**Committee for Risk Assessment**

**RAC**

Annex 2

# Response to comments document (RCOM)

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

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

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

CLH-O-0000003395-72-02/F

**Adopted**
**14 March 2014**

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**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON BRODIFACOUM (ISO); 4-HYDROXY-3-(3-(4**

**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: brodifacoum (ISO); 4-hydroxy-3-(3-(4'-bromo-4-biphenylyl)-1,2,3,4-tetrahydro-1-naphthyl)coumarin**
**CAS number: 56073-10-0**
**EC number: 259-980-5**
**Dossier submitter: Italy**

**GENERAL COMMENTS**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Italy | Activa srl | Company-Manufacturer | 1 |
| Comment received | | | | |
| we support the documents uploaded | | | | |
| Dossier Submitter's Response | | | | |
| IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.<br><br>In particular IT considers that<br><br>- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome<br>- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.<br>- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.<br>IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.<br><br>In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied. | | | | |
| RAC's response | | | | |
| RAC supports the view of the dossier submitter. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| | | | | number |
| 18.04.2013 | France | | MemberState | 2 |
| Comment received | | | | |
| We agree with the classification proposal except for reproduction.<br>Brodifacoum should be classified Repr. Cat1; R61 (DSD) – Repr. 1A H360D (CLP).<br>The categorisation of skin sensitisation should be specified. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you for the support for the classification proposal.<br>For the classification proposal for reproductive toxicity and the categorisation of skin sensitisation, see below. | | | | |
| RAC's response | | | | |
| Repr 1A is supported by RAC. However, in the opinion of RAC, the data on sensitisation is not sufficiently robust to allow classification for sensitisation. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Denmark | | MemberState | 3 |
| Comment received | | | | |
| Danish CA comments to the CLP report on brodifacoum:<br><br>Denmark agrees with the classifications proposed by the Italian rapporteur for the end-points of acute and repeated dose toxicity as well as for aquatic toxicity.<br><br>With respect to the end-point of developmental toxicity, Denmark supports the arguments of read across to the known human teratogen warfarin, and the recognition that the conventional OECD guideline 414 has limitations in the detection of possible teratogenic effects of coumarin-related compounds.<br><br>However, Denmark's position is that brodifacoum, as all coumarin-derived anticoagulant should be classified as repro cat 1; R61 (DSD)/Repro cat 1A; H 360D (CLP), as the information on which classification of brodifacoum is based match the criteria for this category is "largely based on evidence from humans".<br><br>Denmark supports the proposed specific concentration limits for acute and repeated dose toxicity both in relation to directive 67/458/EC and, for repeated dose toxicity, in relation to CLP regulation 1272/2008. Also, 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 classification proposal.<br>For the classification proposal for reproductive toxicity, see below. | | | | |
| RAC's response | | | | |
| The support is noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Switzerland | Syngenta Crop Protection AG | Company-Manufacturer | 4 |
| Comment received | | | | |
| The 2006 Specialised Experts conclusion was based on a conservative read across from warfarin. There are now new data which show a clear difference between warfarin and the | | | | |

other AVKs/brodifacoum, invalidating this read across and providing reassurance for a lack of any developmental effects for the AVKs/brodifacoum.

*(ECHA note: The text below was provide as a separate attachement)*

Exponent Doc ID 1109091.uk0 EWC0009
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**Brodifacoum**
**Comment on the CLH proposal, 5 March 2013**
**Developmental toxicity:**

Brodifacoum should ***not be classified*** for developmental toxicity.
Careful comparison of the developmental toxicity data for brodifacoum against the classification criteria show:
- Criteria for classification for developmental toxicity are not met.
o There is no evidence of brodifacoum being causally associated with developmental toxicity in humans.
o There is no evidence from acceptable GLP- and guideline-compliant studies, that brodifacoum causes an adverse effect on development in animals.
o The rat study design using OECD guideline 414 has been demonstrated to be sensitive to warfarin-induced foetotoxicity and teratogenicity, thus removing the 'uncertainties' in the CLH report leading to a conservative read-across from warfarin.
- No classification for developmental toxicity is therefore appropriate.

