Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

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

CLH-O-0000003398-66-03/F

Adopted

14 March 2014

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COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: flocoumafen (ISO); reaction mass of: cis-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin;trans-4-hydroxy-3-(1,2,3,4-tetrahydro-3-(4-(4-trifluoromethylbenzyloxy)phenyl)-1-naphthyl)coumarin
EC number: 421-960-0
CAS number: 90035-08-8
Dossier submitter: the Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	1

Comment received

We disagree with the classification proposal for human health: Repr. Cat 3; R63- Repr 2 H361d

☐ Environmental hazards:
We agree with the current proposal for consideration by rac:

CLP regulation:
Aquatic acute 1 (M=10);
Aquatic chronic 1 (M=10);
H400 – very toxic to aquatic life;
H410 – very toxic to aquatic life with long lasting effects.
DSD:
N; R50-53 – very toxic to organisms, may cause long-term adverse effects in the aquatic environment.

Dossier Submitter's Response

Thank you for the support for the classification proposal for environmental hazards.
For the classification proposal for reproductive toxicity, see below.

RAC's response

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

As there are no data on the outcome of maternal exposure to Flocoumafen in humans, classification in cat 1A is not considered to be applicable for Flocoumafen.
Based on the assumption that all AVK rodenticides, including Warfarin and other anticoagulant coumarin pharmaceuticals (see below) share the same MoA, namely inhibition of vitamin K epoxide reductase (VKOR), the assessment of Flocoumafen includes consideration of the total data base for the AVKs. A weight of evidence assessment resulted in the conclusion that Flocoumafen has the capacity to adversely affect the human *in utero* development. Therefore a classification with cat 1B is proposed with the reasoning given below.
The reasons for this presumption are:

- Flocoumafen shares the same MoA as expressed by other anticoagulant AVK rodenticides and coumarin pharmaceuticals (inhibition of vitamin K epoxide reductase, an enzyme involved with blood coagulation and foetal tissues development, including bone formation, CNS development and angiogenesis)
- Warfarin and 2 other coumarin pharmaceuticals (acenocoumarol, phenprocoumon) have been shown to cause developmental toxicity in humans.
- One of the 2nd generation AVK rodenticides (Brodifacoum) has been shown to cause foetal effects in humans, possibly after one or a few exposures.
- For AVK rodenticides with a long half-life in the body, even single exposures might suffice to trigger developmental effects. However, such studies are normally not conducted and effects of single dose exposure cannot be detected in standard OECD 414 test where rather the repeated exposure may lead to maternal mortality with steep dose-response.
- The standard animal studies will not pick up all developmental toxicity effects of the AVK rodenticides, most notably the face and CNS malformations that are characteristic for Warfarin and other AVK coumarin pharmaceuticals.
- The most sensitive window for face malformations in humans is the first trimester. Thus, also if some AVK rodenticides may have a lower degree of placental transfer than Warfarin, this will not affect the face malformation hazard.

Not all steps of the MoA in the target tissues liver and bone have been proven, thus introducing some uncertainty in the assessment. However, the RAC is of the opinion that the uncertainty is not sufficiently big to warrant a cat 2 classification.
Reliable evidence of an adverse effect on reproduction in humans, which is required for Repro 1A, was not available for Flocoumafen, but a potential for human developmental toxicity is presumed based on the above stated weight of evidence assessment, and RAC thus proposes classification with Repr. 1B, i.e. "presumed human reproductive toxicant".

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Denmark		MemberState	2
Comment received				
Danish comments to the CLP report on flocoumafen: Denmark agrees with the classifications proposed by the rapporteur for the end-points of acute and repeated dose toxicity as well as for aquatic toxicity for flocoumafen. With respect of classification for reproductive toxicity, Denmark agrees with the proposal that flocoumafen needs not be classified for effects on fertility. However, concerning classification for developmental toxicity, Denmark does not agree with the conclusion that the classification of flocoumafen for should as repro cat 3; R63/Repro cat 2; H361d. Denmark's position is that flocoumafen should be classified as repro cat 1;				

R61/Repro cat 1A; H 360D.

Anticoagulant rodenticides of the coumarin-family have all been agreed in 2007 in the TC C&L group to be classified as R61 (DSD) (corresponding to H360D according to CLP criteria) due to their structural and mechanistical similarity with warfarin, which is a known human teratogen classified as Repr. Cat 1; R61 (DSD) recognising that the OECD 414 guideline has limitations as to showing the teratogenic effects seen in humans, amongst other due to the window of exposure.

In the new study conducted according to OECD 414 on warfarin, which includes two prenatal dosing windows, an extra high dose group was added some time after the beginning of the study. The time shift makes it difficult to fully include this dose group in the assessment of the study outcome and may have impeded on the results. Also the findings of the study, although showing some developmental effects in the rats, do not mirror the embryopathy picture seen in humans. Due to the differences in development of the neonate rat and human, postnatal dosing would be required in order effect as one of the human effects of warfarin, nasal hypoplasia, to be detected.
Therefore, the concern that the OECD 414 protocol is not adequate to show developmental effects of AvKs remains.

Studies of plasma levels and placenta transfer and kinetics in the rat indicate that flocoumafen passes the placenta but that the plasma levels in the foetus are lower for flocoumafen than warfarin. This indicates that there are differences in the plasma levels between the different AvK-substances, as it is also the case with respect of toxicity aspects between the substances across the AvKs. However, these potency aspects do not affect the relevance of the common mechanism of action for all AvKs, leading to the use of read across to warfarin for evaluation of the capability of inducing developmental effects.

In conclusion, the Danish CA therefore supports that read-across to the known developmental toxicant warfarin should be applied and that flocoumafen, as all AvKs, should be classified as Repr cat 1; R61 (DSD)/Repro cat1A; H360D (CLP).

Denmark supports the setting of lower specific concentration limits for repeated dose toxicity both in relation to directive 67/458/EC and in relation to CLP regulation 1272/2008 in order to reflect the potency of flocoumafen. However, further discussion in order to harmonise the method used across the substances is needed.

The Danish CA agrees with the proposed M-factors for acute and chronic aquatic toxicity.

Dossier Submitter's Response

Thank you for the support for the proposal for acute and repeated dose toxicity and aquatic toxicity.

