Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Warfarin (ISO); 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one

EC number: 201-377-6
CAS number: 81-81-2 [racemic mixture]

CLH-O-0000003175-78-11/F

Adopted

14 March 2014

14 March 2014

CLH-O-0000003175-78-11/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 harmonized classification and labelling (CLH) of:

Chemicals name: **Warfarin(ISO); 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one**

EC number: **201-377-6**

CAS number: **81-81-2 [racemic mixture]**

The proposal was submitted by **Ireland** and received by the RAC on **07 December 2012**. All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonized System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

Ireland 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 **05 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: **Boguslaw Barański**

Co-rapporteur, appointed by the RAC: **Jose 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 harmonized 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 Warfarin (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 and Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	607-056-00-0	warfarin (ISO); [1] (*S*)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone; [2] (*R*)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone [3]	201-377-6 [1] 226-907-3 [2] 226-908-9 [3]	81-81-2 [1] 5543-57-7 [2] 5543-58-8 [3]	Repr. 1A STOT RE 1 Aquatic Chronic 3	H360D *** H372 ** H412	GHS08 Dgr	H360D *** H372 ** H412		
Dossier submitters proposal	607-056-00-0	warfarin (ISO); 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one	201-377-6	81-81-2	**Add:** Acute Tox. 1 Acute Tox. 1 Acute Tox. 2 **Modify:** Aquatic Chronic 2	**Add:** H330 H310 H300 **Remove:** ** for H372 **Modify:** H411	**Add:** GHS06 GHS09	**Add:** H330 H310 H300 **Remove:** ** for H372 **Modify:** H411		**Add:** Repr. 1A; H360D: C ≥ 0,0003 % STOT RE 1; H372: C ≥ 0,2 % STOT RE 2; H373: 0,02% ≤ C < 0,2 %

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
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RAC opinion	607-056-00-0	warfarin (ISO); 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one	201-377-6	81-81-2	**Add:** Acute Tox. 1 Acute Tox. 1 Acute Tox. 2 **Modify:** Aquatic Chronic 2	**Add:** H330 H310 H300 **Remove:** ** for H372 **Modify:** (blood) for H372 H411	GHS06 GHS08 GHS09 Dgr	**Add:** H330 H310 H300 **Remove:** ** for H372 **Modify:** (blood) for H372 H411		**Add:** Repr. 1A; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,5 % STOT RE 2; H373: 0,05 % ≤ C < 0,5 %
Resulting Annex VI entry if agreed by COM	607-056-00-0	warfarin (ISO); 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one [1] (*S*)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone; [2] (*R*)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone [3]	201-377-6 [1] 226-907-3 [2] 226-908-9 [3]	81-81-2 [1] 5543-57-7 [2] 5543-58-8 [3]	Repr. 1A Acute Tox. 1 Acute Tox. 1 Acute Tox. 2 STOT RE 1 Aquatic Chronic 2	H360D *** H330 H310 H300 H372 (blood) H411	GHS06 GHS08 GHS09 Dgr	H360D *** H330 H310 H300 H372 (blood) H411		Repr. 1A; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,5 % STOT RE 2; H373: 0,05 % ≤ C < 0,5 %

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

Warfarin 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 current Annex VI entry of warfarin includes the racemic mixture of warfarin as well as the individual R- and S-enantiomers. The dossier submitter (IE) evaluated racemic warfarin (50:50 R:S) under the Biocides Directive (Dir. 98/8/EC) and the Plant Protection Product Directive (Dir. 91/414/EC). The dossier submitter used the Biocide and PPP EU evaluations as the basis for the CLH Report.

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

Warfarin has no harmonised classification for acute toxicity. The dossier submitter (DS) proposed to add a classification for acute toxicity via oral, dermal and inhalation routes of exposure.

Acute oral toxicity
Two studies were presented by the Dossier Submitter (DS) in the CLH report. The first acute oral toxicity study (Bai *et al*. 1992) was not conducted according to GLP or recognised guidelines. In particular, only Wistar female rats were used. However, the study meets basic scientific principles and is considered suitable for acute oral toxicity classification. The LD_{50} in female rats was calculated as 5.62 mg/kg bw (95 % confidence limit: 4.95 – 6.07 mg/kg bw). In this study, signs of toxicity were observed 2 – 3 days after administration of the test compound, and intensified within 4 – 5 days. Thereafter, signs of toxicity in surviving animals reversed gradually. Signs of intoxication were haemorrhages observed under the skin near the neck region, and around the nose, eyes and mouth. Animals were found dead between days 4 – 6 after administration.
The second acute oral toxicity study carried out with male and female Sprague-Dawley rats (Back *et al*., 1978) was viewed as supportive only due to methodological shortcomings (LD_{50} of 112 mg/kg bw ± 15.9 mg/kg bw for males, and 10.4 mg/kg bw ± 1.1 mg/kg bw for females).

The two studies assessed indicate that the acute oral toxicity of Warfarin is dependent on strain and sex.

Further supporting data on acute oral toxicity (expressed as LD_{50}) based on the results of a literature search were presented in the Biocides CAR. The reliability of the data and the level of documentation were considered insufficient for a comparison with the CLP criteria.

In the studies where male and female rats were tested, the LD_{50} values for females (range: 5–58 mg/kg bw) were lower than for males (range: 1.6–323 mg/kg bw.). In contrast, the LD_{50} for other species were significantly higher, for example mice 374–675 mg/kg, rabbits ca. 800 mg/kg, dogs ca. 200–300 mg/kg. These data indicate the particular sensitivity of the rat to Warfarin.

Acute dermal toxicity
The acute dermal toxicity of Warfarin was tested in Wistar rats according to EC method B.3 and OECD Test Guideline (TG) 402. In that study rats (5/sex) per dose were exposed to dose levels of 0.5, 2, 8, 25, 40, 65 and 100 mg/kg in propylene glycol for 24 hours. Animals showed clinical signs such as lethargy, hunched posture, ventro-lateral recumbency, laboured respiration, emaciation, swelling of the head, dark eyes, ptosis of both eyes, pale skin, piloerection, bleeding, scabs and dried blood in ears, dried blood on the head, chromodacryorrhea and adhesion of left eyelids. At the treated skin sites, erythema, scales and scabs were observed. Several animals showed haematomas (blue or green cutaneous areas) on back, shoulder, head, cheek and snout. All deaths occurred between days 5 and 14. Several animals were killed *in extremis*.
The dermal LD_{50} of the test compound in rats was 40 mg/kg for combined sexes and females alone. For males, the estimated LD_{50} was between 20 and 80 mg/kg.

