Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin

EC number: 259-978-4

CAS number: 56073-07-5

CLH-O-0000003392-78-03/F

Adopted

14 March 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON DIFENACOUM (ISO); 3-(3-BIPHENYL-4-YL-1,2,3,4-TETRAHYDRO-1- NAPHTHYL)-4-HYDROXYCOUMARIN

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: difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin
CAS number: 56073-07-5
EC number: 259-978-4
Dossier submitter: Finland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Denmark		MemberState	1
Comment received				
Danish comments to the CLP report on difenacoum Denmark agrees with the classifications proposed by the Finnish rapporteur for the end-points of acute and repeated dose toxicity for reproductive toxicity as well as for aquatic toxicity for difenacoum. With respect to classification for reproductive toxicity, toxicity for development, Denmark agrees with the proposed classification for difenacoum of Repr. Cat 1; R61 (DSD)/Repr. Cat 1 A; H360D (CLP). 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 mechanistic similarity with warfarin, which is a known human teratogen classified as Repr. Cat 1; R61 (DSD), recognising that OECD 414 guideline studies have limitations as to showing the teratogenic effects seen in humans of anticoagulant rodenticides. New data including a new study according to OECD TG 414 on warfarin showed some developmental effects in the rats, but it was not able to detect all warfarin human embryopathy effects, as the window of exposure seems to be very important, and differences in development of the neonate rat and human, would require dosing of the rat postnatally in order for one of the human effects of warfarin, nasal hypoplasia, to be detected. Also, it appears that the human developing foetus is more vulnerable than the rat foetus. Therefore, the concern that the OECD TG 414 protocol is not adequate to show developmental effects of AvK's remains, and classification of difenacoum for developmental toxicity should be based on read across to warfarin, leading to the proposed Repr. Cat 1; R61 (DSD)/Repr. Cat 1 A; H360D (CLP). 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. The Danish EPA also agrees on the M-factors proposed by the Finnish dossier submitter for acute and aquatic toxicity for difenacoum.				
Dossier Submitter's Response				

Thank you for your support.
RAC's response
Environment: Noted. Health harzards: Noted.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	2
Comment received				
We agree with the current proposal for the environment part 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 your support.				
RAC's response				
Environment: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Germany		MemberState	3
Comment received				
The German CA supports the proposed classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Environment: Noted. Health harzards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Italy	Activa s.r.l	Company-Manufacturer	4
Comment received				
We support the documents uploaded (*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.

[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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ECHA note: The second provided document "Difenacoum, Comment on the CLH proposal, 5 March 2013" (File name: Difenacoum classification - developmental EWC0009) was also submitted by Exponent International on behalf of CEFIC RDDG and is copied under comment 6.

Dossier Submitter's Response

The dossier submitter does not agree with comments given regarding classification for reproductive toxicity. Please see the attached document for details.

(ECHA note: The attachment provided is copied below)

Substance name: difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin
CAS number: 56073-07-5
EC number: 259-978-4
Dossier submitter: Finland

Response to comments received during public consultation on difenacoum

The dossier submitter for difenacoum does not agree with the comments received concerning classification for reproductive toxicity in terms of

- method sensitivity of OECD 414 being shown for warfarin in the Kubaszky 2009 study
- Special experts' group conclusion 2006 being no more valid
- classification using read-across from warfarin being incorrect

In the following detailed reasoning is given.

Method sensitivity of the OECD 414 TG, the warfarin study on rat (Kubaszky 2009) and relevance of the results in terms of other AVKs

- As the developmental toxicity tests on difenacoum were carried out in accordance with the old OECD 414 TG, where the exposure was 6-15 gestation days we can only compare the results with those obtained by the TP1 regimen of the new warfarin study

- Regarding the observed fetal hemorrhages in the warfarin study and the absence of similar observations in the difenacoum studies, we have no clear cut explanation why no

hemorrhages were recorded for difenacoum (or other AVKs). One explanation could be that the correct dosing of second generation AVKs is extremely critical due to steep dose-response curve for maternal toxicity. Therefore there could be a very narrow margin between the effective dose for the conceptus and the maternally lethal dose. Also, in only one study it is specifically mentioned that there were no hemorrhages observed in the fetuses; other studies do not mention the absence of fetal hemorrhages therefore the assumption of the absence of hemorrhages is implicit (and leaves room for speculation whether all observations were recorded).

