.25, 0.32, 0.40, and 0.50 mg/kg of flocoumafen in corn oil orally by gavage. Mortalities occurred on day 3 or between days 5 and 8. A 21-day LD_{50} was calculated using a method based on probit analysis at 0.43 mg/kg for males and 0.31 mg/kg for females (combined:

0.37 mg/kg). Overt reactions to the treatment were first apparent on day 4. Among the rats that survived the treatment, two developed a transient pallor of the eyes and/or skin and another three animals showed an unkempt appearance. The great majority of rats surviving oral administration of flocoumafen did not develop overt changes of appearance or behaviour. The principal signs of reaction to treatment among decedents were pallor of the skin and eyes, abasis/ataxia and an unkempt appearance. Other less common clinical signs included hypothermia and prostration immediately before death, swelling of the jaw or feet, increased lachrymation and bleeding from the ear-marks. Necropsy findings of the decedents were considered to be direct or indirect indications of substantial haemorrhage: pallor of lungs, liver and kidneys was a common finding, darkening of the contents of various levels of the gastro-intestinal tract probably due to the presence of blood was observed and clots were found in or around the meninges, thymus, lungs, urinary bladder, testes, seminal vesicles, locomotor musculature and peri-orbital tissues. All surviving rats gained weight relative to their day 1 body weights upon study termination. Although not performed according to any guideline, the method used was consistent to EC method B.1 (92/69/EEC) and OECD 401 in all important aspects. Therefore, the study is considered to be acceptable for classification and labelling purposes.

The acute oral toxicity of flocoumafen was tested in Fischer 344 rats (A6.1.1/02) (see Table 5.4). Groups of 5 male and 5 female rats received a single dose of 0.06, 0.13, 0.25, and 0.50 mg/kg of flocoumafen (purity: >99%; cis-trans isomer ratio not reported) in corn oil by gavage. Mortalities occurred after 5 to 7 days. The acute oral LD_{50} value of the test material, administered to rats as a solution in corn oil, was approximately 0.25 mg/kg for the sexes combined. Based on the obtained mortalities, the LD_{50} values were in the range of 0.25–0.5 mg/kg for males and 0.13–0.25 mg/kg for females. All animals were free of overt clinical signs for the first few days after dosing. Thereafter lethargy, piloerection, hunched back, pale eyes and skin, blood in urine and around nose, and chromodacryorrhea were observed. Some animals were apparently unable to use their hind limbs prior to death. Surviving animals did not show any significant treatment-related abnormality upon necropsy at study termination. Necropsy of animals that died during the study revealed internal haemorrhages. All surviving rats gained weight relative to their day 1 body weights upon study termination. Although this study was not performed to any guideline, the method used was consistent to EC method B.1 (92/69/EEC) and OECD 401 in all important aspects.

Supportive data

In addition to the two oral toxicity studies in rats, one oral toxicity study in rabbits (A6.1.1/03) and one oral toxicity study in gerbils (A6.1.1/04) were available. In male rabbits, the results of an acute oral toxicity study indicated that the LD_{50} of flocoumafen is in the range of 0.10 – 0.464 mg/kg bw. In male gerbils, the results of an acute oral toxicity study indicated that the LD_{50} of flocoumafen is 0.18 mg/kg. Both studies in rabbits and gerbils are considered supportive, as both studies there were methodological and reporting deficiencies. Furthermore, various acute oral toxicity studies with rats, mice, rabbits and guinea pigs were submitted as supportive data (Table 5.3). Studies were not considered relevant for classification and labelling of flocoumafen and did not result in lower LD_{50}'s than reported above.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Acute oral toxicity
The acute oral toxicity of Flocoumafen (purity: 97.6%; cis-trans isomer ratio=57:43) was tested in Fischer 344 rats . Groups of 5 male and 5 female rats received a single dose of 0.20, 0.25, 0.32, 0.40 or 0.50 mg/kg of Flocoumafen in corn oil orally by gavage. Mortalities occurred on day 3 or between days 5 and 8.

A 21-day LD_{50} was calculated using a method based on probit analysis at 0.43 mg/kg for males and 0.31 mg/kg for females (LD_{50} for males and females combined: 0.37 mg/kg).

The acute oral toxicity of Flocoumafen was tested in Fischer 344 rats. Groups of 5 male and 5 female rats received a single dose of 0.06, 0.13, 0.25 or 0.50 mg/kg of Flocoumafen (purity: >99%; cis-trans isomer ratio not reported) in corn oil by gavage. Mortalities occurred after 5 to 7 days. The acute oral LD_{50} value of the test material, administered to rats as a solution in corn oil, was approximately 0.25 mg/kg for the sexes combined. Based on the obtained mortalities, the LD_{50} values were in the range of 0.25–0.5 mg/kg for males and 0.13–0.25 mg/kg for females.

Acute dermal toxicity
The acute dermal toxicity of Flocoumafen was tested in New Zealand White rabbits. Groups of 5 male and 5 female rabbits received 0.2, 0.4, 0.8 or 1.6 mg/kg of Flocoumafen (purity: 96.1%) wetted with 1 ml of water on their skin (semi-occlusive; no information on application area provided) for 24 h. Animals were observed for 28 days. Deaths occurred mainly in week two and three of the study. The test material administered elicited a LD_{50} of 0.65 mg/kg in males and 1.14 mg/kg in females (LD_{50} for males and females combined**:** 0.87 mg/kg).

The acute dermal toxicity of Flocoumafen was also tested in Fischer 344 rats. The method used was in accordance with EC method B.3 (92/69/EEC) and OECD 403 with the following deviations: the test compound was held in contact with the skin by an aluminium foil instead of a porous gauze dressing, necropsy was only reported for two animals instead of all animals. Groups of 5 male and 5 female rats (50-160 mg/kg doses) and 10 male and 10 female rats (125 and 200 mg/kg doses) received 50, 80, 100, 125, 160 or 200 mg/kg of 0.5% Flocoumafen (purity: 0.48% of a.i.) in corn oil on their skin (semi-occlusive; size of the application area not reported) for 24 h. Animals were observed for 14 days. For male rats, an LD_{50} value of 104 mg/kg (equivalent to 0.56 mg a.i./kg) was calculated by probit analysis. The results obtained for female rats indicated an LD_{50} in a range of 80-100 mg/kg (equivalent to 0.43-0.54 mg a.i./kg). The combined LD_{50} was 100 mg/kg (equivalent to 0.54 mg a.i./kg). Deaths occurred between days 5 and 10. Signs of anticoagulant poisoning were delayed, but all affected animals died. Clinical signs observed prior to death included bruising and swelling of the limbs, bleeding from the ear marks, pale eyes and skin, lethargy and gait abnormalities.

Acute inhalation toxicity
The acute inhalation toxicity of a commercial product containing 0.5% Flocoumafen (generated as a particulate dust aerosol) was tested in CD-1 mice. Groups of 5 male and 5 female mice were exposed by inhalation for 4h to 0.07, 0.12, 0.42, or 0.97 mg/l master mix containing 0.5% m/m Flocoumafen. The mass mean diameter of the aerosol particles were 2.2, 3.0, 2.9, and 2.8 µm, respectively. Animals were observed for 14 days. Flocoumafen exposure resulted in an LC_{50} within the range of 0.12 to 0.42 mg/l, corresponding to 0.0006-0.002 mg a.i./l. All animals exposed to 0.42 or 0.97 mg/l of test substance and from which blood sample was obtained showed no coagulation. The majority of animals exhibited classical signs of anticoagulant poisoning, e.g. pale extremities and eyes, green coloured faeces, piloerection, body tremors laboured respiration, hypothermia, hypokinesia. The observed signs were first noted on either day 4 or 5 and became so severe that a number of animals were sacrificed on humane ground.

Groups of five male and five female Cobs Wistar rats were exposed for 4 hours to inhalable dust atmospheres of technical concentrate containing 0.5% Flocoumafen . Rats were exposed to 0.04, 0.16, and 1.4 mg/l 0.5% WL108366 technical/bait concentrate with mass median aerodynamic diameter of the aerosol particles of 3.4±2.1 µm and 4.2±2.3 µm for the two higher doses. Animals were observed for 13 to 15 days. Flocoumafen exposure resulted in an LC_{50} within the range of 0.16 to 1.4 mg/l, corresponding to 0.0008-0.007 mg a.i./l. The animals exposed to a concentration of 1.4 mg/l of test substance exhibited classical signs of anticoagulant poisoning, e.g. bruised appearance of the feet with bleeding, pale skin, eyes and ears, and green coloured faeces. The increased susceptibility of males compared to females was also apparent in the lethargic signs of high dose animals.

Classification proposed by the Dossier Submitter
Oral: Based on the oral LD_{50} for rats (range from 0.13-0.5 mg/kg bw), the DS proposed to classify Flocoumafen as Acute Tox. 1 H300 (criterion: LD_{50}, oral, rat $\leq$ 5 mg/kg).

Dermal: Based on the dermal LD_{50} for rats (range from 0.43-1.14 mg/kg bw), the DS proposed to classify Flocoumafen as Acute Tox. 1 H310 (criterion: LD_{50}, dermal, rat or rabbit $\leq$ 50 mg/kg).

Inhalation: Based on the inhalatory LD_{50} values of 0.0006-0.002 mg/l/4h for the mouse and 0.0008-0.007 mg/l/4h for the rat (both sexes combined) , the DS proposed to classify Flocoumafen as Acute Tox. 1 H330 (criterion: LD_{50}, inhalation, rat, for dusts and mists $\leq$ 0.05 mg/l/4h).

The proposed classifications for acute toxicity for the oral and inhalation routes are a revision of the minimum classifications currently in Annex VI of CLP.

Comments received during public consultation

One Member State (MS) agreed with the classifications proposed by the Dossier Submitter (DS) for acute toxicity for Flocoumafen.

Assessment and comparison with the classification criteria

In the opinion of RAC Flocoumafen warrants classification:

according to Regulation EC 1272/2008

- as Acute Tox. 1 H300 (criterion: LD_{50}, oral, rat $\leq$ 5 mg/kg) based on the oral LD_{50} for rats (range from 0.13-0.5 mg/kg bw;.
- Acute Tox. 1 H310 (criterion: LD_{50}, dermal, rat or rabbit $\leq$ 50 mg/kg) based on the dermal LD_{50} for rats (range from 0.43-1.14 mg/kg bw;
- Acute Tox. 1 H330 (criterion: LD_{50}, inhalation, rat, for dusts and mists $\leq$ 0.05 mg/l/4h) based on the inhalatory LD_{50} values of 0.0006-0.002 mg/l/4h for the mouse and 0.0008-0.007 mg/l/4h for the rat (both sexes combined).

This is in agreement with the DS proposal for classification of Flocoumafen for acute toxicity.

Table 5.3: Supportive data for acute oral toxicity

The following studies are considered to contain additional information concerning acute oral toxicity and are thus presented in tabular format as supportive data:

Study number	Title	System	Results
A6.1.1/05	The acute oral toxicity of WL 108366 in C57BL/10 mice	Male and female C57BL/10 mice (5 per sex and group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male mice = 0.79 mg/kg LD_{50}, female mice = 1.47 mg/kg
A6.1.1/06	The acute oral toxicity of a series of novel anticoagulants in Wistar rats and C57BL/10 mice	Wistar rats (4 males per group); C57BL/10 mice (5 males per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male rats = 0.46 mg/kg LD_{50}, male mice = 0.79 mg/kg
A6.1.1/07	The acute oral toxicity of WL 108366 in female Wistar rats	Female Wistar rats (4 females per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 14 days. LD_{50}, female rats = 0.56 mg/kg
A6.1.1/08	Determination of the acute oral LD_{50} of flocoumafen against the ship rat (*Rattus rattus*)	Male and female rats (4 per sex and group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male rats = 1.78 mg/kg LD_{50}, female rats = 1.0 mg/kg
A6.1.1/09	Summary of protocol for determining acute oral LD_{50} of flocoumafen ('366) for *Rattus rattus*	Male and female rats (4 per sex and group)	summary of A6.1.1/08 as listed above.

The following studies are considered to contain additional information concerning acute oral toxicity and are thus presented in tabular format as supportive data:			
Study number	**Title**	**System**	**Results**
A6.1.1/10	Toxicology of rodenticides: the acute oral toxicity of WL 108366 in mice	Male and female CF1 mice (5 per sex and group)	The test substance was dissolved in corn oil and administered once orally by gavage. The observation period was 42 days. LD_{50}, male mice = 2.9 mg/kg LD_{50}, female mice = 2.0 mg/kg
A6.1.1/11	Toxicology of rodenticides: the acute oral toxicity of WL 108366 in rabbits and hamsters	Male and female Syrian hamsters (5 per sex and group) male and female New Zealand White rabbits (2 per sex and group)	The test substance was administered enclosed in gelatine capsules to rabbits and by gavage as a 0.5% solution in corn oil to the hamsters. The observation period was 35 days for rabbits and 28 days for hamsters. LD_{50}, rabbits = 0.7 mg/kg LD_{50}, hamster > 50 mg/kg
A6.1.1/12	The acute oral toxicity of a series of novel anticoagulants in Syrian hamsters	Syrian hamsters (4 males per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male hamsters > 46.40 mg/kg
A6.1.1/13	The acute oral toxicity of WL 108366 in Dunkin-Hartley guinea pigs	Dunkin-Hartley guinea pigs (4 males per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male Guinea pigs > 10 mg/kg

5.2.2 Acute toxicity: inhalation

The acute inhalation toxicity of Storm master mix containing 0.5% flocoumafen (generated as a particulate dust aerosol) was tested in CD-1 mice (A6.1.3/01) (see Table 5.4). Groups of 5 male and 5 female mice were exposed by inhalation for 4h to 0.07, 0.12, 0.42, and 0.97 mg/l master mix containing 0.5% m/m flocoumafen. The mass mean diameter of the aerosol particles were 2.2, 3.0, 2.9, and 2.8 µm, respectively. Animals were observed for 14 days. Flocoumafen exposure resulted in an LC_{50} within the range of 0.12 to 0.42 mg/l, corresponding to 0.0006-0.002 mg a.i./l. All animals exposed to 0.42 or 0.97 mg/l of test substance and from which blood sample was obtained

showed no coagulation. The majority of animals exhibited classical signs of anticoagulant poisoning, e.g. pale extremities and eyes, green coloured faeces, piloerection, body tremors laboured respiration, hypothermia, hypokinesia. The observed signs were first noted on either day 4 or 5 and became so severe that a number of animals were sacrificed on human ground. These effects were attenuated in the two lower dose groups. No treatment-related pathological changes were found in low dose animals and survivors of the mid and high dose group upon necropsy. The cause of death in the mid and high dose groups was widespread severe haemorrhaging. Lung/body weight ratios were considered to be within normal limits. The majority of the animals exposed to 0.42 or 0.97 mg/l test substance lost body weight following exposure. Body weight loss was still evident for several of the surviving animals at the end of the 14 day observation period. Animals exposed to 0.07 or 0.12 mg /l maintained body weight during the study.) The study was performed according to EPA guidelines and Japanese MHW guidelines for acute toxicity testing (1984). Deviations from the prescribed guidelines were not stated. The method was in accordance with EC B.2 and OECD 403. Normally, for classification and labelling purposes, an acute inhalation study should be performed in rats instead of mice. As the active ingredient would require classification with 'very toxic by inhalation" (by way of read-across, and assuming that the particle size of commercially available flocoumafen is comparable to that of the master mix), the study is considered suitable for evaluation.

Groups of five male and five female Cobs Wistar rats were exposed for 4 hours to inhalable dust atmospheres of technical concentrate containing 0.5% flocoumafen (A6.1.3/02, A6.1.3/03) (see Table 5.4). Rats were exposed to 0.04, 0.16, and 1.4 mg/l 0.5% WL108366 technical/bait concentrate with mass median aerodynamic diameter of the aerosol particles of 3.4±2.1 μm and 4.2±2.3 μm for the two higher doses. Animals were observed for 13 to 15 days. Flocoumafen exposure resulted in an LC_{50} within the range of 0.16 to 1.4 mg/l, corresponding to 0.0008-0.007 mg a.i./l. The animals exposed to a concentration of 1.4 mg/l of test substance exhibited classical signs of anticoagulant poisoning, e.g. bruised appearance of the feet with bleeding, pale skin, eyes and ears, and green coloured faeces. These effects were attenuated in the two lower dose groups and in the case of the group exposed to a concentration of 0.04 mg/l; only one male exhibited a bruised appearance of its feet. The increased susceptibility of males compared to females was also apparent in the lethargic signs of high dose animals. All males were lethargic in the post-exposure period on the day of exposure, whereas it was not until the fifth day following exposure that three out of five females became lethargic. Similarly three males exhibited a hunched back stance on day 4 but only one female showed the same signs on day 12 following exposure. The first signs of bruising of the feet occurred in the majority of the animals on day 2 and most of the animals exhibiting paleness of the skin were affected on day 4. Few or no symptoms were shown on the day following exposure. No treatment-related pathological changes were found in low dose animals and survivors of the mid dose group upon necropsy. The cause of death in the mid and high dose groups was widespread severe haemorrhaging. There were similar findings in the surviving female of the high dose group. All surviving rats in the low and mid dose group gained weight relative to their day 0 body weights in a similar manner as the control upon study termination, except for one animal in each group. The surviving female in the high dose group lost weight dramatically during the observation period. Although not performed to any guideline, the method used was consistent to EC method B.2 (92/69/EEC) in all important aspects with the following deviation: the mass median diameter could not be determined for the lowest concentration atmosphere. Therefore, the study is considered acceptable for classification and labelling purposes.

5.2.3 Acute toxicity: dermal

The acute dermal toxicity of flocoumafen was tested in New Zealand White rabbits (A6.1.2/01) (see Table 5.4). Groups of 5 male and 5 female rabbits received 0.2, 0.4, 0.8, and 1.6 mg/kg of flocoumafen (purity: 96.1%) wetted with 1 ml of water on their skin (semi-occlusive; no information on application area provided) for 24 h. Animals were observed for 28 days. Deaths occurred mainly in week two and three of the study. The test material administered elicited a LD_{50} of 0.65 mg/kg in males and 1.14 mg/kg in females (combined: 0.87 mg/kg). Most of the animals which died during the course of the study showed anorexia, lethargy or haemorrhaging prior to death. No signs of toxicity were observed in survivors, except for one female of the high dose group, which lost weight during the second week of the study. This animal recovered during week 3. Upon necropsy, treatment-related findings were noted at all dose levels and were essentially all haemorrhagic in nature. Negligible effects were recorded in the low dose group and in survivors of the 0.4 mg/kg dose group. More severe effects were noted at 0.8 mg/kg and 1.6 mg/kg. The method used was according to Japanese testing guidelines for toxicological studies (Japan/MAFF, 1985) and consistent to EC method B.3 (92/69/EEC) and OECD 402 in all important aspects. Therefore, the study is considered acceptable for classification and labelling purposes.

The acute dermal toxicity of flocoumafen was tested in Fischer 344 rats (A6.1.2/02, A6.1.2/03) (see Table 5.4). The method used was in accordance with EC method B.3 (92/69/EEC) and OECD 403 with the following deviations: the test compound was held in contact with the skin by an aluminium foil instead of a porous gauze dressing, necropsy was only reported for two animals instead of all animals. Groups of 5 male and 5 female rats (50-160 mg/kg doses) and 10 male and 10 female rats (125 and 200 mg/kg doses) received 50, 80, 100, 125, 160, and 200 mg/kg of 0.5% flocoumafen (purity: 0.48% of a.i.) in corn oil on their skin (semi-occlusive; size of the application area not reported) for 24 h. Animals were observed for 14 days. For male rats, an LD_{50} value of 104 mg/kg (equivalent to 0.56 mg a.i./kg) was calculated by probit analysis. The results obtained for female rats indicated an LD_{50} in a range of 80-100 mg/kg (equivalent to 0.43-0.54 mg a.i./kg). The combined LD_{50} was 100 mg/kg (equivalent to 0.54 mg a.i./kg). Deaths occurred between days 5 and 10. Signs of anticoagulant poisoning were delayed, but all affected animals died. Clinical signs observed prior to death included bruising and swelling of the limbs, bleeding from the ear marks, pale eyes and skin, lethargy and gait abnormalities. Animals treated with 50 or 80 mg/kg showed no signs of toxicity. One male of the 160 mg/kg group was sacrificed non-scheduled and showed a blood clot on one testis, pale lungs and brain haemorrhage upon necropsy. One female of the 125 mg/kg group was also sacrificed non-scheduled and necropsy revealed pale coloured liver, lungs, spleen and kidneys and subcutaneous haemorrhages in cranial and ventral regions. It was not reported, if further gross pathological examinations were performed. All surviving rats gained weight relative to their day 0 body weights upon study termination. Despite the small deviations from EC method B.3 (92/69/EEC) and OECD 403, the study is considered acceptable for classification and labelling purposes.

Supportive data

One supportive acute dermal toxicity study on Fischer 344 rats was submitted testing bait concentrate powder containing 0.5% flocoumafen (A6.1.2/04). LD_{50}'s of 2.3 and 1.8 mg a.i./kg for males and females, respectively were obtained.

5.2.4 Acute toxicity: other routes

No data.

5.2.5 Human data

No data.

5.2.6 Summary and discussion of acute toxicity

Key studies relevant for classification are summarized in Table 5.4.

Table 5.4: Acute oral, dermal and inhalation toxicity studies, relevant for classification

Route	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD50/LC50	Remarks	Study number
Oral	In accordance with EC B.1 and OECD 401	Rat, Fischer 344, 5/sex/dose	0.20, 0.25, 0.32, 0.40 and 0.50 mg/kg; single acute exposure	M: 0.43 mg/kg; F: 0.31 mg/kg; Combined: 0.37 mg/kg	Vehicle corn oil	A6.1.1/01

Route	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD50/LC50	Remarks	Study number
Oral	In accordance with EC B.1 and OECD 401	Rat, Fischer 344, 5/sex/dose	0.06, 0.13, 0.25, and 0.50 mg/kg; single acute exposure	M: 0.25–0.5 mg/kg; F: 0.13–0.25 mg/kg	Vehicle corn oil	A6.1.1/02
Dermal	In accordance with EC B.3 and OECD 402	Rabbit, New Zealand White, 5/sex/dose	0.2, 0.4, 0.8 or 1.6 mg/kg 24 hours	M: 0.65 mg/kg; F: 1.14 mg/kg; Combined: 0.87 mg/kg	Vehicle water 24 hours exposure	A6.1.2/01
Dermal	In accordance with EC B.3 and OECD 402	Rat, Fischer 344, 5 males and 5/sex/dose the two highest doses with 10 individuals of each sex	50, 80, 100, 125, 160 and 200 mg/kg of 0.5% flocoumafen in corn oil	M: 0.56 mg a.i./kg; F: 0.43–0.54 mg a.i./kg; Combined: 0.54 mg a.i./kg	Vehicle corn oil 24 hours exposure	A6.1.2/02, 03
Inhalation	In accordance with EC B.2 and OECD 403	CD-1 mice, 5/sex/dose	0.07, 0.12, 0.42, 0.97 mg/l master mix containing 0.5 % m/m flocoumafen	0.0006 –0.0021 mg a.i./l	Powder MMSD±GSD Group 2: 2.2 µm (2.3) Group 3: 3.0 µm (1.6) Group 4: 2.9 µm (1.6) Group 5: 2.8 µm (1.6) 4h exposure Nose only	A6.1.3/01
Inhalation	In accordance with EC B.2 and OECD 403	Rat, Cobs Wistar, 5/sex/dose	0.04, 0.16, 1.4 mg/l 0.5% WL108366 technical/bait concentrate	0.0008 – 0.007 mg a.i./l	Powder MMSD±GSD 0.04 mg/l: indeterminable 0.16 mg/l: 3.4 ± 2.1 µm 1.4 mg/l: 4.2 ± 2.3 µm 4h exposure Nose only	A.6.1.3/02 and 03

5.2.7 Comparison with criteria

Classification proposals according to 67/548/EEC

Classification

Oral: Based on the oral LD_{50} for rats (range from 0.13-0.5 mg/kg bw in key studies A6.1.1/01 and A6.1.1/02), it is proposed to classify flocoumafen with the symbol T+ and with R28 'very toxic if swallowed' (criterion: LD_{50}, oral, rat ≤ 25 mg/kg).

Dermal: Based on the dermal LD_{50} for rats (range from 0.43-1.14 mg/kg bw in key studies A6.1.2/01 and A6.1.2/02,03), it is proposed to classify flocoumafen with the symbol T+ and with R27 'very toxic in contact with skin' (criterion: LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg).

Inhalation: Based on the inhalatory LD_{50} values of 0.0006-0.002 mg/l/4h for the mouse and 0.0008-0.007 mg/l/4h for the rat (both sexes combined) (key studies A6.1.3/01 and A6.1.3/02,03), it is proposed to classify flocoumafen with the symbol T+ and R26 'very toxic by inhalation' (criterion: LD_{50}, inhalation, rat, for aerosols or particulates ≤ 0.25 mg/l/4h).

TC-C&L conclusion
T+; R26/27/28 was agreed by the TC C&L, November 2006 (ECBI/20/27, Rev. 1).

The proposed classification is in line with the harmonised classification in Annex VI of CLP. However, currently there are no SCL for acute toxicity in Annex VI.

Specific Concentration Limits

Oral: SCLs for acute oral toxicity of ≥0.8% for R28, 0.1 - 0.8% for R25 and 0.01 – 0.1% for R22. These SCLs are based on an LD50 for flocoumafen of approximately 0.2 mg/kg bw and the percentage of flocoumafen in a mixture at which the classification criteria are reached. Example: 0.2 mg/kg bw (LD50) / 25 mg/kg bw (limit R28) * 100% = 0.8% (SCL for R28).