Acute inhalation toxicity
The acute inhalation toxicity of Warfarin (technical) was tested in Sprague-Dawley rats according to EPA guidelines for testing of pesticides (1982). The concentrations were 0, 0.005, 0.021, 0.044 mg/L (actual concentration). All animals appeared normal immediately following exposure (duration of exposure not stated in the CLH report) until day 3 of the study. Significant incidences of decreased activity, increased respiration rate, bleeding at the ear notch, discoloration of ears and pale appearance were observed. All males of the mid and high dose groups died by day 7. In the low dose group, deaths occurred between day 6 and 9. All females died between days 10 and 11.
Significant incidences of haemorrhages were observed in the axillary region, cranium, muscles, gonads, ears and abdominal and thoracic cavities.

The LC_{50} value for inhalation toxicity of Warfarin was the lowest aerosol concentration of 0.005 mg/L.

Classification proposed by the Dossier Submitter
Oral: Based on the oral LD_{50} for female rats (5.62 mg/kg bw), the DS proposed to classify Warfarin as Acute Tox. 2; H300 (criterion: oral, rat, $5 < LD_{50} \leq 25$ mg/kg bw).
Dermal: Based on the dermal LD_{50} for rats (40 mg/kg for combined sexes), the DS proposed to classify Warfarin as Acute Tox. 1; H310 (criterion: LD_{50}, dermal, rat or rabbit $\leq$ 50 mg/kg).

Inhalation: Based on the inhalatory LC_{50} value of 0.005 mg/Lfor the rat (both sexes combined), the DS proposed to classify Warfarin as Acute Tox. 1; H330 (criterion: LC_{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 DS for acute toxicity for Warfarin.

Assessment and comparison with the classification criteria

The RAC supported the proposal from DS to classify Warfarin according to CLP as follows:

- acute Tox. 2; H300 (Fatal if swallowed, criterion: oral, $5 < LD_{50} \leq 25$ mg/kg bw) based on the oralLD_{50} of 5.62 mg/kg bw from the most reliable study in female rats (Bai *et al.,* 1992);
- acute Tox. 1; H310 (Fatal in contact with skin, criterion: LD_{50}, dermal, rat or rabbit $\leq$ 50 mg/kg) based on the dermal LD_{50} for rats (40 mg/kg for combined sexes);

- acute Tox. 1; H330 (Fatal if inhaled, criterion: LC_{50}, inhalation, for dusts and mists ≤ 0.05 mg/l/4h) based on the inhalatory LC_{50} values of 0.005 mg/Lfor the rat (both sexes combined).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One skin irritation/corrosion study conducted in rabbit according to OECD TG 404 was considered by the DS. No classification is proposed by the DS as also agreed by the TC C&L in 2006/2007.

Comments received during public consultation

One MS supported the conclusion of non-classification of Warfarin as a skin irritant.

Assessment and comparison with the classification criteria

In the opinion of RAC the results of the study presented by the DS do not warrant classification of Warfarin for skin irritation/corrosion according to CLP criteria.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

One eye irritation/corrosion study conducted in rabbit according to OECD TG 405 was considered by the DS. No classification is proposed by the DS as also agreed by the TC C&L in 2006/2007.

Comments received during public consultation

One MS supported the conclusion of the DS on the non-classification of Warfarin as an eye irritant.

Assessment and comparison with the classification criteria

In the opinion of RAC the results of the study presented by the DS do not warrant classification of Warfarin for eye corrosion/irritation according to CLP criteria.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

One skin sensitisation study conducted in Guinea pigs according to OECD TG 406 (guinea pig maximisation test, GPMT) was considered by the DS. No classification is 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, the results of the GPMT do not warrant classification for skin sensitisation, because the observed effects do not meet the CLP classification criteria.

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

Summary of the Dossier submitter's proposal

Detailed short/medium term repeat dose oral toxicity studies are not available due to the high susceptibility and sensitivity of experimental animals to the anticoagulant effects of Warfarin. A few repeat dose toxicity studies are available on Warfarin in rats and mice (Hayes and Gaines, 1959; Hayes, 1967). However, these studies are not suitable for hazard evaluation. Rodents are the target species for Warfarin and in comparison to humans are particularly susceptible to the substance.

In the available rat repeat dose Warfarin toxicity studies it is indicated that there is difficulty conducting the studies at low Warfarin levels. For example, the oral uptake of a dose of 0.077 mg/kg bw/day led to a mortality of 50% of test animals in a 90-day study despite the haemorrhaging that also occurred in surviving rats at the levels tested (Hayes, 1967). This dose for a 60 or 70 kg person would correspond to approximately 5 mg/day; for comparison the average therapeutic dose is 2-10 mg/person/day. It is also noted that the response to Warfarin is subject to individual variation or susceptibility. In a therapeutic setting, this dose level should lead to a prothrombin time prolongation factor of between 1.5 and 2.5. The relevance of repeat dose oral toxicity studies conducted in animal models for extrapolation to humans, given the interspecies differences in pharmacological behaviour would be highly questionable even if testing was possible.

The DS did not report NOAEL or LOAEL based on animal studies of repeated toxicity testing.

Warfarin is widely used in humans for both short-term (weeks-months) and long-term (years) oral anticoagulation therapy. It is also documented that Warfarin can have adverse effects in humans. The most frequent adverse effects noted are bleeding episodes, which can be regulated based on monitoring prothrombin times. Only a small number of incidences of Warfarin-induced skin necrosis are described in the literature. Other non-haemorrhagic skin conditions have also been described. Reduced bone mass in patients with long-term anticoagulant therapy (not Warfarin) has also been reported.

Dossier Submitter's conclusion on classification

The DS proposed that Warfarin be classified for Specific Target Organ Toxicity – Repeated Exposure (STOT-RE Category 1) under CLP on the basis of evidence from a limited number of studies in experimental animals, including the repeat rat oral dose 90-day. In this study, death occurred at low doses (50% mortality at 0.077 mg/kg bw/day). In addition, evidence from human cases in which significant toxicity occurred at low doses was taken into account.

