Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

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

CLH-O-0000003395-72-02/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-0000003395-72-02/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:

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

EC number: 259-980-5

CAS number: 56073-10-0

The proposal was submitted by **Italy** and received by the RAC on **14 February 2013.** 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

Italy 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: **Bert-Ove Lund**

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 **Brodifacoum** 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	607-172-00-1	brodifacoum (ISO); 4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin	259-980-5	56073-10-0	Acute Tox. 1 Acute Tox. 2 * STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H310 H300 H372** H400 H410	GHS06 GHS08 GHS09 Dgr	H310 H300 H372 ** H410		
Dossier submitters proposal	607-172-00-1	brodifacoum (ISO); 4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin	259-980-5	56073-10-0	**Modify:** Acute Tox. 1 (oral) **Add:** Acute Tox. 1 (inhalation) Skin Sens. 1 Repr. 1B	**Modify:** H372 (blood) **Add:** H330 H317 H360D	**Add:** GHS07	**Modify:** H372 (blood) **Add:** H330 H317 H360D		**Add:** STOT RE 1; H372 (blood) C ≥ 0,25% STOT RE 2; H373 (blood): 0,025% ≤ C < 0,25% M=10 M=10
RAC opinion					Repr. 1A Acute Tox. 1 (oral) Acute Tox. 1 (inhalation)	H360D H330 H372 (blood)		H360D H330 H372 (blood)		**Add:** Repr. 1A; H360D: C ≥ 0,003% STOT RE 1; H372 (blood): C ≥ 0,02% STOT RE 2; H373 (blood): 0,002% ≤ C < 0,02% M=10 M=10
Resulting Annex VI entry if agreed by COM	607-172-00-1	brodifacoum (ISO); 4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)	259-980-5	56073-10-0	Repr. 1A Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1	H360D H300 H310 H330 H372 (blood)	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372		Repr. 1A; H360D: C ≥ 0,003% STOT RE 1; H372 (blood): C ≥ 0,02% STOT RE 2; H373

	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)	
		coumarin			Aquatic Acute 1 Aquatic Chronic 1	H400 H410		H410		(blood): 0,002% ≤ C < 0,02% M=10 M=10

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

Brodifacoum 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

Brodifacoum presently has a harmonised classification according to CLP for acute toxicity via the dermal route in category 1 and a minimum classification for the oral route in category 2. The dossier submitter (DS) proposed to modify the acute toxicity classification via the oral route to category 1 and to add a classification for acute toxicity via inhalation in category 1. The proposal for classification via the oral route was based on data from one rat study and one mouse study, where the LD_{50}s were <5 mg/kg/day and 0.40 mg/kg/day, respectively. Classification for acute toxicity via the inhalation route is supported by one study in rats, giving a LC_{50} of 3.0 mg/m^3.

Comments received during public consultation

One Member State supported the proposal, and there were no objections.

Assessment and comparison with the classification criteria

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

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

However, a Buehler test in guinea pigs was positive. The induction was intended to be performed using three weekly topical administrations of 1% Brodifacoum in corn oil, but the concentration had to be reduced to 0.1% at the last induction treatment due to signs of toxicity in one animal. The challenge was performed 2 weeks after the last induction using topical administration of either 0.05 or 0.1% Brodifacoum. With both challenge concentrations, roughly 40% of the animals showed allergic reactions. The symptoms were described as scattered mild redness at the concentration of 0.05% (no signs in controls), and a mix of scattered mild redness and moderate diffuse redness at 0.1%. However, as 3 out of 8 control animals in the 0.1% group showed scattered mild redness, the difference in incidence between the group exposed to 0.1% and its control group is only 4%, and the effects at 0.1% did not fulfil the criteria for classification. The finding of redness in the controls indicates that there is some source of skin irritation which interfered with the assay. In contrast, the incidence of 39% at the challenge concentration of 0.05% and induction concentration of 1% does in principle fulfil criteria for classification (the incidence is between 15 and 60% at a topical induction dose of 0.2-20%). However, the reaction was very modest, and since irritation was noted at 0.1%, it is difficult to rule out a contribution of irritation to this reaction. Although the negative LLNA and Magnusson & Kligman assays were performed at much lower concentrations of Brodifacoum, these assays are generally more sensitive than the Buehler assay, and the absence of positive reactions in these two assays argue against a sensitisation potential. Although Brodifacoum was weakly positive at (only) one concentration in the Buehler test, a weight of evidence assessment does not support classification for sensitisation.