Dermal: SCLs for acute dermal toxicity of ≥1% for R27, 0.125 - 1% for R24 and 0.025 – 0.125% for R21. These SCLs are based on a LD50 for flocoumafen of approximately 0.5 mg/kg bw and the percentage of flocoumafen in a mixture at which the classification criteria are reached.

Inhalation: SCLs for acute inhalatory toxicity of ≥0.4% for R26, 0.1 – 0.4% for R23 and 0.02 – 0.1% for R20. These SCLs are based on a LC50 for flocoumafen of approximately 0.001 mg/L and the percentage of flocoumafen in a mixture at which the classification criteria are reached.

The formulas used above for setting SCLs are identical to the formulas given in Regulation EC 1272/2008 for the calculation of the ATE of mixtures.

Proposed SCLs:

R28 above 0.8%, R25 between 0.1 and 0.8%, R22 between 0.01 and 0.1%

R27 above 1%, R24 between 0.125 and 1%, R21 between 0.025 and 0.125%

R26 above 0.4%, R23 between 0.1 and 0.4%, R20 between 0.02 and 0.1%

Based on worst-case simplifications and taken into account the SCLs for both acute and repeated dose toxicity (see chapter 5.6), the following limits are proposed:

$C \geq 0.8\%$: R26/27/28 – 48/23/24/25

$0.4\% \leq C < 0.8\%$: R26-24/25 – 48/23/24/25

$0.1\% \leq C < 0.4\%$: R23/24/25 – 48/23/24/25

$0.01\% \leq C < 0.1\%$: R20/21/22 – 48/20/21/22

Previous discussion

Alternative SCLs were agreed by the TC C&L, May 2007 (Follow-up II, Ispra, 9 July 2007) awaiting the general discussion on the method to be used for setting SCLs. This discussion was not finalised at the TC C&L, May 2007 (Follow-up V, Ispra, 29 May 2008). In a response on follow-up IV of this meeting (ECBI/53/06 Add.18), the Netherlands supported a continuation of the discussion on the method, however, using the method provisionally agreed by TC C&L (use the order of magnitude; go down from 7 % for the limit for classification), the following SCLs for acute toxicity would apply:

Acute oral toxicity:

T+: 7% / 25 (limit) * 0.2 (LD50) = 0.056%

T: 7% / 200 (limit) * 0.2 (LD50) = 0.007%

Xn: 7% /2000 (limit) * 0.2 (LD50) = 0.0007%

Acute dermal toxicity:

T+: 7% / 50 (limit) * 0.5 (LD50) = 0.07%

T: 7% / 400 (limit) * 0.5 (LD50) = 0.00875%

Xn: 7% /2000 (limit) * 0.5 (LD50) = 0.00175%

Acute inhalation toxicity:

T+: 7% / 0.25 (limit) * 0.001 (LD50) = 0.028%

T: 7% / 1 (limit) * 0.001 (LD50) = 0.007%

Xn: 7% / 5 (limit) * 0.001 (LD50) = 0.0014%

Based on worst-case simplifications and taken into account the SCLs for both acute and repeated dose toxicity, this would result in the following limits:

C > 0.06%: T+; R26/27/28 – 48/23/24/25

0.03% < C < 0.06%: T+; R26-24/25 - 48/23/24/25

0.007% < C < 0.03%: T; R23/24/25 – 48/23/24/25

0.0007% < C < 0.007%: Xn; R20/21/22 – 48/20/21/22

Classification proposals according to Regulation EC 1272/2008

Classification

Oral: Based on the oral LD_{50} for rats (range from 0.13-0.5 mg/kg bw in key studies A6.1.1/01 and A6.1.1/02), it is proposed to classify flocoumafen with Acute Tox. 1 H300 (criterion: LD_{50}, oral, rat ≤ 5 mg/kg).

Dermal: Based on the dermal LD_{50} for rats (range from 0.43-1.14 mg/kg bw in key studies A6.1.2/01 and A6.1.2/02,03), it is proposed to classify flocoumafen with Acute Tox. 1 H310 (criterion: LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg).

Inhalation: Based on the inhalatory LD_{50} values of 0.0006-0.002 mg/l/4h for the mouse and 0.0008-0.007 mg/l/4h for the rat (both sexes combined) (key studies A6.1.3/01 and A6.1.3/02,03), it is proposed to classify flocoumafen with Acute Tox. 1 H330 (criterion: LD_{50}, inhalation, rat, for dusts and mists ≤ 0.05 mg/l/4h).

The proposed classification is a change from the minimal classification for acute toxicity for the oral and inhalation route currently in Annex VI of CLP.

Specific Concentration Limits

Specific concentration limits are not required for acute toxicity under Regulation EC 1272/2008 because the classification of mixtures for this endpoint is not based on the classification of the ingredients but on the acute toxicity estimates of the ingredients.

5.3 STOT SE

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

Heamorrhagic effects due to inhibition of the coagulation were observed at relevant dose levels in the acute toxicity studies through all three routes.

5.3.2 Comparison with criteria

Classification with STOT SE is required when a substance induces significant toxicity in humans or significant toxicity in animals at or below certain dose levels after a single exposure.

There is no human data. These effects in itself would fulfil the criteria for classification with STOT SE 1. However, these effects are also the mechanism by which the lethalities occur. Flocoumafen is already classified for acute toxicity for all three routes. Classifying also for STOT SE would therefore be a double classification for the same effect. This is not required as described in chapter 3.8.1 of the guidance on CLP.

5.3.3 Conclusions on classification and labelling

No classification for STOT SE is proposed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

Haemorrhagic effects due to inhibition of coagulation were observed at relevant dose levels in the acute toxicity studies following exposure to Flocoumafen via all three routes.

Classification with STOT SE is required when a substance induces significant toxicity in humans or significant toxicity in animals at or below certain dose levels following a single exposure.

No human data were available. The effects observed in animals on their own would fulfil the criteria for classification as STOT SE 1. However, these effects seemed to be concurrent with the lethalities. Flocoumafen is already classified for acute toxicity for all three routes. Classifying also for STOT SE would therefore be a double classification for the same effect. Classification for STOT SE is therefore according to the DS not required based on chapter 3.8.1 of the CLP Guidance.

No classification for STOT SE is proposed by the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the opinion of RAC, after single exposure to Flocoumafen the blood coagulation system is adversely affected, and this is the main cause of mortality. However, this does not warrant classification of Flocoumafen for specific target organ toxicity – single exposure, because it is already covered by the classification as Acute Tox. 1.

5.4 Irritation

5.4.1 Skin

As supportive data a skin irritation study (A6.1.4/01) with a 1% solution of flocoumafen in PEG was submitted. No erythematic reaction was observed. The skin irritation study is not considered suitable for classification and labelling purposes for flocoumafen, since the study was performed with a 1% solution of flocoumafen in polyethylene glycol (PEG). Furthermore, no data on the purity is available. However, considering the most recent OECD 404 guideline, additional skin irritation testing with flocoumafen is not considered necessary, due to the results (highly toxic) of the acute dermal toxicity studies with flocoumafen.

Another skin irritation study (A6.1.4/03), as supportive data, was performed with Storm manufacturing master mix containing 0.5% flocoumafen. No skin irritation was observed. However, the study is not considered suitable for the evaluation of skin irritation properties of flocoumafen.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Two skin irritation/corrosion studies were considered by the DS as not suitable for classification and labelling of Flocoumafen. Taking into account the CLP Regulation (Annex I, section 3.2.2.3) and the most recent OECD 404 guideline, additional skin irritation testing, and thus corrosivity testing, is considered not necessary, due to the results (highly toxic) of the acute dermal toxicity studies with Flocoumafen.

No classification is currently included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed by the DS as also agreed by the TC C&L in 2006/2007.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the opinion of RAC there are no data which would warrant classification of Flocoumafen for skin corrosion/irritation. The view of the Dossier Submitter that additional skin irritation/corrosion testing is not necessary, due to the results (highly toxic) of the acute dermal toxicity studies with Flocoumafen, is supported by the Committee.

5.4.2 Eye

The acute eye irritation potential of flocoumafen (purity: 99.5%) was tested in New Zealand White rabbits (3 males/group) according to OECD 405 (1987) and EC method B.5 (92/69/EEC) (A6.1.4/02). A volume of approx. 0.1 ml with 22.8 to 22.9 mg of the test substance was instilled for 24 h. No mortality or clinical signs of toxicity were observed in any of the animals during the study period. Redness, chemosis and discharge of the conjunctiva were observed starting one hour after application and had resolved at 48 to 72 hours after instillation. There was no evidence of ocular corrosion. No iridic irritation or corneal opacity was observed, and treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals. Flocoumafen technical material was considered to be non-irritating to the rabbit eye. The eye irritation study is acceptable for classification and labelling purposes.

The eye irritation study with flocoumafen is presented in Table 5.5.

Table 5.5: Eye irritation study with flocoumafen

Species	Method/Guideline	Mean scores per animal from the 24, 48 and 72 h observations				Reversibility yes/no	Result	Reference
		Cornea	Iris	Redness Conjunct	Chemosis			
Rabbit	In accordance with EC B.5 and OECD 405	0,0,0	0,0,0	0.3, 0.7, 0.7	0, 0.7, 0	yes	Non-irritating	A6.1.4/02

Another eye irritation study (A6.1.4/03), as supportive data, was available performed with Storm manufacturing master mix containing 0.5% flocoumafen. Based on the results of the study, Storm manufacturing master mix should be considering eye irritating. However, the study is not considered suitable for the evaluation of eye irritation properties of flocoumafen.

Also as supportive data an eye irritation study with a 1% solution of flocoumafen in PEG was submitted (A6.1.4/04). No eye irritation was observed.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The acute eye irritation potential of Flocoumafen (purity: 99.5%) was tested in New Zealand White rabbits (3 males/group) according to OECD 405 (1987) and EC method B.5 (92/69/EEC). This study was considered by the DS to be acceptable for classification and labelling purposes.. Approx. 0.1 ml containing 22.8 to 22.9 mg of the test substance was instilled for 24 h. No mortality or clinical signs of toxicity were observed in any of the animals during the study period. Redness, chemosis and discharge of the conjunctiva were observed starting one hour after application and these had resolved at 48 to 72 hours after instillation. There was no evidence of ocular corrosion. No iridic irritation or corneal opacity was observed, and treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals. Flocoumafen technical material was therefore considered to be non-irritating to the rabbit eye.

Another eye irritation study, submitted as supportive data, was performed with a commercial product containing 0.5% Flocoumafen. However, the study was not considered suitable for the evaluation of eye irritation properties of Flocoumafen. Based on the results of the study, this commercial product could be considering eye irritating. Also as supportive data an eye irritation study with a 1% solution of Flocoumafen in PEG was submitted. No eye irritation was observed.

No classification is currently included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed by the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the opinion of RAC, the results of a study in New Zealand White rabbits (3 males/group) conduct in accordance with OECD TG 405 (1987) and EC method B.5 (92/69/EEC) does not

warrant classification for eye corrosion/irritation, because the observed effects did not meet the CLP classification criteria.

5.4.3 Respiratory tract

No data.

5.4.4 Summary and discussion of irritation

Flocoumafen is considered not irritating to the eyes (key study A6.1.4/02) according to the criteria given in Annex IV of
Directive 2001/59/EC.

Considering the results (highly toxic) of the acute dermal toxicity study, additional skin irritation testing is not warranted according to the most recent OECD 404 guideline. Therefore, no classification according to 67/548/EEC is proposed.

For the same reason classification for skin irritation and serious eye damage/eye irritation according to the criteria in Regulation EC 1272/2008 is not necessary (see Annex I. Chapter 3.2.2.3).

5.4.5 Comparison with criteria

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007.

TC-C&L conclusion

The TC-C&L concluded in November 2006 (ECBI/20/07, Rev. 1) that classification for skin irritation and serious eye damage/eye irritation was not required.

5.5 Corrosivity

The skin irritation/corrosion studies mentioned in section 5.3.1 (A6.1.4/01, A6.1.4/03) were considered not suitable for classification and labelling purposes for flocoumafen. Considering the most recent OECD 404 guideline, additional skin irritation testing, and thus corrosivity testing, is considered not necessary, due to the results (highly toxic) of the acute dermal toxicity studies with flocoumafen.

5.5.1 Summary and discussion of corrosivity

No suitable study on skin corrosivity was available. However, additional skin corrosivity testing is not warranted, considering the results (highly toxic) of the acute dermal toxicity study. Therefore no classification according to 67/548/EEC is proposed.

For the same reason classification for skin irritation and serious eye damage/eye irritation according to the criteria in Regulation EC 1272/2008 is not necessary (see Annex I. Chapter 3.2.2.3).

5.5.2 Comparison with criteria

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007

TC-C&L conclusion

The TC-C&L concluded in November 2006 (ECBI/20/07, Rev. 1) that classification for skin corrosivity was not required.

5.6 Sensitisation

5.6.1 Skin

The skin sensitizing potential of flocoumafen (97.6%) was tested using the guinea pig maximisation test (A6.1.5/01). Although not a guideline study, the method used was similar to method B.6 (96/54/EC). For induction, 0.05% test substance (slight redness) in corn oil (intra-dermal) was used followed one week later by 50% test substance (highest concentration achievable; non-irritating) in petroleum jelly (topical). Animals were challenged two weeks after topical induction using 50% test substance in petroleum jelly (topical) for 24 h. Although animals were treated with a non-irritating concentration of 50% flocoumafen (highest concentration achievable) without pre-treatment with sodium lauryl sulphate, the test was considered acceptable: seven of the test animals died or were killed for humane reasons between day 8 and 12 (after application of the topical induction patches but before the challenge state) indicating that the test substance was systemically available. The body weight gain in surviving test animals was noticeably less than in the controls. None of the 13 surviving test animals showed positive responses at 24 or 48 hours after removal of the challenge patches. Thus, the test material was considered to be non-sensitising to the skin of guinea pigs.

The skin sensitization study with flocoumafen is presented in Table 5.6.

Table 5.6: Skin sensitisation study with flocoumafen

Species	Method	Number of animals sentitized/total number of animals	Result	Reference

Guinea pig	Maximisation In accordance with EC B.6 and OECD 406 Intra-dermal: 0.05% test substance in corn oil Topical and challenge: 50% test substance in petroleum jelly (no positive control)	0/13[1]	Not sensitising	A6.1.5/01

[1] seven animals were found dead or were killed for humane reasons between day 8 and 12 of the study (flocoumafen was systemically available)

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The skin sensitizing potential of Flocoumafen (97.6%) was tested using the guinea pig maximisation test. Although not a guideline study, the method used was similar to method B.6 (96/54/EC). For induction, 0.05% test substance (slight redness) in corn oil (intra-dermal) was used followed one week later by 50% test substance (highest concentration achievable; non-irritating) in petroleum jelly (topical). Animals were challenged two weeks after topical induction using a preparation containing 50% test substance in petroleum jelly (topical) for 24 h. Although animals were treated with a non-irritating concentration of 50% Flocoumafen (highest concentration achievable) without pre-treatment with sodium lauryl sulphate, the test was considered acceptable: seven of the test animals died or were killed for humane reasons between days 8 and 12 (after application of the topical induction patches but before the challenge state) indicating that the test substance was systemically available. The body weight gain in surviving test animals was noticeably less than in the controls. None of the 13 surviving test animals showed positive responses at 24 or 48 hours after removal of the challenge patches. Thus, the test material was considered to be non-sensitising to the skin of guinea pigs.

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed by the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the opinion of RAC, the results of the guinea pig maximisation test do not warrant classification for skin sensitisation, because the observed effects do not meet the CLP classification criteria.

5.6.2 Respiratory system

No data.

5.6.3 Human data

No data.

5.6.4 Other relevant data

None.

5.6.5 Summary and discussion of sensitisation

Flocoumafen is considered not skin sensitising (key study A6.1.5/01) according to the criteria given in Annex VI of Directive 67/548/EEC. No classification proposed for respiratory sensitisation because no data were available.

Also according to the criteria in Regulation EC 1272/2008 no classification for skin and respiratory sensitisation is necessary.

5.6.6 Comparison with criteria

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007.

TC-C&L conclusion

The TC-C&L concluded in November 2006 (ECBI/20/07, Rev. 1) that classification for sensitisation was not required.

5.7 Repeated dose toxicity

5.7.1 Repeated dose toxicity: oral

Short-term toxicity

Oral short-term (28-days) exposure of rats to 0, 0.01, 0.05, 0.1 or 0.2 mg/kg food (equivalent to 0.0005, 0.0025, 0.005 and 0.01 mg/kg bw/day; based on conversion factor of 20 (JMPR[1])) resulted in increased mean prothrombin and activated partial thromboplastin times in females at 0.2 mg/kg food (A6.3.1/01). At 0.1 mg/kg food, a slight statistically non-significant increase in activated partial thromboplastin time was noted in females. Decreased levels of plasma protein, alkaline phosphatase and cholesterol were noted in females at 0.1 and 0.2 mg/kg food. A statistically significant decrease in calcium and a statistically significant increase in chloride were noted in

[1] The conversion factor of 20 used to convert the dose expressed as mg/kg food to mg/kg bw/day for rats is based on the average food consumption of rats per day and is according to "Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticides Residues (JMPR). See also appendix I of http://www.who.int/foodsafety/chem/jmpr/en/prst_wp_gls.pdf

males at 0.2 mg/kg food. At histopathology, a slight reduction of cytoplasmatic vacuolation of glycogenic type in the periportal parenchymal cells in livers of males at 0.2 mg/kg food was noted.

Based on the decreased levels of plasma protein, alkaline phosphatase and cholesterol and increased activated thromboplastin times in females at 0.1 mg/kg food, the NOAEL was established at 0.05 mg/kg food (equivalent to 0.0025 mg/kg bw/day).

The 28-day oral toxicity study is acceptable for the toxicological evaluation of flocoumafen.

Semichronic toxicity

In a 90-day oral toxicity study, rats were given diets containing 0, 0.01, 0.02, 0.05, 0.1, 0.25 or 0.6 mg/kg food (equivalent to 0.0005, 0.001, 0.0025, 0.005, 0.0125 and 0.03 mg/kg bw/day; based on conversion factor of 20 (JMPR)) (A6.4.1/01). All animals given 0.25 and 0.6 mg/kg food died during the study. Animals found dead or sacrificed during the study showed typical anticoagulant signs are pale eyes and skin, dark or swollen areas on the body, blood around nose and eyes and blood in the urine. Increased mean prothrombin and activated thromboplastin times were noted in males and females at 0.1 mg/kg food. In females an increased platelet count and plateletcrit were noted at 0.1 mg/kg food. Decreases in monocytes were noted in males at 0.05 and 0.1 mg/kg food (61 and 59% of control values, respectively), however, these changes were not accompanied by further haematological changes. Cholesterol levels were increased at 0.05(109% and 106% of control values in males and females, respectively) and at 0.1 mg/kg food (111% and 114% of control values in males and females, respectively). The increased cholesterol levels at 0.05 and 0.1 mg/kg food were not toxicologically relevant, since observed changes were within the same range as the historical control values.

Slightly increased albumin values (2.1%) and slightly decreased chloride values (2%) were noted in males at 0.1 mg/kg food. At urinalysis, increased urine volume was noted in males at 0.1 mg/kg food and increased glucose was noted in females at 0.1 mg/kg food. Absolute and relative heart weights were increased in males at 0.1 mg/kg food (108% of control), in the absence of histopathological correlates. At histopathological examination, increased incidences of haemorrhages were noted in several organs in the two highest dose groups, mainly in males (e.g. testes, prostate, epididymides, and urinary bladder). Centrilobular degeneration was noted at 0.25 and 0.60 mg/kg food in males and females and multifocal necrosis in liver of males at 0.6 mg/kg food. Haematopoiesis was noted in spleen of males at 0.25 and 0.6 mg/kg food and of females at 0.1 and 0.25 mg/kg food. A statistically significant increase in haemorrhages in lymph nodes was noted at 0.10, 0.25 and 0.60 mg/kg food. The incidence of lymph node haemorrhages is within the natural background of historical controls at 0.01, 0.02 and 0.05 mg/kg food. Based on the haemorrhages in lymph nodes, a NOAEL of 0.05 mg/kg food is established (equivalent to 0.0025 mg/kg bw/day).

The 90-day oral toxicity studies is acceptable for the toxicological evaluation of flocoumafen, because it may safely be assumed that the quantity of vitamin K3 added to the experimental diets would be insufficient to counteract any haemorrhagic effects caused by the test substance.

The short-term and semi-chronic oral toxicity studies with flocoumafen are presented in Table 5.7.

Long-term toxicity

Based on the expected exposure pattern, for trained and non-trained professional users chronic primary and secondary exposure cannot be excluded. However, performance of a chronic toxicity study with rodents might be difficult due to technical problems (extremely low doses necessary) and

might induce unnecessary harm to laboratory animals. Considering the differences between the NOAELs of the available subacute and semi-chronic toxicity studies with flocoumafen, an effect of exposure duration cannot be excluded. Therefore, an additional factor for exposure duration should be considered at chronic risk assessment.

Supportive data

Supportive data for repeated dose oral toxicity are reported in Table 5.7 (short-term) and Table 5.8 (subchronic).

Table 5.7: Supportive data for short-term oral toxicity

The following studies are considered to contain additional information concerning short-term repeated dose toxicity and are thus presented in tabular format as supportive data: (all studies were non-GLP studies)			
Study number	**Title**	**System**	**Results**
A6.3.1/02	Toxicology of rodenticides WL108366: a five day range finding feeding study in rats.	Male and female Fischer 344 rats (5 per group and sex)	The study was conducted as a range finding study to reference A6.3.1/1. Groups of five male and five female rats were fed diets containing 0, 0.2, 0.4 or 0.8 ppm flocoumafen for five days and were maintained for a recovery period of ten days. Four females and four males in the high dose group died during the study. No toxicological significant signs were observed in animals fed diets containing 0.4 ppm or less.
A6.3.1/03	The sub-acute oral toxicity of WL108366 in Wistar rats.	Male Wistar rats	Groups of 4 male rats were administered orally by gavage with 0.215, 0.10, 0.0464 and 0.0215 mg flocoumafen/kg in PEG/TEA daily for five days. Following dosing the animals were maintained for a 21 day observation period. In the two highest dose groups, all animals died during the study. Thus, the sub-acute oral LD_{50} was determined to be: LD_{50} = 0.34 mg/kg b.w.[1]

[1] This value does not correspond to the dosages used.

Table 5.8: Supportive data for subchronic oral toxicity

The following study is considered to contain additional information concerning subchronic repeated dose toxicity and is thus presented in tabular format as supportive data: (non-GLP study)			
Study number	**Title**	**System**	**Results**

A6.4.1/02	An evaluation of the long term sub-acute oral toxicity of WL108366 in Wistar rats.	Male Wistar rats (12 per group)	Groups of male rats received oral doses of 0, 0.0125, 0.0625 or 0.125 mg/kg by gavage once a week for up to 12 weeks. Prothrombin times were determined in rats sacrificed at 3, 6, 9 or 12 weeks. It was considered that a significant amount of the weekly administered dose was eliminated. Thus, showing that the long term sub-acute oral toxicity of flocoumafen is less than additive.

5.7.2 Repeated dose toxicity: inhalation

Repeated dose inhalation studies are not considered necessary due to the vapour pressure of flocoumafen (< 1x 10^{-3} Pa) and based on the type of formulation of Storm (was bound block bait).

5.7.3 Repeated dose toxicity: dermal

Route specific repeated dose toxicity studies were not submitted as indicated in A6.3.2 and A6.4.2 Repeated dose dermal toxicity studies are not considered necessary, since route specific effects are not to be expected (based on acute oral and dermal toxicity data) and since there is no evidence of enterohepatic circulation or a first-pass effect (based on ADME studies).

5.7.4 Other relevant information

None.

5.7.5 Summary and discussion of repeated dose toxicity:

Studies relevant for classification are summarized in Table 5.9.

Table 5.9: Repeated dose toxicity studies, relevant for classification

Route	Duration of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Study number

Route	Duration of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Study number
Oral (diet)	28-days	Rat, Fischer 344, 8/sex/dose	0, 0.0005, 0.0025, 0.005, 0.01 mg/kg bw/day	0.0005 and 0.0025 mg/kg bw/day: no treatment related changes. 0.005 and 0.01 mg/kg bw/day: increase in APPT, plasma protein, ALP and cholesterol. 0.01 mg/kg bw/day: increased PTT, reduction of cytoplasmatic vacuolation of in periportal parenchymal cells in livers of males	0.005 mg/kg bw/day	0.0025 mg/kg bw/day	A6.3.1/01

Route	Duration of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Study number
Oral (diet)	90-days	Rat, Fischer 344, 10/sex/dose	0, 0.0005, 0.001, 0.0025, 0.005, 0.0125, 0.03 mg/kg bw/day	0.0005 mg/kg bw/day: no treatment related changes 0.001 mg/kg bw/day: haemorrhages in lymph nodes 0.0025 mg/kg bw/day: haemorrhages in lymph nodes, increased cholesterol 0.005 mg/kg bw/day: increased PTT, APTT and platelet count, increased cholesterol and albumin, haematopoiesis spleen (females), haemorrhages in lymph nodes 0.0125 and 0.03 mg/kg bw/day: all animals died during study, necrosis and centrilobular degeneration liver, haematopoiesis spleen, haemorrhages in various organs (lymph nodes, testes, prostate, epididymides). The incidence of lymph node haemorrhages at 0.0005, 0.001 and 0.0025 mg/kg bw/day and the increased cholesterol levels at 0.0025 and 0.005 mg/kg bw/day are within the natural background of historical controls.	0.005 mg/kg bw/day	0.0025 mg/kg bw/day	A6.4.1/01

In the available repeated dose toxicity studies (28- and 90-days) in rats, flocoumafen induced effects on the coagulation systems, including increased (activated) prothrombin times and haemorrhages in various organs.