Specific concentration limits

The formula set out in the CLP Guidance for setting of specific concentration limits for repeated exposure (STOT-RE) is as follows:

$$\text{SCL Cat. 1 for STOT-RE} = \frac{ED_{10} \text{ mg/kg body weight /day (oral)} \times 100\%}{10 \text{ mg/kg body weight /day (GV1)}}$$

and

$$\text{SCL Cat. 2 for STOT-RE} = \frac{ED_{10} \text{ mg/kg body weight /day (oral)} \times 100\%}{100 \text{ mg/kg body weight /day (GV2)}}$$

A limited number of short and medium-term repeat dose oral toxicity studies are available for assessment of repeat dose toxicity. However, these studies do not enable setting a LOAEL or

NOAEL or determination of an ED_{10} value for use in the above formulae due to the high susceptibility and sensitivity of rat model species to the anticoagulant effects of Warfarin.

In addition to the limitations of the oral repeat dose data, no data are available for assessment of repeat dose toxicity *via* the dermal and inhalation routes. The available rat oral studies show that a specific toxicity profile occurs in repeat dose rat studies at a dose / concentration between 2 and 3 orders of magnitude lower than the guidance values (GV) of 10 and 100mg/kg bw/day.

On the other hand, robust evidence indicates that other species and human patients differ in sensitivity or susceptibility to the effect observed in the rat studies. The clinical dose range for humans reported in the submitted literature is from 2.5 to 20 mg/day. The dose prescribed depends on the prothrombin clotting times in individual patients and the dose is tailored specifically in each case.

An estimated (or surrogate) value for the ED_{10} can be derived for Warfarin in mg/kg bw/day by dividing the lowest reported dose with clinical effect of 2.5 mg/day by 60kg (= 0.04 mg/kg bw/day).

This value is used in setting the specific concentration limits for repeat exposure (STOT-RE) classification as follows:

$$\text{SCL Cat. 1 for STOT-RE} = \frac{0.04 \text{ mg/kg bw/day} \times 100\%}{10 \text{ mg/kg body weight /day (GV1)}} = 0.4 \%$$

and

$$\text{SCL Cat. 2 for STOT-RE} = \frac{0.04 \text{ mg/kg bw/day} \times 100\%}{100 \text{ mg/kg body weight /day (GV2)}} = 0.04 \%$$

The resulting SCL values are rounded down (as described in section 3.9.2.6 of the CLP Guidance), resulting in an SCL for Cat. 1 of ≥ 0.2% w/w, and an SCL for Cat. 2 between 0.02% and 0.2% w/w (0.02 % ≤ Cat. 2 < 0.2 %).

Proposed SCLs:

STOT RE 1; H372 above 0.2% and STOT RE 2; H373 between 0.02 and 0.2%.

Comments received during public consultation

One MS supported the classification of Warfarin as STOT RE 1 and the specific concentration limits for STOT RE proposed by the DS.

Assessment and comparison with the classification criteria

In humans Warfarin is used as oral anticoagulation drug at doses of 2 - 10 mg/person/day and this dose level is expected to lead to prolongation of prothrombin time by a factor between 1.5 and 2.5. The most frequent adverse effects in humans noted during repeated therapeutic exposure were bleeding episodes. There was also a low incidence of Warfarin-induced skin necrosis.

In the 90-day repeated oral toxicity study in rats with Warfarin, 50% mortality was observed at a very low dose (0.077 mg/kg bw/day).

The median lethal dose level for rats exposed orally to Warfarin during 90 days at 0.077mg/kg bw/day corresponds to a daily dose of 5.4 mg/person (70 kg person). In humans, this dose is used as a therapeutic dose to reduce prothrombin time by a factor between 1.5 and 2.5. This comparison suggests that rats are more sensitive to repeated exposure to Warfarin than humans.

In the opinion of RAC Warfarin warrants classification as STOT RE 1 with the hazard statement H372: Causes damage to organs through prolonged or repeated exposure. Classification is warranted because lethality is observed in animals at prolonged exposure well below the guidance value (≤ 10mg/kg bw/day, table 3.9.2 of the CLP Regulation).

RAC is of the opinion that Warfarin also fulfils the classification criteria by the dermal route. Indeed, taking into account the median lethal oral dose and the dermal absorption of Warfarin (14%) mortality would result as consequence of repeated dermal exposure at dose level of 0.55 mg/kg bw/day (0.077mg/kg bw/day x 100/14 = 0.55 mg/kg taking 100% for absorption of

Warfarin by the oral route). This dose level is thus well below a GV of 10 mg/kg bw/day which fulfils the criteria for classification as STOT RE 1; H372.

Therefore, it is the opinion of RAC that Warfarin warrants classification as STOT RE 1 with hazard statement; H372: Causes damage to blood through prolonged or repeated exposure according to CLP criteria.

Specific Concentration Limits

In the opinion of RAC the specific concentration limit for STOT RE for Warfarin should be based on the 90 - day oral study on rats with 90-day The median lethal dose level in rats = 0.077mg/kg/day.

An SCL for STOT RE 1 of 0.5% is proposed based on serious effects (death) seen at 0.077 mg/kg in the 90-day study in rats. Calculation: 0.077 mg/kg bw/day (adverse effect dose) / 10 mg/kg bw/day (GV for cat. 1) * 100% = 0.77% rounded down to 0.5% as required by the Guidance on the Application of the CLP Criteria.

An SCL for STOT RE 2 is proposed between 0.05% and 0.5% using the same data and method of calculation using guidance value of 100 mg/kg bw/day for Cat. 2. This calculation is performed according to the method described in the Guidance on the Application of the CLP Criteria.

RAC evaluation of reproductive toxicity

The DS did not propose any change to the existing Annex VI classification of toxicity to reproduction of warfarin, noting as follows (p4 of CLP report). *"The developmental toxicity classification of Warfarin has been finalised and is not open for further discussion. Relevant background information on developmental toxicity data for Warfarin is included in this dossier to facilitate the discussion on read-across from this classification to the second generation rodenticides."* They did however propose Specific Concentration Limits (SCL). The proposed SCL is dealt with below, followed by an evaluation of the available data on the reproductive toxicity of warfarin in support of the aforementioned evaluations of 7 other anticoagulant rodenticides evaluated by RAC for toxicity to reproduction at the same time.