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

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

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

Regarding the routes of exposure, repeated dose toxicity studies were only available for the oral route. However, the acute toxicity studies indicated that toxicity via the inhalation and dermal routes was also significant. The RAC therefore supported not specifying the exposure routes in the hazard statement.

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

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

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

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

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

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

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

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

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

Although there are only 3 human cases described for Brodifacoum, two of them indicate similar effects of Brodifacoum and Warfarin in humans, and more severe effects in the fetus than in the

mother. Thus, they support the position that Brodifacoum may exert similar developmental toxicity as Warfarin in humans.

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

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

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

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

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

Detailed discussion by RAC

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

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

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

theoretically either be direct via inhibited enzymes in the foetus or indirect via inhibition in the dam resulting in low circulating concentrations of vitamin K.

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

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

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

Mehlhaff *et al.* (2013) reported a case with bleeding diathesis (spontaneous mucosal bleeding) in the mother after oral ingestion of Brodifacoum. After correction of the coagulopathy, the patient was taken for urgent cesarean delivery. The 32nd week neonate showed evidence of foetal coagulopathy and died at 4 days of life.

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

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

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

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

<u>Developmental toxicity - haemorrhage</u>

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

AVK rodenticides act via inhibiting the formation of vitamin K, which in the next step acts by regulating carboxylases, and the AVK rodenticides therefore have effects on the processes (e.g., coagulation, bone formation) regulated by these carboxylases. It is noted that the expression of carboxylases in the foetal liver, which is responsible for the coagulation system, starts at day 16 (Romero *et al.*, 1998), so it is unexpected that haemorrhages are found at rather similar incidences in foetuses exposed until day 15 as in foetuses exposed until day 19. In both cases foetuses were dissected at day 20.

However, a (poorly reported) study on Warfarin by Mirkova and Antonov (1983; see CLH report on Warfarin) also reported foetal haemorrhages, and James *et al.* (1989; see CLH report and CAR on Flocoumafen) reported a low incidence of haemorrhage in controls that did not increase with increasing exposure to another AVK rodenticide, flocoumafen.

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

Brodifacoum data: No foetal haemorrhages were reported in the rat studies on Brodifacoum and there are different opinions regarding how to interpret the absence of fetal haemorraghes in these studies. In contrast, a case report (Munday and Thompson, 2003) describes how an apparently healthy dog gave birth to pups, where 8 out of 13 pups died of haemorrhage within 2 days. The finding of Brodifacoum in the pup livers was taken as evidence of AVK-intoxication.

Developmental toxicity – bone effects

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

Developmental toxicity – ocular effects

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

Developmental toxicity – general foetal toxicity

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

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

Dose-effect relationship between haemorrhages and nose/bone defects

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

Based on available literature, one may rather speculate that the opposite may be true in humans, i.e., that bone effects may precede haemorrhagic effects of AVKs. Cases of Warfarin-induced teratogenicity with no reported haemorrhagic event are reported. For example, Baillie (1980) described "*a term infant with a hypoplastic nose due to failure of development of the nasal septum. No other abnormality was detected on routine clinical examination. X-rays of pelvis and femora showed stippling in the greater trochanters and left pubis and also abnormal vertebral bodies at S4/5*". A similar case with slightly enlarged head and flattened face with a depressed nasal bridge and small nose, stippling of the vertebrae and femoral epiphyses was noted in a stillborn neonate

in the 26th week of gestation where no abnormalities other than mild hydrocephalus, nasal hypoplasia, foetal growth restriction were revealed (by autopsy) (Tongsong *et al.*, 1999). Van Driel et al. (2002) summarised the foetal outcome in cohort studies on use of coumarins during pregnancy and reported two-fold higher prevalence of embryopathy (22 cases of skeletal anomalies seen after in utero exposure to coumarins) than bleeding (11 cases), if coumarins were given from the beginning and throughout the pregnancy. For the cases reported for Acenocoumarol and Phenprocoumon, 85% involved the nose and only one case mentions bleedings (7%) (van Driel et al., 2002).

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

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

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

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

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

particularly considering that there are a few cases of developmental toxicity seen in humans exposed to Brodifacoum and other AVK coumarins used therapeutically.

Toxicokinetics and transplacental transfer

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

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

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

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

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

REFERENCES

Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW. Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr. 2000 Dec;72(6):1523-8.

Baillie M. *et al.* The congenital warfarin syndrome: a case report. Br J Ophthalmol. 1980;64:633-5

EHC document 175, 1995

Hetzel *et al.* 2006, Eur J Pediatr. 165(6):358-60.