Based on available toxicological data with non-rodent species (acute toxicity studies with cats, pigs and dogs), a higher sensitivity of non-rodent species when compared to rats is not to be expected. In both rodent and non-rodent species flocoumafen acts as an anticoagulant. Therefore semichronic toxicity testing with non-rodent species is not considered necessary.

In the above mentioned 28-day oral toxicity study in rats, serious effects (increased APTT; increased levels of plasma protein, alkaline phosphatase and cholesterol) were seen from a dose level of 0.1 mg/kg food, equivalent to 0.005 mg/kg bw/day. In the 90-day oral toxicity study in rats, effects (haemorrhages in lymph nodes) were seen from 0.02 mg/kg food, equivalent to 0.001 mg/kg bw/day. Serious effects were seen from 0.1 mg./kg food equivalent to 0.005 mg/kg bw/day. Mortality was found at the two highest dose levels of 0.25 and 0.6 mg/kg food, equivalent to 0.0125 and 0.03 mg/kg bw/day. These values were clearly below the LD_{50} (range from of 0.13-0.5 mg/kg bw, see section 5.2.1).

A chronic toxicity study with flocoumafen was not available. Performance of a chronic toxicity study with rodents might be difficult due to technical problems (extremely low doses necessary) and might induce unnecessary harm to laboratory animals.

Repeated dose dermal toxicity studies were not submitted. However, they are not considered necessary, since route specific effects are not to be expected based on acute oral and dermal toxicity data: the acute toxicities after oral and dermal exposure were comparable indicating comparable absorptions. Furthermore, there is no evidence of enterohepatic circulation or a first-pass effect (based on ADME studies). For the dermal exposure route, oral toxicity data can be used for classification.

Repeated dose inhalation studies were not available. However, they are not considered necessary due to the vapour pressure of flocoumafen ($< 1x\ 10^{-3}$ Pa) and based on the type of formulation of Storm (was bound block bait). The acute toxicities after oral and inhalation exposure were comparable indicating comparable absorptions. Therefore, oral toxicity data can be used for classification.

5.7.6 Comparison with criteria

Conclusion on classification according to 67/548/EEC

Classification

Oral: The lowest serious effect level of 0.005 mg/kg bw/day (key study A6.4.1/01) is far below the criterion of "oral, rat ≤ 5 mg/kg bw/day for 90-days" used for classification with R48/25 for the oral route.

Dermal, inhalation: For classification for the dermal and inhalatory routes, oral data can be used. The acute data indicate a comparable absorption for all three routes. Further, there is a large margin

between the oral dose levels indicating severe effects and the limit value for R48/25. Also, the acute LD50 values for all three routes were already below the limits for classification as toxic after repeated exposure. Based on these findings, we propose to classify flocoumafen as 'Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed (R48/23/24/25).

TC-C&L conclusion T; R48/23/24/25 was agreed at the TC C&L, November 2006 (ECBI/20/07, Rev. 1).

Specific Concentration Limits

Oral: An SCL for R48/25 is proposed of 0.1% based on serious damage seen at 0.1 mg/kg food (= 0.005 mg/kg bw/day) in the longest study in rats. Calculation: 0.005 mg/kg bw/day / 5 mg/kg bw/day * 100% = 0.1%. R48/22 is proposed between 0.01% and 0.1% using the same data and method of calculation.

Dermal: No dermal repeated dose studies are available. Therefore, extrapolation of the oral route to the dermal route is performed using the differences in LD50 between these routes to compensate for differences in absorption. 0.005 mg/kg bw/day (oral RDT) * 0.5 mg/kg bw (dermal LD50) / 0.2 mg/kg bw (oral LD50) = 0.0125 mg/kg bw/day. An SCL for R48/24 is proposed of 0.125% based on serious damage expected at 0.0125 mg/kg bw/day in a 90-day study in rats. R48/21 is proposed between 0.0125% and 0.125% using the same data and method of calculation.

Inhalation: No inhalatory repeated dose studies are available. Therefore, extrapolation of the acute inhalatory route to the repeated dose inhalatory route is performed using the factor of 40 between the oral LD50 of 0.2 mg/kg bw and the oral repeated dose reference dose of 0.005 mg/kg bw/day . In addition a correction is performed for the differences in exposure duration between the studies (oral single dose versus daily but inhalation single 4-hour versus 5 days a week 6 hours per day) using a factor of 1.07 (6/4 * 5/7 = 1.07). This results in a concentration of 0.001 mg/L / 40 /1.07 = 0.00002 mg/L which is expected to cause serious damage in rats in a 90-day inhalation study. An SCL for R48/23 is proposed of 0.1%. R48/20 is proposed between 0.01% and 0.1%.

The formulas for setting SCLs used above are identical to the formulas given under the guidance on the application of the CLP criteria for the setting of SCLs for STOT-RE.

Proposed SCLs:

R48/25 above 0.1% and R48/22 between 0.01 and 0.1%

R48/24 above 0.125% and R48/21 between 0.0125 and 0.125%

R48/23 above 0.1% and R48/20 between 0.01 and 0.1%

Based on worst-case simplifications and taken into account the SCLs for both acute (see chapter 5.2) and repeated dose toxicity, the following simplified SCLs are proposed:

C ≥ 0.8%: R26/27/28 – 48/23/24/25

0.4% ≤ C < 0.8%: R26-24/25 – 48/23/24/25

0.1% ≤ C < 0.4%: R23/24/25 – 48/23/24/25

0.01% ≤ C < 0.1%: R20/21/22 – 48/20/21/22

Previous discussion

Alternative SCLs were agreed by the TC C&L, May 2007 (Follow-up II, Ispra, 9 July 2007) awaiting the general discussion on the method to be used for setting SCLs. This discussion was not finalised at the TC C&L, May 2007 (Follow-up V, Ispra, 29 May 2008). In a response on follow-up IV of this meeting (ECBI/53/06 Add.18), the Netherlands supported a continuation of the discussion on the method, however, using the method provisionally agreed by TC C&L (use the order of magnitude; go down from 10 % for the limit for classification), the following SCLs for repeated dose toxicity would apply:

Repeated dose oral toxicity:

T: 10% / 5 (limit) * 0.005 (serious effect concentration) = 0.01%

Xn: 10% / 50 (limit) * 0.005 (serious effect concentration) = 0.001%

The SCLs are based on serious damage seen at 0.1 mg/kg food (= 0.005 mg/kg bw/day) in the longest study in rats.

As no data are available for dermal and inhalation, the same values are used for these routes.

Based on worst-case simplifications and taken into account the SCLs for both acute and repeated dose toxicity, this would result in the following limits:

C > 0.06%: T+; R26/27/28 – 48/23/24/25

0.03% < C < 0.06%: T+; R26-24/25 - 48/23/24/25

0.007% < C < 0.03%: T; R23/24/25 – 48/23/24/25

0.0007% < C < 0.007%: Xn; R20/21/22 – 48/20/21/22

Conclusion on classification according to Regulation EC 1272/2008

Classification

Serious effects were observed in the 90-day rat study at levels (0.005 mg/kg bw) (key study A6.4.1/01) below the criterion of "oral, rat ≤ 10 mg/kg bw/day for 90-days" used for classification with STOT Rep. 1 H372 for the oral route. For classification for the dermal and inhalatory routes,

oral data can be used. The acute data indicate a comparable absorption for all three routes. Further, there is a large margin between the oral dose levels indicating severe effects and the limit value for STOT Rep. 1. Also, the acute LD50 values for all three routes were already below the limits for classification as toxic after repeated exposure. Based on these findings, we propose to classify flocoumafen with STOT Rep. 1 without a specific route and stating the blood as the main affected organ: H372: "Causes damage to the blood through prolonged or repeated exposure".

Specific concentration limits

An SCL for STOT Rep. 1 is proposed of 0.05% based on serious damage seen at 0.1 mg/kg food (= 0.005 mg/kg bw/day) in the longest study in rats. Calculation: 0.005 mg/kg bw/day (effective dose) / 10 mg/kg bw/day (limit) * 100% = 0.05%. STOT Rep. 2 is proposed between 0.005% and 0.05% using the same data and method of calculation (limit: 100 resp. 10 mg/kg bw/day). This calculation is performed according to the method described in the Guidance on the Application of the CLP Criteria.

Proposed SCLs:

STOT Rep. 1 H372 above 0.05% and STOT Rep. 2 H373 between 0.005 and 0.05%.

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

Summary of the Dossier submitter's proposal

Short-term toxicity

Short-term daily exposure of rats to Flocoumafen in a 28d dietary study at 0, 0.01, 0.05, 0.1 or 0.2 mg/kg food (equivalent to 0.0005, 0.0025, 0.005 or 0.01 mg/kg bw/day; based on a conversion factor of 20 (JMPR[2]) resulted in increased mean prothrombin and activated partial thromboplastin times in females at 0.2 mg/kg food. At 0.1 mg/kg food, a slight statistically non-significant increase in activated partial thromboplastin time was noted in females. Decreased levels of plasma protein, alkaline phosphatase and cholesterol were noted in females at 0.1 and 0.2 mg/kg food. A statistically significant decrease in calcium and a statistically significant increase in chloride were noted in males at 0.2 mg/kg food. Histopathology, revealed a slight reduction of cytoplasmatic vacuolation of glycogenic type in the periportal parenchymal cells in livers of males at 0.2 mg/kg food.

Based on the decreased levels of plasma protein, alkaline phosphatase and cholesterol and increased activated thromboplastin times in females at 0.1 mg/kg food, the NOAEL was established at 0.05 mg/kg food (equivalent to 0.0025 mg/kg bw/day).

The 28-day oral toxicity study was considered acceptable by the DS for the toxicological evaluation of Flocoumafen.

Semi-chronic toxicity

In a 90-day oral toxicity study, rats were given diets containing Flocoumafen at 0, 0.01, 0.02,

[2] The conversion factor of 20 used to convert the dose expressed as mg/kg food to mg/kg bw/day for rats is based on the average food consumption of rats per day and is according to "Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticides Residues (JMPR). See also appendix I of http://www.who.int/foodsafety/chem/jmpr/en/prst_wp_gls.pdf

0.05, 0.1, 0.25 or 0.6 mg/kg food (equivalent to 0.0005, 0.001, 0.0025, 0.005, 0.0125 or 0.03 mg/kg bw/day; based on a conversion factor of 20 (JMPR)). All animals given 0.25 and 0.6 mg/kg food died during the study. Animals found dead or sacrificed during the study showed typical signs of anticoagulant toxicity, which included pale eyes and skin, dark or swollen areas on the body, blood around nose and eyes and blood in the urine. Increased mean prothrombin and activated thromboplastin times were noted in males and females at 0.1 mg/kg food. In females, an increased platelet count and plateletcrit were noted at 0.1 mg/kg food. Decreases in monocytes were noted in males at 0.05 or 0.1 mg/kg food (61 and 59% of control values, respectively), however, these changes were not accompanied by further haematological changes. Cholesterol levels were increased at 0.05 mg/kg food (109% and 106% of control values in males and females, respectively) and at 0.1 mg/kg food (111% and 114% of control values in males and females, respectively). The increased cholesterol levels at 0.05 and 0.1 mg/kg food were not considered toxicologically relevant, since observed changes were within the same range as the historical control values.

Slightly increased albumin values (2.1% relative to controls) and slightly decreased chloride values (2% relative to controls) were noted in males at 0.1 mg/kg food. Urinalysis revealed increased urine volume was noted in males at 0.1 mg/kg food and increased glucose was noted in females at 0.1 mg/kg food. Absolute and relative heart weights were increased in males at 0.1 mg/kg food (108% of control), in the absence of histopathological correlation. Upon histopathological examination, increased incidences of haemorrhages were noted in several organs in the two highest dose groups, mainly in males (e.g. testes, prostate, epididymides, and urinary bladder). Centrilobular degeneration was noted at 0.25 and 0.60 mg/kg food in males and females and multifocal necrosis in liver of males at 0.6 mg/kg food. Haematopoiesis was noted in spleen of males at 0.25 and 0.6 mg/kg food and of females at 0.1 and 0.25 mg/kg food. A statistically significant increase in haemorrhages was noted in lymph nodes at 0.10, 0.25 and 0.60 mg/kg food. The incidence of lymph node haemorrhages was within the range of historical controls at 0.01, 0.02 or 0.05 mg/kg food. Based on the haemorrhages seen in lymph nodes, a NOAEL of 0.05 mg/kg food was established (equivalent to 0.0025 mg/kg bw/day).

The 90-day oral toxicity study was considered by the DS to be acceptable for the toxicological evaluation of Flocoumafen, because it could safely be assumed that the quantity of vitamin K3 in the diet would be insufficient to counteract any haemorrhagic effects caused by the test substance.

Long-term toxicity

Based on the expected exposure pattern, for trained and non-trained professional users chronic primary and secondary exposure cannot be excluded. However, performance of a chronic toxicity study with rodents might be technically difficult (extremely low doses necessary) and might induce unnecessary harm to laboratory animals.

Repeated dose dermal toxicity studies were not considered necessary, since route specific effects were not to be expected (based on acute oral and dermal toxicity data) and since there was no evidence of enterohepatic circulation or a first-pass effect (based on ADME studies).

Dossier Submitter's conclusion on classification

Serious effects were observed in the 90-day rat study at levels (0.005 mg/kg bw) (key study) below the criterion of "oral, rat $\leq$ 10 mg/kg bw/day for 90-days" used for classification as STOT RE 1; H372 for the oral route. Oral data can be used for classification of STOT-RE via the dermal and inhalatory routes. The acute toxicity data indicated comparable absorption for all three routes. Furthermore, there was a large margin between the oral dose levels indicating severe effects and the limit value for STOT RE 1. Also, the acute LD_{50} values for all three routes were already below the limits for classification as toxic after repeated exposure. Based on these findings, the DS proposed to classify Flocoumafen as STOT RE 1 without a specific route and stating the blood as the main affected organ: H372: "Causes damage to the blood through prolonged or repeated exposure".

Specific concentration limits

An SCL of 0.05% for STOT RE 1 was proposed based on serious damage seen at 0.1 mg/kg food (= 0.005 mg/kg bw/day) in the longest study in rats. Calculation: 0.005 mg/kg bw/day (effective dose) / 10 mg/kg bw/day (limit) * 100% = 0.05%. An SCL for STOT RE 2 between 0.005% and 0.05% was proposed using the same data and method of calculation. This calculation was performed according to the method described in the Guidance on the Application of the CLP Criteria.

Proposed SCLs:

STOT RE 1; H372 above 0.05% and STOT RE 2; H373 between 0.005 and 0.05%.

Comments received during public consultation

One MS agreed with the classifications proposed by the DS for the end-points of repeated dose toxicity for Flocoumafen.

Assessment and comparison with the classification criteria

In the opinion of RAC the existing data warrant classification of Flocoumafen as proposed by the DS as STOT RE 1 without a specific route and stating the blood as the main affected organ: H372: "Causes damage to the blood through prolonged or repeated exposure" according to CLP criteria.

Death of all exposed animals due to anticoagulation effect of Flocoumafen was observed in the 90-day rat study at levels (0.0125 and 0.03 mg/kg bw/day) (key study) which is well below the CLP criterion of "oral, rat ≤ 10 mg/kg bw/day for 90-days" used for classification with STOT RE 1; H372 for the oral route.

Taking into account a high absorption of Flocoumafen through skin and in respiratory system as indicated by comparison of oral LD_{50} with dermal LD_{50} and inhalation LC_{50} in rats the classification based on results of 90-day oral exposure should be extended to include the other routes.

An SCL for STOT RE 1 (H372) of 0.05%, as proposed by the DS, is supported by RAC based on serious damage seen at 0.1 mg/kg food (ED 0.005 mg/kg, haemorrhage in lymph nodes, rat, after 90 days) in the 90-day study in rats. Calculation: 0.005 mg/kg bw/day (effective dose) / 10 mg/kg bw/day (limit) * 100% = 0.05%.

An SCL for STOT RE 2 (H373), as proposed by the DS, between 0.005% and 0.05% using the same data and method of calculation is supported by RAC. This calculation is performed according to the method described in the Guidance on the Application of the CLP Criteria.

5.8 Mutagenicity

5.8.1 In vitro data

Flocoumafen was not mutagenic in a *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (A6.6.1/01). In an *in vitro* cytogenetic assay flocoumafen was not genotoxic with the restriction that no control chemical was tested to indicate the metabolic activation of the rat liver (RL4) cells (A6.6.1/01). Flocoumafen is non mutagenic in an *in vitro* gene mutation study (HPRT) with Chinese hamster lung fibroblasts (A6.6.3/01).

In vitro genotoxicity studies are presented in Table 5.11.

Supportive data

Supportive data for *in vitro* gene mutation study in mammalian cells are presented in Table 5.10.

Table 5.10: Supportive *in vitro* gene mutation study

The following study is considered to contain additional information concerning in vitro genotoxicity and is thus presented in tabular format as supportive data (non-GLP study):			
Study number	**Title**	**System**	**Results**
A6.6.3/02	Genotoxicity studies with WL108366 (rodenticide): in vitro cell transformation studies	Mouse embryo fibroblasts (C3H10T ½)	It was concluded that flocoumafen did not induce in vitro cell transformation in C3H10T ½ fibroblasts, either in the presence or in the absence of metabolic activation under the experimental conditions of the study.

5.8.2 In vivo data

Flocoumafen did not induce chromosome aberrations in an *in vivo* chromosome study with rat bone marrow cells (A6.6.4/01). The *in vivo* chromosome aberration study is presented in Table 5.12.

The effects of Flocoumafen on the incidence of chromosomal damage was tested in rats receiving 0.25 or 1000 mg/kg b.w by gavage (vehicle corn oil). The test method was based on OECD 475 (1984) and EEC Directive 79/831 Annex V (1982) Part B. to be. No mitotic index determined as a measure of cytotoxicity was reported. 50 cells instead of at least 100 cells were analysed for each animal. Increases in polyploidy or endoreduplication were not reported. Bone marrow of 5 SD rats per sex and group at 6, 24 and 48 hours after treatment were analysed (50 cells) for number and types of structural aberrations.

No animals died during the course of the study. Clinical signs commonly observed in animals treated with the test substance were slight to moderate diarrhoea, pilo-erection and hunched posture. In addition, lethargy, decreased respiratory rate, ptosis, pallor of extremities, gasping, cyanosis or thin appearance were observed in animals sacrificed 48 hours after treatment. Results are provided in table 5.12.

5.8.3 Human data

Not available.

5.8.4 Other relevant information

None.

5.8.5 Summary and discussion of mutagenicity

Studies relevant for classification are summarized in Table 5.11 and Table 5.12.

Table 5.11: Genotoxicity studies, *in vitro*, relevant for classification

Test system Method Guideline	Organism/ strain(s)	Concentra-tions tested	Result		Remark	Study number
			+ S9	- S9		
Bacterial reverse mutation test, in accordance with EC B.13/14 and OECD 471	S. typhimurium: TA 98, TA 100, TA 1535, TA 1537, TA 1538; E. coli: WP2 uvr-A pKM 101	31.25–2000 µg/plate	-	-	No evidence of cytotoxicity. Acceptable	A6.6.1/01
In vitro chromosome aberration test, predominantly in accordance with EC B.10 and OECD 473	Rat liver (RL4) cells	5–37.5 µg/ml	n.d.	-	Acceptable	A6.6.1/01
In vitro mammalian cell gene mutation test (HPRT), in accordance with EC B.17 and OECD 476	Chinese hamster lung fibroblasts (V79)	5–150 µg/ml	-	-	Acceptable	A.6.6.3/01

n.d. :no data available

Table 5.12: Genotoxicity study, *in vivo*, relevant for classification

Type of test Method/ Guideline	Species Strain Sex no/group	Frequency of application	Sampling times	Dose levels	Results	Remarks	Study number
Mammalian bone marrow chromosome aberration test, in accordance with OECD 475	Rat, Sprague-Dawley, 15/sex/dose	Single (24 hrs)	6, 24 and 48 h (post exposure)	0.25 and 1000 mg/kg b.w.	Positive contr., 24 hrs: + Vehicle contr., 6 hrs: - Vehicle contr. 24 hrs: - Vehicle contr. 48 hrs: - 0.25 mg/kg bw, 6 hrs: - 0.25 mg/kg bw, 24 hrs: - 0.25 mg/kg bw, 48 hrs: - 1000 mg/kg bw, 6 hrs: - 1000 mg/kg bw, 24 hrs: - 1000 mg/kg bw/, 48 hrs: -	Acceptable	A6.6.4/01

Classification based on 67/548/EEC

In vitro, flocoumafen did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 and the *E. coli* strain WP2uvrA, both with and without metabolic activation. Flocoumafen was negative in a chromosome aberration study with rat liver cells and in a gene mutation test using V79 hamster cells. In addition, flocoumafen was negative in an *in vivo* rat

chromosome aberration test (key study A6.6.4/01). In conclusion, flocoumafen is considered to be non-genotoxic. Therefore, it is proposed not to classify for mutagenicity.

Classification based on Regulation EC 1272/2008

In vitro, flocoumafen did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 and the *E. coli* strain WP2uvrA, both with and without metabolic activation. Flocoumafen was negative in a chromosome aberration study with rat liver cells and in a gene mutation test using V79 hamster cells. In addition, flocoumafen was negative in an *in vivo* rat chromosome aberration test (key study A6.6.4/01).. In conclusion, flocoumafen is considered to be non-genotoxic. Therefore, we propose not to classify for mutagenicity.

5.8.6 Comparison with criteria

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Flocoumafen was not mutagenic in a *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay. In an *in vitro* cytogenetic assay Flocoumafen was not genotoxic with the restriction that no control chemical was tested to indicate the metabolic activation of the rat liver (RL4) cells. Flocoumafen was not mutagenic in an *in vitro* gene mutation study (HPRT) with Chinese hamster lung fibroblasts.

Flocoumafen did not induce chromosome aberrations in an *in vivo* chromosome study with rat bone marrow cells.

The effects of Flocoumafen on the incidence of chromosomal damage were tested in rats receiving 0.25 or 1000 mg/kg b.w by gavage (vehicle: corn oil). The test method was based on OECD 475 (1984) and EEC Directive 79/831 Annex V (1982) Part B. No mitotic index was determined as a measure of cytotoxicity was reported. Increases in polyploidy or endo-reduplication were not reported. Bone marrow from 5 SD rats per sex/dose group at 6, 24 and 48 hours after treatment were analysed (50 cells per animal, instead of 100 cells as required by the TG) for number and types of structural aberrations.

No animals died during the course of the study. Clinical signs commonly observed in animals treated with the test substance were slight to moderate diarrhoea, piloerection and hunched posture. In addition, lethargy, decreased respiratory rate, ptosis, pallor of extremities, gasping, cyanosis or thin appearance were observed in animals sacrificed 48 hours after treatment.

In vitro, Flocoumafen did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 and the *E. coli* strain WP2uvrA, both with and without metabolic activation. Flocoumafen was negative in a chromosome aberration study with rat liver cells and in a gene mutation test using V79 hamster cells. In addition, Flocoumafen was negative in an *in vivo* rat chromosome aberration test (key study). In conclusion, Flocoumafen is considered to be non-genotoxic. Therefore, the DS proposed no classification for mutagenicity.

No classification is currently included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the opinion of RAC the reported mutagenicity studies do not warrant classification of Flocoumafen for germ cell mutagenicity; no genotoxic effects were observed in experimental studies.

TC-C&L conclusion

The TC-C&L concluded in November 2006 (ECBI/20/07, Rev. 1) that classification for mutagenicity was not required.

5.9 Carcinogenicity

Based on the expected exposure pattern, for trained and non-trained professional users chronic primary and secondary exposure cannot be excluded. However, performance of a carcinogenicity study with rodents might be difficult due to technical problems (extremely low doses necessary) and might induce unnecessary harm to laboratory animals. Flocoumafen is considered to be non-genotoxic. Therefore, it is concluded that a carcinogenicity study with flocoumafen is not considered necessary for the registration of flocoumafen according to Directive 91/414/EEC.

5.9.1 Summary and discussion of carcinogenicity

No data available. Read-across with warfarin and other structural related coumarin derivatives was not done for carcinogenicity. Flocoumafen is considered to be non-genotoxic. No classification is proposed for both legislation 67/548/EEC and the new regulation EC 1272/2008.

5.9.2 Comparison with criteria

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007.

TC-C&L conclusion

The TC-C&L concluded in November 2006 (ECBI/20/07, Rev. 1) that classification for carcinogenicity was not required.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No data on carcinogenicity of Flocoumafen were available. Performance of a carcinogenicity study with rodents might be technically difficult (extremely low doses necessary) and might induce unnecessary harm to laboratory animals. Flocoumafen is considered to be non-genotoxic. Therefore, it was concluded by the DS that a carcinogenicity study with Flocoumafen is not considered necessary for the registration of Flocoumafen according to Directive 91/414/EEC.

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed by the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

There is no human or animal evidence suggesting that Flocoumafen has carcinogenic properties. Taking into account the high repeated dose toxicity of Flocoumafen in rats, a carcinogenicity study might be very difficult to carry out due to high mortality of animals exposed even at very low doses. RAC therefore supported the DS proposal for no classification for carcinogenicity of Flocoumafen.

5.10 Toxicity for reproduction

5.10.1 Effects on fertility

A two-generation reproduction study (A6.8.2) was not available as it was waived by the notifier of the biocide-dossier of flocoumafen. Instead the notifier had submitted a reference from the open literature.