We agree that for classification of flocoumafen the available dataset for warfarin (due to the same mode of action) should be taken into account, however also the data available for flocoumafen itself should be included in the weight of evidence approach.
It is clear that the OECD 414 study is not appropriate to detect nasal hypoplasia, since the development of the nasal cartilage in rats occurs after birth. Since the mode of action of warfarin and flocoumafen is the same, it might be expected that flocoumafen will also cause nasal hypoplasia in rats, when they are exposed in the right time-window. However, in humans, the development of the nasal cartilage occurs in the 3rd trimester of the pregnancy and therefore, placental transfer is relevant for this effect. The study on placental transfer in rats shows that, due to a high 1st pass effect, the amount of flocoumafen that reaches the pup is very low (and lower than with warfarin). This might explain why no hemorages are observed in the developmental studies with flocoumafen and

why such effects are observed with warfarin. It could be argued that, due to the 1st pass effect, concentrations reaching the fetus will also be too low to induce nasal hypoplasia. Because this raises doubt on the relevance of the warfarin data for the effects that flocoumafen can cause in humans, the evidence is not sufficiently convincing to classify flocoumafen as Repr. Cat 1 and Repr. Cat 2 is considered a more appropriate classification. See also our response to comment 3 on the relevance of potency and kinetic aspects for read-across.

With regard to the SCL for repeated dose toxicity, we agree that we should use a harmonised method for all anticoagulant rodenticides.

RAC's response

Health Hazards –

Thank you for support to the classifications of acute and repeated dose toxicity and for effects on fertility .

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification that has been provided jointy under Comment number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across approach defined in section 1.5 of Annex XI of REACH Regulation.

Regarding SCL for repeated dose toxicity RAC also supported proposed by the DS an SCL for STOT Rep. 1 of 0.05% based on serious damage seen at 0.1 mg/kg food (ED 0.005 mg/kg, haemorrhage in lymph nodes, rat, 90 d) in the 90-day study in rats. Calculation: 0.005 mg/kg bw/day (effective dose) / 10 mg/kg bw/day (limit) * 100% = 0.05%.

STOT Rep. 2 is proposed between 0.005% and 0.05% using the same data and method of calculation (limit: 100 resp. 10 mg/kg bw/day).

The calculations of SCL were harmonized by using the method described in the Guidance on the Application of the CLP Criteria.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	Exponent International on behalf of CEFIC RDDG	Industry or trade association	3

Comment received

4.11 Toxicity to reproduction
The proposal to classify for developmental toxicity is not agreed. Data are conclusive and not sufficient for classification. Please see the attached document (Exponent DocID 1109091.uk0 EWC0008)

(*ECHA note: The attachment provided is copied below)*

Teratogenicity of AVK Rodenticides
Classification by Read-Across from Warfarin is not Correct

Summary
The conclusion of the Specialised Experts ("SE Conclusion") that the classification of all anti-Vitamin K (AVK) rodenticides as teratogens should be read-across from warfarin is no longer valid.
- The SE Conclusion is inadequate by modern standards, since it lacks a clear comparison of the data against the classification criteria.
- New data overturn a key consideration on which the SE Conclusion was based (i.e., doubt on the ability of the OECD 414 study design to detect AVK embryopathy). A new OECD 414 study of warfarin now demonstrates method sensitivity.
- The SE Conclusion was not based on the most appropriate endpoint, being concerned with teratogenicity when more recent epidemiological data show foetotoxicity in human pregnancies to be of greater incidence.
The CEFIC teratogenicity study of warfarin demonstrates developmental and foetotoxicity, and therefore confirms sensitivity of the OECD 414 study design. There is clear evidence of specific foetal sensitivity to haemorrhage; borderline evidence of an increase of small foetuses (10-day group only) in the absence of maternal toxicity, and adequate evidence of malformation. The incidences of foetal haemorrhage at the low dose demonstrates the ability of the OECD 414 study design to detect specific foetal sensitivity to warfarin, and therefore the same ability to detect specific foetal sensitivity to the AVKs.
The basis for read-across for developmental toxicity from warfarin to the non-warfarin AVK rodenticides, is therefore invalid.
Careful comparison of the guideline developmental toxicity data for each of the non-warfarin AVKs against the classification criteria therefore show:
- Criteria for classification as CLP Cat 1A are not met. There is no evidence that any of the non-warfarin AVK rodenticides are associated with adverse pregnancy outcomes in humans.
- Criteria for classification as CLP Cat 1B are not met. There is no "clear evidence", from valid GLP- and guideline- compliant studies, that any of the non-warfarin AVK rodenticides cause an adverse effect on development in animals. Indeed, with the multiplicity of good and reliable studies (for which validity of the model is demonstrated) there is strong evidence that they do not.
- Criteria for classification as CLP Cat 2 ("some evidence") are not met. There is no evidence from GLP- and guideline- compliant studies, that any of the non-warfarin AVK rodenticides cause an adverse effect on development in animals. Indeed, with the multiplicity of acceptable and reliable studies (for which validity of the model is demonstrated) there is strong evidence that they do not.
- No classification for developmental toxicity is therefore appropriate.

Introduction:

Exponent International Ltd has been retained by the CEFIC RDDG[1] to:
1. Review the Specialised Experts[2] conclusion of September 2006 which recommends the AVK rodenticides be classified as Category 1 developmental toxicants on the basis of read-across from warfarin;
2. Review additional data provided by the CEFIC RDDG (a teratogenicity study of warfarin following OECD Test Guideline 414);
3. Deliver an opinion on the validity of the proposed read-across (from warfarin as a Category 1 developmental toxicant, to therefore all AVKs as Category 1 developmental toxicants);

1. Review of the Specialised Experts Conclusion

a) The SE Conclusion is no longer adequate for modern purposes since it lacks a clear comparison with modern (DSD or CLP) criteria.
b) In addition, recent data amend some of the assumptions from which the conclusion is derived; in particular:
c) The OECD 414 study of warfarin demonstrates sensitivity of the method; it is therefore appropriate to base classification on the actual results achieved in OECD 414 teratogenicity studies with each of the AVKs.
d) Teratogenicity is not the most appropriate human or animal endpoint. It is unusual for teratology to occur in the complete absence of other toxicity. A more usual picture is that teratology occurs as a particularly notable feature, among a spectrum of other foetotoxic change. This would appear to be the clinical picture among the therapeutic AVKs including warfarin. A multicentre prospective clinical trial (Schaefer et al, 2006[3]) examined 666

pregnancies to mothers receiving anticoagulant treatment (with warfarin, phenprocoumon, acenocoumarol, fluindione, or phenindione); birth defects were rare but the more numerous findings were of foetotoxicity – prematurity, miscarriage, decreased mean gestational age at delivery, decreased mean birth weight of term infants. Embryotoxicity (of which the teratology would be only one factor) is more meaningful for protection of the foetus; and is identified in the CEFIC warfarin study. The epidemiology of therapeutic AVKs shows that among human pregnancies foetotoxicity is of higher incidence than teratogenicity; the OECD 414 study of warfarin predominantly shows foetotoxicity. The warfarin-related incidence of foetotoxicity in human pregnancies (as stillbirth, prematurity, small at term) is mentioned in a number of the CLH reports, without drawing appropriate parallels to the warfarin study.