Hoyer. Coumarin Embryopathy after Intrauterine Exposure to Vitamin K Antagonists within the First 10 Postmenstrual Weeks Ultraschall in Med 2010; 31(4): 411-413

Howe AM, Webster WS. The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology. 1992 Oct;46(4):379-90.

Howe and Webster, 1994. Vitamin K—its' essential role in craniofacial development. A review of the literature regarding vitamin K and craniofacial development. Aust Dent J. 39(2):88-92
Howe *et al.* 1995 Am J Med Genet 11(58)3, 238-44.
Keppler-Noreuil and Wenzel, 2012, J Craniofac Surg 21(5):1339-45.
Mehlhaff *et al.*, 2013, Obstet Gynecol 122:500-2.
Munday and Thompson, 2003, Brodifacoum toxicosis in two neonatal puppies. Vet. Pathol. 40:216-219.
Price PA, Kaneda Y. Vitamin K counteracts the effect of warfarin in liver but not in bone. Thromb Res. 1987;46:121-31.
Rane A. and Lindh JD. Pharmacogenetics of anticoagulants. Human Genomics and Proteomics, 2010: doi 10.4061/2010/754919.
Revel-Vilk S. The conundrum of neonatal coagulopathy. Hematology Am Soc Hematol Educ Program. 2012;2012:450-4
Romero *et al.*, 1998, Experimental Cell Research, 243, 334-346
Shearer MJ *et al.* Lancet. 1982;2:460-3
Shepherd *et al.*. Acute, unintentional pediatric brodifacoum ingestions. Pediatr. Emerg. Care 2002, 18(3) 174-178.
Teichert M, Visser L.E., van Schaik R.H.N., Hofman A, Uitterlinden A.G., De Smet P.A.G.M., Witteman J.C.M., Stricker B.HCh. Vitamin K Epoxide Reductase Complex Subunit 1 (VKORC1) Polymorphism and Aortic Calcification. The Rotterdam Study. Arterioscler Thromb Vasc Biol. 2008;28:771-6
Tongson T, Wanapirak C, Piyamongkol W. Prenatal ultrasonographic findings consistent with fetal warfarin syndrome. J Ultransound Med 1999;18:577-80.
Wallin R, Cain D, Sane D.C. Matrix Gla protein synthesis and gamma-carboxylation in the aortic vessel wall and proliferating vascular smooth muscle cells--a cell system which resembles the system in bone cells. Thromb Haemost. 1999 Dec;82(6):1764-7.
van Driel D, Wesseling J, Sauer PJ, Touwen BC, van der Veer E, Heymans HS. Teratogen update: fetal effects after in utero exposure to coumarins overview of cases, follow-up findings, and pathogenesis. Teratology 2002 Sep:66(3):127-40.
Verhoef *et al.* Pharmacogenetic-guided dosing of coumarin anticoagulants: algorithms for warfarin, acenocuomarol and phenprocoumon. Br. J. Clin. Pharmacol. 2013 Aug 7, doi: 10.1111/bcp.12220
Vermeer and Braam. Vitamin K and tissue calcification. J Bone Miner Metab (2001) 19:201–206.
Xie *et al.*, 2013 J Pediatr Hematol Oncol. 35(6):e265-8.
Yan J *et al.*, 2013, Int J Gynaecol. Obstet. 122(2):162-163.Zurawski and Kelly, 1997 Obstet Gynecol 90(4:2)672-4.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

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

<u>Degradation</u>

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

The DS considered Brodifacoum as hydrolytically stable (DT_{50} > 1 yr) and rapidly photodegradable with an experimental half-life < 1 day. It was degraded rapidly in the atmosphere by reaction with

OH radicals, although the presence of this compound in air is not expected due to its low vapour pressure.

In the CLH report, table 21 summarised all relevant information on degradation, including data about ready and inherent degradation. However, the DS did not provide a detailed evaluation of these tests in the report. According to these data, Brodifacoum is not readily or inherently biodegradable under test conditions. In the ready biodegradability tests according to the OECD 301B, OECD 301D, and ISO 11734 guidelines, the level of degradation was between 0-3.5%, being therefore below the ready biodegradability pass levels of 60 or 70%. In the inherent biodegradation test according to the OECD 302D draft guideline, the degradation was 2%.

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

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

Bioaccumulation

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

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

Aquatic toxicity

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

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

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

Degradation

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

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

Bioaccumulation

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

Aquatic toxicity

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

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

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

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

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).