**Reasoning**

**1. Relevance of the 2006 Specialised Experts Conclusion[1]**

The 2013 CLH proposal to classify brodifacoum for developmental toxicity follows the 2006 Specialised Experts Conclusion (SE Conclusion). The SE Conclusion lacks a clear comparison of evidence with current (DSD or CLP) criteria. The conclusion was limited to teratogenicity when epidemiological data show foetotoxicity in human pregnancies to be of greater incidence and concern than teratogenicity. The conclusion relies on a now outdated assumption (uncertainty that the teratogenicity of warfarin can be detected in pre-natal developmental toxicity studies using OECD guideline 414); which is proven incorrect by a more recent OECD 414 study demonstrating developmental toxicity of warfarin. The SE Conclusion is therefore no longer effective.
More details are offered in Exponent's EWC0008.

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

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[3]). 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 this 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 brodifacoum, two teratogenicity studies in rats are available, of which at least one study examines developmental toxicity at a clearly maternally toxic dose based on mortality. No teratogenic effects or foetotoxicity were observed; 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.

**3. Comparison with CLP Criteria**

In the CLH report for brodifacoum, comparison with criteria is not presented. The CLH discusses (p49) 'limitations of the current protocol' without mention of the CEFIC study of warfarin, and states 'areas of uncertainties make it difficult to rule out the developmental toxicity'. The last statement clearly does not meet criteria (i.e. evidence) for classification.

Based on all the current evidence, including some new data (i.e, the CEFIC study of warfarin), a detailed comparison with criteria is therefore offered as follows:

DSD Category 1 CLP Category 1A

| DSD Category 1 | CLP Category 1A |
|---|---|
| **Substances *known* to cause development toxicity in humans.** (Sufficient evidence from human exposure). Placement in Category 1 is done on the basis of epidemiologic evidence. | **Chemicals known to be a human reproductive toxicant.** The placing of the substance in this category is largely based on evidence from humans. |

There is no epidemiological evidence that brodifacoum 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 brodifacoum.

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

| DSD Category 2 | CLP Category 1B |
|---|---|
| **Substances which *should be regarded* as if they cause developmental toxicity in humans.**<br><br>There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:<br><br>- clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects<br><br>- even when clear effects have been demonstrated in animal studies, the relevance for humans may be doubtful because of the doses administered, for example when effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist. For these or similar reasons it may be that classification in category 3, or even no classification, will be warranted. | ***Chemicals presumed to be a human reproductive toxicant***<br><br>The placing of substances in this category is largely based on evidence from experimental animals. Data from animal studies should provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate. |

There is no evidence that brodifacoum 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 brodifacoum is intrinsically different to warfarin, based on absence of both foetotoxicity and developmental toxicity in teratogenicity studies of brodifacoum in both rats and rabbits. The method used to test brodifacoum is appropriate and sufficient to detect developmental toxicity of warfarin.

Negative results in adequate animal studies of brodifacoum are meaningful, and placement in DSD Category 2/ CLP Category 1B is not appropriate.

| DSD Category 3 | CLP Category 2 |
|---|---|
| ***Substances which cause concern for humans owing to possible developmental toxic effects***<br><br>Generally on the basis of:<br><br>- results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary nonspecific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2,<br><br>- other relevant information. | ***Chemicals suspected of being human reproductive toxicants***<br><br>This category includes substances for which there is some evidence from humans or experimental animals - possibly supplemented with other information – of an adverse effect on sexual function and fertility, or on development, in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects, and where the evidence is not sufficiently convincing to place the substance in Category 1. For instance, deficiencies in the study may make the quality of evidence less convincing, and in view of this Category 2 be the more appropriate classification. |

There is no evidence that brodifacoum 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 brodifacoum is intrinsically different to warfarin, based on absence of both foetotoxicity and developmental toxicity in teratogenicity studies in both rats and rabbits. The method used to test brodifacoum is appropriate and sufficient to detect developmental toxicity of warfarin.
Negative results in adequate animal studies of brodifacoum are meaningful.
Placement in DSD Category 3/ CLP Category 2 is not appropriate. No classification for developmental toxicity is appropriate.