- Regarding the observed case of cataract in the warfarin study, there was 1 fetus out of 99 (1 %) in the dose group 0.200 mg/kg in the TP1 regimen with cataract. This is a rare finding not recorded in the historical data and thus could be related to warfarin-treatment. There are human warfarin embryopathy cases where cataract is one manifestation of teratogenicity however the incidence is probably low. Similarly, human cases with microphthalmia has been recorded and associated with warfarin embryopathy (van Driel et al. 2002). In a study on difenacoum in rat one fetus with microphthalmia of both eyes was observed in the dose group 0.09 mg/kg (the highest dose), however the RMS or applicant's study summary concluded that there was no evidence of teratogenicity and this conclusion was also written in the CLH proposal for difenacoum. However, taking all evidence into account we now tend to think that the microphthalmia finding could be related to difenacoum treatment.

- There were more fetuses with cataract in the TP2 regimen of the warfarin study: 2/124 (1.6%; fetuses in one litter) in the 0.150 mg/kg group, 4/132 (3%; fetuses in 2 litters) in the 0.200 mg/kg group and 0 cases in the 0.250 mg/kg group. However as pointed out before, the exposure duration is not similar to the difenacoum study and thus the studies should not be compared to each other.

- Regarding the most common findings concerning warfarin-evoked congenital anomalies in humans, the skeletal and facial effects were not observed in the warfarin study. However, these anomalies can be observed if the rats are exposed postnatally. Therefore the warfarin study is comparable to the difenacoum studies where no skeletal or facial anomalies were recorded.

- Regarding the relevance of concern for teratogenicity instead of other type of fetotoxicity, it is clear that these phenomenon are not directly linked to each other, they do not exclude each other nor are they "altenates" to each other. Instead, haemorrhages and developmental abnormalities are independent endpoints of serious nature each. If no bleeding was observed in the difenacoum studies (or other AVK studies) the concern of reproductive toxicity still remains since it has not been proven that teratogenic findings are not relevant for other non-warfarin AVKs.

Mode of action

- Whether the mode of action is indirect or direct, has not been proven for warfarin. Warfarin can cross placenta and the recent studies show that flocoumafen can cross placenta too.

- There is a case report of a toxicosis in neonatal puppies where the dam was intoxicated by brodifacoum (Munday and Thompson, 2003). Eight out of 13 puppies were born dead or died within 48 hours of birth. Two puppies that were born alive but which died in 6 hours had hemorrhages in the thoracic and peritoneal cavities, intestinal serosa and meninges. Brodifacoum was found in the livers of these two puppies. The dam was unaffected. This is a prove that brodifacoum can cross placenta. Since difenacoumn differs from brodifacoum in

terms of a Br-substituent and thus the compounds are very similar, it is very likely that difenacoum can cross placenta as well.

- The mechanism of warfarin-evoked teratogenicity can also be indirect due to the disturbance of vitamin K cycling. It is striking that all the treatments (other pharmaceutical coumarins, indandiones, anticonvulsants), physiological states (severe vomiting, biliary lithiasis) and genetic disorders that affect vitamin K balance cause similar congenital anomalies.

- In the flocoumafen CLH report it is stated that warfarin treatment does not affect plasma vitamin K concentrations (Nakamura et al. 1994) and therefore the mechanism of teratogenicity of warfarin cannot be the reduction of fetal vitamin K levels. However there are other studies showing that warfarin-treatment lowers plasma vitamin K levels (Sato et al. 1997; Yamanaka et al. 1990). Vitamin K1 can cross placenta (Suzuki et al. 2001) and fetal vitamin K levels follow maternal levels.

- About maternal toxicity and reproductive toxicity classification criteria under CLP: According to the Annex I, part 3, section 3.7.2.4 of the CLP Regulation, maternal toxicity does not by default exclude classification for developmental effects. The effect of warfarin and other AVKs on the vitamin K recycling should not be considered as a non-specific secondary effect and therefore should be taken into account in classification.