e) The essential evaluation of animal developmental toxicity studies is to assess whether a chemical is able to produce adverse effects in the foetus of experimental animals and whether the foetus is directly affected and/or is more susceptible than the mother. It is not generally expected that the same effects occur across species. It is however generally accepted that if a chemical is able to produce adverse effects on embryos of experimental animals, it could be a hazard also for human embryos, independently of the specific features of the effect. In the case of the CEFIC study of warfarin, results show that the test was able to identify warfarin as a substance toxic for the conceptus, inducing embryofetal mortality, haemorrhages, and malformations i.e. cataract. It appears to be a reliable test to identify a risk for human foetuses.

f) A placental transfer study demonstrated that there was foetal exposure to both warfarin and flocoumafen (which may also be the case for the other AVKs). These data identify foetal exposure in this study yet there is still a significant difference in the foetotoxic effects observed with warfarin compared to those observed with the other AVKs. For all of the nonwarfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies despite clear exposure.

g) It is unclear how maternal toxicity is taken into account in the classification process for the AVKs. From the Regulation, classification should address the foetus as an especially sensitive target for toxicity. All evidence of warfarin teratogenicity and foetotoxicity in humans is at levels of maternal 'toxicity' (i.e., therapeutic anticoagulation). Further, comments from at least one MS appear to use a potential concern of maternal Vitamin K depletion leading to the embryopathy, as a reason to discount arguments of the AVKs reaching the foetus. A mechanism dependant entirely on maternal toxicity is however justification to not classify.

2. Comments on the CEFIC teratogenicity study of warfarin[4]

The study is reviewed in the CLH proposal for warfarin, and for that reason a detailed description is not given here. The following observations are however offered:

The study carefully examines dose levels around the limit of maternal toxicity. This is important, since the dose-response curve for teratogenicity can be steep (Schardein, 2000[5]). This might be particularly so with the AVKs, since the dose-response for maternal toxicity is also particularly steep. The study also examines two different periods of exposure: days 6-15 of pregnancy ("TP1", corresponding to the pre-2001 OECD 414 guideline) and days 6-19 of pregnancy ("TP2", corresponding to the revised 2001 OECD 414 guideline).

The warfarin study provides clear evidence (for classification purposes) of specific foetal sensitivity to haemorrhage (i.e., foetal haemorrhage is a dose-related finding, found at the lowest dose level which was not maternally toxic, thus demonstrating detection of specific foetal sensitivity). Both exposure periods (10- and 14-day) were adequate to demonstrate foetotoxicity.

In the opinion of this reviewer, the study also showed: borderline evidence of an increase of small foetuses (10-day treatment group only) in the absence of maternal toxicity; and adequate evidence of malformation (cataract, which has been noted in human foetuses from mothers administered warfarin during pregnancy [Hall *et al*., 1980[6]]). Although this study examines dose levels very closely spaced in the maternally toxic range, the incidence of foetal haemorrhage at the low dose is clear demonstration of the ability of the standard "OECD 414" design to detect specific foetal sensitivity to warfarin and the AVKs.

In summary: the study showed maternotoxic effects primarily due to haemorrhages in different organs and mortality. The No Adverse Effect Level (NOAEL) for maternal toxicity was 0.125 mg/kg bw/day.

At the level of conceptus warfarin treatment induced:

- an increase of foetal mortality with a NOAEL of 0.150 mg/kg bw/day;
- a dose related increase of foetal haemorrhages even at the lowest dose tested of 0.125 mg/kg

bw/day;
- central ocular cataract (typical malformation of warfarin embryopathy) even at the lowest dose tested of 0.125 mg/kg bw/day.

Warfarin is seen to be embryotoxic and teratogenic in the rat.
For each of the non-warfarin AVK rodenticides, at least one teratogenicity study in rats examines developmental toxicity within the maternally toxic range; in total, nine studies in rats of seven non-warfarin AVKs appear adequate for classification purposes, and demonstrate absence of any form of developmental toxicity. For each of the non-warfarin AVK rodenticides, further adequate studies in rabbit also demonstrate absence of developmental toxicity.

Additional Observations on Reasoning for Read-across from the CLH Reports

Most CLH proposals (March 2013) consider the results of the new OECD 414 study of warfarin, and available placental transfer data.
For all of the non-warfarin AVK rodenticides (with the possible exception of bromadiolone), the animal data are concluded to show no evidence of teratogenicity. In cases where classification is recommended, proposals therefore remain entirely based on the common position of read-across from warfarin.
Current proposals for reproductive classification from the seven non-warfarin AVK CLH proposals range from CLP 1A (4 substances), 1B (one), 2 (one) and no classification (one).
In the CLH report for brodifacoum, comparison with criteria is not considered (no entry).
For bromadiolone, the CLH report concludes teratogenicity in the rabbit, based on dissimilar findings in 3 foetuses at two dose levels. The evaluation however appears inconsistent within the CLH report (evaluated as "may constitute a possible risk" on p48, or "some effects" on p51, or "inconclusive" then "teratogenic" on p 53) and there is no evaluation of "strength" (the reader cannot determine if the evaluation constitutes "clear" or "some" animal evidence). This review notes that the findings fall within the range of spontaneous incidence and show no syndrome. There is no evident consideration of warfarin effects other than teratogenicity (i.e. foetotoxicity) or consideration of human foetotoxicity.
The CLH recommendation for chlorophacinone accepts the new data as adequate to not classify.
For coumatetralyl, the CLH report offers a comparison with criteria. The comparison states "*However, due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to coumatetralyl, no clear conclusion can be drawn from the standard guideline studies.*" This statement is inconsistent with the CEFIC warfarin study results; no explanation is offered as to how the studies of coumatetralyl might significantly differ from the warfarin study design. There is no discussion as to the relevance of foetoxicity in the warfarin study with respect to the human epidemiology. The CLH report postulates that a study including Vitamin K supplementation might be meaningful, and that post-natal exposure (after Howe & Webster, 1994[7]) might also be necessary; neither of which were features of the warfarin study design. It must be noted that the design of Howe & Webster (1992)[8], examining bone growth post-natally in rats, probably differs fundamentally from the process of embryonic cell death and remodeling that occurs during the period of major organogenesis and that is the target of teratogenicity studies. Further, in the teratogenicity studies with coumatetralyl, to overcome the fact that developing rodent fetus is typically evaluated at a time when ossification of the skeleton is incomplete (at gestation day 20 in the rat), the skeletons are double-stained (Alizarin red S and Alcian blue) for a thorough assessment of skeletal development including both ossified and cartilaginous structures.
The CLH report for difenacoum offers no comparison with criteria. The warfarin study is assessed as not having shown malformation using the typical TP1 dosing regimen. There is no consideration of the relevance of embryotoxicity in the warfarin study or in humans. Teratogenicity studies of difenacoum were considered not suitable for determination of teratogenicity, citing a need for postnatal exposure (after Howe & Webster, 1992).
The CLH report for difethialone offers a comparison with criteria. The comparison states: "*Due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to difethialone, no clear conclusion can be drawn from these studies*". This statement is inconsistent with the warfarin study results; no explanation is offered as to how the studies of difethialone might significantly differ from the warfarin study design. The difethialone rat study is also criticized for absence of maternal toxicity at the highest dose (50 µg/kg bw/day), with mortality having been observed only in a pilot study (at 70 µg/kg bw/day); this review notes the dose spacing to be within the range of the (effective) warfarin study. There is no discussion of the relevance of foetotoxicity as seen in the warfarin study and in humans.
The CLH report for flocoumafen contains a comparison with criteria, and notes that the absence of

