**Committee for Risk Assessment**

**RAC**

Annex 2

**Response to comments document (RCOM)**

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

# Coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin

**EC number: 227-424-0**

**CAS number: 5836-29-3**

CLH-O-0000003397-68-03/F

**Adopted**

**14 March 2014**

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1- naphthyl)coumarin**

**COMMENTS AND RESPONSE TO COMMENTS FROM DANISH EPA ON CLH PROPOSAL AND JUSTIFICATION ON COUMATETRALYL**

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: coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin**
**EC number: 227-424-0**
**CAS number: 5836-29-3**
**Dossier submitter: Denmark**

## GENERAL COMMENTS

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 1 |
| Comment received | | | | |
| FR is in accordance with the environmental classification proposal :<br>- CLP Regulation:<br>• Aquatic Chronic. 1 (M=10); H410 – very toxic to aquatic life with long lasting effects<br>- Directive 67/548/EEC:<br>• N; R52-53 – Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.<br><br>The method to determine the SCLs for acute and chronic toxicity should be harmonised with other anticoagulant rodenticides. Difenacoum approach to set SCLs could be used. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the French CA for the support on the environmental classification, including the choice of M factor.<br>The method to determine the specific concentration limits (environment) used is the same as for difenacoum, and is in accordance with the CLP regulation and the DPD/99/45/EC. | | | | |
| RAC's response | | | | |
| A harmonised approach to set SCLs has been used by RAC for the AVKs. | | | | |

## TOXICITY TO REPRODUCTION

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Norway | | MemberState | 2 |
| Comment received | | | | |
| The Norwegian CA agrees with the proposal to classify coumatetralyl as Repr. 1A; H360D (CLP) /Repr. Cat. 1; R61 (DSD) based on the rationale put forward in the CLH report.<br>We agree that no clear conclusions can be drawn from the performed teratogenicity studies because of the recognized limitations of the conventional OECD 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 supported as coumatetralyl contains the same chemical moiety and has the same mechanism of action responsible for the teratogenicity of warfarin.<br>We agree that the possibility of setting specific concentration limits for reprotoxicity should | | | | |

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| be explored as potential developmental effects would be expected at very low doses. |
| Dossier Submitter's Response |
| Thank you to the Norwegian CA for the support to the classification as Repr. 1A; H360D (CLP) /Repr. Cat. 1; R61 (DSD) based on read across to the human teratogen warfarin, and the |
| RAC's response |
| The support is noted. See also the response to comment number 4. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 3 |
| Comment received | | | | |
| SCL for reprotoxicity should be harmonized with warfarin. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the French CA for bringing up the subject of setting of SCL. We agree that the different AvK should be considered to be highly potent, and welcome the discussion on setting SCL when classification of coumatetralyl is in place. However, Denmark has not taken stance on the method to apply. | | | | |
| RAC's response | | | | |
| A harmonised approach to set SCLs has been used by RAC for the AVKs. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | France | | Company-Manufacturer | 4 |
| Comment received | | | | |
| In the CLH report for Coumatetralyl, the Danish EPA has proposed to classify Coumatetralyl for developmental toxicity as Reprotoxicity Cat. 1, R61 (Directive 67/548/EEC), or Reprotoxicity 1A H360D (Regulation EC 1272/2008). This proposal is based on doubts about the ability of the OECD 414 prenatal developmental toxicity studies to adequately detect potential teratogenic effects of Vitamin K-dependent anticoagulants (AVKs) such as coumatetralyl and warfarin. These concerns were expressed by the Specialised Experts Group (SEG) for classification and labelling under directive 67/548/EC in 2006. At that time there were no robust animal data for Warfarin, a known human teratogen. Therefore, the absence of adverse findings in the rat and rabbit teratogenicity studies with Coumatetratlyl and the other AVK rodenticides lead the SEG to conclude that the OECD 414 guideline was not capable of detecting developmental effects of AVKs. They considered that it was appropriate to read across from the known human effects of warfarin to all the other AVK rodenticides and to classify all of them as Cat 1 or Cat 2 R61 for reprotoxicity.<br>In order to clarify this equivocal situation a study was commissioned by the CEFIC Rodenticide Data Development Group (RDDG) to test warfarin according to the OECD 414 guideline protocol. The results of this study showed that warfarin is toxic for the conceptus, inducing embryo-foetal mortality, haemorrhages and malformations like cataract. These data also demonstrate that OECD 414 (prenatal developmental toxicity) studies are adequate to detect potential teratogenic effects of AVKs and should be considered as a reliable animal testing method to identify a risk for human conceptus with these types of chemicals.<br>When Coumatetralyl was tested following the same OECD 414 study design, at dose levels adequately chosen with respect to maternal toxicity and during in utero exposure period | | | | |