Route of exposure

- In one comment industry states that concern is reduced because warfarin is an oral therapeutic and the operator exposure to biocide is via dermal route and the skin presents a considerable barrier. As a response we would like to point out that there are no indications on differences in toxicokinetics and toxicodynamics via different exposure routes, in other words route-to-route extrapolation is justified. This comment also totally ignores the nature of especially second generation AVKs whose half-life is longer than that of warfarin and they also accumulate in the body. These features make them even more potent than warfarin. The absorption via dermal route is not negligible. Also, classification as Acute Tox. 1 via dermal route and as STOT RE 1 is proposed.

Comparison with CLP criteria and read-across and SE conclusion

- According to the CLP Regulation, classification as a reproductive toxicant 1A is largely based on human evidence. The CLP Regulation allows use of read-across for classification (article 5(1) point c). The Regulation supports weight of evidence evaluation of the available data (Annex I, section 1.1.1.3).

- Classification of warfarin as Repr. 1A for developmental effects relies entirely on human evidence. The available evidence shows that warfarin and other AVKs share similar toxicity profile and physiochemical properties. It has not been proven that non-warfarin AVKs do not have human relevance in terms of developmental effects. Although the SE conclusion lacks clear comparison with criteria, the fundamental reasoning for the remaining concern has not changed.

- In conclusion, the criteria for classification of difenacoum as Repr. 1A are fulfilled since the classification is based on read-across from warfarin, a well-known human teratogen.

References

Driel Van, D., Wesseling, J., Sauer, P.J.J., Touwen, B.C.L., Veer Van Der, E. and Heymans,

H.S.A. (2002).Teratogen update: fetal effects after in utero exposure to coumarins . Overview of cases, follow-up findings, and pathogenesis.Teratology, 66: 127-140

Munday, J.S. and Thompson, L. J. (2003). Brodifacoum toxicosis in two neonatal puppies. Vet. Pathol., 40:216-219
Nakamura, K., Toyohira, H., Kariyazano, H., Ishibashi, M., Saigenji, H., Shimokawa, S. and Taira, A. (1994). Anticoagulant effects of warfarin and kinetics of K vitamins in blood and feces. Artery, 21: 148-160.

Sato, Y., Honda, Y., Kunoh, H. and Oizumi, K. (1997). Long-term anticoagulation reduces bone mass in patients with previous hemispheric infarction and nonrheumatic atrial fibrillation. Stroke, 28: 2390-2394.
Suzuki, S., Iwata, G.n and Sutor, A.H. (2001). Vitamin K deficiency during the perinatal and infantile period. Semin. Thromb. Hemost., 27: 93-98.

Yamanaka, Y., Yamano, M., Yasunaga, K., Shike, T. and Uchida, K. (1990). Effect of warfarin on plasma and liver vitamin K levels and vitamin K epoxide reductase activity in relation to plasma clotting factor levels in rats. Thromb. Res.,57: 205-214.

--- End of attachment ----

RAC's response
Health hazards: Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	5
Comment received				
SCL: Specific Concentration Limits (SCL) for reprotoxicity are necessary for Difenacoum, it should be discussed and harmonized between all the anticoagulant CLH reports.				
Dossier Submitter's Response				
We agree.				
RAC's response				
Health hazards: 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	6
Comment received				
Section 4.11 Toxicity for reproduction. Difenacoum should not be classified for developmental toxicity. Data are conclusive and not sufficient for classification. Please see attached document (Exponent docID 1109091.uk0 EWC0009 - Difenacoum) (*ECHA note: The attachment provided is copied below)*				

Difenacoum
Comment on the CLH proposal, 5 March 2013
Developmental toxicity:

Difenacoum should ***not be classified*** for developmental toxicity.
Careful comparison of the guideline developmental toxicity data for Difenacoum against the classification criteria show:
- Criteria for classification for developmental toxicity are not met.
  - There is no evidence of difenacoum being causally associated with developmental toxicity in humans.
  - There is no evidence from acceptable GLP- and guideline-compliant studies, that difenacoum causes an adverse effect on development in animals.
  - The rat study design is demonstrated to be sensitive to warfarin.
- No classification for developmental toxicity is therefore appropriate.