Specific concentration limits

Summary of the Dossier submitter's proposal

The DS proposed setting an SCL for reproductive toxicity according to the then Draft Guidance on the setting of concentration for reproductive toxicants with the CLP Regulation (Draft 2, Feb 2010).

Their argumentation was that Warfarin at doses of 2.5 mg/day (0.04 mg/kg bw/day, female bodyweight of 60kg) have been reported to result in nasal hypoplasia and vertebral stippling. Higher doses have resulted in a high percentage of embryofoetal mortality (A NOAEL cannot be set and the value of 0.04 mg/kg bw/day represents a LOAEL which in turn approximates to an ED_{10} value.

Based on the Guidance for Setting Specific Concentration Limits for Reproductive Toxicants within the CLP Regulation (EC/1272/2008), substances with an ED_{10} value less than or equal to 4 mg/kg bw/day in animal studies are considered as high potency substances. Warfarin is considered to have very high potency in terms of developmental toxicity simply because its LOAEL is approximately 2 orders of magnitude below the upper limit value for high potency classification. The general concentration limit (GCL, 0.3% w/w) is applied to all medium potency reproductive toxicants. For high potency substances, the ECHA Guidance for CLP has proposed an SCL of 0.03% w/w. Furthermore, extremely potent developmental toxicants with ED_{10} values deviating 10-fold or greater, below the upper limit value of 4 mg/kg bw/day must lead to a further revision

of the SCL value. The high potency SCL must be reduced by a factor of 10 for each 10-fold disparity between the ED10 and the upper limit value of 4 mg/kg bw/day.

Warfarin has a LOAEL of 0.04 mg/kg bw/day which approximates to the ED_{10}. This value is about 2 orders of magnitude below the upper limit value for high potency classification. Based on the draft guidance, a value of 0.03/100 = 0.0003% w/w is calculated for Warfarin. Any preparation containing Warfarin equal to or in excess of 0.0003% w/w shall be classified with respect to reproductive toxicity, Repr. 1A – H360D, i.e. C ≥ 0.0003% leads to Repr. 1A.

Comments received during public consultation

Comments from industry disagreed with the proposed SCL (comments with detailed justification were submitted as a confidential attachment). Two MS agreed that the current classification of Warfarin as Repr. 1A (developmental toxicity) was appropriate

Assessment and comparison with the classification criteria

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 ED_{10} level. This human ED_{10} 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 ED_{10} < 4 mg/kg/day, the SCL is 0.03%, and for ED_{10} below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from an ED_{10} value obtained from animal studies (0.125 mg/kg/day; Kubaszky et al. 2009), it would qualify Warfarin for the high potency group and this would result in a SCL of 0.003%. Thus, the RAC has concluded on a SCL on 0.003% for the developmental toxicity of Warfarin.

Data on reproduction toxicity in support of other anticoagulant rodenticide classifications

Summary of information supplied by the Dossier Submitter

Fertility
No multi-generation study data are available from published literature. However, the conduct of such studies is not considered feasible due to the particular sensitivity of the model species (rodents). For example, sodium Warfarin, administered 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 (Feteih *et al*., 1990), which, for a 70 kg person would be calculated to equate to an exposure of 12 mg/day, which corresponds to a dosage within the usual therapeutic range (2.5 – 15 mg/day).
Warfarin did not show any effect on fertility after many years of human use or in a two generation reproduction study in rats with Vitamin-K supplementation. Therefore no classification has been proposed for fertility.

Developmental toxicity
NOTE: The classification for developmental toxicity of Warfarin is already harmonised and the substance has an entry in Annex VI of the CLP Regulation as toxic to reproduction, Repr. 1A; H360D. Harmonised classification for developmental toxicity of Warfarin was neither subject of the current DS proposal, nor of the RAC discussion. Relevant background information on developmental toxicity data for Warfarin was included in the CLH report in order to facilitate the weight of evidence assessment and the discussion on harmonised classification for developmental toxicity of other anticoagulant rodenticides and to allow for setting of specific concentration limits.
Developmental toxicity: Human evidence

Warfarin, which is also used as an oral anticoagulant drug in order to prevent formation of clots in the blood of people with mechanical heart valves or with deep vein thrombosis, has been found to cause death of embryos or foetuses and malformations, mainly nasal hypoplasia in humans. Since deformation of the naso-maxial part of the face is very specific, it is also referred to as "Warfarin embryopathy".

The evidence that Warfarin has the intrinsic property to induce alterations of development in humans doesn't come from cohort or case-control studies, but mostly from clinical reports each

describing one or a few cases with various effects on the health of neonates, abortions or stillbirths where pregnant women were treated with Warfarin as an anticoagulant drug.

The DS presented summaries of over 10 case reports of pregnancy outcomes in women treated with Warfarin as an oral anticoagulant as well as two reviews (Schardein, 1985; Hall et al., 1980) of case reports, in which the administration of Warfarin during pregnancy induced birth defects. The daily dose of Warfarin was usually between 5-10 mg/day.

Such exposure during the first trimester may cause a well-defined complex of malformations, with hypoplastic nose being the most characteristic feature. 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.

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, but details of how this estimate was derived are not available. More recently, a review of the maternal and foetal risks (Chan, 2000) associated with oral anticoagulants indicated that the use of oral anticoagulants 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). In the more recent literature survey provided by industry (BASF, 2010) in support of the Flocoumafen CLH dossier, which has been summarised by the DS in the respective CLH report, the risk of 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).

In addition to skeletal malformation there are other hazards caused by the toxic properties of Warfarin:

- spontaneous abortion (14 - 47 %, 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),
- neonatal death (1.4 – 4.5 %; 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 (4.6 – 13.9 %; Blickstein & Blickstein, 2002; Hall, Pauli, & Wilson, 1980),
- ocular atrophy (Greaves, 1933; Hall, Pauli, & Wilson, 1980).

In summary, it is noted that oral administration of Warfarin leads to various developmental toxicity effects in humans, spontaneous abortion and stillbirth being the most frequent ones (ca. 27% of pregnancies), and naso-maxial hypoplasia being the most frequent malformation among alive births (ca. 5% of pregnancies). Substitution of Warfarin by Heparin during first trimester of pregnancy removes the risk of naso-maxial hypoplasia.