covering the organogenesis and foetal development until delivery, there was no evidence of teratogenic potential in both rats and rabbits. These results show that although Warfarin and Coumatetralyl share the same mode of action, they have different effects on the embryo development. Warfarin provokes teratogenic effects, while Coumatetralyl does not. In conclusion, the proposal to classify Coumatetralyl in Reprotoxicity category 1A based on read across from Warfarin is not justified because the two substances produce different results in terms of developmental effects. The classification of Coumatetralyl should be based on the results of the OECD 414 studies performed with this test substance, which show no evidence in valid animal studies.

Dossier Submitter's Response

The Danish CA does not agree with the arguments of the French manufacturer.

The core point in the evaluation from the specialised experts group in 2006 was that the mode of action of the anticoagulants is the same, and that the inhibition of the vitamin K, leading to disrupted coagulation and is probably also responsible for the developmental toxic effects, as seen in human foetus in treatment of the mother with warfarin, it be it a direct effect on the foetus or not. The specific mechanism of action for the developmental effects of warfarin was not established. Therefore, the experts concluded that read-across to warfarin should be used in the classification of other AvKs, amongst other coumatetralyl. The mechanism of action is still not established. Therefore, concern that the same mechanism of action is used by the other AvKs remains, and the Danish CAs stance is that the proposed read-across from known human teratogen warfarin to the other anticoagulant rodenticides should still be used for classification of amongst other coumatetralyl.

The Specialised Experts were also concerned that the standard OECD 414 protocol had not been able to demonstrate embryopathy in coumarin-derived anticoagulants without the administration of vit K. Denmark does not agree that the results of the recent OECD 414 studies on warfarin in rats, showing some developmental effects at non-maternally toxic doses are sufficient to conclude that the guideline is capable of demonstrate any teratogenic effect in any other anticoagulant and refuke the validity of the conclusion of the specialised experts. It appears from the warfarin OECD 414 studies that other effect than the warfarin embryopathy, nasocartilage hypoplasia, cannot be reproduced in the animal model due to differences in the development intrautero of rat and human foetus. In the new OECD 414 studies with warfarin, it can be noted that the malformation effects seen with warfarin are not seen in the study with short exposure time, but only the prolonged exposure time. It is also noted that even for warfarin, which has been studied much more extensively that other coumarin derivates, the dose levels used in the new OECD 414 has to be revised after the start of the study is order to acertain toxicity. Therefore, it appears that there are many elements challenging the suitability of the protocol, amongst other the window of exposure, the balance with vitamin K and dose levels. Based on the above arguments, the Danish CA maintains its position that the OECD 414 protocol is not suited for testing coumarin-derivatives.

In conclusion, the classification as repr.Cat 1A; H360D is maintained for coumatetralyl

RAC's response

Based on the assumption that all AVK rodenticides, including Warfarin and other anticoagulant coumarin pharmaceuticals (see below) share the same MoA, namely inhibition of vitamin K epoxide reductase (VKOR), the assessment of Coumatetralyl includes consideration of the total data base for the AVKs. A weight of evidence assessment resulted in the conclusion that Coumatetralyl has the capacity to adversely affect the human in utero development. Therefore a classification with cat 1B is proposed with the reasoning given below.

The reasons for this presumption are:

- Coumatetralyl shares the same MoA as expressed by other anticoagulant AVK rodenticides and coumarin pharmaceuticals (inhibition of vitamin K epoxide reductase, an enzyme involved with blood coagulation and foetal tissues development, including bone formation, CNS development and angiogenesis)
- Warfarin and 2 other coumarin pharmaceuticals (acenocoumarol, phenprocoumon) have been shown to cause developmental toxicity in humans.
- One of the 2nd generation AVK rodenticides (Brodifacoum) has been shown to cause foetal effects in humans, possibly after one or a few exposures.
- The standard animal studies will not pick up all developmental toxicity effects of the AVK rodenticides, most notably the face and CNS malformations that are characteristic for Warfarin and other AVK coumarin pharmaceuticals.
- The most sensitive window for face malformations in humans is the first trimester. Thus, also if some AVK rodenticides may have a lower degree of placental transfer than Warfarin, this will not affect the face malformation hazard.