Supportive data

In this reference from the open literature (Sangha et al., 1992), one group of rats received a single oral dose (17 females per dose group, gavage) of 0.08, 0.11 or 0.14 mg/kg bw. After one week ovaries were weighted and investigated histopathologically. A second group received 0 and 0.14 mg/kg (13 animals per dose group, gavage); after one week levels of total lipids, total cholesterol, phospholipids, free fatty acids, glycolipids and triglycerides were determined in the ovaries of half of the animals. The other half of the animals was paired and the breeding time and litter size were recorded.

In the first group, ovarian cyclicity was disturbed in all the treated rats, in the two highest dose groups most of them remained in di-oestrous stage. Decreased ovary weights, artretic follicles and degenerating corpora lutea with pyknotic granules were noted at 0.14 mg/kg bw.

In the second group increased levels of total lipids, triglycerides and cholesterol and decreased levels of phospholipids, free fatty acids and glycolipids were noted. Of the paired animals, control animals bred after 30 days and gave seven or eight pups per litter. Treated animals bred after 60 days and gave two to four pups per litter. 45 days after parturition, the second breeding was normal with an equivalent litter size (five to eight pups) for both groups.

In this study on female rats with flocoumafen, effects on ovary and fertility were noted after a single dose of flocoumafen. However, this study did not fulfil requirements of guideline studies, and

reporting of methods and results were very limited (no attention was paid to clinical symptoms and haematological and pathological parameters). Furthermore, the study indicated that effects on ovary and fertility occurred after single oral dosing with flocoumafen with doses close to the LD50 values (range: 0.13-0.5 mg/kg bw) possibly causing internal bleedings. For these reasons, it cannot be excluded that the effects on fertility were secondary to haemorrhages.

In the 90-day oral rat study (A6.4.1/01, see section 5.6.1) effects on male reproductive organs (haemorrhages in testes, prostate and epididymides) were observed in the animals of the two highest dose groups (0.25 and 0.6 mg/kg food, equivalent to 0.0125 and 0.03 mg/kg bw per day). But it should be noted that all animals of these dose groups died during the study and haemorrhages were noted in several organs.

5.10.2 Developmental toxicity

The notifier of the biocides-dossier on flocoumafen submitted three teratogenicity studies, one in rabbits, and two in rats.

In a teratogenicity study with rabbits (A6.8.1/01, A6.8.1/02), animals were dosed with 0, 0.001, 0.002 or 0.004 mg/kg bw/day by gavage, from day 6 to day 18 post mating. Maternal animals at 0.004 mg/kg bw/day showed abortions (3 of 14 animals), and the presence of blood on the tray paper on days 19 to 29 in 6 out of 11 dams with live young, and a slight increased incidence of fur loss in the post dosing period. The NOAEL for maternal effects is established at 0.002 mg/kg bw/day. Since no toxicologically relevant developmental effects were observed and no teratogenic effects were reported, the NOAEL for developmental and teratogenic effects was set at >0.004 mg/kg bw/day.

In a teratogenicity study with rats (A6.8.1/03), pregnant animals were given doses of 0, 0.01 or 0.04 mg/kg bw, from day 8 to 17 post mating. At the high dose level, females showed clinical signs of toxicity (pale eyes, lethargy and haemorrhage from vulva), indicative of anti-coagulant poisoning. At necropsy, animals showed internal haemorrhage. Maternal animals at 0.01 mg/kg bw showed no signs of toxicity. No effects on number of live pups, litter weight and surviving pups were noted. No external abnormalities were observed. As animals delivered naturally, the number of corpora lutea could not be reported and pre- and post implantation loss was not calculated. Pups were not examined for skeletal and soft tissue alterations. Under the circumstances of the study, flocoumafen did not induce developmental effects in rats at dose levels up to 0.04 mg/kg bw. However, considering the limited study design, a NOAEL for developmental and teratogenic effects is not established. The NOAEL for maternal effects is established at 0.01 mg/kg bw/d.

In a teratogenicity study (A6.8.1/04, A6.8.1/05), 55 rats per group were mated and were given flocoumafen (in corn oil) at levels of 0, 0.01, 0.02 and 0.04 mg/kg bw/day from day 7 to 17 post mating. Many animals were found non-pregnant (16, 18, 11, and 3 in the control, low, mid and high dose group) and were discarded. Ten dams per dose level were allowed to litter and to rear their offspring to weaning, and the remaining females were allocated to day 20 sacrifice and their foetuses were preserved for visceral and skeletal examination. From the dams that were allowed to litter the F1 offspring were examined in specific behavioural tests and excess pups were sacrificed and examined for abnormalities. F1 offspring were mated at 12 weeks and females were allowed to litter and rear their offspring to weaning. F2 pups and F1 adults were sacrificed and examined for abnormalities.
At 0.04 mg/kg bw/day P females showed mortality (10 females in the 20 day sacrifice group and one female in the group that was allowed to litter), anticoagulant signs and/or haemorrhages at

necropsy. One female of the mid-dose group with life young at day 20 also showed clinical signs of toxicity (unsteady walking, pale extremities and bleeding from vagina on day 20). Malformations (M) or abnormalities were observed in some foetuses of the 20 day sacrifice group in the control and treated groups. Two small foetuses (< 2.3 g) were found in the high dose group, one of which had a small left eye and one small foetus was found in the mid-dose group, which also showed microphthalmia. The incidence of the most relevant findings, are depicted in the table below:

	control	Low dose	Mid dose	High dose
Visceral malformations:				
haemorrhage	6	8	5	5
Cleft palate (M)	1			
Hydrocephaly (M)	1			
Incomplete vena cava+ absent artery	1			
Diaphragmatic hernia (M)		1		
Retro-oesophageal aortic arch		1		
Microphthalmia or small eye			1**	1**
Skeletal malformations				
multiple irregularities *		1		
umbilical hernia		1		
Distortions/ossification irregularities		1		
scoliosis			1	
Interventricular septal defect				1

*: a.o. cleft palate, brachygnathia, etc
** found in small foetus

Based on these observations the NOAEL for maternal toxicity was established at 0.02 mg/kg bw/day. No toxicological relevant effects were observed on F1 and F2 litter. Pre-weaning development was similar in offspring from all groups. Therefore, the NOAEL for developmental toxicity was established at >0.04 mg/kg bw/day.
Since no teratogenic effects were reported, the NOAEL for teratogenic effects was set at >0.04 mg/kg bw/day.

The teratogenicity studies are presented in Table 5.13.

5.10.3 Human data with flocoumafen

No data available.

5.10.4 Other relevant information

Information on structural analogues and possibilities for read-across

Flocoumafen belongs to the group of coumarins and as such is a structural analogue of warfarin, the most well-known coumarin. The coumarins are used as rodenticides and are known as anti-vitamin K (AVK) rodenticides. These rodenticides have a chemical structure resembling vitamin K and inhibit the coagulation, most likely via the same mechanism, namely inhibition of the vitamin K (epoxide) reductase complex. Vitamin K epoxide reductase (VKOR) is an integral membrane protein that catalyzes the reduction of vitamin K 2,3-epoxide and vitamin K to vitamin K hydroquinone, a cofactor required for the gamma-glutamyl carboxylation reaction. VKOR is highly

sensitive to inhibition by warfarin. Warfarin inhibition of VKOR decreases the concentration of reduced vitamin K, which reduces the rate of vitamin K-dependent carboxylation and leads to under-carboxylated, inactive vitamin K-dependent proteins (Tie and Stafford, 2008). There are several proteins requiring carboxylation to become active including several coagulation factors and bone proteins (Furie et al., 1999). Inhibition of VKOR results in effects on coagulation and bone formation (Howe and Webster, 1999; Hall *et al*., 1980).

Warfarin is an anticoagulant compound which has been used in patients for many years to avoid or reduce blood coagulation. No effects on fertility in humans have ever been observed, and also in a two generation reproduction study in rats with vitamin K supplementation warfarin did not show any effect on fertility. Warfarin is therefore not classified for effects on fertility. It is, however, classified as Repro. Cat. 1; R61, because warfarin was found to induce teratogenicity in humans.

The mechanism for the developmental effects of warfarin in humans is probably caused by VKOR inhibition. However, other mechanisms such as reduced transfer of vitamin K from the mother through the placenta to the foetus cannot be excluded. A reduction in foetal vitamin K levels due to lower maternal plasma levels caused by the inhibitory effect of warfarin is unlikely to be part of the mechanism as warfarin treatment in patients does not reduce the vitamin K plasma levels (Nakamura et al., 1994).

When discussing flocoumafen in 2006, TC C&L took note of the negative results in the three developmental toxicity studies (see section 5.9.2), the absence of human data on flocoumafen, and the fact that, given the similarities in mechanism, flocoumafen could potentially induce the same developmental effects as warfarin when present in the foetus at relevant concentrations. It was thus questioned whether the results from the data on warfarin in humans should be used for read-across to flocoumafen and also to other anticoagulant rodenticides. This question was forwarded to the Specialised Experts on reprotoxicity. Their conclusion was (ECBI/121/06):

Warfarin is an established human teratogen classified as Repr. Cat. 1; R61. It is uncertain whether teratogenicity of warfarin can be detected in pre-natal developmental toxicity studies (including OECD guideline 414). The teratogenic mechanism of warfarin is likely to involve maternal Vitamin K depletion and/or direct effects on embryo/foetus via transplacental exposure. Given the vitamin K inhibition, there is concern that other anti-vitamin K (AVK) compounds could cause similar teratogenic effects as warfarin in humans.

The other AVK rodenticides have not shown teratogenic effects in conventional rat and rabbit developmental studies and there is no data in humans. Given the uncertainties surrounding the ability of the standard pre-natal developmental toxicity studies to detect warfarin teratogenicity the predictive value to humans of these studies is uncertain.

On the basis of currently available data, there are no convincing arguments that other AVKs including the second generation compounds could not pass the placenta. Both the mechanism of action and the possible placental passage give reason for concern of possible teratogenicity in human.

Considering all the available information the Specialised Experts unanimously agreed that the AVK rodenticides should collectively be regarded as human teratogens. Therefore the other AVK rodenticides should be classified as Repr. Cat. 1; R61.

Based on the advice of the Specialised Experts, the TC C&L (2007) agreed with R61: however, Repr. Cat. 1 or Cat. 2 was still under discussion (for flocoumafen no human data exist and animal data were negative). (Follow-up V, Ispra, 29 May 2008).

As industry did not agree with a simple read-across from the classification of warfarin, further rat studies on developmental toxicity of warfarin and on placental transfer of warfarin and flocoumafen have been conducted by industry as a follow-up to the TC C&L discussions. These studies were provided to the RMS in the year 2010, together with some additional references from the open literature on human data. These studies were not available to the Specialised Experts and the TC&CL in May 2007, and are presented below. Apart from these studies the MSCA added some additional references from open literature.

Human data on warfarin (based on the Irish CLH proposal for warfarin)

The following extract from Pesticide DAR summarises the human clinical data ,and reviews of clinical data, which are key to the current classification and labelling of Warfarin as Toxic for reproduction Category 1, T; R 61 May cause harm to the unborn child:
In two reviews (Schardein, 1985; Hall et al., 1980), retrospective summaries of case reports in which the administration of Warfarin during pregnancy induced birth defects were presented, together with a description of the encountered malformations or other effects, and the dosage of Warfarin involved. The duration of exposure in most of the 22 cases reviewed in detail by Hall et al. (1980) extends far beyond the first trimester (> week 30 of gestation). The daily dose of Warfarin was usually between 5-10 mg/day, only in one case at 2.5-5 mg/day. The following case reports were submitted and represent a selection from the published literature of warfarin-associated adverse developmental outcomes.

Reference	Patient Treatment	Time of treatment	Outcome
Kerber, I. J. et al. (1968)	*Warfarin (7.5 mg/day)* Digitalis Penicillin	Preconception to 31 weeks	Nasal hypoplasia Mental retardation Brachydactyly Scoliosis and other skeletal abnormalities

Reference	Patient Treatment	Time of treatment	Outcome
Bloomfield, D. K.; Rubinstein, L.I. (1969)	*Warfarin sodium (av. 6.25 mg/day)* Penicillin Digoxin	Preconception to 36 weeks	Normal female.
Becker, M. H. et al. (1975)	1. *Warfarin (-)* Digoxin Sulfisoxazole Erythromycin 2. *Warfarin (7.5 mg/day)* Digoxin	Preconception to 26 weeks. Throughout pregnancy	Nasal hypoplasia Optic atrophy Mental retardation Kyphoscoliosis Shortened proximal extremities Nasal hypoplasia Opacification of optic lens. Poorly developed ears Punctate calcification of vertebra and epiphyseal regions.
Shaul, W. L. et al. (1975)	*Warfarin sodium (2.5-5 mg/day)* Diazapam (briefly) Furosemide (2 wks at 26 weeks)		Nasal hypoplasia Vertebral stippling
Fourie, D. T. ; Hay, I. T. (1975)	*Warfarin sodium (5 mg/day)* Digoxin Furosemide Pottassium Isoptin	Preconception to week 36.	Nasal hypoplasia Choanal stenosis Short fingers, dysplastic nails Chondrodysplasia punctata
Barr, M.; Burd, A. R (1976)	*Warfarin sodium (7.5 mg/day)* Propanolol	Preconception to 17 weeks (elective abortion)	Nasal hypoplasia Large protuberant eyes Short fingers Hypertelorism

Reference	Patient Treatment	Time of treatment	Outcome
Carson, M.; Reid, M. (1976)	Warfarin (20 mg –3 mg – 4.5 mg/day)	Wk 12.5 to wk 36	Microcephaly Bifrontal narrowing Mental retardation Spastic
Holzgreve, W. et al. (1976)	*Warfarin (-)*	6mths preconception to wk 12 of gestation.	No abnormalities apparent at birth Retarded psycomotor development at 5 mths.
Abbott, A. et al. (1977)	*Warfarin* (6-7 mg/day)	Preconception to 24 wks.	Nasal hypoplasia Epiphyseal stippling Chonrodysplasia punctata.
Smith, M. F.; Cameron, M. D. (1979)	*Warfarin (-)*	Throughout pregnancy	Nasal hypoplasia Hypertelorism Tachycardia Hepatomegaly Generalised oedema
Stevenson, R. E. et al. (1980)	*Warfarin (5 mg/day)*	Throughout	Nasal hypoplasia Optic atrophy Developmental retardation

The administration of Warfarin to women during pregnancy has been shown to cause a well-defined complex of malformations in some of the offspring. This occurs as a result of exposure during the first trimester. This syndrome has been designated as “warfarin embryopathy” or “foetal warfarin syndrome’ (FWS). The risk of malformation to the foetus of a mother treated with warfarin is not known with certainty. Schardein (1985) assessed the risk of malformation due to exposure to Warfarin during pregnancy as in the order of 1:5. More recently, a review of the maternal and foetal risks associated with oral anticoagulants (OA) indicated that the use of OA throughout pregnancy was associated with embryopathy in 6.4% (95% confidence interval [CI], 4.9% - 8.9%) of live births. Substitution with heparin at or prior to 6 weeks and up to 12 weeks was reported to remove this risk (Chan, 2000). Such malformations are still being reported in the literature, due to the necessity of treatment of patients (with e.g., mechanical heart valves), with warfarin, even after pregnancy has been detected (Howe, et. al., 1997 , Chan and Ginsberg, 20002, Gohlke-Barwolf, 2001 , Ginsberg and Hirsh, 2001 ,).

The most consistent feature of FWS is a hypoplastic nose, caused by underdeveloped nasal cartilage. The degree of severity is varied from mild abnormality to severe breathing and feeding

difficulties. Bone abnormalities of the axial and appendicular skeleton (radiological stippling of the vertebral column) often also occur. Punctate calcification of other bone sites may also be present. Kyphoscoliosis, abnormal skull development, and brachydactyly have been observed as associated skeletal effects. It is believed that avoidance of exposure to OA during weeks 6-12 of gestation should avoid warfarin embryopathy. It should be noted that exposure to coumarins during the first trimester was associated with a high rate of spontaneous abortions, in addition to the incidences of specific embryopathy. Likewise, exposure during the first and second trimester was also associated with a high rate of spontaneous abortion, stillbirths and warfarin-related complications (developmental abnormality) (Hall et. al.,1980).
Exposure after this time interval (first trimester) is associated with an apparently separate series of warfarin-related adverse effects, not related to warfarin embryopathy, per se. Adverse effects on the central nervous system predominate and include hydrocephaly or microcephaly, microphthalmia, various eye abnormalities, Dandy-Walker malformation and other CNS malformations often associated with degrees of mental retardation (Kaplan, 1985, Pati and Helmbrecht, 1993).

A more recent literature survey has been provided by industry (BASF, 2010) in support of the floucoumafen CLH dossier, which includes literature published since that submitted for the Warfarin Plant Protection DAR and the Biocide CAR (up to 1994) and also the some of older literature. An extract of industry summary of this survey is included below:
The risk of adverse foetal effects due to Warfarin treatment in humans is difficult to estimate, due to the inhomogeneous data base: Some review articles evaluate complication rates in relation to pregnancies, others to live births, and this cannot always be resolved, due to incomplete information given in some articles. Nevertheless, since the number of pregnancies is predominantly referred to, this approach is adopted for the current overall evaluation. In case of significant overlap between review articles only the most comprehensive and reliable one was considered for deriving an overall foetal complication rate based on most recent data, resulting in the selection presented in Table 1. Furthermore, the data base has been restricted to Warfarin exposures only (ignoring other anticoagulants, e.g. Acenocoumarol) where possible. For details also see discussion of individual articles above.
Accordingly, based on the available data the risk for embryopathy due to Warfarin treatment in sensitive periods of gestation is 4.3 %, relative to the number of pregnancies. This is in agreement with other authors, estimating the malformation risk to be “probably below 5 %” (De Swiet, 1987), or otherwise frequently in the range of 4–7 %, with some studies even reporting 0 % (Chan, Anand & Ginsberg, 2000; Hung & Rahimtoola, 2003; van Driel et al., 2002; Oakley, 1955; Hall, Pauli, & Wilson, 1980; Schaefer et al., 2006).
Other significant risks to the foetus or the newborn are associated with Warfarin treatment: Spontaneous abortion (27.3 %%, aggregated figure based on Blickstein && Blickstein, 2002; Chan, Anand & Ginsberg, 2000; Oakley & Doherty, 1976; Arnaout et al., 1998; Khamooshi et al., 2007; Shannon et al., 2008), stillbirth (27..1 %, based on the same articles except Oakley,, 1976), neonatal death (3.1 %; Blickstein & Blickstein, 2002; Oakley & Doherty, 1976; Arnaout et al., 1998; Khamoshi et al., 2007), CNS defect (4.33 %; Hall, Pauli & Wilson, 1980; Oakley & Doherty, 1976), premature delivery (66.2 %; Blickstein & Blickstein, 2002; Hall, Pauli, & Wilson, 1980), haemorrhage (2.2 %; Hall, Pauli, & Wilson, 19 80), and ocular atrophy (Greaves, 19933; Hall, Pauli, & Wilson, 1980).

Table 1. Compilation and analysis of literature on Warfarin embryopathy in humans (a number of papers cited were not considered, either due to extensive overlap with the selected articles, or since merely citing and reiterating conclusions from other evaluations, Bates et al.

(2008)), Srivastava et al. (20007), Ginsberg et al. (2003), van Driel et al. (2002), Oakley (1995), and Pauli (1988)).

Reference	No.	Embryopathy	
		No.	%
Hung and Rahimtoola, 2003 -pregnancies -live births	637 472 84	28 44 2	4.4 9.3 2.4
Blinckstein & Blickstein, 2002 -pregnancies -live births	792 549 224	35 35 16	4.4 6.4 7.1
Hall, Pauli & Wilson, 1980	224	16	7.1
Schafer, et al., 2006 -warfarin -All AVKs (live births)	66 356	0 2	0 0.6
Cotrufo et al., 2002 -pregnancies	71	4	5.6
Oakley & Doherty, 1976 -pregnancies	11	1	9.1
Arnaout et al., 1998	18	0	0.0
Srivastava et al., 2002	30	3	10
Geelane et al., 2005	150	0	0
Khamooshi et al., 2007	142	7	4.9
Akhtar et al., 2007	43	0	0
Shannon et al., 2008	11	1	9.1
Total, relative to number of pregnancies	**2279**	**97**	**4.3**

Animal data on warfarin

1. Teratogenicity study in rats

Characteristics

Reference/notifier	:	Kubaszky (2009)	Exposure	:	day 6-15 (TP 1) or day 6-19 (TP 2)
Type of study	:	Teratogenicity	Doses	:	0, 0.125, 0.150, 0.200, 0.250 mg/kg bw per day
Year of execution	:	2007	Vehicle	:	aqueous CMC
Test substance	:	Warfarin sodium	GLP statement	:	Yes

Route	:	Oral by gavage	Guideline	:	OECD 414
Species	:	Rat Crl:(Wi) BR-Wistar	Acceptability	:	yes
Group size	:	25 dams per group, except high dose group 12 dams/group	$NOAEL_{mat}$	:	0.125 mg/kg bw per day
			$NOAEL_{dev}$	:	< 0.125 mg/kg bw per day

Study design

Study was performed according to OECD 414. There were two treatment regimens. In the first group (TP1) the sperm positive females were exposed to warfarin from days 6-15 post coitum (i.e. during the period of organogenesis according to the old guideline) and the second group from days 6-19 post coitum (treatment according to the new (2001) guideline).

One deviation from the guideline was that in both treatment regimens the high dose group (0.250 mg/kg bw per day) was added after the other groups had started. This dose group was added to demonstrate clear maternal toxicity. From the test report it is not clear when exactly the high dose group had started, but it seems to be about 4-6 weeks later. As a consequence the following deviations from the OECD guideline are inevitable:

- the high dose group females paired with the same male were not always allocated to different treatment groups.

- the high dose group females were older thus body weights were higher.

- it should be noted that no extra control group was started together with the extra high dose group.

These deviations may affect the dose response relation.

Furthermore, it should be noted that in this study the interval between the dose levels is very small: 4 dose levels are chosen within a factor 2! Therefore, it is difficult to detect a dose relation in the effects observed in the treated groups.

Results

Table B.6.6.2-1 Results from study TP1: exposure during days 6-15 of gestation

	Dose (mg/kg bw per day)	0	0.125	0.150	0.200	0.250	dr
Maternal effects	Mortality (incl. moribund)			2/25	2/25	5/12	
	Clinical signs	0/25	1/25	3/25	7/25	8/12	dr
	- furless		1				
	- piloerection			3	4	5*	dr
	- paleness			2	3	5*	dr
	- reduced activity			2	4	5*	dr
	- vaginal bleeding			1	4	6*	dr
	- open vaginal orifice			1			
	- other [a]					1	
	Pregnant animals %	84	88	92	92	92	
	Abortions	-	-	-	-	-	
	Gravid uterine weight	No effect					
	Corpora lutea	No effect					
	Body weight	No effect					
	Food consumption	No effect					
	Water consumption	Not determined					
	Pathology						
	- reddish mottled lungs/point-like haemorrhages on lungs[b]	6/25	9/25	11/25	12/25	0/12	
	- uterine horn and/or uterus filled with blood	0	0	3/25	5/25	5/12	dr
Litter response	No of litters examined	18	20	19	17	6	

	Dose (mg/kg bw per day)	0	0.125	0.150	0.200	0.250	dr
	Live foetuses	251	292	267	221	84	
	Foetal weight	No effect					
	Pre implantation loss (%)	9	8	4	6	16	
	Post implantation loss (%)	7	6	9	19*	3	
	early embryonic death (%)	5	4	7	6	3	
	Total intrauterine mortality (%)	16	13	13	23	19	
	Late embryonic death (%)	3	1	3	11*	0	
Foetal examination	No. of dead foetuses	0	1	0	5*	0	
	No. of abnormal foetuses	5	14	14	14	2	
	Sex ratio (m/f) [c]	0.74	1.06	0.98	1.0	1.2	
	External examination (no. foetuses) - variations - malformations - retarded in bw - placenta: greenish discolouration - placenta: pale - haemorrhages [e]	 5 0 5 (2.4%) 3 3 6 (2%)	 13 1[d] 14 (4.8%) 2 0 25 (8.6%)	 14 0 14* (5.2%) 12* 1 25 (9.3%)	 14 0 14* (6.3) 37* 7 21 (9.5%)	 2 0 2 (2.4%) 5* 4* 9 (10.7%)	
	visceral examination (no. foetuses/total examined) - variations short brachiocephalic trunk haemorraghes - malformations cataract [f]	 8/108 7/108 2/108 4/108 0	 7/129 6/129 2/129 3/129 0	 8/117 6/117 0/117 1/117 0	 7/99 4/99 6/99 2/99 1/99	 1/37 1/37 0/37 2/37 0	
	Skeletal examination (no. foetuses/total examined) - variations - malformations	 28/107 2/107	 38/124 5/124	 38/112 6/112	 25/89 3/89	 7/35 1/35	

* statistically significant

(a) 'other' clinical signs': one animal only showed signs of haemorrhage on tail, left hind limb and on both side of face.

(b) haemorrhages in lungs and reddish mottled lungs are findings associated with the method of euthanasia

(c) No effects on sex ratio were observed except in the control group where the ratio males : females was 43 : 57!

(d) foetus with absent tail, closed anus, and absent caudal vertebrae.

(e) haemorrhages of different sizes and number were recorded during external examination of foetuses, classified as follows: one or more pinhead-sized or smaller, one or more bigger than pinhead sized (0.5-1.5 cm or bigger); less severe haemorrhages were described as slight. In the table only the total number of haemorrhages per dose group are presented. A statistical significance cannot be added in the table because this can only be established when the haemorraghes are presented per size.