When compared with the classification criteria for developmental toxicity, the existing evidence in humans is sufficient for category Repr. 1A with hazard statement H360D: 'May damage the unborn child'.

Developmental toxicity: Animal evidence
The DS used 6 experimental studies to evaluate the developmental toxicity in animals: Mirkova & Antov, 1983; Howe & Webster, 1992; Feteih *et a*l. 1990 (containing 2 studies); Kronick *et al*., 1974 and Kubaszky, 2009).
The study of Kubaszky (2009) was carried out in accordance with the OECD TG 414 in order to address the doubts of Specialised Experts (September 2006 Commission Doc ECBI/121/06) regarding the capability of OECD 414 compliant teratogenicity protocols to detect adverse effects on embryos and foetuses due to maternal exposure to Warfarin.

Four other studies (Mirkova & Antov, 1983, Feteih *et al*., 1990 and Kronick *et al*., 1974) followed the general design of OECD 414, because the animals were exposed during various periods of pregnancy, but with considerable deviations in methodology.

A sixth study (Howe & Webster, 1992) did not follow the OECD 414 design, since treatment of mothers after parturition of offspring was applied in order to deliberately produce malformations of facial skull (mostly maxillonasal hypoplasia) resembling those being induced by Warfarin in humans.

Kubaszky, 2009

In the study of Kubaszky (2009), teratogenicity of Warfarin was investigated by oral administration (gavage) of Warfarin sodium (vehicle: carboxymethylcellulose) to groups of twenty-five pregnant female Wistar rats at dose levels of 0, 0.125, 0.150 or 0.200 mg/kg bw/day according to two test protocols (TP):

TP1, groups 1–4 - from gestation day (GD) 6 to 15

TP2, groups 5–8 - from GD 6 to 19

Two other groups of twelve pregnant female Wistar rats were dosed with 0.250 mg/kg bw/day (GD 6–15, TP1, group 9, and GD 6–19, TP2, group 10).

Thus, dosing regimens following both the most recent and earlier test guideline versions were covered.

Maternal mortality (0, 0, 2/25, 2/25, 5/12) and clinical signs of toxicity occurred in the 0.150, 0.200 and 0.250 mg/kg bw/day TP 1 treatment. In total, 8 dams died or were sacrificed at 0.250 mg/kg bw/day in the TP 2 treatment. The majority of mortalities (and sacrifices) occurred between gestation days 14 and 17 (one dam was sacrificed on GD 19). Clinical signs (piloerection, pallor, reduced activity, vaginal bleeding and an open vaginal orifice), death and morbidity were considered to be treatment-related and consistent with the pharmacological action of the substance. In the opinion of the DS, there was clear maternal toxicity from 0.0150 mg/kg bw/day (LOAEL). This was demonstrated as treatment-related clinical signs and mortalities. 0.125 mg/kg bw/day was an NOEL for maternal toxicity.

In the groups exposed from day 6 to day 21of gestation the mortality of dams was observed only at dose of 0.250 mg/kg bw/day but not at lower doses. Thus, severe maternal toxicity was observed at a dose of 0.250 mg/kg bw/day, mild or moderate maternal toxicity was observed at doses 150 and 0.200 mg/kg bw/day and no maternal toxicity was seen at dose of 0.0125 mg/kg bw/day.

A single litter (TP1, 0.150 mg/kg bw/day), which had been excluded from the statistical analysis due to uncertainty about the exact day 0 of gestation) had four foetuses showing abnormally high body weights and facial skeletal malformations. In total, 2/7 foetuses had malformed skulls with wide nasal and/or frontal bone/cartilage. One foetus had unossified nasal bone, while another one had malformed vertebra and both of them had malformed sternum. The malformations were considered attributable to treatment by the study director. However, it is noted that these foetuses were part of a single mid dose litter. There is no evidence of such effects in other litters at any dose levels. It must be concluded that a relationship with treatment is equivocal, at best.

Warfarin at a dose of 0.2 mg/kg bw/day given by gavage to female rats from day 6 to day 15 of pregnancy caused a significant increase in late embryonic death (11% vs 3% in control females), increased number of dead foetuses (5 foetuses vs 0 in control group), increased intrauterine mortality (23% vs 16% in control group), increased percentage of post-implantation loss (19% vs 7% in the control group) and number of runts (6% at 0.2 mg/kg bw/day, 5% at 0.15mg/kg bw/day vs 2% in the control group. Warfarin given at 0.2 mg/kg bw/day by gavage to female rats from day 6 to day 19 of pregnancy (TP 2) caused an increase in bloody infiltration in viscera (21% vs 5% in control group). In addition, at doses of 0.125; 0.125 and 0.200 mg/kg bw/day Warfarin caused cataracts in 1 up to 4 animals per a dose group of ca. 200 foetuses. This malformation is reported as very rare for Wistar rats (not seen in >5000 foetuses in this test laboratory and >17 000 foetuses of Charles River data base) and so was attributed to an effect of treatment with Warfarin sodium by the study director.

Mean foetal weights were similar in all groups and were thus unaffected by Warfarin treatment.

There was an increase in incidence of foetal external haemorrhages in all treatment groups in comparison with the controls, but a clear steep dose-response relationship was not observed due to low dose increment. When dosed at 0.2 mg/kg bw/day Warfarin given by gavage to female rats from day 6 to day 19 of pregnancy (TP 2) caused an increase in bloody infiltration in viscera (21% vs 5% in the control group).

Mirkova & Antov, 1983

In the study of Mirkova & Antov (1983), the application of Warfarin at a dose of 0.32 mg/kg bw/day during the period of organogenesis from days 8-16, caused increased incidences of post implantation loss (551,8%), overall embryonic mortality (525%) and an increased incidence in foetuses (182.7%) of haematoma and haemangioma (foetal haemorrhagic syndrome). In addition, increased incidences of structural malformations of the rear limbs (*pes varus*), internal hydrocephalus, intracerebral haematomas, massive haemorrhages into the abdominal cavity and delayed ossification of the parietal skull bones were observed.

In the other experimental groups in the study of Mirkova and Antov (1983), a daily application of Warfarin at doses 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%, respectively) in comparison to the control. The post implantation loss was increased by 1074 and 501.8% for these dose levels, respectively. 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 incidences of delayed ossification of the parietal skull bones were increased statistically significantly by 21.6 and 15.7%, respectively.