teratogenicity seen with flocoumafen, and placental transfer data, give reason to base a classification on the (negative) animal data. However, the report also states that the placental barrier is not absolute (transfer is diminished, not prevented) and the rat model is not an exact model for humans; hence there remains a possibility for developmental effects in humans. The comparison does not discuss the significance of foetotoxicity as seen in the warfarin study and in humans.
It would therefore appear that none of the CLH reports address the significance of foetotoxicity, as seen in humans and in the rat study of warfarin; and therefore they all fail to address the most appropriate endpoint.

3. Comparison with Criteria

This review offers a detailed comparison with criteria, under the assumption that all of the nonwarfarin AVKs show a clear absence of developmental toxicity in animal studies (i.e. dismissing the bromadiolone interpretation as discussed earlier).
Classification should be based on evidence, not hypothesis.
In comparison to the criteria for DSD Cat 1/ CLP Cat 1A:
There is no epidemiological evidence that the non-warfarin AVK rodenticides cause developmental toxicity in humans.
There is clear epidemiologic evidence that warfarin causes developmental toxicity in humans; and that other AVK anticoagulants used as therapeutics (which do not include the non-warfarin AVK rodenticides) also cause developmental toxicity in humans. However, the criterion for "sufficient epidemiologic evidence" is not met for the non-warfarin AVK rodenticides.
There is evidence to support that, due to absence of effect in appropriately-sensitive teratogenicity studies, the non-warfarin AVK rodenticides are intrinsically different to warfarin.
Because the criterion for "sufficient epidemiologic evidence" is not met for the non-warfarin AVK rodenticides, classification into DSD Cat 1/ CLP Cat 1A is not appropriate.
With respect to DSD Cat 2/CLP Cat 1B:
There is no evidence that the non-warfarin AVK rodenticides cause developmental toxicity in animals.
There is a concern, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans. However, there is evidence that the non-warfarin AVK rodenticides are intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits.
Both warfarin and flocoumafen are seen to cross the placenta. Only warfarin induces clear anticoagulant and developmental effects in the foetus. In contrast, flocoumafen clearly does not.

Therefore, for all of the non-warfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies.
In the absence of relevant effect in animal studies, and with the demonstration of method sensitivity to warfarin, read-across of warfarin developmental toxicity to the other rodenticidal AVKs becomes a scientifically unjustified extrapolation.
Negative results in adequate studies of the AVK rodenticides are meaningful, and placement in DSD Category 2/ CLP Category 1B is not appropriate.
With respect to DSD Cat 3/ CLP Cat 2:
There is no evidence that the non-warfarin AVK rodenticides cause developmental toxicity in animals.
There is a concern, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans. However, there is evidence that the non-warfarin AVK rodenticides are intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits.
Both warfarin and flocoumafen are seen to cross the placenta. Only warfarin induces clear anticoagulant and developmental effects in the foetus. In contrast, flocoumafen clearly does not.
Therefore, for all of the non-warfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies.
In the absence of relevant effects in animal studies, and with the demonstration of method sensitivity to warfarin, read-across of warfarin developmental toxicity to the other rodenticidal AVKs becomes a scientifically unjustified extrapolation.
Negative results in adequate studies of the non-warfarin AVK rodenticides are meaningful.
Concern is reduced in that warfarin as a therapeutic is administered to humans orally; operator exposure to rodenticidal biocidal products is dermal; and the skin presents a considerable and effective barrier to the AVK rodenticides.
Placement in DSD Category 3/ CLP Category 2 is not appropriate.

By comparison of evidence with the criteria, no classification for developmental toxicity is appropriate.
In conclusion, ample evidence is provided that a read-across from warfarin teratogenicity to the nonwarfarin AVK rodenticides is not justified from a scientific point of view, based on the results of valid and good quality data. When compared with the criteria for classification, there is inadequate evidence for classification of the non-warfarin AVKs for developmental toxicity.

Simon Warren
18 April 2013

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1 The CEFIC RDDG is comprised of the following companies: Activa, Babolna-Bio, BASF, Bayer, Bell Laboratories, Hentschke & Sawatzki KG, Laboratorios Agrochem, Liphatech, PelGar and Syngenta who each have joint ownership of this document
2 Commission Working Group of Specialised Experts on Reproductive Toxicity. ECBI/121/06. Ispra, 19-20 September 2006
3 Schaefer C, Hannemann D *et al* (2006) Vitamin K antagonists and pregnancy outcome. A multi-centre prospective study. Thromb.Haemost. 95(6) 949-57.
4 Kubaszky R (2009) Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
5 Schardein J (2000) Chemically induced birth defects. Third edition revised and expanded. Marcel Dekker: New York. ISBN: 0-8247-0265-4
6 Hall *et al*. (1980). Maternal and fetal sequelae of anticoagulation during pregnancy. *Am J. Med.* 68: 122-140.
7 Howe AM & Webster WS (1994): Vitamin K – its essential role in craniofacial development. Australian Dental Journal, **39**(2) 88-92.
8 Howe AM & Webster WS (1992): The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances, Teratology, **46**(4) 379-90

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Dossier Submitter's Response

We do not agree that the Specialised Experts conclusion is not valid, however, it does need to be updated with the new data and should be compared with the current classification criteria. Further, the requirements and justification for read-across have developed since 2007 and these developments should also be taken into account for the classification of the coumarins including flocoumafen.

The Warfarin teratogenicity study indeed shows that part of the effects of the AVK rodenticides can be detected with the OECD414 study, i.e. haemorrhages. However, nasal hypoplasia, which is an important effect that is observed in humans, can not be detected with the OECD414 study, since the nasal cartilage development in rats takes place after birth. This shows that there are differences between humans and the test model.