Developmental Toxicity

1. Relevance of the Specialised Experts Conclusion[1]

The CLH proposal to classify difenacoum for developmental toxicity follows the SE Conclusion. However, the SE Conclusion lacks a clear comparison of evidence with modern (DSD or CLP) criteria. The conclusion is based on an inappropriate endpoint (malformation, not foetotoxicity). The conclusion relies on an assumption (uncertainty that the teratogenicity of warfarin can be detected in pre-natal developmental toxicity studies including OECD guideline 414) for which however no evidence is provided; and which is proven incorrect by a more recent OECD 414 study demonstrating developmental toxicity of warfarin.
The SE Conclusion also cites an absence of data addressing placental transfer for the rodenticidal AVKs. A recent study comparing the transplacental radiolabel distribution of flocoumafen and warfarin in pregnant rats and their litters is described in the CLH report for flocoumafen, demonstrating that the foetus is substantially better protected from flocoumafen compared to warfarin. It is appropriate to read-across from flocoumafen to difenacoum (EBRC, 2010[2]) since key pharmacokinetic parameters are comparable, and conclude that the foetus will be as effectively protected from difenacoum as from flocoumafen.
The SE Conclusion is therefore no longer scientifically valid.
More details are offered in Exponent's EWC0008.

2. Relevance of the CEFIC teratogenicity study of warfarin[3]

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[4]). 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 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 a clear demonstration of the ability of the standard "OECD 414" design to detect specific foetal sensitivity to warfarin and the AVKs.
For difenacoum, two teratogenicity studies in rats examine 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.

3. Comparison with Criteria

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) which was not a feature of the effective warfarin study.
Since the discussion does not address the significance of foetotoxicity seen in the warfarin study (without a post-natal exposure period) and in human pregnancies, the reasoning is incomplete.
A detailed comparison with criteria based on evidence is therefore offered as follows:

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

There is no epidemiological evidence that difenacoum 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 difenacoum.
Because the criterion for "sufficient epidemiologic evidence" is not met for difenacoum, 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 difenacoum 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 difenacoum is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies of difenacoum in both rats and rabbits. The method used to test difenacoum is appropriate and sufficient to detect developmental toxicity of warfarin. By read-across from flocoumafen, the pharmacokinetic properties of difenacoum provide effective protective of the foetus.
Negative results in adequate animal studies of difenacoum 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 difenacoum 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 difenacoum is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits. The method used to test difenacoum is appropriate and sufficient to detect developmental toxicity of warfarin. By readacross from flocoumafen, the pharmacokinetic properties of difenacoum provide effective protective of the foetus.
Negative results in adequate studies of difenacoum are meaningful.
Placement in DSD Category 3/ CLP Category 2 is not appropriate. No classification for developmental toxicity is appropriate.

Conclusion

Ample evidence is provided that the basis for a read-across from warfarin teratogenicity to difenacoum is not valid.
When compared with the criteria for classification, there is inadequate evidence for any classification of difenacoum for developmental toxicity.

[1] ECBI/121/06, 20 September 2006. ECB, Ispra.
[2] EBRC Consulting GmBH (2010) Difenacoum: Applicant's statement on the pending classification proposal for developmental toxicity (R61) by read-across from Warfarin. BASF Doc ID2010/1065256
[3] Kubaszky R (2009) Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
[4] 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

The dossier submitter does not agree with comments given regarding classification for reproductive toxicity. Please see the attached document for details.

(ECHA note: The attachment provided is copied under comment 4)

RAC's response

Health hazards: Noted.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON DIFENACOUM (ISO); 3-(3-BIPHENYL-4-YL-1,2,3,4-TETRAHYDRO-1- NAPHTHYL)-4-HYDROXYCOUMARIN

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Norway		MemberState	7
Comment received				
The Norwegian CA agrees with the proposal to classify difenacoum as Repr. 1A; H360D (CLP) /Repr. Cat. 1; R61 (DSD) based on the rationale put forward in the CLH report. We support the argument that no clear conclusions can be drawn from the performed teratogenicity studies because of the limitation of the conventional OECD TG 414 studies in detection of coumarin-specific developmental effects. No human data on teratogenicity exists for the substance. Read across to the established human teratogen, warfarin, is considered appropriate as difenacoum contains the same chemical moiety as warfarin and has the same mechanism of action responsible for the teratogenicity of warfarin. As potential developmental effects would be expected at very low doses, the possibility of setting specific concentration limits for reprotoxicity should be considered, as proposed, using a common approach for all relevant AVK rodenticides.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Health hazards: 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	8
Comment received				
4.11, Toxicity for 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)				
Dossier Submitter's Response				
The dossier submitter does not agree with comments given regarding classification for reproductive toxicity. Please see the attached document for details. (ECHA note: The attachment provided is copied under comment 4)				
RAC's response				
Health hazards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	9
Comment received				
No clear developmental toxicity was observed in rabbits or rats. However, due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to difenacoum, no clear conclusion can be drawn from these studies. Difenacoum contains the same chemical moiety responsible for the teratogenicity of warfarin and it has the same mode of action (inhibition of vitamin K cycle				