There was also no information on maternal toxicity at the doses at which developmental effects were observed.

Kronick et al., (1974)

In the Kronick, *et al*. study (1974)_Warfarin sodium (salt) (Coumadin drug) was administered intraperitoneally (i.p.) at doses of 1, 2, 3 and 4 mg/kg bw/day at various stages of pregnancy in mice. Control animals received physiological saline or distilled water.

In female mice 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 fold compared to controls 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. None of the doses administered in this study from 3 to 11 day of pregnancy led to an increase of the frequency of malformations, probably due to high foetal death rates.

In female mice treated with single daily i.p. injection of Warfarin at 4 mg/kg on day 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 of pregnancy there was an increase of foetal death rate in mice treated on days 10 and 11 of pregnancy.

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

In female mice given a single daily i.p. injection of Warfarin at a dose of 1, 2, 3 or 4 mg/kg on gestation day 8, or 9, or 10 or 11 (period of organogenesis) no increased incidence of foetal or placental haemorrhage was observed. Statistical analysis revealed that there was a significant linear regression of over-all foetal deaths on log dose, suggesting a dose-dependent increase of foetal mortality irrespective of the day of gestation. Analysis of malformation data showed a significant difference between the Warfarin-treated mice and the controls. However, 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).

Feteih et al., 1990

In the first study of Feteih, et al. (1990), sodium Warfarin was administered subcutaneously at a daily dose of 0.175 mg/kg bw to Sprague-Dawley rats from gestational day 8 to day 22 to examine the effects of this compound on the developing foetal skeleton and on the Vitamin K-dependent bone and cartilage proteins. At a dose of 0.175 g/mg bw/day, Warfarin induced significant maternal toxicity leading to a mortality of 43% among dams, whereby maternal prothrombin times were only slightly (but not significantly) elevated. Mean litter size and foetal weights, although reduced for Warfarin-exposed animals, were not significantly different from controls. The mean numbers of resorptions were also not significantly different from control litters.

There was also no significant difference in the numbers of ossification centers examined between controls and Warfarin-exposed foetuses. First analysis of craniofacial dimensions showed significant decreases in measures of mandibular length and depth and maxillary length, but when these proportions were adjusted for foetal body weight no significant differences were found. The morphologic defects in the development of bone were associated with biochemical effects of Warfarin on the skeleton as seen by analysis of the bones for gamma-carboxyglutamic acid (Gla) and osteocalcin (bone Gla protein).

As noted by the DS, 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 (MPG) that predominates in embryonic bone and cartilage extracellular matrix. Osteocalcin, also known as bone Gla-containing protein (BGLAP), is a non-collagenous protein found in bone and dentin. Matrix Gla protein (MGP) is a protein found in numerous body tissues that requires Vitamin K for its optimum function. It is present in bone (together with the related vitamin K-dependent protein osteocalcin). Osteocalcin and MPG are both calcium-binding proteins that may participate in the organisation of bone tissue. Both have glutamate residues that are post-translationally carboxylated by the enzyme gamma-glutamyl carboxylase in a reaction that requires Vitamin K hydroquinone. This process also occurs with a number of proteins involved in coagulation: prothrombin, factor VII, factor IX and factor X, protein C, protein S and protein Z.

In the second study of Feteih *et al*. (1990), the effects of prenatal treatment with Warfarin were investigated on bone histology and morphology, and associated biochemical effects (levels of osteocalcin and Gla protein) in the rat.

On GD 21, Gla was decreased from 46 to 53% of controls based on the number of residues per 10^3 moities of glutamic acid. When Gla concentration was normalised to bone dry weight the decrease was even greater (65-67% of control). In contrast to serum osteocalcin values, which were not statistically different between the two groups, osteocalcin levels in the calvariae of Warfarin-exposed pups were decreased by 23-43% of control values by day 22. In the long bones of these foetuses, osteocalcin was decreased from 25 to 50%. The osteocalcin concentration in both long bone and calvariae was highly correlated to the foetal body weight of controls only.

Since foetal bones contain two known vitamin K-dependent proteins, osteocalcin and matrix Gla protein (MPG), a calculation was carried out to determine quantitatively how much Gla in foetal bone is accounted for by the presence of immunoreactive osteocalcin. The results indicated that, at most, 3% of the Gla content in foetal long bone and 6% of the Gla content of foetal calvariae can be accounted for by the presence of osteocalcin. A large portion of the remainder of Gla-containing protein is likely to be the matrix Gla protein, although as yet unidentified proteins may also be present. Since matrix Gla protein is present in much greater quantities than osteocalcin in embryonic bone and cartilage extracellular matrix, the large reduction in Gla content of the Warfarin-exposed bones suggests that this protein was inhibited by the prenatal Warfarin exposure.

In spite of the reduction of level of osteocalcin and Gla in foetal bones the ossification centres revealed by staining with alizarin were not different in Warfarin treated animals from the control animals. However, analysis of the tibial growth showed several changes compared to control that included (1) widened hypertrophic zones, (2) increased calcification of the hypertrophic zones, and (3) disorganization of the hypertrophic cells. These results suggest that the growth plate abnormalities seen in foetal rats prenatally exposed to Warfarin are related to the inhibition of the vitamin K-dependent proteins of the skeletal system.

Howe & Webster, 1992

The study of Howe & Webster (1992) was not performed in accordance with OECD TG 414 but was designed to investigate the developmental toxicity of Warfarin, which 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 post-natal developmental toxicity of Warfarin was studied in new born rats given sub-cutaneous injection of Warfarin in combination with Vitamin K starting on post-natal day 1. Thus, the extra-hepatic vitamin K deficiency induced by Warfarin is maintained, whereas vitamin-K-dependant processes of the liver are not disturbed.

The following groups were created:

- Warfarin group: 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;
- Vit. K1 group: three litters were treated only with Vit. K1, and 11 males and 10 females were subjected to the final sacrifice;
- Control group: four litters served as untreated control. 13 males and 14 females were sacrificed upon study termination.

In Warfarin-treated male and female offspring, 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. The measurements of alizarin-stained skulls after 12 weeks of treatment also revealed a small but statistically significant reduction in length of nasal bone length, frontal bone length, maxilla length and reduction of transfrontal width, width of snout and facial height. The forelimb bones length of Warfarin-treated rats were slightly shorter (4-5% reduction in both sexes) than Vit. K1 and/or untreated control animals.