We do agree that the conclusion on the classification of flocoumafen should not only be based on read across, but also on the data available for flocoumafen itself in a weight of evidence determination (Annex 1 1.1.1) (which we do in the CLH proposal). Warfarin and flocoumafen have the same mode of action and therefore, in principle, read across should be considered. The basic concept behind read-across is that if two substances have a comparable chemical structure, they can induce comparable effects. For most type of effects, the amount of the substance that reaches the site of first interaction of the substance with the target, determined by distribution and metabolism, determines the potency of a substance (dose) to induce such effects. However, for developmental effects the dose that can be achieved in the fetus may also be limited due to the maternal toxicity. In principle every substance that reaches the fetus will have a developmental effect when the dose level gets high enough (Paracelsus). However, in practice (animal studies), the fetal dose that can be achieved is limited by the maternal toxicity (else every substance would be a developmental toxicant when tested at a sufficiently high dose). Therefore, in the case of read-across for developmental effects it is not only important to justify that both

substances can interact with the molecular target in a comparable way but also that a dose level sufficient to induce developmental effects can reach the fetus without marked toxicity/lethality of the dams. In our opinion, there is sufficient evidence that flocoumafen and the other coumarines can interact with the molecular target in a way comparable to warfarine. However, there are indications that the fetal concentration of flocoumafen may be limited due to the maternal toxicity to levels which may not be sufficient to induce developmental toxicity.

The study on placental transfer on rats shows that, due to a high 1st pass effect, the amount of flocoumafen that reaches the pup is very low (and lower than with warfarin). This might explain why no embryotoxicity is observed in the developmental studies with flocoumafen and why such effects *are* observed with warfarin. It is possible that embryotoxicity will be induced by higher doses of flocoumafen, and maybe only at doses that are toxic to the dams. This raises doubt on the relevance of the effects for classification and therefore, classification as Repr. 1A or B is not appropriate. Because there are no data on placental transfer or 1st pass effect in humans, it can not be excluded that higher concentrations of the substance will reach the fetus in humans and embryotoxicity will occur, with or without maternal toxicity (for example due to a lower 1st pass effect or a higher placental transfer). In addition, no data are available with regard to developmental effects following inhalation or dermal exposure to flocoumafen. Further, there is no indication that the skin is an effective barrier for flocoumafen as shown by the dermal LD_{50} which is only slightly higher than the oral LD_{50}. Since after such exposure the 1st pass effect is not relevant, the concentration flocoumafen reaching the offspring might be substantially higher and therefore, embryotoxic effects might occur (possibly even without maternal toxicity). This is considered a difference in distribution but not an intrinsical difference. As a result, it is difficult to extrapolate this differences in maternal to fetal concentration from rats to humans especially as there are known differences between humans and the test model rat. In addition, as the maternal effect (anticoagulation) is induced via a reduced formation of anticoagulation proteins in the liver, it could also be considered that the concentration in the liver is a beter indicator of the maternal exposure than the concentration in the maternal blood. Based on the study by Johnson (2009), the ratio between maternal liver concentration and fetal plasma concentration is 13.7 times higher for flocoumafen compared to warfarine. As for maternal plasma/fetal plasma concentration ratio, also this ratio may not apply to other routes of exposure. Seen these uncertainties, no classification is also not appropriate. In conclusion, because flocoumafen and warfarin share the mode of action and both substances can pass the placenta, the warfarin data (both human and animal data) provide some evidence that flocoumafen might also induce embryotoxicity. However, due to the difference in 1st pass effect between flocoumafen and warfarine and the negative developmental studies of flocoumafen, the evidence is not sufficiently convincing to classify flocoumafen as Repr. Cat 1 and Repr. Cat 2 is a more appropriate classification.

RAC's response

Health Hazards –

Thank you for comments.

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification has been provided jointy under Comment number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across

approach defined in section 1.5 of Annex XI of REACH Regulation.

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	Exponent International on behalf of CEFIC RDDG	Industry or trade association	4

Comment received

Secrtion 4.11: Toxicity for reproduction
Flocoumafen should not be classified for developmental toxicity. Data are conclusive but not sufficient for classification. Please see attached document (Exponent DocID 1109091.uk0 EWC0009 - flocoumafen)

(ECHA note: The attachment provided is copied below)

Flocoumafen
Comment on the CLH proposal, 5 March 2013
Developmental toxicity:
Flocoumafen should ***not be classified*** for developmental toxicity.
Careful comparison of the developmental toxicity data for flocoumafen against the classification criteria show:
- Criteria for classification for developmental toxicity are not met.
  - There is no evidence of flocoumafen being causally associated with developmental toxicity in humans.
  - There is no evidence from acceptable GLP- and guideline-compliant studies, that flocoumafen causes an adverse effect on development in animals.
  - The rat study design is demonstrated to be sensitive to warfarin.
  - Pharmacokinetic evidence shows that the foetus is considerably less exposed to flocoumafen than to warfarin
- No classification for developmental toxicity is therefore appropriate.

Reasoning
1. Basis for classification from the CLH report
The CLH proposal to classify flocoumafen as Repr. Cat 2 (CLP) and Repro Cat 3 (DSD) is: "both the flocoumafen teratogenicity study and the placental transfer study seem to indicate that foetal availability of flocoumafen is lower than foetal availability of warfarin. This may be a reason not to read-across from warfarin to flocoumafen, and to base the decision for classification for developmental toxicity on the (negative) animal data. This would result in no classification for developmental toxicity. Then again, some transplacental transfer of flocoumafen has been shown in the rat. In the rat this transplacental transfer is not high enough to induce developmental effects even at maternally toxic dose levels. However, as the rat model is not an exact model for humans it cannot be excluded that there is a possibility for induction of developmental effects in humans at exposure levels that are not severely maternally toxic. Given this uncertainty, it is proposed to classify flocoumafen as Repr. 2 – H361d (Regulation EC 1272/2008) and Repro Cat. 3 – R63 (Directive 67/548/EEC)."
It must be noted that "uncertainty" does not meet evidence requirements for classification, although uncertainty may be used to reduce concern.
In terms of evidence required for classification, there is no animal evidence on which to base classification of flocoumafen, in a study design which is demonstrated to be sensitive to the developmental effects of warfarin. Evidence is provided that pharmacokinetics provide an effective protection to the foetus from flocoumafen.
In a study of radiolabel distribution in pregnant rats and their fetuses, ^{14}C-flocoumafen and ^{14}Cwarfarin were administered at similar equimolar doses daily between days 6 and 19 of pregnancy.
Rats were sacrificed on day 19 of pregnancy at the maternal plasma level maximum (T_{max}); and

radiolabel distribution into blood and liver from dams, and liver, blood and placenta from fetuses, was measured. Metabolites were identified. In interpreting the results, distribution into liver is considered particularly meaningful since both chemical bind to liver; while plasma values are more variable with time and the T_{max} may vary between parent and foetus. On the basis of liver concentrations, the concentration of flocoumafen attained in the liver of pups after 10 days exposure during pregnancy was 22-fold lower than was attained with warfarin. When metabolism was taken into account (flocoumafen was seen to be metabolically degraded, warfarin not) flocoumafen residues in foetal liver were 38-fold lower than with warfarin. The difference may be due to a placental barrier effect although it would appear more likely that the maternal liver is more effectively binding flocoumafen than warfarin, such that less flocoumafen is distributed to the foetus. The study was conducted to GLP and although there is no specific guideline, appears scientifically rigorous.