Not all steps of the MoA in the target tissues liver and bone have been proven, thus introducing some uncertainty in the assessment. However, the RAC is of the opinion that the uncertainty is not sufficiently big to warrant a cat 2 classification.

Reliable evidence of an adverse effect on reproduction in humans, which is required for Repro 1A, was not available for Coumatetralyl, but a potential for human developmental toxicity is presumed based on the above stated weight of evidence assessment, and RAC thus proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant".

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | United Kingdom | Exponent International for CEFIC RDDG | Industry or trade association | 5 |

Comment received

Section 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)

**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

Dossier Submitter's Response

The Danish CA does not agree with the consultant to the CEFIC Rodenticides group Exponent International that the conclusion of the Specialised Experts that all anti-Vitamin K should be classified as Repr cat 1; R61 (DSD) are "no longer valid".

Obviously, the conclusion of the SE do not meet the format of a Annex XV dossier. However, this group of independent academic experts appointed by the Member States to advised the Commission working group on classification on carcinogenicity, genotoxicity and reproductive toxicity by giving expert guidance on how to interprete the toxicological data available for classification, using all the criteria laid down in directive 67/548/EC, general as well as specific criteria, for the end-point of reproductive toxicity.

Based on the all data available the Specialised Experts expressed concern that AvKs were developmental toxicant in humans, as they all use the same mode of action as the human teratogen warfarin. To date, no data have been tabled to demonstrate a different mechanism of action for developmental toxicity by warfarin compared to other coumarin-derived AvKs substances. Therefore, the concern that other AvKs using the same mechanism of action as warfarin also have a developmental toxicant effect is still valid.

The Danish CA disagrees that the new OECD 414 study(ies) on warfarin can be used as proof that this standard test protocol is able to detect developmental effects for any other anticoagulant. It appears from the warfarin OECD 414 studies that the window of exposure for effects on developmental seen in humans cannot be reproduced in the animal model. This is due to differences in the development intrautero of rat and human foetus. In the new OECD 414 studies with warfarin, it can be noted that the malformation effects seen with warfarin are not seen in the study with short exposure time, but only the prolonged exposure time. It is also noted that even for warfarin, which has been studied much more extensively that other coumarin derivates, the dose levels used in the new OECD 414 needed to be revised after the start of the study is order to acertain toxicity. Therefore, it appears that there are many elements challenging the suitability of the standard OECD 414 protocol, amongst other the window of exposure, the balance with vitamin K and dose levels in order to demonstrate an effect of warfarin.
The negative results of the standard OECD 414 for the other AVKs (as well as for previous studies with warfarin) may be due to differences in developmental toxicity potency between the AVKs, choices of doses, of treatment duration, of delopmental differences in time between rat and humans.
Therefore, the Danish CA still has strong concern that the OECD 414 in rodents is not suited for the detection of developmental toxicity of coumarin-derived substances.

Classification shall be performed according to the rules of regulation 1272/2008. The comparison with the criteria by Eksponent should include consideration to the overall criteria given in the introduction to Annex I of regulation 1272/2008, and not only refer to the end-point specific criteria. In Annex I point 1.1.1.3, it is stated that all available information should be considered, amongst other grouping, read-across, and human experience.
Thus, the following information should be taken into account in the classification of the coumarin derived anticoagulants:
- Warfarin is a potent human reproductive toxicant, classified in category 1A. There is

evidence that warfarin is associated with adverse effects on the human foetus.
- The AvK are coumarin-derivatives, which are structurally related to warfarin.
- There is evidence that couramin-derivatives use the same mode of action with respect of inhibition of vit K for their anticoagulant effect.
- No demonstration of the mechanism of action for developmental toxicity for warfarin is available.
- The mechanism of action for the developmental toxicity has been proposed for all coumarin-derivatives by the specialized experts also to be related to vitamin K inhibition.
- As no other mechanism of action has been proposed, explaining that only one of a structural similar group would odd out in this respect.
- The standard OECD 414 is not suited for testing coumarin-derivatives for developmental effects due to differences in developmental toxicity potency between the AVKs, challenge in the choices of doses, of treatment duration, and because of timing differences in the delopment of the rat and human foetus.