(f) the incidence of cataract in the historic control data of the performing laboratory was none out of more than 5000 foetuses and has also not been recorded in the Charles River database on Wistar rats (> 5000 foetuses).

Table B.6.6.2-2 Results from study TP2: exposure during days 6-19 of gestation

	Dose (mg/kg bw per day)	0	0.125	0.150	0.200	0.250	dr
Maternal effects	Mortality (incl. moribund)			1/25		8/12	
	Clinical signs - piloerection - paleness, - reduced activity - vaginal bleeding	0/25	0/25	1/25 1 1 1	5/25 1 2	8/12 8* 8* 8* 8*	dr dr dr dr dr

	Dose (mg/kg bw per day)	0	0.125	0.150	0.200	0.250	dr
	- open vaginal orifice - other [a]			1	1 2		
	Pregnant animals %	80	92	84	92	100	
	Abortions		No	effect			
	Gravid uterine weight		No	effect			
	Corpora lutea		No	effect			
	Body weight		No	effect			
	Food consumption		No	effect			
	Water consumption		Not	determined			
	Pathology - reddish mottled lungs/point-like haemorrhages on lungs[d] - uterine horn and/or uterus filled with blood - pale or pale area on liver	15/25 0 1/25	16/25 0 3/25	9/25 1/25 6/25	15/25 1/25[b] 6/25	2/12 8/12[c] 0	- dr
Litter response	Live foetuses	296	306	281	300	60	
	Foetal weight		No effect				
	Pre implantation loss (%)	5	8	5	4	0	
	Post implantation loss (%)	7	6	8	8	5	
	Late embryonic death (%)	0	1	0	4	2	
Foetal examination	No. of abnormal foetuses	7	7	6	6	2	
	No. of dead foetuses	1	0	0	0	0	
	Sex ratio (m/f)	0.97	1.03	0.95	1.11	0.94	
	External examination (no. foetuses) - variations - malformations - retarded in bw - placenta: greenish discoloration - placenta: fibrinoid degenerated - haemorrhages (total) [f]	 6 1 [e] 7 11 20 7 (2.4%)	 7 0 7 13 9* 24 (7.8%)	 6 0 6 16 4* 22 (7.8%)	 6 0 6 27* 6* 19 (6.3%)	 2 0 2 0 2 8 (13.3%)	
	visceral examination (no. foetuses/total examined) - variations short brachiocephalic trunk haemorrhages - malformations cataract [g]	 2/129 7/129 0/129	 3/134 8/134 1/134	 2/124 10/124 2/124	 8/132* 28/132* 4/132*	 0/26 0/26 0/26	
	Skeletal examination (no. foetuses/total examined) - variations - malformations	 45/126 8/126	 37/127 4/127	 21/116 * 1/116 *	 25/126 * 6/126	 13/26 1/26	

* statistically significant

a 'other' clinical signs: one animal showed brown discoloration on forelimbs and around mouth; another showed brown discoloration around eye. None of these animals showed other signs.

b Uterus was filled up with dark brown colouration (this animal had shown an open vaginal orifice from days 13-15 and on gestation day 17)

c The pathological finding in these animals was bleeding in the uterus rather than uterus filled with blood

d Haemorrhages in lungs and reddish mottled lungs are findings associated with the method of euthanasia and not considered treatment related

e this foetus showed hydrops foetalis.

f haemorrhages of different sizes and number were recorded during external examination of foetuses, classified as follows: one or more pinhead-sized or smaller, one or more bigger than pinhead sized (0.5-1.5 cm or bigger); less severe haemorrhages were described as slight. In the table only the total number of haemorrhages per dose group are presented. A statistical significance cannot be added in the table because this can only be established when the haemorraghes are presented per size.

g the incidence of cataract in the historic control data of the performing laboratory was none out of more than 5000 foetuses and has also not been recorded in the Charles River database on Wistar rats (> 5000 foetuses).

Effects in the TP 1 group:

Mortality and signs of maternal toxicity were observed in the 0.15, 0.20 and 0.25 mg/kg dose groups. These signs were predominantly observed in dams that died or were moribund. Some surviving animals also showed clinical signs, like piloerection, vaginal bleeding, signs of haemorrhage or reduced activity. There was no effect on body weight, weight gain and/or gravid uterine weight of the pregnant dams.

An increase (not stat. sign.) in early embryonic death (%) was observed in the 0.15 and 0.20 mg/kg groups. Late embryonic death (%) was increased in the 0.20 mg/kg group. Also the number of dead foetuses, post implantation loss (%) and total intrauterine mortality (%) was increased in this dose group. The study authors considered this as "unlikely to be treatment related since these effects were not observed in the added high dose group". However, in the added high dose group the pre-implantation loss was increased and thus the subsequent effects could have been "lost".

At external examination, one malformed foetus was observed in the low dose group with absent tail, closed anus, and absent caudal vertebrae. The malformations in this single animal are considered as spontaneous and not related to treatment.

Also at external examination the number of foetuses that were retarded in bodyweight was increased in the low, mid and high dose groups (not in the extra high dose group) with statistical significance in the mid and high dose group, however without a dose relationship. But, when expressed in percentage there is a dose relation visible: 2.4, 4.8, 5.2 and 6.3% in the control, low, mid and high dose group but only 2.4% in the extra high dose group.
A clear effect of warfarin was the increased number of foetuses showing haemorrhages in all treated groups.
A greenish discolouration of the placenta was found in all groups including the controls, and the incidence was increased in the mid and high dose group in relation to the dose and was also observed in the extra high dose group. Further the number of pale placentas was increased in the high and significantly increased in the extra high dose group.

There was no increase in the incidence of variations.

At visceral examination there was one foetus in the high dose group with yellowish discolouration in the lens which was diagnosed as central cataract, a teratogenic effect.
As an additional observation, the incidence of bloody infiltrations (haemorrhage) was recorded during the visceral examination. The incidence was 2, 2, 0, 6, and 0 respectively in the control, low, mid, high and extra high dose group.

At skeletal examination there were several foetuses with skeletal malformations, but there was no clear dose related effect. The malformations observed were:

- extremely marked incomplete ossification or irregular calcification of the skull in 1 mid dose foetus, but this was also observed in 2 control foetuses of the TP 2 group.

- short and/or bent scapula and/or clavicle. The incidence was 1, 4, 4, 2 and 0 in the control, low, mid, high and extra high dose group.

- 1 low dose foetus had several malformations: malformed vertebrae and absent ribs, caudal regression syndrome, unossified thoracic vertebral central Th I-III, absent vertebrae from thoracic vertebra III with cartilage according to S I, L III, LVI, and absent ribs from rib 5 on both sides.

- short and/or thickened and/or bent humerus: incidence 1, 2, 4, 2, and 1 in the control, low, mid, high and extra high dose group.

One litter in the mid dose group was excluded from evaluation because the foetal weights were higher than normal (3-6 g) which could indicate that some pups were one day more advanced in development. Since this finding is rare, the study authors considered it possibly related to treatment. Furthermore, in this litter there were 4/7 foetuses with short nose and wide frontal bone, a malformation which is also found in humans after warfarin exposure.

Effects in the TP 2 group:

Mortality occurred in the mid dose (one dam) and in the extra high dose group (8/12 dams). Signs of maternal toxicity were observed in the 0.15, 0.20 and 0.25 mg/kg dose groups. There was no effect on body weight, weight gain and/or gravid uterine weight of the pregnant dams.

The only finding in the reproduction data was an increased incidence (not statistically significant) in late embryonic death (%) that was observed in the 0.20 mg/kg group. According to the authors the value was at a normal historical control level, but these control data were not provided. No effects were observed on the other litter parameters.

At external examination no malformed foetuses were found, except for one foetus in the control dose group with hydrops foetalis. This malformation was not considered treatment related. A clear effect of warfarin was the increased number or foetuses showing haemorrhages in all treated groups.

A greenish discolouration of the placenta was found in all groups including the controls, but the incidence was doubled in the high dose group. No discolouration was observed in the extra high dose group. The lower incidence of fibrinoid placentas is considered an incidental finding. No other treatment related findings for placentas were observed.

At visceral examination an increased number of foetuses with a short brachiocephalic trunk (variation) was observed. However, since this incidence was in the same range as the control value in the TP 1 group, it is not considered treatment related.

A dose related increase in central cataract (by histopathologic evidence) was observed in the TP2 group in the low, mid and high dose group. The effect was not observed in the extra high dose group, but is considered a treatment related malformation.

As an additional observation, the incidence of bloody infiltrations (haemorrhage) was recorded during the visceral examination. The incidence was 7, 8, 10, 28 and 0 in the control, low, mid, high and extra high dose group, with statistical significance in the high dose group. These signs were most probably related to the anticoagulant effect of warfarin and to the administration of the test item for a longer treatment period in the TP2 group (the incidence in the TP1 dose group was 2, 2, 0, 6, and 0 respectively).

At skeletal examination there were several foetuses with skeletal malformations, but there was no clear dose related effect. The malformations observed were:

- short and/or bent scapula and/or clavicle. The incidence was 5, 3, 1, 2 and 1 in the control, low, mid, high and extra high dose group.

-short and/or thickened and/or bent humerus: incidence 6, 3, 0, 5, and 0 in the control, low, mid, high and extra high dose group.

- short and/or bent femur was recorded in 1 foetus of the high dose group, but a similar malformation, short bent radius and or ulna, was recorded in 3 foetuses in the control group.

Acceptability

The study was performed in compliance with OECD 414. However, several weeks after the start of the experiment an extra high dose group was added in order to demonstrate clear maternal toxicity. However due to high mortality in this group and the higher body weights at the start of the treatment, plus the fact that there was no extra control group included, the results of this group are difficult to interpret.

The interval between the dose levels in this study is very small and thus dose related effects are not easy to detect.

Conclusions

Warfarin caused adverse effect in dams at dose levels of 0.150 mg/kg bw and higher in a dose related manner. These effects included piloerection, paleness, reduced activity, vaginal bleeding and open vaginal orifice. Treatment was associated with increased incidence of maternal death and bleeding from the vagina, with necropsy findings of blood in the uterus.
The finding of haemorrhages at external observation of the foetuses from warfarin treated dams in either treatment regimen was an obvious effect of warfarin. It was however not a dose related effect. It should be noted however, that due to the small steps in dose levels, a dose related effect is difficult to detect.

At visceral examination an increased incidence of haemorrhage was also observed in foetuses of the high dose group, especially in the TP 2 group (longer treatment group), but not in the extra high dose group.

Cataracts (malformation) were found in one animal of the 0.200 mg/kg TP1 dose group, and in all dose groups (except the extra high dose group) of the TP2 dose group.
No other clear indications of malformations were observed. From the litter data that were included in the examination of the study there were no indications of skeletal abnormalities. However, one litter of the mid-dose group of the TP1 treatment group (which was excluded from statistical analysis) had 4 of 7 foetuses with short nose and wide frontal bone at external examination. Skeletal examination was performed on two of these foetuses and the malformations were confirmed.

Although the study has several shortcomings, the findings show that warfarin induces cataracts and haemorrhages and, in view of the cataracts, should be considered a teratogenic compound. The incidence of possible other teratogenic effects which are specific in humans, like skull malformations, are not convincingly seen in this rat study. The incidental finding of a litter with some foetuses with short nose and wide frontal bone is of too low incidence and in only one group and cannot be related to warfarin.

Remark:

In light of the doubt expressed by the Specialised Experts whether the standard OECD 414 test can detect coumarin-specific developmental effects, this new study shows that some of the developmental effects induced by warfarin are also induced in rats, but others are not. For further discussion, see section 5.9.6.

2. Other studies on warfarin (added for completeness)

Howe and Webster (1990) exposed Sprague-Dawley rats orally to warfarin at 100 mg/kg and concurrent intramuscular injections of vitamin K1. Exposure from day 1 to day 12 of pregnancy did not affect the dams or the foetuses. Similar treatment from day 9 to day 20 caused haemorrhage in the foetuses examined on day 21. No bone or facial defects were observed, probably because the vitamin K dependent components of bone development occur postnatally in the rat.

Another study by Howe and Webster (1992) is summarised as follows, based on the Irish CLH proposal for warfarin:

Howe, A.M.; Webster, W.S. (1992): The Warfarin embryopathy: A rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology 46, 379-390.
Guidelines: Not presented
GLP: No
Material and methods: This study was designed to investigate the developmental toxicity of warfarin, which in anticoagulant therapy in humans during the first trimester of pregnancy is known to cause various degrees of nasal hypoplasia and other anomalies known as Warfarin embryopathy. However, conventional studies in pregnant mice, rats or rabbits were not considered feasible since there appears to be a very narrow margin between the no-effect dose for the conceptus and the maternal lethal dose. Thus, in this investigation, the developmental toxicity of Warfarin was studied by dosing rats with Warfarin in combination with Vitamin K. Thus, the extrahepatic Vitamin K deficiency induced by Warfarin is maintained, whereas Vitamin-K-dependant processes of the liver are not disturbed.
Test material: Warfarin, sodium-salt (Boots Company, North Rocks, Sydney); Batch: no data; Purity: no data; Species: Sprague-Dawley rats; No. of animals: a total of 13 litters (5 males and 5 females if possible); Administration: subcutaneous injection; Dose level: Group 1: 100 mg/kg Warfarin + 10 mg/kg Vitamin K1, Group 2: 10 mg/kg Vit. K1; Vehicle: distilled water; Controls: untreated litters; Duration of treatment: 12 weeks; Sacrifice: at various times throughout the study.
Group 1 (six litters) were given daily s.c. injections of Warfarin and Vit. K1, and the dams were also treated with Vit. K1 (10 mg/kg) to prevent haemorrhages from Warfarin ingestion by coprophagy. 11 males and 12 females from these litters were treated for 12 weeks until the final sacrifice.
Group 2 (three litters) were treated only with Vit. K1, and 11 males and 10 females were subjected to the final sacrifice.
Group 3 (four litters) served as untreated control. 13 males and 14 females were sacrificed upon study termination.
Findings: All rats survived without any signs of haemorrhage. For Warfarin treated males and females, there was a statistically significant reduction in tail length (12-17%), nasal length (7-13%), overall length (6-12%) and weight (7-13%) upon study termination (week 12). The snout of these animals was shorter and broader, and the pinnae of the ears were reduced in size. These features

were particularly evident after 3 weeks of treatment. The growth parameters upon study termination are presented in Table 7.9.2.1.2b-1.

Table 7.9.2.1.2b-1: Growth measurements in male and female rats upon study termination (week 12)

Group	Sex	No. of litters	No. of rats	Weight [g]	Body length [mm]	Tail length [mm]	Nasal length [mm]
Warfarin	male	6	11	$311.1 \pm 20.2^{1,2}$	$380 \pm 11.8^{2,3}$	$168.5 \pm 8.1^{2,3}$	$21.4 \pm 1.2^{2,3}$
Vit. K1	male	3	11	334.6 ± 22.8	406.8 ± 9.0^{2}	190.9 ± 8.6^{2}	23.8 ± 0.6
Control	male	4	13	344.8 ± 34.2	424.0 ± 18.3	201.8 ± 9.6	24.5 ± 0.8
Warfarin	female	6	12	207.3 ± 18.1^{3}	$338.8 \pm 14.8^{3,2}$	$152.8 \pm 9.4^{3,2}$	$20.8 \pm 1.0^{3,2}$
Vit. K1	female	3	10	237.4 ± 37.2^{4}	380.5 ± 9.0	184.5 ± 7.6	22.7 ± 0.5
Control	female	4	14	224.2 ± 23.0	384.3 ± 13.9	186.0 ± 8.2	22.4 ± 0.6

1) significantly different from Vit. K group ($p<0.05$)
2) significantly different from untreated group ($p<0.01$)
3) significantly different from Vit. K group ($p<0.01$)
4) significantly different from untreated group ($p<0.05$)

Warfarin treatment had a differential effect on the growth of various skull bones (skull length reduced by 5-6% in male and 5% in females). The results of measurements of alizarin-stained skulls after 12 weeks of treatment are presented in Table 7.9.2.1.2b-2, the anterior third of the skull was most affected. The results of forelimb bone length in Table 7.9.2.1.2b -3. The bones from treated rats were slightly shorter (4-5% reduction in both sexes) than controls.

Table 7.9.2.1.2b-2 : Skull measurements [mm] upon study termination (week12)

	Males			Females		
	Warfarin	Vit. K1	Control	Warfarin	Vit. K1	Control
No of rats	11	11	13	12	10	14
Skull length	42.7 ± 1.0[1,2]	45.2 ± 0.9	45.5 ± 1.8	41.3 ± 1.2[1,2]	43.6 ± 1.4	43.3 ± 1.6
Nasal bone length	15.2 ± 1.1[1,2]	17.5 ± 0.6	17.3 ± 0.6	14.6 ± 0.7[1,2]	17.0 ± 0.7	17.0 ± 0.6
Frontal bone length	13.2 ± 0.7[3]	13.8 ± 0.6	13.5 ± 0.6	12.4 ± 0.5[1,4]	13.1 ± 0.4	12.8 ± 0.5
Parietal bone length	7.8 ± 0.8	7.6 ± 0.6	7.7 ± 0.7	7.5 ± 0.3	7.1 ± 0.7	7.4 ± 0.4
Interparietal bone length	6.3 ± 0.6[4]	6.7 ± 0.4	6.8 ± 0.5	6.3 ± 0.3	6.4 ± 0.6	6.6 ± 0.6
Premaxilla length	10.7 ± 0.5	11.5 ± 0.4	11.5 ± 1.6	10.4 ± 0.6[3]	11.1 ± 0.5	10.9 ± 1.0
Maxilla length	15.9 ± 1.2[1,4]	17.7 ± 0.7	17.2 ± 1.7	14.9 ± 1.5[1,4]	17.0 ± 0.7[4]	15.9 ± 0.7
Mandibular length	24.0 ± 0.9	24.5 ± 1.1	24.8 ± 1.3	24.1 ± 1.0[4]	23.5 ± 0.9	23.7 ± 1.4
Bizygamatic width	23.1 ± 0.8	23.3 ± 0.5	23.5 ± 0.7	22.0 ± 0.8	22.6 ± 0.8	22.3 ± 0.6
Width of snout (max.)	8.5 ± 1.0[2,3]	9.3 ± 0.8	9.7 ± 0.4	8.4 ± 0.4[2,3]	8.9 ± 0.4	9.1 ± 0.6
Transfrontal width (min.)	6.5 ± 0.2[1,2]	6.9 ± 0.3	6.9 ± 0.3	6.4 ± 0.3[4]	6.6 ± 0.3	6.7 ± 0.3
Facial height	13.4 ± 0.4[1,2]	14.0 ± 0.5	14.1 ± 0.3	12.8 ± 0.3[1,4]	13.4 ± 0.3	13.1 ± 0.4
Max. nasal height	8.1 ± 0.4[4]	8.5 ± 0.5	8.7 ± 0.7	7.9 ± 0.3[3]	8.3 ± 0.4	8.0 ± 0.5

1) significantly different from Vit. K group ($p<0.01$)
2) significantly different from untreated group ($p<0.01$)
3) significantly different from Vit. K group ($p<0.05$)
4) significantly different from untreated group ($p<0.05$)

Table 7.9.2.1.2b-3: Forelimb bone length [mm] upon study termination (week 12)

Group	No.	Sex	Scapula	Humerus	Ulna	Meta-carpal	Prox. phalanx	Middle phalanx	Distal phalanx
Warfarin	11	male	25.3 ± 1.0[1]	25.8 ±0.6[1,2]	30.2 ±0.6[3,4]	8.2 ± 0.7[3,4]	5.2 ± 0.2[1,3]	2.8 ± 0.1[3]	2.7 ± 0.1
Vit. K1	11	male	25.8 ± 1.2	27.0 ± 0.5	31.6 ± 0.6	8.5 ± 0.2	5.6 ± 0.2	3.0 ± 0.1[4]	2.7 ± 0.2
Control	13	male	26.8 ± 1.5	26.9 ± 1.5	31.3 ± 0.9	8.5 ± 0.3	5.5 ± 0.3	2.9 ± 0.2	2.7 ± 0.1
Warfarin	12	female	23.4 ± 1.2	24.0 ±0.8[3,4]	28.2 ±1.7[3,4]	7.9 ± 0.2[3,1]	5.2 ± 0.3	2.6 ± 0.1[3,4]	2.5 ± 0.1[2]
Vit. K1	10	female	24.4 ± 0.9	25.2 ± 0.4[3]	30.6 ± 0.4	8.2 ± 0.2	5.3 ± 0.3	2.8 ± 0.1	2.6 ± 0.1
Control	14	female	24.3 ± 1.3	25.0 ± 0.7	30.5 ± 0.8	8.1 ± 0.3	5.2 ± 0.2	2.8 ± 0.1	2.5 ± 0.2

1) significantly different from untreated group ($p<0.05$)
2) significantly different from Vit. K group ($p<0.05$)
3) significantly different from Vit. K group ($p<0.01$)
4) significantly different from untreated group ($p<0.01$)

The alizarin-stained nasal septa from Vit. K1 and control rats did not show evidence of calcification in the septal cartilage. In contrast, all septal cartilages from Warfarin-treated rats showed extensive areas of calcification. The calcification appeared 2 weeks after the start of Warfarin administration, and increased progressively during the following weeks. This calcification remained visible up to 15 months after cessation of treatment. There were no abnormal calcifications in the limbs or axial skeleton that might correspond to the "stipplings" described in the human Warfarin embryopathy. The growth plates from the femur and tail vertebrae showed many calcium bridges which transverse the growth plate from postnatal day 10 onwards. Similar structures were not seen in the controls. The primary and secondary ossification centres appeared to be normal.

Study Conclusion:
Under the conditions of this test, Warfarin treatment (100 mg/kg bw) in combination with Vit. K1 (10 mg/kg bw) induced extrahepatic Vit. K deficiency in the neonatal rat which caused differential growth retardation of the developing skull resulting in maxillonasal hypoplasia, calcium deposits in the cartilage of the nasal septum, and calcium bridges in the epiphyseal cartilage of vertebrae and long bones. These findings indicate a generalised disturbance in the maintenance of uncalcified cartilage. The authors concluded that it was unclear whether calcification of the nasal septum is the cause of maxillonasal hypoplasia, by reducing the longitudinal growth of the septum, or if they were the result of another more fundamental disturbance of the chondrocytes.

Reviewer's conclusion:
The study appeared to be carried out to a good standard and the data well reported. It is considered to be acceptable. The test substance purity was not reported (not known). This was a hypothesis testing study in which the apparent lack of teratogenicity of warfarin (nasal hypoplasia and bone stippling) in animal models was explored. It was proposed that the reason for the difference in response may relate to the critical periods for nasal and skeletal development, which are prenatal (during the first trimester) in man and occur during late foetal and early postnatal life in the rat. The

study clearly demonstrated a warfarin induced effect on nasal cartilage and facial/skull bone growth, not dissimilar to the human embryopathy but significantly less marked. This effect may be linked to the vitamin K-dependent bone protein-matrix gla (γ-carboxyglutamic acid) protein (MGP). This protein is synthesised in the growth plate cartilage and has a role in prevention of calcification. In the presence of warfarin, the MGP remains decarboxylated and therefore unable to prevent calcification of the cartilage. The study provided evidence that abnormal calcification of the nasal septum may be the underlying cause of the warfarin embryopathy.

A third study is summarised as follows, based on the Irish CLH proposal for warfarin :

Mirkova, E.; Antov, G. (1983): Experimental evaluation of the risk of prenatal pathology under effect of warfarin – coumarine rodenticide. Hig. Zdrav. 25, 476-482.
Guidelines: Not presented
GLP: No
Test material: Warfarin, Na-salt, technical pure;
Batch: No data;
Purity: No data;
No. of animals: A total of 260 pregnant Wistar rats;
Administration: Oral per gavage;
Vehicle: Water;
Controls: Included but not specified;
Sacrifice: Day 21;
I. single application: 8 mg/kg bw on days: 10, 11, 12, 13, 14 and 17;
II. repeated appl.: 0.32 mg/kg bw; Application period: days 1-7;
III. repeated appl.: 0.32 mg/kg bw; Application period: days 8-16;
IV: repeated appl.: 0.32, 0.16, 0.08, 0.04 mg/kg bw on days days 1-21.

Findings:
Following single applications of warfarin to groups of animals on days 10, 11, 12, 13, 14 and 17 of pregnancy, toxicological signs, abortion and massive vaginal haemorrhages (for an average of 3 days) were observed. Mortality rates of pregnant females were between 93.3 and 100%, except for the group dosed on day 10. In this group, mortality of dams was 48%. The overall and postimplantation loss was increased by 393.3 and 472%, and the mean foetal weights were decreased by 37%.

I. A significant increase of the incidences of depressed ossification of the skull bones (44.8%), and abnormal ossification of the sternum were observed in rat foetuses. A total absence of ossification was found in 31%, and lack of ossification of xiphoid in 41.4%.

II. Application of 0.32 mg/kg bw during the pre implantation stage from day 1-7 caused an increased incidence of haemorrhages (198%). No further signs of toxicity were described. Foetuses showed predominantly damage of cerebral vessels. Intracerebral haematoma were found in 42.8%, whereas the incidence of structural malformations of the rear limbs (pes varus) was only 5.4%.

III. Following application of 0.32 mg/kg bw during the period of organogenesis from days 8-16, increased incidences of post implantation loss (551,8%), and overall embryonic mortality (525%) were noted, and foetuses showed an increased incidence (182.7%) for the development of the haemorrhagic syndrome (haematoma and haemangioma).

Profound teratogenic effects such as increased incidences of structural malformations of the rear limbs (pes varus), internal hydrocephalus, intracerbral haematomas, massive haemorrhages into the abdominal cavity and delayed ossification of the parietal skull bones were found.