Pharmacokinetic protection is seldom absolute (there is almost always some exposure), so a judgment is necessary as to how effective the protection might be. For flocoumafen the pharmacokinetic difference appears entirely effective in protecting the rat foetus, as judged by the absence of foetotoxicity at dose levels that are severely toxic to the dam. These factors show flocoumafen to be intrinsically different to warfarin, such that no classification for developmental toxicity is appropriate.

2. Relevance of the CEFIC teratogenicity study of warfarin[1]

The study is reviewed in the CLH proposal for warfarin, and for that reason a detailed description is not given here. The following observations are however offered:

The study carefully examines dose levels around the limit of maternal toxicity. This is important, since the dose-response curve for teratogenicity can be steep (Schardein, 2000[2]). This might be particularly so with the AVKs, since the dose-response for maternal toxicity is also particularly steep. The study also examines two different periods of exposure: days 6-15 of pregnancy ("TP1", corresponding to the pre-2001 OECD 414 guideline) and days 6-19 of pregnancy ("TP2", corresponding to the revised 2001 OECD 414 guideline).

The warfarin study provides clear evidence (for classification purposes) of specific foetal sensitivity to haemorrhage (i.e., foetal haemorrhage is a dose-related finding, and found at the lowest dose level which was not maternally toxic). Both exposure periods (10- and 14-day) were adequate to demonstrate foetotoxicity. In the opinion of this reviewer, the study also showed: borderline evidence of an increase in small foetuses (10-day treatment group only) in the absence of maternal toxicity; and adequate evidence of malformation (cataract). Although this study examines dose levels very closely spaced in the maternally toxic range, the incidence of foetal haemorrhage at the low dose is clear demonstration of ability of the standard "OECD 414" design to detect specific foetal sensitivity to warfarin and the AVKs.

For flocoumafen, at least one teratogenicity study in rats examines developmental toxicity at a clearly maternally toxic dose based on mortality; further adequate studies in rabbit also demonstrate absence of developmental toxicity. There was no evidence of foetotoxicity, in studies closely comparable in design to the effective study of warfarin.

4. Comparison with Criteria

The CLH report for flocoumafen contains a comparison with criteria, and notes that the absence of teratogenicity seen with flocoumafen, and placental transfer data, give reason to base a classification on the (negative) animal data. However, the report also states that the placental barrier is not absolute (transfer is diminished, not prevented) and the rat model is not an exact model for humans; hence there remains a possibility for developmental effects in humans. The comparison does not discuss the significance of foetotoxicity as seen in the warfarin study and in humans.

Classification is, however, based on evidence. Absence of data is accepted as reason not to classify; it is therefore inappropriate to propose classification on the basis of data showing a clear absence of effect.

Based on evidence, a detailed comparison with criteria is therefore offered as follows:

In comparison to the criteria for DSD Cat 1/ CLP Cat 1A:

There is no epidemiological evidence that flocoumafen causes developmental toxicity in humans.

There is clear epidemiologic evidence that warfarin causes developmental toxicity in humans; and that other AVK anticoagulants used as therapeutics also cause developmental toxicity in humans.

However, the criterion for "sufficient epidemiologic evidence" is not met for flocoumafen.

Because the criterion for "sufficient epidemiologic evidence" is not met for flocoumafen, classification into DSD Cat 1/ GHS Cat 1A is not appropriate.

In comparison to the criteria for DSD Cat 2/CLP Cat 1B:

There is no evidence that flocoumafen causes developmental toxicity in animal studies.

There is a *concern*, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans.
However, there is *evidence* that flocoumafen is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies of flocoumafen in both rats and rabbits, and the demonstration of an effective pharmacokinetic barrier. The method used to test flocoumafen is appropriate and sufficient to detect developmental toxicity of warfarin.
Negative results in adequate animal studies of flocoumafen are meaningful, and placement in DSD Category 2/ CLP Category 1B is not appropriate.
In comparison to the criteria for DSD Cat 3/ CLP Cat 2:
There is no evidence that flocoumafen causes developmental toxicity in animal studies.
There is a *concern*, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans.
However, there is *evidence* that flocoumafen is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits, and the demonstration of an effective pharmacokinetic barrier to flocoumafen. The method used to test flocoumafen is appropriate and sufficient to detect developmental toxicity of warfarin.
Negative results in adequate animal studies of flocoumafen are meaningful.
Concern is reduced in that warfarin as a therapeutic is administered to humans orally; biocidal exposure to rodenticides is dermal; and the skin presents a considerable and effective barrier to the AVK rodenticides.
Placement in DSD Category 3/ CLP Category 2 is not appropriate. No classification for developmental toxicity is appropriate.

Conclusion
When compared with the criteria for classification, there is inadequate evidence for any classification of flocoumafen for developmental toxicity.

Simon Warren *DABT DIBT DipRCPath*
18 April 2013

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[1] Kubaszky R (2009) Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
[2] Schardein J (2000) Chemically induced birth defects. Third edition revised and expanded. Marcel Dekker: New York. ISBN: 0-8247-0265-4

--- End of attachment ---

Dossier Submitter's Response

See reponse to comment number 3

RAC's response

Health Hazards –

Thank you for comments.

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification has been provided jointy under Comment number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across approach defined in section 1.5 of Annex XI of REACH Regulation.

Date	Country	Organisation	Type of Organisation	Comment number

19.04.2013	Sweden		MemberState	5
Comment received				

(*ECHA note: The comment below has been submitted as a separate attachment)*

The Swedish CA does not support the classification proposal for flocoumafen regarding reproductive toxicity. We propose that the classification for flocoumafen (as well as for the other AVK rodenticides) should be based on read across to human data for Warfarin (i.e warfarin embryopathy). Therefore, flocoumafen should be classified in regards to its developmental toxicity as a reproductive toxicant in category 1A.

The AVK rodenticides and warfarin share a common mechanism of action, i.e they inhibit the recycling of vitamin K by inhibiting vitamin K epoxide reductase. As a consequence of this, the post-translational carboxylation of coagulation proteins is affected and an increase in coagulation time is observed.