**Conclusion**

With the provision of the warfarin rat study, there is now sufficient evidence that the basis for a readacross of developmental toxicity classification from warfarin to brodifacoum is not valid.
When compared with the criteria for classification, there is clear and adequate evidence that brodifacoum should not be classified for developmental toxicity.
Simon Warren *DABT DIBT DipRCPath*
18 April 2013

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1 ECBI/121/06, 20 September 2006. ECB, Ispra.
2 Kubaszky R (2009) Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
3 Schardein J (2000) Chemically induced birth defects. Third edition revised and expanded. Marcel Dekker: New York. ISBN: 0-8247-0265-4

## 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 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 p[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.rospective study. Thromb.Haemost. 95(6) 949-57.

[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

Dossier Submitter's Response

IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.

In particular IT considers that

- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome
- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.
- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.

IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.

In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied.

RAC's response

The issues raised by Syngenta have been thoroughly discussed in the opinion, and it is noted that for brodifacoum there are 2 human cases supporting that brodifacoum, just like warfarin, may exert developmental toxicity by the same mode of action. Repr 1A is supported by RAC.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Sweden | | MemberState | 5 |
| Comment received | | | | |
| Comments have been submitted in the attachment. | | | | |

*ECHA note: The comment below has been submitted as a separate attachement*
***Reproductive toxicity*** :
The Swedish CA does not support the classification proposal for brodifacoum regarding reproductive toxicity. We propose that the classification for brodifacoum (as well as for the other AVK rodenticides) should be based on read across to human data for Warfarin (i.e warfarin embryopathy). Therefore, brodifacoum 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

IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.

In particular IT considers that

- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome
- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.
- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.
IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.

In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied.

RAC's response

The support for Repr 1A is noted.

**TOXICITY TO REPRODUCTION**

| Date | Country | Organisation | Type of Organisation | Comment |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| | | | | number |
| 19.04.2013 | United Kingdom | PelGar International Limited - on behalf of the Activa/PelGar Brodifacoum and Difenacoum Task Force | Company-Manufacturer | 6 |
| Comment received | | | | |
| The attached paper was developed by Syngenta and shared with the Activa/PelGar Brodifacoum and Difenacoum Task Force. The Task Force members (Activa S.r.l. and PelGar International Limited) are in full agreement with the conclusions of the paper.<br><br>*ECHA note: the attachment is provided as a separate document* | | | | |
| Dossier Submitter's Response | | | | |
| IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.<br><br>In particular IT considers that<br><br>- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome<br>- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.<br>- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.<br>IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.<br><br>In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied. | | | | |
| RAC's response | | | | |
| The issues raised by Syngenta have been thoroughly discussed in the opinion, and it is noted that for brodifacoum there are 2 human cases supporting that brodifacoum, just like warfarin, may exert developmental toxicity by the same mode of action. Repr 1A is supported by RAC. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | United Kingdom | PelGar International Limited | Company-Manufacturer | 7 |
| Comment received | | | | |
| We strongly agree with the position in the attached papers. | | | | |

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|---|
| Dossier Submitter's Response |
| IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.<br><br>In particular IT considers that<br><br>- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome<br>- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.<br>- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.<br>IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.<br><br>In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied. |
| RAC's response |
| The issues raised by Syngenta have been thoroughly discussed in the opinion, and it is noted that for brodifacoum there are 2 human cases supporting that brodifacoum, just like warfarin, may exert developmental toxicity by the same mode of action. Repr 1A is supported by RAC. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 8 |
| Comment received | | | | |
| We support the read-across for the developmental toxicity based on the structural similarity and the same mode of action (vitamin K deficiency).<br><br>Based on the read-across, we support a classification Repr. Cat. 1A as the warfarin.<br><br>Editorial comments: No references are mentioned in the table 4.11.1 Pg 50 (the developmental toxicity), the lines 1 and 3. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you for the support for the classification proposal.<br>Agreed for Editorial comments. | | | | |
| RAC's response | | | | |

| |
|---|
| The support for Repr 1A is noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | United Kingdom | Exponent International, on behalf of CEFIC RDDG | Industry or trade association | 9 |