The alizarin-stained nasal septa from Vit. K1 and control rats did not show evidence of calcification in the septal cartilage, while all nasal 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.

Assessment of the toxicity to reproduction of warfarin in support of other anticoagulant rodenticide classifications

Although the classification of developmental toxicity of Warfarin is based on humans studies it is of great importance to note that in the studies of developmental toxicity in rats carried out with methodology compliant with OECD TG 414 (Kubaszky, 2009) or with a design resembling that methodology (Mirkova & Antov, 1983) and also in mice (Kronick *et al*. 1974) Warfarin induced clear developmental toxicity at doses within the same order of magnitude as therapeutic doses used in humans.
In the opinion of RAC Warfarin has caused developmental toxicity (intrauterine death of foetuses , increased postimplantation loss, internal and subcutaneous haemorrhages, intracerebral haematomas, cataract of lens) resembling the effects of developmental toxicity of Warfarin in humans except the naso-maxial hypoplasia which is observed only in humans due to difference in time of skull ossification between humans and rats. In the Kronick, et al. study (1974) Warfarin induced a very high incidence of haemorrhaged placentae and foetal deaths (including both dead and resorbed foetuses) when female mice were treated i.p. from days 3-11 of gestation with 2 and 4 mg/kg bw/day. These effects warrant classification of Warfarin as a developmental toxicant according to CLP criteria, although these effects were not systematically seen in all studies.

The developmental toxicity in humans has been observed at dose levels of 2-18 mg/person (equivalent to 2/60 = 0.033mg/kg/day – 18/60= 0.3 mg/kg/day), which in rats is probably lethal (the median lethal dose in a 90-day rat study is 0.077 mg/kg bw/day).

The developmental toxicity in humans and animals is linked with relatively effective transplacental transport of Warfarin to the foetus. The concentration of radioactive Warfarin residues in the liver of foetal rats are ca. 5 times lower than in maternal liver, in foetal blood cells and plasma, less than 2 times lower than in maternal blood, and in the foetal carcass slightly lower than in foetal plasma but higher than in blood cell fraction.

It is noted that the developmental toxicity of Warfarin has been demonstrated in humans at the dose levels of 2- 18 mg/person at which this substance exerts clear anticoagulation effects leading to substantial prolongation of prothrombin time by a factor of 1.5 to 2.5.

There are no data on developmental toxicity of Warfarin in humans at lower doses or data on dose response-relationship between developmental effects and level of exposure since the dose given to individual patients is adjusted to avoid the development of complications. The level of exposure is controlled to maintain level of anticoagulation required by the therapy.

Therefore, taking into account available evidence, it is concluded that Warfarin has to affect the coagulation system leading to moderate prolongation of prothrombin time through alteration of the Vitamin K cycle in order to induce developmental toxicity. In the opinion of RAC the existing evidence from case studies on humans is sufficient to establish a causal relationship between human exposure to Warfarin and subsequent developmental toxic effects in the progeny.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

The current harmonised environmental classification of Warfarin is Aquatic Chronic 3 (H412) according to CLP. The DS proposed to harmonise the classification for Warfarin as Aquatic Chronic 2 (H411) according to CLP.

Degradation
Degradation was studied in a ready biodegradability test (OECD TG 301D), and based on this', Warfarin can be classified as readily biodegradable. The criterion of 60 % removal of ThOD within a 10 day window was exceeded. A degradation of 92.7% was determined for a 28 day period.

No other degradation tests were submitted.

Based on the available data rapid degradation was proposed for Warfarin.

Bioaccumulation
The experimental log K_{ow} of Warfarin is 0.7 at pH 7 and the calculated log K_{ow} is 2.23. These values are lower than the cut-off values of log $K_{ow} \geq 4$. Furthermore, the experimental bioconcentration factor (BCF) was obtained following OECD TG 305E and the resulting BCF was 21.6 L/kg, which is lower than the cut-off value of 500 L/kg. This value was not indicated in the CLH report, but the DS reported it after public consultation.

In conclusion, since the low log K_{ow} as well as the low experimental BCF indicated low potential for bioaccumulation, the DS concluded that warfarin has no potential for bioaccumulation.

Aquatic toxicity
Three acute toxicity studies in fish (*Oncorhynchus mykiss*), three in invertebrates (*Daphnia magna*) and two in algae (*Desmodesmus subspicatus*) were reported by the DS. There are also chronic tests available for the same species of fish and invertebrates following OECD TG204 and 202 part II, respectively. However, chronic endpoints for algae have not been included in the CLH

report.

All the acute endpoints ($L(E)C_{50}$) reported in the CLH dossier for the three trophic levels are higher than 1 mg/L: fish (*Oncorhynchus mykiss,* LC_{50}(96h) = 65-88 mg/L); invertebrate (*Daphnia* magna, one study for which an EC_{50}(48h) > 105 mg/L and two other studies for which LC_{50}(48h) = 130 mg/L and LC_{50}(24h) = 180 mg/L were reported) and algae (*Desmodesmus subspicatus,* E_rC_{50}(72h) > 83.2 mg/L). Fish and invertebrate toxicities ($L(E)C_{50}$) were based on nominal concentrations because the concentrations were not measured during the tests, while algae toxicity was based on measured concentrations. *Oncorhynchus mykiss* is the most sensitive species in the acute studies with an LC_{50} value of 65 mg/L from the key study. This report of test was dated 1984 following guideline EPA-660/3-75-009. Acetone was used as the vehicle due to the low solubility of warfarin. However, the concentration of acetone as a vehicle was well above the recommended 100 mg/L (OECD TG 203). In addition, the mortality in the 100 mg/L solvent (acetone) control was 40%.

Regarding the chronic toxicity, *Daphnia magna* is the most sensitive species with a NOEC value of 0.059 mg/L while fish showed lower toxicity with a NOEC value of 2 mg/L. The toxicity of both tests was based on mean measured concentrations and the both studies were considered valid by the DS.

An LC_{50} of 65 mg/L (*Oncorhynchus mykiss*) and a NOEC of 0.059 mg/L (*Daphnia magna*) were used to establish the classification by the DS.