IV. Daily application of 0.32 and 0.16 mg/kg bw during the entire gestation period from day 1- 21 resulted in a statistically significant increase of the total embryonic mortality (725.5 and 388.8%) in comparison to control. The post implantation loss was increased by 1074 and 501.8% for these dose levels, respectively. This dose-related effect was combined with the development of the haemorrhagic syndrome. Subcutaneous haematoma at different parts of the trunk and extremities, and haemangioma around the great vessels and the neck regions were observed. The incidence increased the rates of spontaneous occurrence and the control incidence by 193.8% for the 0.32 mg/kg bw dose, and 123.8% for the 0.16 mg/kg dose level.

In foetuses, significant increased incidences of structural malformations of the rear limbs (pes varus), internal hydrocephalus, intracerebral haematoma, and massive haemorrhages into the abdominal cavity were found. At 0.32 mg/kg and 0.16 mg/kg, the incidence of delayed ossification of the parietal skull bones was increased statistically significantly by 21.6 and 15.7%, respectively.

Following application of 0.32, 0.16 and 0.08 mg/kg bw, changes of biochemical parameters in livers of foetuses were observed. The activity of the cytochrome-oxidase ($P=0.818$, $p<0.01$) and succinate-dehydrogenase ($P=0.301$) was decreased. At 0.32 and 0.16 mg/kg bw, the activities of lactate-dehydrogenase ($P=0.956$, $p<0.05$) and glucose-6-phosphat-dehydrogenase ($P=0.980$, $p<0.02$) were statistically significantly increased. ATP and the amount of soluble proteins were significantly decreased in all dose level compared to control.

Conclusion:
The embrytoxic and teratogenic effects of the test compound warfarin resulted from the direct action of metabolites on embryonic cells following transplacental transfer. A certain role in the pathogenesis is probably related to the circulatory changes and vasotoxic effects of warfarin that resulted in thrombosis of foetal blood vessels followed by tissue necrosis. The structural malformations of the limbs were explained by the formation of spot chondrodystrophy of the calcaneal epiphysis.

Reviewer's conclusion:
This submission is a translation of an (apparently) Russian language paper. There was very limited reporting of data in the original paper, none for most parameters. The test substance purity is unknown, other than that it is a technical pure a.s. The data can be considered as limited at best, but can be used to extract some general information.
-A single dose of 8 mg/kg/day on days 10, 11, 12, 13, 14, and 17 was highly toxic, causing up to 100% mortality, except on day 10 (48%). Foetal observations where mortality is so high are not meaningful.
-The incidence of intra-cerebral haematoma was increased in foetuses following administration of 0.32 mg/kg/day from days 1-7. The relevance of the data on malformations of the hind-limbs cannot be evaluated because of the lack of documentation of incidence data and the lack of background data.
-0.32 mg/kg/day administered from days 8-16 caused greatly increased embryonic loss, increased incidence of haemorrhage and greatly increased incidences of structural malformations. There was no information on maternal effects at this dose level. When the a.s. was administered throughout gestation, the same effects on the foetus were seen at $\geq$ 0.16 mg/kg/day. There was no information

on maternal effects at this dose level. As the data were not reported, it is not possible to make any evaluation of the effects seen/not seen at doses lower than 0.16 mg/kg/day.
- 0.32 mg/kg/day from days 1-7, 8-16 or 10-21 of gestation have profound adverse effects on the foetus. 0.16 mg/kg/day caused similar effects when administered from days 1-21 of gestation. The maternal effects are not known at these dose levels.

Summary of the study of Feteih et al. (1990), based on the Irish CLH proposal of warfarin:
Feteih, R. et al. (1990): Effect of Sodium Warfarin on Vitamin K-dependent proteins and skeletal development in the rat foetus, Journal of Bone and Mineral Research 5 (8), 885-894.
Guidelines: Not presented
GLP: No
Material and methods: The purpose of this study was to investigate the effects of Warfarin administered to rats on gestational days 8-22 on the developing foetal skeleton and on vitamin-Kdependant bone and cartilage proteins. Pregnant Sprague-Dawley rats received daily subcutaneous doses of 175 μg/kg bw of Sodium Warfarin on days 8 or 10-22 of gestation. Control animals received physiological saline. Litters were examined on days 20, 21 and 22, the number of fetuses and resorptions was recorded, and each foetus was examined for gross abnormalities. Individual maternal and foetal blood samples were assayed for prothrombin time and osteocalcin levels. Foetuses were further subjected to whole skeletal, biochemical or histological evaluation at random.
Findings
Sodium Warfarin administration at a dose of 175 μg/kg bw/day to Sprague-Dawley rats on gestational days 8-22 led to a mortality of 43% among dams, whereby maternal prothrombin times were only slightly (but not significantly) elevated. In the surviving litters, foetal bone osteocalcin and γ –carboxyglutamic acid were significantly (ca. 50%) reduced compared to control pups. Analysis of tibial growth showed changes such as widened hypertrophic zones, increased calcification of these zones and disorganisation of the hypertrophic cells, suggesting that the growth plate abnormalities seen with prenatal warfarin exposure relate to the inhibition of vitamin-K dependant proteins of the skeletal system.

Table 7.9.2.1.2d-1. Summary of levels of Gla in long bone and Calvaria (mean ± SE *(n)*)

Day	*Long bone*		*Calvaria*	
	Gla residues/1000glu	*Gla NM/mg*	*Gla residues/1000glu*	*Gla NM/mg*
20 Control	1.80 ± 0.22 (7)	0.34 ± 0.08 (7)	1.05 ± 0.13 (7)	0.28 ± 0.07
21 Control Warfarin-treated *% reduction*	2.21 ± 0.21 (16) 1.27 ± 0.09 (26) *43.9*	0.35 ± 0.03 (16) 0.12 ± 0.04 (25) *65*	1.34 ± 0.06 (16) 0.62 ± 0.01 (15) *53.8*	0.31 ± 0.01 (16) 0.10 ± 0.02 (14) *67*
22 Control Warfarin-treated *% reduction*	2.40 ± 0.09 (17) 1.29 ± 0.14 (7) *46.1*	0.28 ± 0.05 (17) 0.18 ± 0.03 (7) *52*	1.50 ± 0.08 (12) 0.81 ± 0.11 (8) *46*	0.38 ± 0.04 (12) 0.16 ± 0.04 (8) *57*

Reviewer's conclusion:
The pharmacological action of warfarin involves inhibition of vitamin K-dependent synthesis of γ -carboxyglutamic (gla) residues in proteins of the liver, bone and other tissues. Two vitamin K dependent proteins have been characterised in the skeleton, i.e., osteocalcin, which is associated with hydroxyapatite crystals in the extracellular matrix, and matrix Gla protein that predominates in

embryonic bone and cartilage extracellular matrix. In the present study, the effects of prenatal treatment with warfarin on bone histology and morphology, and associated biochemical effects (levels of osteocalcin and Gla protein) were investigated in the rat.
Mean litter size and foetal weights were reduced compared to controls (not statistically significant). The mean number of resorptions was not significantly increased. There was no significant difference in ossification centres in warfarin-treated foetuses. Apparent differences in facial dimensions (mandibular length and depth, maxillary length) were not significant when adjusted for foetal body weight. The results clearly demonstrate and adverse effect on the hypertrophic region of the long bones, with marked disruption of the columnar arrangement of the hypertrophic chondrocytes. These morphological defects were correlated with biochemical effects on the skeleton with marked reductions in Gla in calvariae and long bones (by up to 53%). This data suggests that decreased synthesis of this protein in response to warfarin exposure may account for bone abnormalities.
The dose causing effects on the foetus in this study was 175 μ g/kg b.w. At this dose, there was 43% maternal mortality. While the cause of death is not actually stated, it must be assumed that haemorrhage was involved. Maternal prothrombin times were not affected at this dose, except for a single dam whose prothrombin times were increased by 1.6 times normal. The litter from this dam were most profoundly affected by adverse bone change.

Summary of the study of Kronick et al. (1974), based on the Irish CLH proposal of warfarin:
Kronick, J. et al. (1974): Effects of Sodium Warfarin administered during pregnancy in mice, Am.J. Obstet. Gynecol. 118 (6), 819-823.
Guidelines: Not presented
GLP: No
Material and methods: Test design: The purpose of this study was to investigate teratogenic and foetotoxic effects in mice. Virgin mice (F1 generation derived from crossing C3H and A/J strains) were caged with males overnight. Upon detection of vaginal plugs, Warfarin sodium (salt)(Coumadin drug) was administered i.p. at doses of 1, 2, 3 and 4 mg/kg bw/day at various stages of pregnancy. Control animals received physiological saline or distilled. water. Mice were sacrificed at various intervals following treatment, and uterine contents were preserved for later inspection, plus withdrawal of blood samples (prothrombin assay).
Findings:
In groups treated from days 3-11 of gestation with 2 and 4 mg/kg bw/day, there was a very high incidence of haemorrhaged placentae and foetal deaths (including both dead and resorbed foetuses). These doses of Warfarin prolonged the prothrombin time by 3.5-5 times the control at 24 hours after the final injection. In contrast, there was no evidence of haemorrhaged placentae and no significant increase in either prothrombin time or foetal deaths in animals treated with 1 mg/kg bw/day. In addition, none of the doses employed in this study lead to an increase of the frequency of malformations. However, the authors conclude that this may also be due to the high incidence of severely haemorrhaged placentae which subsequently could have resulted in early foetal death, and so obscured any embryotoxic effect of Warfarin. For this reason, the effects of single doses of Warfarin were investigated.

Table 7.9.2.1.2e-1 Effects of sodium warfarin administered to pregnant mice from days 3-1 of gestation (taken from Kronick et. al., 1974)

Treatment group							
days	Dose mg/kg/day	No. pregnant	No. implantations	Haemorrhaged placentas	Foetal deaths (%)	Mat. deaths (%)	Mean prothrombin time
3-11	4	14	92	92.7	92.7	14.3	48.9
3-11	2	8	66	100	71.2	12.5	33.6
3-11	1	11	88	0	13.6	0	10.4
3-11	0	10	88	0	4.5	0	10.1

Single injections of 4 mg/kg from days 5-14 of gestation showed a significant increase of foetal deaths for an administration on days 10 and 11 compared to control. No foetal or placental haemorrhages were observed in this series, and there was only a low incidence of gross foetal malformations (all of which were cleft lip and/or cleft palate). Mean prothrombin times 24 hours after Warfarin administration were elevated by a factor of 2.0 – 3.3 compared to control.
Single doses of 1, 2, 3 and 4 mg/kg were administered on either one of the gestation days 8 through 11 (period of organogenesis), and all animals were sacrificed on day 18. No incidence of foetal or placental haemorrhage was observed. Statistical analysis revealed that there was significant linear regression of over-all foetal deaths on log dose, suggesting a dose-dependant increase of foetal mortality irrespective of the day of gestation.

Treatment group						
days	Dose mg/kg/day	No. litters	No. implantations	Foetal deaths (%)	Live and ext. malformed (%)	Mean prothrombin time
5	4	7	62	4.8	3.2	25.1
6	mg/kg/day	7	56	10.8	0	15.6
7	‘	7	56	1.8	0	20
8	‘	7	60	1.6	0	25.2
9	‘	7	56	9.1	0	24.4
10	‘	14	118	29.6*	2.5	32.6
11	‘	14	112	17.0**	2.7	25.6
12	‘	7	57	7.1	0	27.4
13	‘	7	62	11.3	0	23.8
14	‘	7	59	10.2	1.7	20.2
11	control	8	62	3.2	0	9.4

* $p < 0.001$, ** $p < 0.01$ compared to controls.

Day 10 was found to be the most sensitive day to warfarin induced embryotoxicity. Analysis of malformation data showed a significant difference between the Warfarin-treated and the control groups. However, the low incidence of malformations is only suggestive of a teratogenic effect, and the majority of malformations were described as very minor (open eyelid, skeletal and ossification abnormalities.

Treatment; Day 10 (pooled groups)	No. of litters	Implantations	Dead/resorbed foetuses	Foetal deaths (%)
Warfarin, 4 mg/kg/day	31	278	70	25.2
Warfarin, 4 mg/kg/day + Vit K1	11	93	11	11.8
Control	9	84	7	8.3

Co-administration of 8 mg/kg Vitamin K together with 4 mg/kg Warfarin on day 10 of gestation prevented Warfarin-induced foetal death.

Conclusion.
It was concluded by the authors that the developmental effects of warfarin could be classified into three categories; foetal death associated with haemorrhaged placentas, foetal death not associated with haemorrhage and foetal malformation. The haemorrhaged placentaes were associated with high mortality rates and increased prothrombin times of 3.5 to 5 times controls.

3. Study on placental transfer

A study on placental transfer (Johnson, 2009) was provided in order to quantify differences of warfarin and flocoumafen in their potential to cross the placental barrier.

Study design

The ^{14}C-radiolabelled test substances flocoumafen and warfarin were administered orally as a single daily dose from gestation day 6 through 19 to time-pregnant Wistar (Crl:WI[HAN]) rats.
Flocoumafen, in corn oil, was administered to two groups of 5 rats at dose levels of 0.006 and 0.013 mg/kg/day (groups 2 and 3) and to one group of 2 rats (group 6) at 0.013 mg/kg/day.
Warfarin, in 0.5% carboxymethylcellulose (CMC), was administered to two groups of 5 rats at dose levels of 0.016 and 0.033 mg/kg bw per day (group 4 and 5) and to one group of 2 rats at 0.033 mg/kg bw per day (group 7). One control group (group 1) of 5 rats received corn oil. The rats were observed daily for clinical signs and survival, and maternal body weights were recorded daily on gestation days 5-19.
The animals in the groups of five rats were euthanised on gestation day 19 at either 0.5 h (warfarin), 4 h (control) or 6 hours (flocoumafen) following the last dose, which corresponds with the highest plasma levels (Tmax) for warfarin and flocoumafen. Following euthanasia, a blood sample (from vena cava) was collected from each dam and separated into plasma and cellular fractions. The liver and uterus were collected from each dam. Foetuses and placentas were collected from gravid uteri. Blood, livers and remaining carcasses of the foetuses of each dam were collected and were pooled per item by litter and also the placentas were pooled by litter. All dams were analysed for total ^{14}C using liquid scintillation (LS) techniques.
The animals in the groups with 2 rats were designated for WBA (whole body autoradiography) and were euthanised on gestation day 19 at 6 h following the last dose for flocoumafen treated rats, and at 40 and 62 minutes following final dose for the warfarin treated animals (due to technical problems the protocol specified euthanasia time (30 min) for these animals was not achieved). Blood samples were collected by heart puncture and the carcasses of the animals were frozen and then processed for qualitative analysis by WBA. A section from a warfarin-treated animal and from a flocoumafen-treated animal were imaged for visual evaluation of tissue distribution of radioactivity.

Results

The pregnant dams did not show any adverse clinical signs during the exposure period of the study. The overall weight change over the exposure period (13 days) ranged for the control rats from 55-98 g and for most of the treated rats from 50-83 g. There were a few exceptions: one animal of group 4 had a bw change of only 12 g, and both a group 5 and a group 7 animal of only 14 g. These three animals were all treated with warfarin. The overall weight gain of the warfarin treated rats was lower than the flocoumafen treated rats.

The concentrations of radiolabelled flocoumafen and warfarin equivalents in maternal and foetal tissues are indicated in following tables.

Residue levels (µg/g) of ^{14}C-flocoumafen equivalents in maternal and foetal tissues

Tissue type	Dose group 2 0.0059 mg/kg bw per day		Dose group 3 0.013 mg/kg bw per day	
	Maternal (ug/g)	Foetal (ug/g)	Maternal (ug/g)	Foetal (ug/g)
Plasma	0.003	0.007	0.006	0.014
Blood cell fraction	0.001	0.001	0.003	0.004
Placenta	0.018	NA	0.050	NA
Liver	0.913	0.007	1.903	0.017
carcass	NA	0.003	NA	0.007

NA = not applicable

Residue levels (µg/g) of ^{14}C-warfarin equivalents in maternal and foetal tissues

Tissue type	Dose group 4 0.016 mg/kg bw per day		Dose group 5 0.033 mg/kg bw per day	
	Maternal (ug/g)	Foetal (ug/g)	Maternal (ug/g)	Foetal (ug/g)
Plasma	0.209	0.115	0.403	0.354
Blood cell fraction	0.061	0.036	0.104	0.086
Placenta	0.102	NA	0.169	NA
Liver	1.141	0.223	1.857	0.387
carcass	NA	0.091	NA	0.185

At similar dose levels, 0.013 and 0.016 mg/kg bw per day for flocoumafen and warfarin respectively the flocoumafen treated dams had approximately 20-35 fold lower concentrations of r.a. residue in plasma and blood cellular fraction and approx. 2 fold lower concentrations in placenta than dams treated with warfarin.

Warfarin metabolites were not found at significant levels in tissues:: parent warfarin accounted for about 98% of the maternal plasma and 94% of the maternal liver. In the liver there was one metabolite accounting for 5%. The three other peaks were less than 1%.
In the foetus warfarin equivalents, i.e. warfarin and its metabolites were mainly found in the carcass (about 80% of the residue in foetuses) and the other 20% was found in the foetal liver. In the foetal carcass next to warfarin, 2 minor more polar metabolites were found, accounting for 0.8 and 0.6% of the r.a. in the carcass. In the foetal liver only warfarin was found.
The common metabolites of warfarin (2,3-dihydro-2-methyl-4-phenyl-5-oxo-γ-pyrano(2,3-c)(1)benzopyran and hydroxy-warfarin) were not detected in any of the maternal and foetal tissue extracts.

For flocoumafen there were 6 residues characterised in the plasma of the dams. Flocoumafen accounted for 25% of the total residue in the plasma and of the remaining 5 more polar residues there were 2 metabolites which accounted for 23 and 31% and the rest for less than 10% each. In the liver there were less metabolites: the main residue was flocoumafen (95%) and further two more

polar metabolites were found.
In the foetus flocoumafen equivalents were mainly found in the remaining carcass (about 80% of the residue in foetuses) and the other 20% was found in the liver. In the foetal carcass next to flocoumafen (20% of r.a. in carcass), there were 3 more polar metabolites found, accounting for 0.7, 63 and 16 % of the r.a. in the carcass. In the foetal liver the metabolite that was most abundant in the carcass was not found. Flocoumafen in liver accounted for 61% and the other 2 metabolites for 26 and 13%.

WBA analysis for both compounds showed that both warfarin and flocoumafen equivalents were able to cross the maternal-foetal placental barrier. Radioactivity was widely distributed throughout the placenta and foetus, with concentration in the foetal liver higher than in the other foetal tissues. Furthermore it was found that both warfarin and flocoumafen did not cross the foetal blood-brain barrier and did not appear to be eliminated from the foetus into the amniotic fluid.

Conclusion

The only sign of toxicity during the study was that the dams of the warfarin treated groups had a lower body weight gain during pregnancy, with the same but a lesser effect in the flocoumafen treated groups.
The study shows that flocoumafen, like warfarin, is able to pass the placenta. Whereas for warfarin maternal plasma levels were higher than foetal plasma levels, for flocoumafen the foetal plasma concentrations were twice as high as maternal plasma concentrations (at the Tmax of 6 h after dosing). However, the absolute foetal exposure (in µmol/kg, see table presented under 'Remarks') is much lower (about a factor 15) for flocoumafen than for warfarin at similar oral dosing levels, because most of the flocoumafen is retained in the maternal liver during first pass. For warfarin there is hardly a first pass effect.

For warfarin, parent compound accounted for about 98% of the maternal plasma and 94% of the liver. In foetal tissues about 99% of the residue was parent warfarin.

In contrast to warfarin, flocoumafen residues in tissues consist of metabolites to a substantial degree. In the maternal liver the main residue was flocoumafen itself, accounting for 95% of the r.a., and further 2 more polar metabolites. In maternal plasma only 25% of the residue was parent compound. Next to this, 6 other more polar metabolites were found, two of which accounted for 22 and 31% of the total residue in liver, respectively.
In foetal tissues, parent flocoumafen accounted for about 61% of the r.a. residue in foetal liver, the remainder consists of several more polar metabolites.

Remarks

- The vehicles used for flocoumafen and warfarin are different (corn oil vs. CMC) to maximise the bio-availability of each substance. This does not mean that the bio-availability of both substances is the same.

- For each compound only one time point of sacrifice is chosen. A more accurate comparison could have been made if the Area Under the Curve (AUC) of each compound was determined. Tmax varies between animals, so that it is likely that samples are taken sometimes before and sometimes after the Tmax. How much this affects the information on the internal exposure to flocoumafen and warfarin is unknown.

- According to the author of the study, "the molar residue levels in maternal liver allow for the assessment of the critical effect, since the percentage of VKOR molecules blocked by an anticoagulant determines the degree of blood clotting disturbance. The flocoumafen and warfarin dosing regimens of 0.013 and 0.016 mg/kg bw /day respectively indeed resulted in nearly identical molar residues in maternal liver at sacrifice thus providing a suitable basis for comparative evaluation of placental transfer." (see table below).

tissue	**Warfarin**		**Flocoumafen**		**Warfarin vs. flocoumafen (molar basis)**
	mg/kg	µmol/kg	mg/kg	µmol/kg	
maternal					
Oral dose	0.016	0.052	0.013	0.024	2.17
Liver	1.14	3.683	1.90	3.507	1.05
Blood cells	0.06	0.197	0.003	0.005	39.4
Plasma	0.21	0.675	0.006	0.012	56.3
foetal					
Carcass	0.09	0.293	0.007	0.013	22.5
Liver	0.22	0.719	0.017	0.031	38*
Blood cells	0.04	0.116	0.004	0.007	16.6
Plasma	0.12	0.371	0.014	0.025	14.8

- = considering that flocoumafen residues in foetal liver consist of 61% parent compound and 39% transformation products, the warfarin/flocoumafen ratio is 38.0.

In his comparison, the study author concluded on a molar basis that the difference in foetal exposure between warfarin and flocoumafen as evidenced by liver is about a factor 38 or 23 when including the metabolites of flocoumafen. The gradient between maternal and foetal flocoumafen residues in livers is in the range of 113 (including metabolites) - 177 (parent only), whereas this parameter amounts to only approximately 5 for warfarin. Therefore, since the liver is the target organ for anticoagulants, the study author stipulates that this result indicates substantial differences in exposure to flocoumafen between dams and foetuses.

The MSCA is of the opinion that for comparison of transplacental transfer one should look at the ratio plasma/blood in the mother versus the ratio in the foetus. Based on this the following can be concluded:
The major difference between warfarin and flocoumafen is that flocoumafen shows a high first pass effect after oral dosing. Most flocoumafen is retained in the liver, resulting in considerably lower plasma levels for flocoumafen compared to warfarin at a similar dosing level. Due to the lower maternal plasma levels, the foetal exposure to flocoumafen is lower than the foetal warfarin exposure after similar oral warfarin dosing. The results indicate higher foetal than maternal plasma levels of flocoumafen. However, the plasma levels are very low and in the range of the LOQ of 0.008 mg/kg meaning that there is some doubt to this conclusion. If foetal plasma levels were higher than maternal plasma levels this suggests that flocoumafen is possibly actively transported across the placenta to the foetus. In contrast, foetal plasma concentrations of warfarin are lower than

maternal plasma concentrations, suggesting that warfarin is transported across the placenta, but not actively.

The high first pass effect of flocoumafen after oral exposure results in low foetal exposure. However, in a situation in which the first pass effect is for any reason less effective or in a situation of a non oral exposure route, the materal plasma levels of flocoumafen may be significantly higher, resulting in a higher foetal exposure.

Further we do not agree that "the molar residue levels in maternal liver allow for the assessment of the critical effect, since the percentage of VKOR molecules blocked by an anticoagulant determines the degree of blood clotting disturbance" because the relation between the molar residue level and the percentage of blocked VKOR molecules also depends on the affinity of the molecules to VKOR and other molecules in the liver. This means that equimolar levels of warfarin and flocoumafen in the liver do not indicate a comparable level of inhibition of VKOR.

- The data show that foetal exposure to flocoumafen is relatively low compared to warfarin on day 19 of gestation when a fully developed placenta is present. However, some of the effects of warfarin are assumed to be induced in humans during the first trimester. In humans the placenta develops during the first trimester. This may mean that in humans there is no or only a partly developed placenta at the sensitive window for some of the warfarin effects. The rat data on day 19 may therefore not be relevant for these effects. However, as the main difference between flocoumafen and warfarin is the much higher liver retention of flocoumafen after oral exposure, a lower foetal availability of flocoumafen can be assumed also in the absence of a placenta.

5.10.5 Lactation

Neither toxicokinetic studies exist that would indicate any likelihood that flocoumafen would be present in potentially toxic levels in breast milk, nor did an animal teratogenicity study involving treatment of maternal rats with follow-up on the off-spring (F1) provide any indication of adverse effects on offspring mediated through transfer via milk, nor is there any evidence in humans indicating a risk to babies during the lactational period (according to current medical recommendations warfarin is considered to be compatible with breast feeding). In addition, the results of QSAR models predict that transfer of flocoumafen to breast milk would be extremely low. Particularly in comparison with warfarin, flocoumafen is expected to lack in any relevant potential of excretion to milk.

5.10.6 Summary and discussion of reproductive toxicity

This includes a comparison with the criteria.

Fertility

No multigeneration study investigating a potential effect of flocoumafen on fertility was available but effects on reproductive organs have been observed in other studies. In a single dose study with female rats, effects on ovaries and fertility were observed, however, at doses close to the LD50 values and possibly causing internal bleedings. In a 90-day rat study, haemorrhages were observed in male reproductive organs (testes, prostate, epididymes), but not in female ovaries. But also in this study the effects were noted at doses that caused severe generalised toxicity and death.