Comment received

Section 4.11 Toxicity for reproduction:
Brodifacoum should not be classified for developmental toxicity. Data are conclusive, and not sufficient for classification. Please see attached document (Exponent docID 1109091.uk0 EWC0009; Brodifacoum)

Dossier Submitter's Response

IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.

In particular IT considers that

- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome
- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.
- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.
IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.

In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied.

RAC's response

The issues raised in the comments have been thoroughly discussed in the opinion, and it is noted that for brodifacoum there are 2 human cases supporting that brodifacoum, just like warfarin, may exert developmental toxicity by the same mode of action. Repr 1A is supported by RAC.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Germany | | MemberState | 10 |

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). Up today there is no evidence for differences between the vitamin K antagonists in the capacity to pass the placenta and the mechanisms of action in inhibiting vitamin K epoxide reductase. Thus, warfarin, chlorophacinone and the 2nd generation rodenticide anticoagulants should be regarded as human teratogens.
Also in the light of the new study by Johnson, 2009, that demonstrated warfarin and flocoumafen to pass the placenta there is no evidence to assume for 2nd generation rodenticides including Brodifacoum different mechanism of action or different capacity to pass the placenta. It is not possible however to quantitatively extrapolate data on foetal exposure between the AVK rodenticides. The study was evaluated by the Netherlands and is included in the CLH report on flocoumafen.

According to Read-across from warfarin, Brodifacoum should be classified as Repr. Cat. 1; R61 / Repr. 1A H360D

Reference:
1. Johnson, TL (2009. A placental transfer study of warfarin and flocoumafen in rats. Confidential report of BASF: report no. 2009/7000085, dated 16 July 2009. Study).

| Dossier Submitter's Response |
|---|
| Agreed. Thank you for your comment. |
| RAC's response |
| The support for Repr 1A is noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 11 |
| Comment received | | | | |
| SCL for reprotoxicity should be harmonized with warfarin. | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. | | | | |
| RAC's response | | | | |
| The SCL for warfarin has been calculated in two different ways, using animal or human data, but in both cases leading to the same result (SCL=0.003%). The RAC is proposing to use the same SCL for the developmental toxicity of all other AVK rodenticides. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | United Kingdom | Exponent International, on behalf of CEFIC RDDG | Industry or trade association | 12 |

| Comment received |
|---|
| Section 4.11, Toxicity for Reproduction: I disagree the proposal to classify brodifacoum for developmental toxicity. |
| Dossier Submitter's Response |
| IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.<br><br>In particular IT considers that<br><br>- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic syndrome<br>- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.<br>- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.<br>IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.<br><br>In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied. |
| RAC's response |
| The RAC notes this disagreement, but support classification with Repr. 1A. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | United Kingdom | Syngenta Crop Protection AG | Company-Manufacturer | 13 |
| Comment received | | | | |
| The previously proposed read across from warfarin is no longer valid, based on the recent rat warfarin study. Classification of the AVKs/brodifacoum should be based on the negative rodent studies. Therefore there should be no classification for developmental toxicity. | | | | |
| Dossier Submitter's Response | | | | |
| IT gratefully acknowledges the comments received on the classification of Brodifacoum for developmental toxicity.<br><br>In particular IT considers that<br><br>- Warfarin is a recognized human teratogen, producing a distinct dysmoprhogenetic | | | | |