Comments received during public consultation

Four Member States made comments during the public consultation. The main concern raised in the comments was the reliability of the reported acute toxicity studies. For example, the provided studies for fish were old (1984) and high concentrations of solvent were used, resulting in 40% of death in the solvent control in the key study, and furthermore several signs of toxicity were observed in the solvent controls of the supportive studies. Additionally, in the supportive studies, unidentified white precipitates were observed. Moreover, the substance is readily biodegradable, its solubility is low and the endpoints are based on nominal concentrations. It was also pointed out that the known acute toxicity of acetone in fish is well above the applied concentrations but the reported fish studies showed some toxic effects in the solvent controls.

One MS requested to include a missing NOEC value (algae) from the biocidal dossier to the CLH report as well.

In their response to comments received during public consultation the DS agreed that the acute studies are unreliable due to the physical and chemical properties of Warfarin.

Regarding the use of these acute studies to classify Warfarin, the DS noted that it should be discussed by the ECHA experts.

The DS included the following additional information about the NOEC values from the algae tests:
Hertl J. (2001) NOErC = 21.3 mg/L

Dommröse A.-M. (1989-supportive study) NOEC = 8.5 mg/L

Assessment and comparison with the classification criteria

Degradation
Information on hydrolysis and photolysis from the biocides CAR has been included in the additional elements section.

According to this information, Warfarin is hydrolytically stable under environmentally relevant conditions with a DT_{50} possibly higher than 1 year. Direct photolysis in water is not expected to contribute significantly to abiotic degradation in aqueous systems under normal environmental conditions.

Regarding the data which appears in the CLH report, RAC agreed that Warfarin is readily

biodegradable under test conditions (OECD TG 301D), with a level of degradation of 92.7% after 28 days also meeting the criterion of 60% removal within a 10 day window. Therefore, based on these data and the decision scheme in the CLP Guidance, RAC agreed with the DS that warfarin should be considered **rapidly degradable** according to CLP.

Bioaccumulation
The experimental log K_{ow} for warfarin is 0.7 (pH 7) and the experimental BCF (OECD TG 305) is 21.6 L/kg, both values are below the cut-off values of log $K_{ow} \geq 4$ (CLP) and BCF ≥ 500 (CLP). RAC agreed with the DS that warfarin has **low potential for bioaccumulation**.

Aquatic toxicity
The acute toxicity category should be based on the lowest valid L(E)C_{50}. In this case, all the acute endpoints (L(E)C_{50}) reported in the CLH dossier for the three trophic levels are higher than 1 mg/L. The reported three fish studies were based on nominal concentrations and taking into account that the substance is readily biodegradable and shows low solubility, the reported toxicity values could be underestimates of acute toxicity in fish. Furthermore, the three acute fish studies were performed in 1984, and acetone was used as a vehicle in order to dissolve warfarin, but the acetone concentrations used exceeded in some cases the maximum concentration for solvents suggested in the current guideline (100 mg/L). Although there are data which showed that the fish had a high degree of tolerance to acetone, the mortality in the 100 mg/L solvent control of the key study was 40% throughout the test. Although the LC_{50} values of the three studies were fairly consistent (65 mg/L for the key study, 66 and 88 mg/L for the supportive studies), all these tests showed the same deficiencies. In addition in the studies used as supportive tests a white precipitate was formed due to solubility problems, so the reliability of these tests is questionable.

In the CLH report a prolonged toxicity test in fish (*Oncorhynchus mykiss,* OECD TG 204), is reported as a chronic test. This test, as well as the acute tests, used acetone as vehicle to dissolve Warfarin, and for some tested concentrations at a level higher than that allowed in the guidelines (100 mg/L). However, for the NOEC concentration of 3.8 mg/L (nominal concentration), the concentration of acetone was 45.6 mg/L, therefore this value is in agreement with the guideline and may be considered as reliable. Measured concentrations were actually 29-73% below nominal and a 21 day NOEC (mortality, 1.8 mg/L if the geometric mean is considered) of 2.0 mg/L based on mean measured concentrations were reported. This value cannot be used as a chronic value but it strengthens the evidence that the aquatic acute toxicity for fish is higher than 1 mg/L (see "In *depth analysis by RAC*" section).

Regarding the *Daphnia* test used as a key study, although the results are based on nominal concentrations, the concentration of Warfarin was determined in duplicate at 0 and 48 h. The measured concentrations were within ± 20% of the nominal or measured initial concentration, thus the results can be based on nominal concentrations, i.e. the EC_{50} value > 105 mg/L (nominal) is acceptable. Two more acute *Daphnia* tests were also submitted in the CLH report and their results, based on nominal concentration are in agreement with this value, although it is not possible to conclude based on the provided information if the measured concentrations were within the 20% of the nominal concentration throughout the whole tests.

Two algae tests were summarised in the CLH report. Warfarin showed low toxicity to algae with E_rC_{50} and E_bC_{50} > 83.2 and >8.5 mg/L, respectively. After public consultation NOEC values of 21.3 mg/L (NOE_rC) and 8.5 mg/L (NOEC), respectively were submitted by the DS for both tests. The first test was used as a key study for this trophic level and the values are based on measured concentrations.

In conclusion, the acute tests for fish cannot be considered reliable and valid for classification, taking into account the prolonged toxicity test, the reported information suggests that the LC_{50} for fish is higher than 1 mg/L. Reliable acute toxicity tests for algae and daphnia are available and according to the results, no classification for aquatic acute toxicity is justified.

Regarding chronic toxicity, no adequate chronic data is available for all three trophic levels. Chronic information was included only for daphnia in the CLH report and for algae after public consultation. Taking into account the lowest reliable chronic toxicity value (*Daphnia*, NOEC =

0.059 mg/L) and that the substance is rapidly degradable, a classification as Aquatic Chronic 2 (H411) would be applicable for Warfarin.

Due to the lack of chronic data for fish, the surrogate approach must to be applied and compared to the classification based on chronic toxicity. However, the observed low acute toxicity in fish would result in a less stringent classification and therefore, the chronic classification should be based on the chronic toxicity in *Daphnia*.

In conclusion, RAC agreed with the DS's proposal and concluded that the provided data did not justify classification for aquatic acute toxicity but while classification for long-term aquatic hazards is warranted as follows: **Aquatic Chronic 2 (H411)**.

It is recommended to revise the classification if reliable acute and chronic data for fish become available.

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).