Warfarin is a well-known human teratogen and the syndrome caused by exposure during early pregnancy is usually referred to as warfarine embryopathy (nasal hypoplasia, stippled epiphysis and distal digital hypoplasia[1]). The presumed mechanism for these effects is similar to the pharmacological/toxicological MoA for effects on coagulation proteins i.e. inhibition of post-translational carboxylation but in this case it is the carboxylation of matrix-gla protein (MGP) in embryonic bone and cartilage extracellular matrix that is affected. Exposure during the second and third trimesters is mainly associated with anatomical abnormalities of CNS that are thought to be secondary to hemorrhages.

No similar effects on bone formation were observed at fetal examination in studies performed according to OECD TG 414 (new and old version) on warfarin or any other AVK rodenticide. However, as shown by Howe and Webster[2] nasal hypoplasia can indeed be induced in rats, if the pups are dosed postnatally with warfarin. This indicates that the study design of the OECD 414 is not appropriate to detect nasal hypoplasia. Consequently, a possible effect on bone formation process by the six rodenticides has not been properly assessed. The absence of bleedings in the fetuses from OECD TG 414 studies from the AVK rodenticide group (with the exception of warfarin) should thus not be used as an argument to indicate that effect on bone formation process is unlikely. Instead, the absence of reported bleedings in the fetuses treated with the six AVK inhibitors could just as well indicate that it is a very narrow margin between the effect dose for the conceptus and the maternally lethal dose. Interestingly, a case report found in the open literature also supports that larger 2nd generation molecules such as brodifacoum (Mw 523) can cross the placenta and cause bleedings and mortalities in dog neonates seemingly without effect on the mother[3]. Some differences in placental transfer and potency are observed in the available data but not to an extent that the relevance of the proposed mechanism behind the warfarine syndrome to humans can be rejected as not being applicable for these AVK rodenticides. In addition, there are no obvious differences in the mammalian toxicity within the AVK rodenticide group to suggest that any of the substances are to be classified differently than the others (see table 1). Chlorophacinone is larger than warfarin when ranked according to molecular weight but is smaller than brodifacoum. Chlorophacinone fits into the overall toxicity pattern of the AVK rodenticides (see table 1). The absence of bleedings in the pups compared to warfarin is not unique to chlorophacinone and cannot explain the absence of nasal hypoplasia in the rats. The difference in placental transfer and lower availability in fetuses of flocoumafen is also not a sufficient reason not to read-across to the human data for warfarin, since it does not suggest that the proposed mechanism behind the warfarin data is irrelevant. In addition it does not suggest that the inherent overall mammalian toxicity of flocoumafen differ from the other AVK rodenticides.

In summary, annex 1, point 1.1.1.3 of the CLP regulation supports a weight of evidence

evaluation and the available data shows that the physicochemical properties and the mammalian toxicity profile of all the 2nd generation AVK rodenticides is very similar and this supports read across to the animal data for warfarin and also a read across to the human evidence for teratogenicity of warfarin (table 1). Thus classification regarding developmental toxicity of all AVK rodenticides (including brodifacoum, chlorophacionone and flocoumafen) as reproductive toxicants in category 1A is warranted.

1. Pauli, R.M. (1997). Anticoagulants. In: Drug Toxicity in embryonic development II (Editors R.J. Kavlock and G.P. Daston), Springer-Verlag, Berlin. p 191 – 229.
2. Howe, A.M. and Webster, W.S. (1992): The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology. Oct;46(4):379-90.
3. Munday, J.S. and Thompson, L.J. (2003). Brodifacoum toxicosis in two neonatal puppies. Vet. Pathol. 40:216-219

(*ECHA note: Table 1 is provided as a separate attachment to this comments table)*

Dossier Submitter's Response

We agree that for classification of flocoumafen the available dataset for warfarin (due to the same mode of action) should be taken into account, however also the data available for flocoumafen itself should be included in the weight of evidence approach.

We agree that the other coumarines will be able to inhibit vitamin k epoxide reductase in fetusses in the same way as warfarine. However, not only the mechanism should be taken into account for read-across but also pharmacokinetic differences or similarities as described in the RCOM to comment 3 and in the ECHA guidance on read-across (http://echa.europa.eu/en/support/grouping-of-substances-and-read-across).

It is clear that the OECD 414 study is not appropriate to detect nasal hypoplasia, since the development of the nasal cartilage in rats occurs after birth. Since the mode of action of warfarin and flocoumafen is the same, it might be expected that flocoumafen will also cause nasal hypoplasia in rats, when they are exposed in the right time-window. However, in humans, the development of the nasal cartilage occurs in the 3rd trimester of the pregnancy and therefore, placental transfer is relevant for this effect. The OECD study is capable of determining (some) developmental effects relevant for coumarines and should therefore be taken into account in a weight of evidence evaluation. It would be good to know what is the most sensitive effect in humans (nasal hypoplasia versus reduced coagulation) taking into account differences in the sensitive period but probably the data are too limited for such a conclusion. The study on placental transfer in rats shows that, due to a high 1st pass effect, the amount of flocoumafen that reaches the pup is very low (and lower than with warfarin). This might explain why no haemorrhages are observed in the developmental studies with flocoumafen and why such effects *are* observed with warfarin. It could be argued that, due to the 1st pass effect, concentrations reaching the fetus will also be too low to induce nasal hypoplasia. Because this raises doubt on the relevance of the warfarin data for the effects that flocoumafen can cause in humans, the evidence is not sufficiently convincing to classify flocoumafen as Repr. Cat 1 and Repr. Cat 2 is considered a more appropriate classification.

RAC's response

Health Hazards –

Thank you for comments.

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification has been provided jointy under Comment

number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across approach defined in section 1.5 of Annex XI of REACH Regulation.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Germany		MemberState	6

Comment received

Proposal:
Read-across from warfarin with Repr. Cat.1; R61 / Repr. 1A H360D to chlorophacinone and all the 2nd generation rodenticide anticoagulants should be applied.

Justification:
Read-across was agreed in the Specialised Expert Group (September 2006 Commission Doc ECBI/121/06).
The study of Johnson (2009) shows that flocoumafen and warfarin are able to pass the placenta and reached the fetuses. Thus, these AVKs have direct anticoagulant activity to the fetus and can affect proteins levels dependent on Vitamin K levels and related to organogenesis.
Since the treated dams did not show any adverse clinical signs during the exposure period, it remains unclear whether the concentration in the fetus was sufficient to induce any effect, i.e. hemorrhage and or malformations were not detected.
As there is no evidence for differences in the capacity to pass the placenta and mechanisms of action in inhibiting Vitamin K epoxide reductase (VKOR) we suggest to regard flocoumafen as a human teratogen and to classify it accordingly with Repr. Cat.1; R61 / Repr. 1A H360D.