syndrome
- sufficient evidence indicate that the conventional OECD guideline 414 in rodents has limitations in detecting the possible teratogenic effects of coumarin-related anticoagulant rodenticides: thus, a "negative" result in OECD 414 cannot rule out that these compounds may represent a developmental hazard in humans.
- There is no evidence for qualitative differences between warfarin and the 2nd generation vitamin K antagonists, concerning (i) the capacity to pass the placenta leading to exposure of the conceptus and (ii) and the basiv mechanism of action, resulting in the inhibition of extra-hepatic vitamin K epoxide reductase, and consequently impaired post-translational carboxylation of proteins critical for coagulation or (in the conceptus) bone/cartilage matrix. Such biochemical mechanism involved in the adverse effects of warfarin in human pregnancy, occurring both upon exposure during the first trimester (mainly skeletal defects) and the second-thrd trimester (mainly oculo-cerebral) of pregnancy.
IT recognizes that quantitative differences may exist, e.g., as regards placental transfer or potency between different coaguulants, but there is no evidence that such possible quantitative differences would lead to essentially different effects.

In conclusion, IT considers it prudent to apply read-across from warfarin to all the 2nd generation rodenticide anticoagulants as regards the classification for developmental toxicity. Accordingly, Repr. Cat.1; R61 / Repr. 1A H360D should be applied.

RAC's response

The Kubaszky study is discussed in the opinion, and it is clear that the face malformations that are characteristic for the warfarin embryopathy are not picked up in animal studies. In addition, there are two human cases showing that brodifacoum can be a developmental toxicant in humans. The RAC supports classification with Repr. 1A.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Norway | | MemberState | 14 |

Comment received

The Norwegian CA supports classifying brodifacoum for developmental effects.
We agree with the argumentation that no clear conclusions can be drawn from the performed teratogenicity studies because of the limitations of the conventional OECD 414 studies in detection of coumarin-specific developmental effects. Read across to the established human teratogen, warfarin, is supported as brodifacoum has the same chemical moiety as warfarin and the same mechanism of action responsible for the teratogenicity of warfarin.
Being based on human data, the classification of brodifacoum should rather be Repr. 1A; H360D (CLP)/ Repr. Cat. 1; R61 (DSD) than Repr. 1B; H360D (CLP)/Repr. Cat. 2; R61 – in agreement with what is proposed for warfarin (and other AVK rodenticides).
As potential developmental effects would be expected at very low doses, the possibility of setting specific concentration limits for reprotoxicity should be considered.

Dossier Submitter's Response

Agreed. See the IT response for the classification proposal for reproductive toxicity.

RAC's response

The support is noted. The SCL for warfarin has been calculated in two different ways, using animal or human data, but in both cases leading to the same result (SCL=0.003%). The RAC is proposing to use the same SCL for the developmental toxicity of all other AVK rodenticides.

**OTHER HAZARDS AND ENDPOINTS – Acute Toxicity**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 15 |
| Comment received | | | | |
| We support the classification Acute Toxicity Cat. 1 based on the following results<br>- Oral route:LD50 < 5mg/kg,<br>- Inhalation route LC50 : ≤ 0.05mg/L<br>- Dermal route LD50: ≤ 50mg/kg<br>Based on these findings, the criteria for Acute Toxicity Cat1 are fulfilled for each route. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you for your support. | | | | |
| RAC's response | | | | |
| The support is noted, and the RAC also agrees with the proposed classification. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 16 |
| Comment received | | | | |
| Point 1.3, tables 3 and 4, page 9.<br>The approach for the setting of specific concentration limits (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 | | | | |
| Agreed. Thank you for your comment. | | | | |
| RAC's response | | | | |
| The SCL proposed by RAC for STOT RE is set based on the animal data for brodifacoum. Regarding a SCL for developmental toxicity, a SCL for warfarin has been calculated in two different ways, using animal or human data, but in both cases leading to the same result (SCL=0.003%). The RAC is proposing to use the same SCL for the developmental toxicity of all other AVK rodenticides. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Skin Hazard**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 17 |
| Comment received | | | | |
| Point 4.4.1, page 34.<br>The species tested in skin irritation studies should be consistent between table 12 and the summary. | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. Thank you for your comment. | | | | |
| RAC's response | | | | |
| Noted | | | | |