Based on the complete criteria for classification, classification of coumatetralyl should be based on read-across to warfarin, a potent human developmental toxicant, due to structural and mechanistic analogy consideration. To conclude, the Danish CA maintains its position that coumatetralyl should be classified as repr cat 1A.; H360D according o CLP.

RAC's response

See the response to comment number 4.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Finland | | MemberState | 6 |

Comment received

We support the proposed classification for developmental effects as Repr. 1A; H360D for coumatetralyl. There is no substance specific human data, and the results from animal studies are inconclusive. However, the structurally related AVKs share the same mode of action justifying classification based on read-across from warfarin, the known human teratogen. The mode of action of warfarin and other anticoagulant rodenticides is the same, namely causing vitamin K deficiency. There is no evidence that the toxicokinetic differences between individual substances would make a fundamental difference in the disturbing effect on vitamin-K balance which is the underlying reason for the teratogenic effects of warfarin. Therefore, applying read-across from warfarin for classification is justified.

We also agree that the substance should not be classified for fertility. In analogy to teratogenicity and developmental toxicity, read-across to warfarin data is justified. Warfarin has not been classified as toxic to fertility. In literature, there are no indications of adverse fertility effects associated to warfarin or vitamin K deficiency.

Dossier Submitter's Response

Thank you to the Finnish CA for the support to the arguments used for the proposal for classification for reproductive toxicity of coumatetralyl.

RAC's response

The support is noted. See also the response to comment number 4.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | Sweden | | MemberState | 7 |

Comment received

The Swedish CA supports the classification proposal for coumatetralyl regarding reproductive toxicity. We support that the classification for coumatetralyl (as well as for the other AVK rodenticides) should be based on read across to human data for Warfarin (i.e warfarin embryopathy). Therefore, coumatetralyl 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).

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.

Attachment: Table 1. Physicochemical properties and mammalian toxicity summarized from the hydroxyl coumarin AVK dossiers, substances organized according to molecular weight.

| Dossier Submitter's Response |
|---|
| Thank you to the Swedish CA for your support for the classification proposal for coumatetralyl as repr. Cat 1A; H360D. Thank you also for the clear argumentation on read-across to warfarin and weaknesses of the OECD 414 standard protocol in demonstrating developmental effects in AVK and examples of developmental effects which other AVK's. |
| RAC's response |
| The support is noted. See also the response to comment number 4. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 8 |
| Comment received | | | | |
| For developmental toxicity in rat study, we agree that the results in foetus can show no clear evidence of an adverse effects on fertility or on development. This could be explained by the difference in a bone structure development in humans and rats which takes place early in pregnancy in the case of humans and late in the pregnancy or even postnatally in rats.<br><br>We agree with the read across for the developmental toxicity based on the structural similarity and the same mode of action (vitamin K deficiency). Based on the higher vulnerability to the humans foetuses in the warfarin data than rodent foetuses and the classification of warfarin as Reprotoxicity Category 1, H360D, we support the classification Repr. Cat. 1A, H360D for Coumatetralyl. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the Belgian CA for supporting the Danish proposal to classify coumatetralyl as Repr. Cat. 1A, H360D based on read-across to warfarin, based on the structural similarity and the shared mode of action (vitamin K defiency), and the concern that human foetuses are more vulnerable than rodent foetuses to this vitamin K defiency. We agree that the negative results in the standard OECD test amongst other could be due to differences in the time of bone structure development in humans and rats | | | | |
| RAC's response | | | | |
| The support is noted. See also the response to comment number 4. | | | | |

| 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 | | | | |
| 4.11 Toxicity for reproduction: | | | | |

Coumatetralyl should not be classified for developmental toxicity. Data are conclusive but not sufficient for classification. Please see attached document (Exponent DocID 1109091.uk0 EWC0009 - coumatetralyl)

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

Coumatetralyl should ***not be classified*** for developmental toxicity.
Careful comparison of the guideline developmental toxicity data for coumatetralyl against the classification criteria show:
- Criteria for classification for developmental toxicity are not met.
  - There is no evidence of coumatetralyl being causally associated with developmental toxicity in humans.
  - There is no evidence from acceptable GLP- and guideline-compliant studies, that coumatetralyl 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.