leading to vitamin K deficiency) that is a known mechanism of teratogenicity in humans. Moreover, considering that human foetuses seem to be much more vulnerable to vitamin K deficiency than rodent foetuses, classification of difethialone for developmental toxicity with Repr. 1A; H360D (Regulation EC 1272/2008) similar to warfarin, is supported.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Health hazards: Noted.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Germany		MemberState	10
Comment received				
Page 42, last sentence (Relevance of the OECD414 test for AVKs): The study on placental transfer of warfarin and flocoumafen (for further information on the study see CLH report on flocoumafen, ref. Johnson, 2009) was evaluated by the Netherlands and is included in the CLH report on flocoumafen. The study demonstrates that flocoumafen, like warfarin, is able to pass the placenta. It is not possible however to quantitatively extrapolate data on foetal exposure between the AVK rodenticides. Therefore, the proposal to classify difenacoum with Repr. Cat.1; R61 / Repr. 1A; H360D is supported.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Health hazards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Germany	BASF	Company-Manufacturer	11
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 difenacoum should not be classified on the basis of available data. (*ECHA note: The attachment "Teratogenicity of AVK Rodenticides - Classification by Read-Across from Warfarin is not Correct" is copied under comment number 4. The second attachment "Difenacoum (CAS 56073-07-5), BASF comments on the CLH proposal, March 2013" is being provided as a separate document to this table)*				
Dossier Submitter's Response				
The dossier submitter does not agree with comments given regarding classification for reproductive toxicity. Please see the attached document for details. (ECHA note: The attachment provided is copied under comment 4)				
RAC's response				
Health hazards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number

19.04.2013	United Kingdom	PelGar International Limited	Company-Manufacturer	12
Comment received				
We strongly support the positions given in the attached papers. The attached documents are: Teratogenicity of AVK Rodenticides, Classification by Read-Across from Warfarin is not Correct (File name: Read-across rebuttal EWC0008) – copied under comment 4 and Difenacoum, Comment on the CLH proposal, 5 March 2013 (File name: Difenacoum classification - developmental EWC0009) - copied under comment 6.				
Dossier Submitter's Response				
The dossier submitter does not agree with comments given regarding classification for reproductive toxicity. Please see the attached document for details. (ECHA note: The attachment provided is copied under comment 4)				
RAC's response				
Health hazards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Germany	HENTSCHKE & SAWATZKI KG	Company-Manufacturer	13
Comment received				
(ECHA note: The organisation provided 2 attached document. *The attached documents are:* *Teratogenicity of AVK Rodenticides, Classification by Read-Across from Warfarin is not Correct (File name: Read-across rebuttal EWC0008) – copied under comment 4 and Difenacoum, Comment on the CLH proposal, 5 March 2013 (File name: Difenacoum classification - developmental EWC0009) - copied under comment 6.)*				
Dossier Submitter's Response				
The dossier submitter does not agree with comments given regarding classification for reproductive toxicity. Please see the attached document for details. (ECHA note: The attachment provided is copied under comment 4)				
RAC's response				
Health hazards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Sweden		MemberState	14
Comment received				
(*ECHA note: The comment below has been submitted as a separate attachment)* The Swedish CA supports the classification proposal for difenacoum regarding reproductive toxicity. We support that the classification for difenacoum (as well as for the other AVK rodenticides) should be based on read across to human data for warfarin (i.e warfarin embryopathy). Therefore, difenacoum 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 g/mol) 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).