## OTHER HAZARDS AND ENDPOINTS – Eye Hazard

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 18 |
| Comment received | | | | |
| Point 4.4.2, page 35.<br>The species tested in eye irritation studies should be consistent between table 13 and the summary. | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. Thank you for your comment. | | | | |
| RAC's response | | | | |
| Noted | | | | |

## OTHER HAZARDS AND ENDPOINTS – Skin Senzitation Hazard

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 19 |
| Comment received | | | | |
| We support Skin Sensitisation 1 H317 based on the skin sensitization test (a guinea pig test with a percentage response to 39%). | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. Thank you for your support. | | | | |
| RAC's response | | | | |
| The reaction was 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 contradict a sensitisation potential. Although brodifacoum was weakly positive, but only at one concentration in the Buehler test, a weight of evidence assessment do not support classification for sensitisation. Thus, the RAC is of the view that there is not sufficient evidence to support a classification for sensitisation for brodifacoum. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 20 |
| Comment received | | | | |
| Point 4.6.1.5, page 38.<br>The categorisation of skin sensitisation according to Regulation EC 1272/2008 should be specified. Brodifacoum should be classified Skin Sens. Cat.1B; H 317. | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. Thank you for your comment. | | | | |
| RAC's response | | | | |
| The reaction in the Buehler test was 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 contradict a sensitisation potential. Although brodifacoum was weakly positive, but only at one concentration in the Buehler | | | | |

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| test, a weight of evidence assessment do not support classification for sensitisation. Thus, the RAC is of the view that there is not sufficient evidence to support a classification for sensitisation for brodifacoum. |

**OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 21 |
| Comment received | | | | |
| The findings of the repeat dose toxicity test (by oral route, NOAEL between 1 and 40 µg/kg) warrant the classification STOT RE 1. We agree with the extrapolation of oral toxicity data to dermal and inhalation toxicity due to the acute toxicities after oral and dermal, and oral and inhalation, comparable indicating comparable absorptions. Then we support the proposed classification for STOT RE 1 H 372. | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. Thank you for your support. | | | | |
| RAC's response | | | | |
| The support is noted. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 22 |
| Comment received | | | | |
| We support the proposed M-factor for acute toxicity of 10 (most sensitive species Algae Pseudokirchnierella subcapitata : 72hErC50=0.04 mg/l) and toxicity band between 0.01 mg/l and 0.1 mg/l), as well as with the proposed SCLs :<br>N, R50/53 C≥2.5%<br>N, R51/53 0.25%≤C<2.5%<br>R52/53 0.025%%≤C<0.25%<br><br>Based on the most stringent outcome for Aquatic Chronic toxicity (on the basis of the Algae NOEC and the LC50 for the other trophic levels) an M-factor for chronic toxicity of 10 could be assigned . | | | | |
| Dossier Submitter's Response | | | | |
| Agreed. Thank you for your comment. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 23 |
| Comment received | | | | |
| We agree with the current proposal for consideration by rac:<br><br>CLP regulation:<br>Aquatic acute 1 (M factor =10);<br>Aquatic chronic 1 ;<br>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.

Nevertheless we have several comments:
In section 5.6, only specific limit concentrations and M factor are presented. It should be clearer to add the risk sentences for both regulation DSD and CLP?
Moreover, in this same section, the M factor is given for the acute classification. You also propose a M factor of 10 for chronic classification in section 1.2 table 2. So, could you give more detail about this M factor for chronic effects?

In table 23 section 5.4, could you please add the unit (mg/L) used to express the toxicity for fish?

Dossier Submitter's Response

Agreed. Thank you for your comment. For M factor for chronic effects, see below.