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

The CLH proposal to classify coumatetralyl for developmental toxicity follows the conclusion of the 2006 Specialised Experts (SE) meeting.
However, the SE Conclusion lacks a clear comparison of evidence with modern (DSD or CLP) criteria. The conclusion is not based on the most appropriate endpoint (malformation, not foetotoxicity which is more frequently seen in human pregnancy). 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) which has been demonstrated to be incorrect by a more recent OECD 414 study demonstrating developmental toxicity of warfarin. 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[2]**

The study, conducted in accordance with OECD 414, has been 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, 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 clear demonstration of ability of the standard "OECD 414" design to detect specific foetal sensitivity to warfarin and the AVKs.
For coumatetralyl, the teratogenicity study in rats examined developmental toxicity at clearly maternally toxic doses based on mortality. Rats were dosed from days 6 to 20 of pregnancy comparable to the "TP2" of the warfarin study; dose spacing was a factor of 2 with two dose levels showing maternal mortality. A further adequate study in rabbits also demonstrates 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**

For coumatetralyl, the CLH report offers a comparison with criteria which 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 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. The CLH report postulates that a study including Vitamin K supplementation might be meaningful, and that postnatal exposure (after Howe & Webster, 1994[4]) 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)[5], 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. Moreover, 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 developmental toxicity studies the skeletons were doublestained (Alizarin red S and Alcian blue) for a thorough assessment of skeletal development including both ossified and cartilaginous structures

Since the CLH report on coumatetralyl does not address the conflict of interpretation arising from the results of the CEFIC study of warfarin, an alternative comparison must be offered based on evidence as follows:

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

There is no epidemiological evidence that coumatetralyl 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 coumatetralyl.

Because the criterion for "sufficient epidemiologic evidence" is not met for coumatetralyl, 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 coumatetralyl 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 coumatetralyl is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies of coumatetralyl in both rats and rabbits. The method used to test coumatetralyl is appropriate and sufficient to detect developmental toxicity of warfarin.

Negative results in adequate animal studies of coumatetralyl 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 coumatetralyl 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 coumatetralyl is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits. The method used to test coumatetralyl is appropriate and sufficient to detect developmental toxicity of warfarin.

Negative results in adequate animal studies of the non-warfarin AVK rodenticide coumatetralyl are meaningful.

Concern is reduced in that warfarin as a therapeutic is administered to humans orally; biocidal operator exposure to rodenticides is dermal; and the skin presents a considerable and effective barrier to the AVK rodenticides.

Placement in DSD Category 3/ CLP Category 2 is not appropriate. No classification for developmental toxicity is appropriate.

**Conclusion**

Ample evidence is provided that the basis for a read-across from warfarin teratogenicity to coumatetralyl is not valid.

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

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

4 Howe AM & Webster WS (1994): Vitamin K – its essential role in craniofacial development. Australian

Dental Journal, **39**(2) 88-92.
5 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

This comment submitter, the consultant Eksponent International has included comments along the same lines above.

The Danish CA fundamentally disagrees that the new studies on warfarin demonstrate the the standard OECD 414 guideline is suitable for testing all coumarin-derived AvKs. The concern that the substances are structurally close to the human developmental toxicant warfarin, that they use the same mode of action for toxicity, and the fact that no other mechanism of action has been demonstrated for all the other AvKs than warfarin, should lead to classification as repr cat 1A; H 360D for coumatetralyl.

The text herunder is a replication of the comments to the first comment from Eksponent International (comment no 5):
*The Danish CA does not agree with the consultant to the CEFIC Rodenticides group Exponent International that the conclusion of the Specialised Experts that all anti-Vitamin K should be classified as Repr cat 1; R61 (DSD) are "no longer valid".*

*Obviously, the conclusion of the SE do not meet the format of a Annex XV dossier. However, this group of independent academic experts appointed by the Member States to advised the Commission working group on classification on carcinogenicity, genotoxicity and reproductive toxicity by giving expert guidance on how to interprete the toxicological data available for classification, using all the criteria laid down in directive 67/548/EC, general as well as specific criteria, for the end-point of reproductive toxicity.*

*Based on the all data available the Specialised Experts expressed concern that AvKs were developmental toxicant in humans, as they all use the same mode of action as the human teratogen warfarin. To date, no data have been tabled to demonstrate a different mechanism of action for developmental toxicity by warfarin compared to other coumarin-derived AvKs substances. Therefore, the concern that other AvKs using the same mechanism of action as warfarin also have a developmental toxicant effect is still valid.*

