Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU 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

Adopted

14 March 2014

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

14 March 2014

CLH-O-0000003398-66-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:	**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**

The proposal was submitted by **The Netherlands** and received by the RAC on **15 June 2012.** All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 March 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 April 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Bogusław Barański**

Co-rapporteur, appointed by RAC: **José Luis Tadeo**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **14 March 2014** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on **Flocoumafen (ISO)** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	607-375-00-5	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	421-960-0	90035-08-8	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		
Dossier submitters proposal		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	421-960-0	90035-08-8	**Add:** Repr. 2 **Modify:** Acute Tox. 1 Acute Tox. 1	**Add:** H361d **Modify:** H330 H300 **Modify:** ** for H372		**Add:** H361d **Remove:** ** for H372		**Add**: STOT RE 1; H372: C ≥ 0,05% STOT RE 2; H373: 0,005% ≤ C < 0,05% Repr. 2; H361d: C ≥ 0,003%

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
RAC opinion	607-375-00-5	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	421-960-0	90035-08-8	Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 Repr. 1B STOT RE 1	H330 H310 H300 H360D H372				STOT RE 1; H372: C ≥ 0,05% STOT RE 2; H373: 0,005% ≤ C < 0,05% Repr. 1B; H360D: C ≥ 0,003%
Resulting Annex VI entry if agreed by COM					Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H330 H310 H300 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H330 H310 H300 H372 (blood) H410		Repr. 1B; H360D: C ≥ 0,003% STOT RE 1; H372: C ≥ 0,05% STOT RE 2; H373: 0,005% ≤ C < 0,05%

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

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 transferred to 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), were submitted by eight different Dossier Submitters (DS). The dossiers were handled as a group but the Committee for Risk Assessment (RAC) proceeded to evaluate the proposals on a substance by substance basis comparing the human data available for Warfarin (and other AVKs) and relying on a weight-of–evidence approach as required by Regulation 1272/2008 (CLP).

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 ≤ 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.

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.

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.

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.

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.

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[1]) 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, 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

[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

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 ≤ 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.

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.

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.

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.

Effects on 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 Teratogenicity 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

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 Teratogenicity Embryo toxicity	Study number
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 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.

Effects on 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.

ENVIRONMENTAL HAZARD ASSESSMENT

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.**

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).