Since the effects observed in female (single dose) and male rats (repeated dose) could be related to the anticoagulation and were neither specific nor restricted to the reproductive system (effects secondary to severe generalised toxicity, i.e. haemorrhages, death), the data on flocoumafen do not meet the criteria for classification for fertility under both Regulation EC 1272/2008 and Directive 67/548/EEC.

The structural analogue warfarin did not show any effect on fertility after many years of human use and neither in a two generation reproduction study in rats with vitamin-K supplementation. It has therefore no classification for fertility.

Overall, there is insufficient evidence for a potential effect of flocoumafen on fertility, so no classification is proposed.

NB: the TC C&L based its conclusion on the same data as presented in this Annex VI report.

TC-C&L conclusion

The need for classification with Repro. Cat. 3; R62 was discussed at the TC C&L meeting, November 2006. The TC C&L agreed (ECBI/20/07, rev. 1) that the data presented did not trigger classification for fertility effects. This was based on the fact that the fertility effects were possibly non-specific accompaniments to severe generalised toxicity and on read-across from warfarin. For these and humane reasons, a new multi-generation study was not found to be necessary.

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007.

Developmental toxicity

Key studies relevant for classification are summarized in Table 5.13.

Table 5.13: Teratogenicity studies, relevant for classification

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 Teratogenicity Embryotoxicity**	**Study number**

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 Teratogenicity Embryotoxicity	Study number
Oral (gavage)	In accordance with EC B.31 and OECD 414 (1981)	Rabbit, NZW, 16 females/ dose	Day 6-18 post mating	0, 0.001, 0.002 or 0.004 mg/kg bw/day	Dams: Abortions, fur loss Foetuses: no toxicological relevant effects	0.002 mg/kg bw/day	> 0.004 mg/kg bw/day	A6.8.1/01 and A6.8.1/02
Oral (gavage)	Not in accordance with EC B.31 and OECD 414 (1981)[1]	Rat, Fischer 344, 18 females/ dose	Day 8-17 post mating	0, 0.01 or 0.04 mg/kg bw/day	Dams: haemorrhages, clinical signs (pale eyes, lethargy) Foetuses: no toxicological relevant effects	0.01 mg/kg bw/day	Could not be established due to methodological shortcomings.	A6.8.1/03
Oral (gavage)	Teratogeni-city study, non-guideline but shares characteris-tics of EC B.31 and OECD 414[2].	Rat, Crl:CD®, 20-15/females /dose	P-generation day 7-17 post mating[2]	0, 0.01, 0.02 or 0.04 mg/kg bw/day	Dams: mortality, haemorrhages Foetuses: No toxicological relevant effects	0.02 mg/kg bw/day	> 0.04 mg/kg bw/day	A6.8.1/04 and A6.8.1/05

[1] The following methodological deficiencies were noticed: females exposed from day 8-17 post mating instead of 6-15 post mating, females delivered naturally, the number of corpea lutea could not be reported, pre- and post implantation loss were not calculated, pups were not weighed, sex of the foetuses were not reported, pups were not examined for skeletal and soft tissue alterations.

[2] In addition, ten females littered to rear ther offspring. F1 offspring was retained and there performance in specific behavioural tests was assessed. When offpring was appr. 84 days of age, they were mated.

The criteria require classification in category 1A (CLP) or 1 (DSD) in case of clear evidence of developmental effects in humans, 1B (CLP) or 2 (DSD) in case of a clear developmental effect that is not secondary to marked maternal toxicity and not shown to be irrelevant to humans and category 2 (CLP) or 3 (DSD) where there is some evidence in human or animal studies but not sufficient for category 1B or 2.

Flocoumafen did not induce irreversible structural effects in rats and rabbits (key studies A6.8.1/01 and A6.8.1/02, A6.8.1/03, A6.8.1/04 and A6.8.1/05). In rabbits a NOAEL for maternal effects was established at 0.002 mg/kg bw/day, based on abortions and clinical signs. A NOAEL for developmental effects was set at >0.004 mg/kg bw/day, since no toxicological significant effects were observed in foetuses. In rats a NOAEL of 0.02 mg/kg bw/day was established for maternal effects, based on haemorrhages and mortality. As no toxicological significant effects were observed in foetuses, the NOAEL for developmental effects was set at >0.04 mg/kg bw/day.

The structurally related coumarin warfarin induces developmental effects in humans and is classified as Repr. Cat 1. Flocoumafen and warfarin both have a chemical structure resembling

vitamine K and both substances inhibit the coagulation most likely through the same mechanism namely inhibition of the vitamin K (epoxide) reductase complex. This results in effects on coagulation and bone formation. The same mechanism is also considered relevant for the developmental effects of warfarin. Therefore, it is very likely that also flocoumafen can induce the same developmental effects as warfarin if present in the foetus at relevant concentrations.

The fact that warfarin induces developmental effects in humans but flocoumafen and other second generation coumarins do not induce developmental effects in animal studies caused the Specialised Experts in 2006 to doubt whether the OECD 414 developmental study is a reliable model for the determination of the developmental effects of coumarins. The recently performed OECD 414 study with warfarin in rats shows that this model can detect some of the developmental effects induced by warfarin in humans but not all effects. Foetal haemorrhage, an effect observed in humans, was markedly increased in rats at all dose levels. However, other human effects of warfarin such as ocular changes, skeletal variations and malformations were observed at such low incidences in some of the higher exposed rat groups that it is uncertain whether such effects would occur or be observed in a standard OECD 414 study with normal dose intervals. The difference in type of developmental effects between the rat study and the human cases shows that there are differences in response between the rat model and humans. Further it is unlikely that a model like the OECD 414 study would give exactly the same relation for coumarins, including flocoumafen between the levels of maternal toxicity and foetal toxicity as in humans. This is indicated by the differences in effects of warfarin between rats and humans.
When comparing the warfarin rat OECD 414 study with the flocoumafen rat teratogenicity study (A6.8.1/04 and A6.8.1/05), the relative potency (maternal effects versus foetal effects) of flocoumafen to induce developmental effects is lower than for warfarin because in contrast to warfarin, no increase in foetal haemorrhages was observed for flocoumafen.
The recently conducted placental transfer study shows that whereas both flocoumafen and warfarin can cross the placenta and are available in the foetus, the relative amount of the coumarin that reaches the foetus is lower for flocoumafen than for warfarin.
So, both the flocoumafen teratogenicity study and the placental transfer study seem to indicate that foetal availability of flocoumafen is lower than foetal availability of warfarin. This may be a reason not to read-across from warfarin to flocoumafen, and to base the decision for classification for developmental toxicity on the (negative) animal data. This would result in no classification for developmental toxicity. Then again, some transplacental transfer of flocoumafen has been shown in the rat. In the rat this transplacental transfer is not high enough to induce developmental effects even at maternally toxic dose levels. However, as the rat model is not an exact model for humans it cannot be excluded that there is a possibility for induction of developmental effects in humans at exposure levels that are not severely maternally toxic. Given this uncertainty, it is proposed to classify flocoumafen as Repr. 2 – H361d (Regulation EC 1272/2008) and Repro Cat. 3 – R63 (Directive 67/548/EEC).

If flocoumafen induces developmental effects in humans then this will probably occur already at very low dose levels because at somewhat higher dose levels there will be marked maternal toxicity including mortality. Therefore, SCL for Repr. 2; H361d and Repr. Cat 3; R63 are required. Qualitative read-across of the developmental effects from warfarin to flocoumafen is already difficult but this is even more difficult if not impossible for quantitative effects. Therefore, it is proposed to assume that flocoumafen may only induce developmental effects at dose levels just below lethal dose levels. This results in a starting value of 0.005 mg/kg bw/day based on the highest dose level without mortality in the oral 90 day study in rats.

A method for the determination of SCLs for substances toxic to the reproduction is currently developed by a working group of EChA. This method is not finalised or approved by RAC or CARACAL. The method proposes to apply the general concentration limits to substances with an

ED10 (a 10% change in effect compared to controls) for effects warranting classification for reproductive toxicity between 4 to 400 mg/kg bw/day. For substances with an ED10 below 4 mg/kg bw/day SCLs of 0.03% (Cat 1) or 0.3% (Cat 2) are proposed. Substances with an ED10 more then a factor 10 below the limit of 4 mg/kg bw/day will have a 10-fold lower SCL and so on.

For flocoumafen a SCL of 0.003% is proposed because the starting value of 0.005 mg/kg bw/day is within the limit of 0.004 to 0.04 mg/kg bw/day. Although the general concentration limit differs between CLP (3%) and DSD (5%), the same SCL is proposed for both legislations as the difference is only very small.

Effects on or via lactation

Classification

Neither toxicokinetic studies exist that would indicate any likelihood that flocoumafen would be present in potentially toxic levels in breast milk, nor did an animal teratogenicity study involving treatment of maternal rats with follow-up on the off-spring (F1) provide any indication of adverse effects on offspring mediated through transfer via milk, nor is there any evidence in humans indicating a risk to babies during the lactational period (according to current medical recommendations warfarin is considered to be compatible with breast feeding). In addition, the results of QSAR models predict that transfer of flocoumafen to breast milk is extremely low. Particularly in comparison with warfarin, flocoumafen is expected to lack in any relevant potential of excretion to milk.

The available data therefore do not indicate a need for classification for effects on or via the milk.

TC-C&L conclusion

The TC-C&L concluded in November 2006 (ECBI/20/07, Rev. 1) that classification for effects on or via lactation was not required.

No classification is included for this hazard class in Annex VI, Tables 3.1 and 3.2, CLP Regulation, and no classification is currently proposed as also agreed by the TC C&L in 2006/2007.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Effects on fertility

A two-generation reproductive toxicity study was not available as it was waived by the notifier of the biocide-dossier of Flocoumafen.

Supporting data

Sangha et al., 1992, reported that in a non-guideline study, one group of rats received a single oral dose (17 females per dose group, gavage) of 0.08, 0.11 or 0.14 mg/kg bw. After one week ovaries were weighed and investigated histopathologically. A second group received 0 or 0.14 mg/kg (13 animals per dose group, gavage); after one week levels of total lipids, total cholesterol, phospholipids, free fatty acids, glycolipids and triglycerides were determined in the ovaries of half of the animals. The other half of the animals was paired and the breeding time and litter size were recorded.

In the first group, ovarian cyclicity was disturbed in all the treated rats and in the two highest dose groups most remained in the di-oestrous stage. Decreased ovary weights, artretic follicles and degenerating corpora lutea with pyknotic granules were noted at 0.14 mg/kg bw.

In the second group, increased levels of total lipids, triglycerides and cholesterol as well as decreased levels of phospholipids, free fatty acids and glycolipids were noted. Of the paired animals, the controls bred after 30 days and gave seven or eight pups per litter. Treated animals bred after 60 days and gave two to four pups per litter. 45 days after parturition, the second breeding was normal with a similar litter size (five to eight pups) for both groups.

In this study on female rats, effects on ovary and fertility were noted after a single dose of Flocoumafen. However, this study did not fulfil requirements of a guideline study, and the reporting of methods and results was very limited (no attention was paid to clinical symptoms and haematological and pathological parameters). Furthermore, the study indicated that effects on the ovary and fertility occurred after single oral dosing with Flocoumafen with doses close to the LD_{50} values (range: 0.13-0.5 mg/kg bw), possibly causing internal bleeding. For these reasons, it cannot be excluded that the effects on fertility were secondary to haemorrhages.

In the 90-day oral rat study, effects on male reproductive organs (haemorrhages in the testes, prostate and epididymides) were observed in the animals of the two highest dose groups (0.25 or 0.6 mg/kg food, equivalent to 0.0125 or 0.03 mg/kg bw per day). But it should be noted that all animals of these dose groups died during the study and haemorrhages were noted in several organs.

Warfarin is an anticoagulant compound which has been used in patients for many years to avoid or reduce blood coagulation. No effects on fertility in humans have ever been observed, and also in a two generation reproduction study in rats with vitamin K supplementation Warfarin did not show any effect on fertility. Warfarin is therefore not classified for effects on fertility.

Dossier Submitter's conclusion on Fertility

According to the DS there is insufficient evidence for an effect of Flocoumafen on fertility, so no classification was proposed.

Dossier Submitter's conclusion on Lactation

In the opinion of the DS the available data did not indicate a need for classification for effects on or via the milk.

Developmental toxicity

The notifier of the biocides dossier on Flocoumafen submitted three teratogenicity studies, one in rabbits, and two in rats.

In a teratogenicity study with rabbits, animals were dosed daily with Flocoumafen at 0, 0.001, 0.002 or 0.004 mg/kg bw/day by gavage, from day 6 to day 18 post mating. Maternal animals at 0.004 mg/kg bw/day showed abortions (3 of 14 animals), and the presence of blood on the tray paper on days 19 to 29 in 6 out of 11 dams with live young, and a slight increased incidence of fur loss in the post dosing period. The NOAEL for maternal effects was established at 0.002 mg/kg bw/day. Since no toxicologically relevant developmental effects were observed and no teratogenic effects were reported, the NOAEL for developmental and teratogenic effects was set at >0.004 mg/kg bw/day.

In a teratogenicity study with rats, pregnant animals were given daily Flocoumafen doses of 0, 0.01 or 0.04 mg/kg bw, from day 8 to 17 post mating. At the high dose, females showed clinical signs of toxicity (pale eyes, lethargy and haemorrhage from vulva), indicative of anti-coagulant poisoning. At necropsy, animals showed internal haemorrhage. Maternal animals at 0.01 mg/kg bw showed no signs of toxicity. No effects on number of live pups, litter weight and surviving pups were noted. No external abnormalities were observed. As animals delivered naturally, the number of corpora lutea could not be reported and pre- and post- implantation loss was not calculated. Pups were not examined for skeletal and soft tissue alterations. Under the circumstances of the study, Flocoumafen did not induce developmental effects in rats at dose levels up to 0.04 mg/kg bw. However, considering the limited study design, a NOAEL for developmental and teratogenic effects was not established. The NOAEL for maternal effects was established at 0.01 mg/kg bw/d.

Table 1. Key teratogenicity studies, relevant for classification

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Doses	Critical effects dams foetuses	NO(A)EL maternal toxicity	NO(A)EL Terato-genicity Embryo-toxicity	Study number
Oral (gavage)	In accordance with EC B.31 and OECD 414 (1981)	Rabbit, NZW, 16 females/ dose	Day 6-18 post mating	0, 0.001, 0.002 or 0.004 mg/kg bw/day	Dams: Abortions, fur loss Foetuses: no toxicological relevant effects	0.002 mg/kg bw/day	> 0.004 mg/kg bw/day	A6.8.1/01 and A6.8.1/02
Oral (gavage)	Not in accordance with EC B.31 and OECD 414 (1981)[1]	Rat, Fischer 344, 18 females/ dose	Day 8-17 post mating	0, 0.01 or 0.04 mg/kg bw/day	Dams: haemorr hages, clinical signs (pale eyes, lethargy) Foetuses: no toxicological relevant effects	0.01 mg/kg bw/day	Could not be established due to metho-dological short-comings.	A6.8.1/03
Oral (gavage)	Terato geni-city study, non-guideline but shares character ris-tics of EC B.31 and OECD 414[2].	Rat, Crl:CD®, 20-15/ fem ales/dose	P-gene ration day 7-17 post mating[2]	0, 0.01, 0.02 or 0.04 mg/kg bw/day	Dams: mortality, haemorr hages Foetuses: No toxicological relevant effects	0.02 mg/kg bw/day	> 0.04 mg/kg bw/day	A6.8.1/04 and A6.8.1/05

[1] The following methodological deficiencies were noticed: females exposed from day 8-17 post mating instead of 6-15 post mating, females delivered naturally, the number of corpea lutea could not be reported, pre- and post-implantation loss were not calculated, pups were not weighed, sex of the foetuses were not reported, pups were not examined for skeletal and soft tissue alterations.

[2] In addition, ten females littered to rear their offspring. F1 offspring were retained and their performance in specific behavioural tests was assessed. When offspring were approximately 84 days of age, they were mated.

In a teratogenicity study, 55 rats per group were mated and were given Flocoumafen (in corn oil) at levels of 0, 0.01, 0.02 or 0.04 mg/kg bw/day from day 7 to 17 post mating. Many animals were found not to be pregnant (16, 18, 11, and 3 in the control, low, mid and high dose groups, respectively) and were removed from the study. Ten dams per dose were allowed to litter and to rear their offspring to weaning, and the remaining females were allocated to day 20 sacrifice and their foetuses were preserved for visceral and skeletal examination. From the dams that were allowed to litter the F1 offspring were examined in specific behavioural tests and excess pups were sacrificed and examined for abnormalities. F1 offspring were mated at 12 weeks and females were allowed to litter and rear their offspring to weaning.

F2 pups and F1 adults were sacrificed and examined for abnormalities.

At 0.04 mg/kg bw/day, P females showed mortality (10 females in the 20 day sacrifice group and one female in the group that was allowed to litter), signs of anticoagulant toxicity and/or haemorrhages at necropsy. One female of the mid-dose group with live young at day 20 also showed clinical signs of toxicity (unsteady walking, pale extremities and bleeding from vagina on day 20). Malformations (M) or abnormalities were observed in some foetuses of the 20 day sacrifice group in the control and treated groups. Two small foetuses (< 2.3 g) were found in the high dose group, one of which had a small left eye and one small foetus was found in the mid-dose group, which also showed microphthalmia.

Based on these observations the NOAEL for maternal toxicity was established at 0.02 mg/kg bw/day. No toxicological relevant effects were observed on F1 and F2 litter. Pre-weaning development was similar in offspring from all groups. Therefore, the NOAEL for developmental toxicity was established at >0.04 mg/kg bw/day.

Since no teratogenic effects were reported, the NOAEL for teratogenic effects was set at >0.04 mg/kg bw/day.

The DS noted that Flocoumafen is a coumarin derivative and as such is a structural analogue of Warfarin, the most well-known coumarin. Warfarin is classified as Repr. 1A; H360D, because it was found to induce teratogenicity in humans. The coumarins are used as rodenticides and are known as anti-vitamin K (AVK) rodenticides. These rodenticides have a chemical structure resembling vitamin K and inhibit the coagulation of blood, most likely via the same mechanism, namely inhibition of the vitamin K (epoxide) reductase complex. Vitamin K epoxide reductase (VKOR) is an integral membrane protein that catalyzes the reduction of vitamin K 2,3-epoxide and vitamin K to vitamin K hydroquinone, a cofactor required for the gamma-glutamyl carboxylation reaction. VKOR is highly sensitive to inhibition by Warfarin. Warfarin inhibition of VKOR decreases the concentration of reduced vitamin K, which reduces the rate of vitamin K-dependent carboxylation and leads to under-carboxylated, inactive vitamin K-dependent proteins (Tie and Stafford, 2008). There are several proteins requiring carboxylation to become active including several coagulation factors and bone proteins (Furie et al, 1999). Inhibition of VKOR results in effects on coagulation and bone formation (Howe and Webster, 1999; Hall et al, 1980).

In order to compare the existing data on Flocoumafen with those for Warfarin, the DS provided *in extensor* data on reproductive toxicity of Warfarin in humans and animals based on the CLH report submitted to ECHA by the Irish MSCA. These data are summarized in the RAC opinion for Warfarin.

Placental transfer

To facilitate comparison of the mode of action (MoA) of developmental toxicity of Warfarin and Flocoumafen, the DS provided the results of a study on placental transfer (Johnson, 2009). The results enable a comparison of the potential of Warfarin and Flocoumafen to cross the placental barrier.

Study design

^{14}C-radiolabelled Flocoumafen and Warfarin were administered orally as a single daily dose from gestation day 6 through 19 to time-pregnant Wistar (Crl:WI[HAN]) rats. Flocoumafen, in corn oil, was administered to two groups of 5 rats at dose levels of 0.006 and 0.013 mg/kg/day (groups 2 and 3) and to one group of 2 rats (group 6) at 0.013 mg/kg/day. Warfarin, in 0.5% carboxymethylcellulose (CMC), was administered to two groups of 5 rats at dose levels of 0.016 and 0.033 mg/kg bw per day (group 4 and 5) and to one group of 2 rats at 0.033 mg/kg bw per day (group 7). One control group (group 1) of 5 rats received corn oil. The rats were observed daily for clinical signs and survival, and maternal body weights were recorded daily on gestation days 5-19.

The animals in the groups of five rats were sacrificed on gestation day 19 at either 0.5 h (Warfarin), 4 h (control) or 6 hours (Flocoumafen) following the last dose, which correspond to the highest plasma levels (Tmax) for Warfarin and Flocoumafen.

The animals in the groups with 2 rats were designated for WBA (whole body autoradiography) and were sacrificed on gestation day 19 at 6 h following the last dose for Flocoumafen treated rats, and at 40 and 62 minutes following final dose for the Warfarin treated animals (due to technical problems the protocol-specified euthanasia time (30 min) for these animals was not achieved).

The concentrations of radiolabelled Flocoumafen and Warfarin equivalents in maternal and foetal tissues

are indicated in following tables.

Table 2. Residue levels (µg/g) of ^{14}C-Flocoumafen equivalents in maternal and foetal tissues

Tissue type	Dose group 2 0.0059 mg/kg bw per day		Dose group 3 0.013 mg/kg bw per day	
	Maternal (ug/g)	Foetal (ug/g)	Maternal (ug/g)	Foetal (ug/g)
Plasma	0.003	0.007	0.006	0.014
Blood cell fraction	0.001	0.001	0.003	0.004
Placenta	0.018	NA	0.050	NA
Liver	0.913	0.007	1.903	0.017
carcass	NA	0.003	NA	0.007

NA = not applicable

Table 3. Residue levels (µg/g) of ^{14}C-Warfarin equivalents in maternal and foetal tissues

Tissue type	Dose group 4 0.016 mg/kg bw per day		Dose group 5 0.033 mg/kg bw per day	
	Maternal (ug/g)	Foetal (ug/g)	Maternal (ug/g)	Foetal (ug/g)
Plasma	0.209	0.115	0.403	0.354
Blood cell fraction	0.061	0.036	0.104	0.086
Placenta	0.102	NA	0.169	NA
Liver	1.141	0.223	1.857	0.387
carcass	NA	0.091	NA	0.185

At similar dose levels, 0.013 or 0.016 mg/kg bw per day for Flocoumafen and Warfarin respectively, the Flocoumafen treated dams had approximately 20-35 fold lower concentrations of radioactive residues in plasma and blood cellular fraction and approx. 2 fold lower concentrations in placenta than dams treated with Warfarin.

For Warfarin metabolites were not found at significant levels in tissues: parent Warfarin accounted for about 98% of the maternal plasma and 94% of the maternal liver radioactivity. In the liver, one metabolite accounted for 5% of the radioactivity. The three other peaks were less than 1%.
In the foetus, Warfarin equivalents, i.e. Warfarin and its metabolites were mainly found in the carcass (about 80% of the residue in foetuses) and the other 20% was found in the foetal liver. In the foetal carcass, apart from Warfarin, 2 minor more polar metabolites were found, accounting for 0.8 and 0.6% of the radioactivity. in the carcass. In the foetal liver only Warfarin was found.

The common metabolites of Warfarin (2,3-dihydro-2-methyl-4-phenyl-5-oxo-γ-pyrano(2,3-c) (1) benzopyran and hydroxy-Warfarin) were not detected in any of the maternal and foetal tissue extracts.

For Flocoumafen, 6 residues were characterised in the plasma of the dams. Flocoumafen accounted for 25% of the total residue in the plasma and of the remaining 5 more polar residues, there were 2 metabolites which accounted for 23 and 31% and the rest for less than 10% each. In the liver there were less metabolites: the main residue was Flocoumafen (95%) and a further two more polar metabolites were found.

In the foetus, Flocoumafen equivalents were mainly found in the remaining carcass (about 80% of the residue in foetuses) and the other 20% was found in the liver. In the foetal carcass apart from

Flocoumafen (20% of radioactivity in carcass), 3 polar metabolites were found, accounting for 0.7, 63 and 16 % of the radioactivity in the carcass. In the foetal liver the metabolite that was most abundant in the carcass was not found. Flocoumafen in liver accounted for 61% and the other 2 metabolites for 26 and 13%.

WBA analysis for both compounds showed that both Warfarin and Flocoumafen equivalents were able to cross the maternal-foetal placental barrier. Radioactivity was widely distributed throughout the placenta and foetus, with concentrations in the foetal liver higher than in the other foetal tissues. Furthermore, it was found that neither Warfarin nor Flocoumafen crossed the foetal blood-brain barrier and did not appear to be eliminated from the foetus into the amniotic fluid.

According to the author of the study, "the molar residue levels in maternal liver allow for the assessment of the critical effect, since the percentage of VKOR molecules blocked by an anticoagulant determines the degree of blood clotting disturbance. The Flocoumafen and Warfarin dosing regimens of 0.013 and 0.016 mg/kg bw /day respectively indeed resulted in nearly identical molar residues in maternal liver at sacrifice thus providing a suitable basis for comparative evaluation of placental transfer." (see Table 11 below).

Table 4. Comparative evaluation of placental transfer for Warfarin and Flocoumafen

Tissue	**Warfarin**		**Flocoumafen**		**Ratio of Warfarin vs. Flocoumafen concentrations in tissues (molar basis)**
Oral dose administered	mg/kg	µmol/kg	mg/kg	µmol/kg	
	0.016	0.052	0.013	0.024	2.17
maternal					
Liver	1.14	3.683	1.90	3.507	1.05
Blood cells	0.06	0.197	0.003	0.005	39.4
Plasma	0.21	0.675	0.006	0.012	56.3
foetal					
Carcass	0.09	0.293	0.007	0.013	22.5
Liver	0.22	0.719	0.017	0.031	38*
Blood cells	0.04	0.116	0.004	0.007	16.6
Plasma	0.12	0.371	0.014	0.025	14.8

* = considering that Flocoumafen residues in foetal liver consist of 61% parent compound and 39% transformation products, the Warfarin/Flocoumafen ratio is 38.0.