*The Danish CA disagrees that the new OECD 414 study(ies) on warfarin can be used as proof that this standard test protocol is able to detect developmental effects for any other anticoagulant. It appears from the warfarin OECD 414 studies that the window of exposure for effects on developmental seen in humans cannot be reproduced in the animal model. This is due to differences in the development intrautero of rat and human foetus. In the new OECD 414 studies with warfarin, it can be noted that the malformation effects seen with warfarin are not seen in the study with short exposure time, but only the prolonged exposure time. It is also noted that even for warfarin, which has been studied much more extensively that other coumarin derivates, the dose levels used in the new OECD 414 needed to be revised after the start of the study is order to acertain toxicity. Therefore, it appears that there are many elements challenging the suitability of the standard OECD 414 protocol, amongst other the window of exposure, the balance with vitamin K and dose levels in order to demonstrate an effect of warfarin.*
*The negative results of the standard OECD 414 for the other AVKs (as well as for previous studies with warfarin) may be due to differences in developmental toxicity potency between the AVKs, choices of doses, of treatment duration, of delopmental differences in time between rat and humans.*
*Therefore, the Danish CA still has strong concern that the OECD 414 in rodents is not suited*

*for the detection of developmental toxicity of coumarin-derived substances.*

*Classification shall be performed according to the rules of regulation 1272/2008. The comparison with the criteria by Eksponent should include consideration to the overall criteria given in the introduction to Annex I of regulation 1272/2008, and not only refer to the end-point specific criteria. In Annex I point 1.1.1.3, it is stated that all available information should be considered, amongst other grouping, read-across, and human experience.*
*Thus, the following information should be taken into account in the classification of the coumarin derived anticoagulants:*
*- Warfarin is a potent human reproductive toxicant, classified in category 1A. There is evidence that warfarin is associated with adverse effects on the human foetus.*
*- The AvK are coumarin-derivatives, which are structurally related to warfarin.*
*- There is evidence that couramin-derivatives use the same mode of action with respect of inhibition of vit K for their anticoagulant effect.*
*- No demonstration of the mechanism of action for developmental toxicity for warfarin is available.*
*- The mechanism of action for the developmental toxicity has been proposed for all coumarin-derivatives by the specialized experts also to be related to vitamin K inhibition.*
*- As no other mechanism of action has been proposed, explaining that only one of a structural similar group would odd out in this respect.*
*- The standard OECD 414 is not suited for testing coumarin-derivatives for developmental effects due to differences in developmental toxicity potency between the AVKs, challenge in the choices of doses, of treatment duration, and because of timing differences in the delopment of the rat and human foetus.*

*Based on the complete criteria for classification, classification of coumatetralyl should be based on read-across to warfarin, a potent human developmental toxicant, due to structural and mechanistic analogy consideration. To conclude, the Danish CA maintains its position that coumatetralyl should be classified as repr cat 1A.; H360D according to CLP.*

| RAC's response |
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| See the RAC response to comment number 4. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 19.04.2013 | France | | Consultant | 10 |
| Comment received | | | | |
| Teratogenicity of AVK Rodenticides: Classification by Read-Across from Warfarin is not Correct<br>The conclusion of the Specialised Experts ("SE Conclusion") that the classification of all anti-Vitamin<br>K (AVK) rodenticides as teratogens should be read-across from warfarin is no longer valid.<br>- The SE Conclusion is inadequate by modern standards, since it lacks a clear comparison of the data against the classification criteria.<br>- 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<br>- 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.

Dossier Submitter's Response

The Danish CA does not agree with the arguments put forward by this consultant to refuke the proposed classification for toxicity to reproduction as repr cat 1A; H360D. The comments from this consultant are similar to the those from another consultant.
The response from the Danish CA below is therefore repeated from the earlier comment (comment nr 5):

*The Danish CA does not agree that the conclusion of the Specialised Experts that all anti-Vitamin K should be classified as Repr cat 1; R61 (DSD) are "no longer valid".*

*Obviously, the conclusion of the SE do not meet the format of a Annex XV dossier. However, this group of independent academic experts appointed by the Member States to advised the Commission working group on classification on carcinogenicity, genotoxicity and reproductive toxicity by giving expert guidance on how to interprete the toxicological data available for classification, using all the criteria laid down in directive 67/548/EC, general as well as specific criteria, for the end-point of reproductive toxicity.*