Dossier Submitter's Response

Although the dams in the placental transfer study did not show adverse clinical signs,dams in the developmental studies with flocoumafen did (as wel as internal hemorage at necropsy). Therefore the dose used was high enough to induce effects in the mother, while no effects in the fetus were observed. The placental transfer study shows that the concentration flocoumafen that reaches the pup is much lower when compared to warfarin, due to the high 1st pass effect in the dams, which might explain the different outcome of developmental studies with flocoumafen and warfarin. Therefore, we think the evidence from studies with warfarin is not sufficiently convincing to classify flocoumafen as Repr. Cat 1 and Repr. Cat 2 is a more appropriate classification.

RAC's response

Health Hazards –

Thank you for comments.

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification has been provided jointy under Comment number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR

molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across approach defined in section 1.5 of Annex XI of REACH Regulation.

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Germany	BASF	Company-Manufacturer	7

Comment received

BASF refutes the proposal to read-across the classification of warfarin to all other anticoagulant rodenticides and provides specific details as to why flocoumafen should not be classified on the basis of available data.

*(ECHA note: The attachment provided "*Teratogenicity of AVK Rodenticides - Classification by Read-Across from Warfarin is not Correct*" is copied under the Comment number 3. The second attachment "Flocoumafen (CAS 90035-08-8) BASF Comments on the CLH proposal, March 2013" is being provided as a separate document to this table)*

Dossier Submitter's Response

See response to comment 3.

RAC's response

Health Hazards –

Thank you for comments.

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification has been provided jointy under Comment number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across approach defined in section 1.5 of Annex XI of REACH Regulation.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	8

Comment received

5.10.2 Developmental toxicity, page 55
Flocoumafen is part of the same group of chemicals, as warfarin. Flocoumafen has also the same mode of action as warfarin, which is a well documented human teratogen classified as a reproductive toxicant (Repr. Cat1; R61 – Repr. 1A H360D). Warfarin has been shown to cause teratogenicity in humans and in experimental animals. Based on analogy consideration to warfarin classification of Flocoumafen for developmental toxicity, is relevant unlike dog and rabbit.
The study on placental transfer was conducted on rats, Rats were not considered to the most relevant species for this type of study. Further, none data allow to extrapolate the rat's placental transfer to the human's transfer.
Furthermore, reproductive toxicity studies have been performed on difenacoum. No adverse effect were observed on the offspring, however Difenacoun belongs to AVK, is classified

Repr. Cat1; R61 – Repr. 1A H360D, based on read-across from warfarin.

Therefore, Flocoumafen should be classified Repr. Cat1; R61 – Repr. 1A H360D instead of Repr. Cat 3; R63- Repr 2 H361d.
SCL for reprotoxicity should be harmonized with warfarin.

Dossier Submitter's Response

We agree that warfarin and flocoumafen have the same mode of action and that flocoumafen also may cause haemorrhages in offspring at high doses. However, the study on placental transfer on rats shows that, due to a high 1st pass effect, the amount of flocoumafen that reaches the pup is very low (and lower than with warfarin). This might explain why no embryotoxicity is observed in the developmental studies with flocoumafen and why such effects are observed with warfarin. (It should be noted that the size of the 1st pass effect and placental transfer of difenacoum is not known). It seems likely that embryotoxicity will only be induced by high doses of flocoumafen that will also result in extreme toxicity (hemorages) in the dams. This raises doubt on the relevance of the effects for humans. In conclusion, because flocoumafen and warfarin share the mode of action and both substances can pass the placenta, the warfarin data (both human and animal data) provide some evidence that flocoumafen might also induce embryotoxicity. However, due to the difference in 1st pass effect and the negative developmental studies of flocoumafen, the evidence is not sufficiently convincing to classify flocoumafen as Repr. Cat 1 and Repr. Cat 2 is a more appropriate classification.

RAC's response

Health Hazards –

Thank you for comments.

Regarding classification for developmental toxicity please see above because response regaring developmental toxicity classification has been provided jointy under Comment number 1. In addition please note that due to considerable differences in the chemical structures between Warfarin and other AVKs , particularly Flocoumafen, linked with the presence of second type of functional group, the dynamics of interaction with VKOR molecule is different for these substances . This is evidenced by the differences in potency of acute and repeated toxicity as well as in differences in toxicokinetics and metabolism. For these reason the AVK rodenticides do not meet criteria for grouping and read-across approach defined in section 1.5 of Annex XI of REACH Regulation.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	9

Comment received

The method to determine the SCLs for acute and chronic toxicity should be harmonised with other anticoagulant rodenticides. Difenacoum approach to set SCLs could be used.

Dossier Submitter's Response

We agree that we should aim for a harmonised method for SCLs for acute and repeated dose toxicity of all anticoagulant rodenticides.

RAC's response

Thank you for comments. SCLs for acute toxicity is not applicable under CLP.

SCLs derivation for STOT RE for various AVKs has be harmonised based on the Guidance on

the Application of the CLP Criteria.

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	10
Comment received				
Under REACH ref. annex XI, 7.15, it is reported solubility of flocoumafen in organic solvent instead of stability of flocoumafen in organic solvent.				
Dossier Submitter's Response				
You are correct. In the CAR, the stability of flocoumafen in organic solvents is stated as 'not relevant'.				
RAC's response				
Thank you for comment.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Finland		MemberState	11
Comment received				
We support the proposed classification according to Regulation EC 1272/2008 and 286/2011: Aquatic Acute 1; H400, M-factor of 10, Aquatic Chronic 1; H410, M-factor 10 for flocoumafen.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				

ATTACHMENTS RECEIVED:

1. **Teratogenicity of AVK Rodenticides - Classification by Read-Across from Warfarin is not Correct** (Filename: Read-across rebuttal EWC0008), submitted on 19.04.2013 by
   Exponent International on behalf of CEFIC RDDG and BASF. (*ECHA note: This attachment has been copied under the section Toxicity to Reproduction)*

2. **Flocoumafen (CAS 90035-08-8) BASF Comments on the CLH proposal, March 2013** (Filename: 2013_1125920_BASF_Flocoumafen_comments on CLH proposal), submitted on 19.04.2013 by BASF

3. **Flocoumafen - Comment on the CLH proposal, 5 March 2013** (Filename: Flocoumafen classification - developmental EWC0009), submitted on 19.04.2013 by Exponent International on behalf of CEFIC RDDG *(ECHA note: This attachment has been copied under the section Toxicity to Reproduction)*

4. **Comments on Annex XV dossiers proposing harmonised Classification & Labelling** (Filename: COM_CLH_PC_Flocoumafen_SE), submitted on 19.04.2013 by Sweden (*ECHA note: This attachment has been copied under Toxicity to Reproduction, with the exception of Table 1. Physicochemical properties and mammalian toxicity summarized from the hydroxyl coumarin AVK dossiers, substances organized according to molecular weight)*