In his comparison, the study author concluded that based on distribution to the liver on a molar basis, the difference in foetal exposure between Warfarin and Flocoumafen, is about a factor of 38 (or 23 when the metabolites of Flocoumafen are included). The ratio between maternal and foetal Flocoumafen residues in livers is in the range of 113 (including metabolites) - 177 (parent compound only), whereas this parameter amounts to only approximately 5 for Warfarin. Therefore, since the liver is the target organ for anticoagulants, the study author stipulates that this result indicates substantial differences in exposure to Flocoumafen between dams and foetuses.

Study Author Conclusion: The only sign of toxicity during the study was that the dams of the Warfarin treated groups had a lower body weight gain during pregnancy, which was less pronounced in the Flocoumafen treated groups.

The study showed that Flocoumafen, like Warfarin, was able to pass through the placenta. Whereas for Warfarin maternal plasma levels were higher than foetal plasma levels, for Flocoumafen the foetal plasma

concentrations were twice as high as maternal plasma concentrations (at the Tmax of 6 h after dosing). However, the absolute foetal exposure (in µmol/kg, see table 11) is much lower (about a factor 15) for Flocoumafen than for Warfarin at similar oral dosing levels, because most of the Flocoumafen is retained in the maternal liver during first pass. For Warfarin there is hardly any first pass effect.

For Warfarin, the parent compound accounted for about 98% of the maternal plasma and 94% of the liver. In foetal tissues about 99% of the residue was parent Warfarin.

In contrast to Warfarin, Flocoumafen residues in tissues consist of metabolites to a substantial degree. In the maternal liver the main residue was Flocoumafen itself, accounting for 95% of the radioactivity, and a further 2 more polar metabolites. In maternal plasma only 25% of the residue was parent compound. Apart from this, 6 other more polar metabolites were found, two of which accounted for 22 and 31% of the total residue in liver, respectively.

In foetal tissues, parent Flocoumafen accounted for about 61% of the radioactivity residue in foetal liver, the remainder consisting of several more polar metabolites.

The Dossier Submitter for Flocoumafen noted that the major difference between Warfarin and Flocoumafen is that Flocoumafen showed a high first pass effect after oral dosing. The first-pass effect (also known as first-pass metabolism or pre-systemic metabolism) is a phenomenon of drug metabolism whereby the concentration of a drug is greatly reduced before it reaches the systemic circulation.

Most Flocoumafen is retained in the liver, resulting in considerably lower plasma levels for Flocoumafen compared to Warfarin at a similar dosing level. Due to the lower maternal plasma levels, the foetal exposure to Flocoumafen is lower than the foetal Warfarin exposure after similar oral doses of both substances. The results indicate higher foetal than maternal plasma levels of Flocoumafen. However, the plasma levels are very low and in the range of the LOQ of 0.008 mg/kg meaning that there is some doubt about this conclusion. If foetal plasma levels were higher than maternal plasma levels, then this suggests that Flocoumafen is possibly actively transported across the placenta to the foetus. In contrast, foetal plasma concentrations of Warfarin are lower than maternal plasma concentrations, suggesting that Warfarin is transported across the placenta, but not actively.

In addition, the DS noted that they disagree that "*the molar residue levels in maternal liver allow for the assessment of the critical effect, since the percentage of VKOR molecules blocked by an anticoagulant determines the degree of blood clotting disturbance*" because the relationship between the molar residue level and the percentage of blocked VKOR molecules also depends on the affinity of the molecules to VKOR and other molecules in the liver. This means that equimolar levels of Warfarin and Flocoumafen in the liver do not necessarily indicate a comparable level of inhibition of VKOR.

The data show that foetal exposure to Flocoumafen is relatively low compared to Warfarin on day 19 of gestation when a fully developed placenta is present. However, some of the effects of Warfarin are assumed to be induced in humans during the first trimester. In humans the placenta develops during the first trimester. This may mean that in humans there is no or only a partly developed placenta at the sensitive window for some of the Warfarin effects. The rat data on day 19 may therefore not be relevant for these effects. However, as the main difference between Flocoumafen and Warfarin is the much higher liver retention of Flocoumafen after oral exposure, a lower foetal availability of Flocoumafen can be assumed also in the absence of a placenta.

Lactation

There were neither toxicokinetic studies that indicated any likelihood that Flocoumafen would be present in potentially toxic levels in breast milk, nor did an animal teratogenicity study involving treatment of maternal rats with follow-up on the off-spring (F1) provide any indication of adverse effects on offspring mediated through transfer via milk, nor is there any evidence in humans indicating a risk to babies during the lactational period. In addition, the results of QSAR models predict that transfer of Flocoumafen to breast milk in humans would be extremely low. Particularly in comparison with Warfarin, Flocoumafen is expected to be devoid of any potential for excretion to milk.

DS Conclusion on Developmental toxicity

According to the DS, the Flocoumafen teratogenicity study and the placental transfer study seem to indicate that foetal availability of Flocoumafen is lower than foetal availability of Warfarin. This may be a

reason not to read-across from Warfarin to Flocoumafen, and to base the decision for classification for developmental toxicity on the (negative) animal data. This would result in no classification for developmental toxicity. Then again, some placental transfer of Flocoumafen has been shown in the rat. In the rat this placental transfer is not high enough to induce developmental effects even at maternally toxic dose levels. However, as the rat model is not an exact model for humans it cannot be excluded that there is a possibility for induction of developmental effects in humans at exposure levels that are not severely maternally toxic. Given this uncertainty, it is proposed to classify Flocoumafen as Repr. 2 – H361d (Regulation (EC) 1272/2008).

SCL proposed by DS

For Flocoumafen a SCL of 0.003% is proposed because the starting value of 0.005 mg/kg bw/day is within the limit of 0.004 to 0.04 mg/kg bw/day. Although the general concentration limit differs between CLP (3%) and DSD (5%), the same SCL is proposed for both legislations as the difference is only very small.

Comments received during public consultation

During the PC four MS disagreed with the DS proposal to classify Flocoumafen as Repr. 2; H360d and proposed to classify the substance (based on Warfarin data) as Repr. 1A; H360D.

Industry was of the opinion that Flocoumafen should not be classified for developmental toxicity.

Assessment and comparison with the classification criteria

Fertility /Lactation

In the opinion of RAC, due to lack of relevant data, classification of Flocoumafen is not warranted for adverse effects on sexual function and fertility or for effects on or via lactation.

Developmental toxicity

Based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (Repr 1A), the reproductive toxicity of Flocoumafen has been analysed in detail. It is acknowledged that the animal developmental toxicity studies on Warfarin are weakly positive and that the animal developmental toxicity studies on Flocoumafen are negative. However, in comparison with Warfarin, Flocoumafen and other 2nd generation AVKs have higher acute and repeated dose toxicity, steeper dose-response curves, and much longer half-lives in the exposed organisms, making the evaluation of developmental effects of all 2nd generation AVK rodenticides difficult. Thus, repeated exposure to relatively low doses during gestation lead to maternal toxicity and lethality which hinders the detection of developmental toxicity at higher doses.

As there were no data available on the outcome of maternal exposure to Flocoumafen in humans, classification as Repr. 1A was not considered to be applicable for Flocoumafen.

Based on the assumption that all AVK rodenticides, including Warfarin and other anticoagulant coumarin-based pharmaceuticals (see below) share the same MoA, namely inhibition of vitamin K epoxide reductase (VKOR), the assessment of Flocoumafen includes consideration of the total database for the AVKs. A weight of evidence assessment resulted in the conclusion that Flocoumafen has the capacity to adversely affect the human *in utero* development. Therefore a classification as Repr 1B is proposed with the reasoning given below.

The reasons for this conclusion are:

- Flocoumafen shares the same MoA as expressed by other anticoagulant AVK rodenticides and coumarin-based pharmaceuticals (inhibition of vitamin K epoxide reductase, an enzyme involved with blood coagulation and foetal tissues development, including bone formation, CNS development and angiogenesis)
- Warfarin and 2 other coumarin pharmaceuticals (acenocoumarol, phenprocoumon) have been shown to cause developmental toxicity in humans.
- One of the 2nd generation AVK rodenticides (Brodifacoum) has been shown to cause foetal effects in humans, possibly after one or a few exposures.

- For AVK rodenticides with a long half-life in the body, even single exposures might suffice to trigger developmental effects. However, such studies are normally not conducted and effects of single dose exposure cannot be detected in standard OECD 414 test where instead the repeated exposures may lead to maternal mortality with steep dose-response.
- The standard animal studies do not pick up all developmental toxicity effects of the AVK rodenticides, most notably the face and CNS malformations that are characteristic for Warfarin and other AVK coumarin pharmaceuticals.
- The most sensitive window for face malformations in humans is the first trimester. Thus, even if some AVK rodenticides may have a lower degree of placental transfer than Warfarin, this will not affect the face malformation hazard.

Not all steps of the MoA in the target tissues liver and bone have been proven, thus introducing some uncertainty into the assessment. However, the RAC is of the opinion that the uncertainty is not sufficient to warrant a Repr. 2 classification.

Reliable evidence of an adverse effect on reproduction in humans, which is required for Repr. 1A, was not available for Flocoumafen, but potential for human developmental toxicity is presumed based on the weight of evidence assessment above, and RAC thus proposes classification with Repr. 1B, i.e. "presumed human reproductive toxicant".

Specific Concentration Limit

Classification as Repr. 1B for developmental toxicity for Flocoumafen is supported by the RAC. However, only for Warfarin is there 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 be regarded as an ED10 level. This human ED10 value would, if using the guidance for setting SCLs based on animal data, belong to the high potency group (<4 mg/kg/day). The guidance states that for an ED10 <4 mg/kg/day, the SCL is 0.03%, and for ED10 below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from 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 result in a SCL of 0.003%. Thus, the RAC concluded on a SCL on 0.003% for the developmental toxicity of 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 rather to base the SCLs on the SCL 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 all the currently discussed AVK rodenticides, including Flocoumafen.

5.11 Neurotoxicity

A neurotoxicity study was waived by the applicant. From the results of the available toxicity studies with flocoumafen there are no indications for neurotoxicity. Furthermore, flocoumafen is not similar or related to substances capable of inducing (delayed) neurotoxicity.

5.12 Human data

Only a limited amount of human data was available.

A biomedical monitoring study was conducted during the start-up period of a flocoumafen rodenticide formulation plant. However this study has its limitations, apart from small decreases in

prothrombin concentration in two subjects, all or not related with absorption of flocoumafen into the body, no biological or adverse health effects were detected in any plant worker (because no PIVKA II could be detected in the blood of any subject, using the indirect PIVKA II assay, indicating that PIVKA II concentrations, if present, were below 10%).

At a health check at the Sorex Product Development Laboratories, the prothrombin times determined in 17 staff members were in a normal range. However, this medical surveillance of manufacturing plant personnel has a limited study design with limited reporting. No symptoms of intoxication were detected.

5.13 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity, flammability, and oxidising potential

Table 6.1: Physical-chemical properties of flocoumafen required for hazard identification

Parameter	Method/ Guideline	Result	Study number
Flammability and auto-flammability	EC A.10	Not "highly flammable" according to the guideline criteria. No induction of self-ignition.	A3.11/01
	EC A.16		A3.11/02
Explosive properties	Model calculation	No evidence for explosive properties based on structural aspects and thermodynamic properties.	A3.15/01
Oxidising properties	Model calculation	No evidence for oxidising properties based on structural aspects and thermodynamic properties.	A3.16/01

Summary and discussion of human health assessment of physiochemical properties

Flocoumafen does not exhibit any particularly hazardous physical-chemical properties. The substance is thermally stable, not "highly flammable", does not show explosive and/or oxidising properties, and can be stored in commercially available packaging material. Therefore, no classification for physiochemical properties is proposed. Corrosiveness to any type of containers and apparatus has not been observed. The proposed packaging material Lupolen is a high density polythene material manufactured by BASF.

7 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties assessment for flocoumafen is based on the revised Competent Authority Report (CAR) and the draft final CAR, document IIA, prepared in the context of the possible inclusion of flocoumafen in Annex I of Council Directive 91/414/EEC (May 2009 + January 2009, RMS The Netherlands) on the inclusion of flocoumafen in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.

All tables in the present assessment are copied from the draft final CAR. The tables are renumbered in accordance with the paragraph numbers. The assessment report is publicly available at: http://circa.europa.eu/Public/irc/env/bio_reports/library?l=/assessement_directive/assessment_cleanpdf/_EN_1.0_&a=d

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

Table 7.1: Acute toxicity of flocoumafen to fish.

Guideline/ Exposure	Species	Endpoint/ Type of test	Exposure		Results [mg/L]	Remarks	Reference
			Design	Duration [h]	LC50		
OECD 203/ EC C.1	*Oncorhynchus mykiss*	Mortality	Semi-static	96	0.07	None	A7.4.1.1/01
OECD 203/ EC C.1	*Lepomis macrochirus*	Mortality	Semi-static	96	0.11	None	A7.4.1.1/02

Study 1: In an OECD 203 study, carried out under semi-static conditions with test solution renewal every 24 hours, *Oncorhynchus mykiss* (10 fish per group in duplicate) were exposed to flocoumafen at the concentrations 0, 0.01, 0.022, 0.10 and 0.22 mg/L (nominal). Acetone (0.1 mL/L) was used as solvent. Analytical monitoring of flocoumafen concentrations in the water were carried out a 1, 24, 48, 72 and 96 h after start of the test. The mean measured concentrations were within 81-102% of nominal concentrations. The 96 hour LC50, calculated by probit analysis, was 0.07 mg/L based on mean measured concentrations.

Study 2: In an OECD 203 study, carried out under semi-static conditions with test solution renewal every 24 hours, *Lepomis macrochirus* (10 fish per group in duplicate) were exposed to flocoumafen at the concentrations 0, 0.05, 0.1, 0.22, 0.5 and 1.0 mg/L (nominal). Acetone (0.1 mL/L) was used as solvent. Analytical monitoring of flocoumafen concentrations in the water were carried out a 1, 24, 48, 72 and 96 h after start of the test. The mean measured concentrations were within 80% for the nominal concentrations 0.05, 0.1 and 0.22 mg/L but about 72% for the concentrations 0.5 and

1.0 mg/L. The 96 hour LC50, calculated by probit analysis, was 0.11 mg/L based on mean measured concentrations.

Long-term toxicity to fish

No data are available.

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Table 7.2: Acute toxicity of flocoumafen to aquatic invertebrates

Guideline/ Exposure	Endpoint/ Type of test	Exposure		Results [mg/L]	Remarks	Reference
		Design	Duration [h]	EC50		
OECD 202	% immobilisation	Semi-static	48	0.18	None	A7.4.1.2/01

In an OECD 202 study, carried out under semi-static conditions with test solution renewal after 24 hours, *Daphnia magna* were exposed to flocoumafen at the concentrations 0, 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/L (nominal). Acetone, applied at 0.1 mL/L, was used as solvent. Analytical monitoring of flocoumafen concentrations in the water were carried out at 0, 24 (fresh and used solutions) and 48 h after start of the test. Mean measured concentrations were 0, 0.025, 0.068, 0.12, 0.28 and 0.62 mg/L. No *Daphnia* in the blank control were immobilised but one immobilised *Daphnia* was found in the solvent control at 48 hours. No daphnids were trapped at the surface. The 48 hour EC50 was 0.18 mg/L based on mean measured concentrations.

Long-term toxicity to aquatic invertebrates

No data are available.

7.1.1.3 Algae and aquatic plants

Table 7.3: Growth inhibition of flocoumafen to algae, based on mean measured concentrations.

Guideline/ Exposure	Species	Endpoint/ Type of test	Exposure		Results [mg/L]		Reference
			Design	Duration [h]	NOE_rC	E_rC50	
OECD 201, EC C.3	*Pseudo-kirchneriella subcapitata*	Growth inhibition	Static	72	> 18.2	> 18.2	A7.4.1.3/01

In an OECD 201 study, carried out under static conditions with, *Pseudokirchneriella subcapitata* (initial cell concentration 10^4 mg/L) were exposed to flocoumafen at the concentrations 0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L (nominal). Cremophor RH40 (100 mg/L) was used as a solubilising agent. Analytical monitoring of flocoumafen concentrations in the water were carried out at test initiation and termination. The highest nominal test concentration were above the water solubility. Consequently, the measured concentrations deviate significantly from the nominal concentration. Only a minor growth inhibition occurred within the tested concentrations. The 72 hour ErC50 and NOErC were higher than the highest measured concentration of 18.2 mg/L.

7.1.1.4 Sediment organisms

No data are available.

7.1.1.5 Other aquatic organisms

No data are available.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this dossier.

7.2 Terrestrial compartment

Not relevant for this dossier.

7.3 Toxicity test results

7.3.1.1 Toxicity to soil macro organisms

No data are available.

7.3.1.2 Toxicity to terrestrial plants

No data are available.

7.3.1.3 Toxicity to soil micro-organisms

No data are available.

7.3.1.4 Toxicity to other terrestrial organisms

Not relevant for this dossier.

7.3.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of report.

7.4 Atmospheric compartment

See section 4.1.1

7.5 Microbiological activity in sewage treatment systems

7.5.1 Toxicity to aquatic micro-organisms

In a study following OECD209 with 3-h contact time, the toxicity of flocoumafen to micro-organism in sludge was examined. No respiration inhibition was observed at 4 mg/l, the highest concentration tested.

7.5.2 PNEC for sewage treatment plant

Not relevant for this dossier.

7.6 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this dossier.

7.7 Comparison with criteria

Directive 67/548/EEC

Flocoumafen fulfils the criteria for classification with N; R50/53:

- In acute toxicity studies in fish and crustaceans, $L(E)C_{50}$ values < 1 mg/L were obtained. The lowest aquatic acute toxicity value is an LC50 of 0.07 mg/L in fish.
- Flocoumafen is hydrolytically stable. Flocoumafen appears to be susceptible to primary degradation due to photo-transformation in water and photo-oxidation in air. However, flocoumafen is not ready biodegradable and is not degraded under anaerobic conditions. In a simulation test in soil, mineralisation of flocoumafen was only 2.5-15.6% after 120 days. Based on these results, flocoumafen is considered not readily degradable for the purpose of classification and labelling.
- The log Kow of flocoumafen is > 3. No measured BCF values are available.

Specific concentration limits according to Directive 2006/8/EC, amending Directive 1999/45/EC should be set. Based on the LC50 value of 0.07 mg/L obtained for *Oncorhynchus mykiss* in a 96-h study following OECD guideline 203, the following specific concentration limits for flocoumafen are proposed:

$C \geq 2.5\%$	N; R50/53
$0.25\% \leq C < 2.5\%$	N; R51/53
$0.025\% \leq C < 0.25\%$	R52/53

Regulation EC 1272/2008, acute toxicity:

Flocoumafen fulfils the criteria for classification as aquatic acute 1 (H400):

- In acute toxicity studies in fish and crustaceans, $L(E)C_{50}$ values < 1 mg/L were obtained. The lowest aquatic acute toxicity value is an LC50 of 0.07 mg/L in fish.

An acute M-factor of 10 should be applied, based on an LC50 value of 0.07 mg/L obtained for the fresh water fish *Oncorhynchus mykiss* in a 96-h semi static study following guideline OECD 203.

Regulation EC 1272/2008, chronic toxicity:

Flocoumafen fulfils the criteria for classification as Aquatic Chronic 1 (H410):

- Chronic toxicity values are available for algae: a NOErC of ≥ 18.2 mg/L was obtained in an OECD 201 study.
- No chronic toxicity studies have been carried out for invertebrates or fish. In acute toxicity studies in fish and crustaceans, LC_{50} value of 0.07 mg/L and EC50 value of 0.18 mg/L were obtained.
- Flocoumafen is hydrolytically stable. Flocoumafen appears to be susceptible to primary degradation due to photo-transformation in water and photo-oxidation in air. However, flocoumafen is not ready biodegradable and is not degraded under anaerobic conditions. In a simulation test in soil, mineralisation of flocoumafen was only 2.5-15.6% after 120 days. Based on these results, flocoumafen is considered not rapidly degradable for the purpose of classification and labelling.
- The log Kow of flocoumafen is > 4. No measured BCF values are available.

In conclusion, based on surrogate chronic data for invertebrates and fish, flocoumafen is considered to fulfil the criteria for classification as Aquatic Chronic 1 (H410).

A chronic M-factor of 10 should be applied, based on an LC50 value of 0.07 mg/L obtained for the fresh water fish *Oncorhynchus mykiss* in a 96-h semi static study following guideline OECD 203.

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

There is a current environmental classification in Annex VI of CLP for Flocoumafen as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The DS proposed to add an M-factor of 10 to Aquatic Acute 1 and an M-factor of 10 to Aquatic Chronic 1.

Degradation
Degradation was studied in a hydrolysis test, a photolysis test in water, a ready biodegradability test, an anaerobic biodegradation test and finally one degradation test in soil.

The DS considered Flocoumafen as hydrolytically stable (DT_{50}>1 year, 50ºC) and susceptible to primary degradation by photo-transformation in water (DT_{50} = 1.67 days). Four metabolites were detected but only two identified. Flocoumafen 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.

Flocoumafen is not readily biodegradable under test conditions (OECD 301B), with a degradation of 6% after 29 days and it is not degraded under anaerobic conditions. No degradation was observed after 60 days.

In an aerobic simulation test in soil, Flocoumafen showed a very slow degradation with a mean dissipation half-life (DT_{50}) of 213 days at 20 ºC and only a mineralization of 2.5-15.6% after 120 days.

Based on the reported data the DS concluded that Flocoumafen is not rapidly degradable.

Bioaccumulation
The experimental log K_{ow} of Flocoumafen is 6.12 at pH 4, 6.12 at pH 7 and 5.11 at pH 9. All these values are above the cut-off values of log K_{ow}≥4 (CLP). Experimental bioconcentration tests are not available.

In conclusion, based on the high log K_{ow}, the DS concluded that Flocoumafen has potential for bioaccumulation.

Aquatic toxicity
Two acute toxicity studies in fish (*Oncorhynchus mykiss,* LC_{50} = 0.07 mg/L and *Lepomis macrochirus,* LC_{50} = 0.11 mg/L), one in invertebrates (*Daphnia magna,* EC_{50} = 0.18 mg/L) and one in algae (*Pseudokirchneriella subcapitata,* ErC_{50} and NOE_rC > 18.2 mg/L) were reported by the DS. Long-term tests in fish and invertebrates are not available, but for algae the test submitted in the CLH report can be considered as an acute (LC_{50}) and chronic (NOEC) test. All the toxicity values for these tests were based on mean measured concentrations.

Fish (*Oncorhynchus mykiss*) was the most sensitive trophic level in the acute tests, with an EC_{50} value of 0.07 mg/l and the proposed classification Aquatic Acute 1 with an M-factor of 10 was based on the fish toxicity. The only available chronic toxicity value, i.e. NOE_rC value > 18.2 mg/l (*Pseudokirchneriella subcapicatata)* did not lead to long-term hazard classification. However, adequate chronic data was not available for all trophic levels and in this case the surrogate approach was applied for the most sensitive species in the acute studies, i.e. for fish. As a result the DS applied the most stringent outcome, i.e. surrogate approach, to propose Aquatic Chronic 1 with an M-factor 10 for Flocoumafen, taking into account that the substance is not rapidly biodegradable and the log K_{ow} ≥ 4.

Comments received during public consultation

Three Member States supported the environmental classification proposed by the DS without any additional comment.

RAC assessment and comparison with criteria

Degradation
RAC agreed that Flocoumafen could be considered hydrolytically stable and susceptible to primary degradation due to photo-transformation in water based on the information provided in the CLH report but was not readily biodegradable under test conditions with a degradation of 6% after 29 days. Furthermore, in an aerobic soil study Flocoumafen showed only a very slow degradation (DT_{50}=213 days).Therefore, based on these data, RAC agreed with the DS that Flocoumafen should be considered **not rapidly degradable** according to CLP.

Bioaccumulation
The experimental log K_{ow} for Flocoumafen is in the range of 6.12 - 5.11 (pH dependent). These values are above the cut-off values of log K_{ow}≥4 (CLP), therefore RAC agreed with the DS, Flocoumafen has a **high potential for bioaccumulation**.

Aquatic toxicity
Classification of cute toxicity should be based on the lowest $EC_{50.}$ The lowest aquatic acute toxicity value was an LC_{50} of 0.07 mg/l in *Oncorhynchus mykiss* (OECD 203). This value is ≤ 1 mg/l, therefore Flocoumafen classifies as Acute category 1 (H400) with a M-factor of 10, because the LC_{50} is between 0.01 and 0.1 mg/l.
No adequate chronic data was available for all three trophic levels and only chronic data from algae were submitted in the CLH report. According to this, no classification would

result for Flocoumafen based on a NOE_rC > 18.2 mg/L. However, the surrogate approach should be applied due to the lack of chronic data for fish and invertebrates. Taking into account the fact that the substance is not rapidly degradable, the log K_{ow} ≥ 4 and the LC_{50} (fish) ≤ 0.1mg/L (0.07 mg/L), classification as Aquatic Chronic 1 (H410) with an M- factor of 10 is justified.

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

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Flocoumafen is an active substance in the meaning of Directive 98/8/EC and therefore subject to harmonised classification and labelling (Regulation EC 1272/2008 article 36.2).

OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of flocoumafen according to Directive 98/8/EC. The summaries included in this proposal are partly copied from the draft final CAR and CAR document IIA. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the CAR and its document IIA.

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