*Based on the all data available the Specialised Experts expressed concern that AvKs were developmental toxicant in humans, as they all use the same mode of action as the human teratogen warfarin. To date, no data have been tabled to demonstrate a different mechanism of action for developmental toxicity by warfarin compared to other coumarin-derived AvKs substances. Therefore, the concern that other AvKs using the same mechanism of action as warfarin also have a developmental toxicant effect is still valid.*

*The Danish CA disagrees that the new OECD 414 study(ies) on warfarin can be used as proof that this standard test protocol is able to detect developmental effects for any other anticoagulant. It appears from the warfarin OECD 414 studies that the window of exposure*

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| *for effects on developmental seen in humans cannot be reproduced in the animal model. This is due to differences in the development intrautero of rat and human foetus. In the new OECD 414 studies with warfarin, it can be noted that the malformation effects seen with warfarin are not seen in the study with short exposure time, but only the prolonged exposure time. It is also noted that even for warfarin, which has been studied much more extensively that other coumarin derivates, the dose levels used in the new OECD 414 needed to be revised after the start of the study is order to acertain toxicity. Therefore, it appears that there are many elements challenging the suitability of the standard OECD 414 protocol, amongst other the window of exposure, the balance with vitamin K and dose levels in order to demonstrate an effect of warfarin.*<br>*The negative results of the standard OECD 414 for the other AVKs (as well as for previous studies with warfarin) may be due to differences in developmental toxicity potency between the AVKs, choices of doses, of treatment duration, of delopmental differences in time between rat and humans.*<br>*Therefore, the Danish CA still has strong concern that the OECD 414 in rodents is not suited for the detection of developmental toxicity of coumarin-derived substances.*<br><br>*Classification shall be performed according to the rules of regulation 1272/2008. The comparison with the criteria by Eksponent should include consideration to the overall criteria given in the introduction to Annex I of regulation 1272/2008, and not only refer to the end-point specific criteria. In Annex I point 1.1.1.3, it is stated that all available information should be considered, amongst other grouping, read-across, and human experience.*<br>*Thus, the following information should be taken into account in the classification of the coumarin derived anticoagulants:*<br>*- Warfarin is a potent human reproductive toxicant, classified in category 1A. There is evidence that warfarin is associated with adverse effects on the human foetus.*<br>*- The AvK are coumarin-derivatives, which are structurally related to warfarin.*<br>*- There is evidence that couramin-derivatives use the same mode of action with respect of inhibition of vit K for their anticoagulant effect.*<br>*- No demonstration of the mechanism of action for developmental toxicity for warfarin is available.*<br>*- The mechanism of action for the developmental toxicity has been proposed for all coumarin-derivatives by the specialized experts also to be related to vitamin K inhibition.*<br>*- As no other mechanism of action has been proposed, explaining that only one of a structural similar group would odd out in this respect.*<br>*- The standard OECD 414 is not suited for testing coumarin-derivatives for developmental effects due to differences in developmental toxicity potency between the AVKs, challenge in the choices of doses, of treatment duration, and because of timing differences in the delopment of the rat and human foetus.*<br><br>*Based on the complete criteria for classification, classification of coumatetralyl should be based on read-across to warfarin, a potent human developmental toxicant, due to structural and mechanistic analogy consideration. To conclude, the Danish CA maintains its position that coumatetralyl should be classified as repr cat 1A.; H360D according to CLP.* |
| RAC's response |
| See the RAC response to comment number 4. |