RAC's response

Noted and agreed, however a comparison of brodifacoum data with criteria for environmental hazards should have been clearly presented in the CLH report. In the conclusions on classification and labeling section a better explanation about the key endpoints used for the proposal should have been included.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Finland | | MemberState | 24 |

Comment received

We support the proposed classification: Aquatic Acute 1; H400, M-factor of 10 and Aquatic Chronic 1; H410, M-factor of 10. However, the comparison of brodifacoum data with criteria for environmental hazards should be clearly presented in the CLH report (Section 5.5.).

Degradation and bioaccumulation potential:

We agree with the conclusions that brodifacoum is not rapidly degradable and that it fulfills the criteria for bioaccumulation potential based on log Kow.

Aquatic toxicity:

Since, there is only one NOEC value available, it should be clarified whether tiered approach according to 2nd ATP to CLP (EC) Regulation No 1272/2008 has been taken into account.

Dossier Submitter's Response

Agreed. Thank you for your comment. The tiered approach according to 2nd ATP to CLP (EC) Regulation No 1272/2008 has been taken into account for classification Aquatic Chronic 1; H410, M-factor of 10.

RAC's response

Noted and agreed, however a comparison of brodifacoum data with criteria for environmental hazards should have been clearly presented in the CLH report. In the conclusions on classification and labeling section a better explanation about the key endpoints used for the proposal should have been included.

**OTHER HAZARDS AND ENDPOINTS – Physical Hazards**

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON BRODIFACOUM (ISO); 4-HYDROXY-3-(3-(4**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 25 |
| Comment received | | | | |
| p 18, EC number:<br>The EC number is missing. Please add the EC number in the table 5.<br><br>p 25, Dissociation constant:<br>In the CLH report, values have been reported whereas in the combined AR "not applicable" is reported. Please clarify.<br><br>P26, Table 10:<br>The physico-chemical studies relevant for classification should be described in the table 10.<br><br>*ECHA note: Confidential part of the attachement will be provided as a separate document*. | | | | |
| Dossier Submitter's Response | | | | |
| p 18, Sorry for the oversight, the missing EC number will be included in table 5 as required.<br><br>p 25, Disagree. There is no inconsistency between the two documents. The information presented in the CLH report for the dissociation constant is just the same available in the combined AR.<br><br>P26, OK, it will be done.<br><br>*ECHA note: No updates of the text are possible at this stage.* | | | | |
| RAC's response | | | | |
| | | | | |

Non confidential Attachments:

1. **Brodifacoum - Comment on the CLH proposal, 5 March 2013** (File name: Brodifacoum classification - developmental EWC0009.pdf) submitted by:
   Syngenta Crop Protection AG on 19/04/2013,
   Activa srl on 19/04/2013,
   Exponent International, on behalf of CEFIC RDDG on 19/04/2013,
   PelGar International Limited on 19/04/2013

2. **Comments on Annex XV dossiers proposing harmonised Classification & Labelling** (File name: COM_CLH_PC_Brodifacoum_SE.docx ) submitted by:
   Sweden on 19/04/2013

3. **Teratogenicity of AVK Rodenticides - Classification by Read-Across from Warfarin is not Correct**(File name: Read-across rebuttal EWC0008.pdf ) submitted by:
   Syngenta Crop Protection AG on 19/04/2013,
   Activa srl on 19/04/2013,
   PelGar International Limited on 19/04/2013,
   Exponent International, on behalf of CEFIC RDDG on 19/04/2013

4. **BRODIFACOUM – POSITION ON CLASSIFICATION FOR DEVELOPMENTAL TOXICITY, 3 April 2013** (File name: Brodifacoum position on classification for developmental toxicity April 2013.docx) submitted by:

PelGar International Limited - on behalf of the Activa/PelGar Brodifacoum and Difenacoum Task Force on 19/04/2013,

Confidential Attachment:

1. **Classification and labelling of dangerous substances - French comments on Brodifacoum (CAS 56073-10-0)** submitted by:
   France on 18/04/2013