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 "Physicochemical properties and mammalian toxicity summarized from the hydroxyl coumarin AVK dossiers, substances organized according to molecular weight",*

is provided as a separate attachment to this comments table)
Dossier Submitter's Response
Thank you for your support.
RAC's response
Health hazards: Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	15
Comment received				
With regard to the acute toxicity, we support the following classification: - Acute tox. 1, H300 based on LD50 < 5mg/kg observed in rat and mouse studies, - Acute tox.1, H330 based on LC50 ≤ 0.05 mg/l/4h for dusts and mists - Acute tox. 1, H310 based on LD50 < 5mg/kg bw				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Health hazards: Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	16
Comment received				
• Repeated toxicity (p36-37): As the LOAELs are derived from two studies of 42 and 13 days, should the Haber's rule be considered in the derivation of the SCL (as mentioned in the CLP guidance to regulation)?				
Dossier Submitter's Response				
We considered using the Haber's rule however since the effect (increase in PT) occurs early on (in few days) the total length of the study does not play a role in this case and therefore we decided not to use the Haber's rule.				
RAC's response				
Health hazards: Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	17
Comment received				
We support the classification STOT RE Cat.1 based on C≤ 10mg/kg bw/day obtained from the 90 day repeated dose toxicity in rat. We also support the route-to-route extrapolation and therefore we consider classification via all the three routes for repeated dose toxicity justified.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Health hazards: Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	18
Comment received				
We agree with the proposed M-factor for acute toxicity of 10 (most sensitive species Fish Oncorhynchus mykiss with 96h LC50 = 0.064 mg/l and toxicity band between 0.01 mg/l and 0.1 mg/l), as well as with the proposed SCLs : N, R50/53 C≥2.5% N, R51/53 0.25%≤C<2.5% R52/53 0.025%≤C<0.25% Based on the most stringent outcome for Aquatic Chronic toxicity (most sensitive species Fish Oncorhynchus mykiss with 96h LC50 = 0.064 mg/l + substance not rapidly degradable) an M-factor for chronic toxicity of 10 (0.01 mg/l <LC50 ≤ 0.1 mg/l) could be assigned.				
Dossier Submitter's Response				
Thank you for your support. M-factor of 10 is proposed for both aquatic acute and chronic toxicity in the CLH report.				
RAC's response				
Environment. : Noted.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	19
Comment received				
It seems that the template has not been used. The chapter classification for physico-chemical properties is not present. p 13, Water solubility: In the CLH report, "0.483 mg/L at 20°C, pH 6.5" is reported whereas in the combined AR, "0.43 mg/L at 20°C, pH 6.5" is reported. Please clarify.				
Dossier Submitter's Response				
If a substance already has a harmonised classification in Annex VI, it is only required to include those hazard classes in the CLH report that are proposed to be updated. This has been discussed for instance in the 12th CARACAL meeting. Difenacoum has an Annex VI entry and no updating to physical hazard classification is proposed in the CLH report therefore these endpoints are not assessed. p. 13, Water solubility: There is an unfortunate mistake in the Assesment Report, the number should be "0.483 mg/L at 20°C, pH 6.5" as written in the CLH report.				
RAC's response				
Noted				

ATTACHMENTS RECEIVED:

1. **Difenacoum, Comment on the CLH proposal, 5 March 2013** (File name: Difenacoum classification - developmental EWC0009), submitted on 19 April 2013 by:

Exponent International on behalf of CEFIC RDDG
Activa s.r.l

HENTSCHKE & SAWATZKI KG
PelGar International Limited

(ECHA's comment: additional information provided in the document copied under Toxicity to reproduction, comment 6)

2. **Teratogenicity of AVK Rodenticides, Classification by Read-Across from Warfarin is not Correct** (File name: Read-across rebuttal EWC0008), submitted on 19 April by:

Activa s.r.l
HENTSCHKE & SAWATZKI KG
PelGar International Limited

(ECHA's comment: additional information provided in the document copied under Toxicity to reproduction, comment 4)

3. **Teratogenicity of AVK Rodenticides, Classification by Read-Across from Warfarin is not Correct** (Filename: 2013_1125919_RDDG_BASF_Warfarin Read-across rebuttal), submitted on 19 April by BASF

4. **Difenacoum (CAS 56073-07-5), BASF comments on the CLH proposal, March 2013** (File name: 2013_1125921_BASF_Difenacoum_comments CLH proposal), submitted on 19 April 2013 by BASF

5. **Comments on Annex XV dossiers proposing harmonised Classification & Labelling** (File name: COM_CLH_PC_Difenacoum_SE), submitted on 19 April by Sweden (*ECHA's comment: additional information 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)