**OTHER HAZARDS AND ENDPOINTS – Acute Toxicity**

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

| | | | | |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 11 |
| Comment received | | | | |
| We support the proposal to classify coumatetralyl for Acute dermal and inhalation toxicity.<br>☐ For the dermal acute toxicity, due to the LD50 for rats is between 100 and 500 mg/kg for male and 258 mg/kg bw for females. We agree with the classification 3.<br>☐ For the inhalation acute toxicity, based on the LC50 in rats of 0.063 and 0.039 mg/l/4h and in mouse of 0.054 mg/l/4h, we agree with the classification 2. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the Belgian CA for the support to the proposed classifications for acute dermal and acute inhalation toxicity. | | | | |
| RAC's response | | | | |
| The support is noted. The RAC agrees with the proposal. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 12 |
| Comment received | | | | |
| - Acute toxicity : inhalation<br>Point 4.2, page 24. Could you replace the R25 in R26. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the French CA for noticing this error. The R25 on page 24, point 4.2 should read R26. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Skin Hazard**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | France | | MemberState | 13 |
| Comment received | | | | |
| - Conclusion on classification and labelling for irritative effect to the skin:<br>Point 4.2.1, page 25. Could you justify the H311. The lowest value of DL50 is 100 µg/kg b.w. for males that correspond to H310. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the French CA for allowing for clarification on the point of the classification for acute toxicity by the dermal route. Danish proposal for classification for acute dermal toxicity as H311 is based on the one acute dermal study reported in the CAR. (Bomann, W., 1992) with a LD 50 reported as 100<LD50<500 mg/kg bw in males and 258 mg/kg bw in females. 5 males per group were tested, and the number of lethalities were 0,0,4 and 5 males in the dose groups 50, 100, 500 and 2000 mg/kg bw. We agree with France that the true LD50 could be lower than the cut-off of 200 mg/kg bw/day. However, on balance, classification as H311 was considered more appropriate for this substance. | | | | |
| RAC's response | | | | |
| One dermal guideline study in rats gave $LD_{50}$ values of 258 mg/kg for females and 100-500 mg/kg for males. Three non-guideline studies (all three from the same author) gave much lower $LD_{50}$ values (5-62 mg/kg), but there are no study summaries available for the non-guideline studies in document IIIA of the CAR. Since no study summary is available to the RAC, it is difficult to make an | | | | |

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| independent assessment of the data. The CLH dossier bases the classification proposal on the guideline study, and the RAC has to assume that it is more reliable than the other studies. Thus, the LD50 value of 258 mg/kg lies within the range of 200-1000 mg/kg for category 3, indicating that Coumatetralyl should be classified for the dermal route with Acute Tox 3, H311. This classification was also agreed in the former TC C&L group. |

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

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 14 |
| Comment received | | | | |
| We agree with the classification STOT RE Cat.1, indeed the studies show significant and severe toxic effects (increase blood counting time, haemorrhage, labored breathing, ..) which are relevant for the human health and which are produced at low exposure concentration (≤ 10 mg/kg). We agree with the extrapolation of oral toxicity data to dermal and inhalation toxicity due to the mechanism of toxicity of coumatetralyl is independent of the route of exposure. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the Belgian CA for supporting the classification as STOT RE Cat1 and the extrapolation to other routes of exposure. Especially the effects on clotting time occur at very low dose (0.021 mg coumatetralyl/kg bw/day in males). Therefore, in addition to classification as STOR RE cat 1, SCL's are proposed.<br>Thank you for acknowledging that this classification should apply to all routes of exposure. | | | | |
| RAC's response | | | | |
| The support is noted. The RAC also supports STOT RE1. | | | | |

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

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | Belgium | | MemberState | 15 |
| Comment received | | | | |
| We support the proposed M-factor for chronic toxicity of 10 (most sensitive species Fish Oncorhynchus mykiss with 21dNOEC= 0.005mg/l) and toxicity band between 0.001 mg/l and 0.01 mg/l) | | | | |
| Dossier Submitter's Response | | | | |
| Thank you to the Belgian CA for supporting the classification for chronic toxicity including the chosen M factor, and the choice of Fish as the most sensitive animal test group. | | | | |
| RAC's response | | | | |
| Noted. See comment 16. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 18.04.2013 | United Kingdom | | MemberState | 16 |
| Comment received | | | | |
| Whilst the prolonged acute NOEC for fish 0.005 mg/L is not appropriate for chronic classification, a true chronic value may well be lower than this. Reliable chronic endpoints for algae and Daphnia (0.1 mg/L) are also available. We consider, therefore, that for a non-readily degradable substance the lowest Daphnia endpoint should be used - resulting in a | | | | |

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| CLP classification of 'Chronic 1' (M-factor 1). |
| Dossier Submitter's Response |
| The Danish CA does not agree with the conclusion of the UK CA.<br>As the substance is an anticoagulant fish are expected to be the most sensitive group and algae and crustacean less sensitive. Basing the M-factor on the crustacean chronic NOEC as proposed by the UK would clearly underestimate the M-factor.<br>As pointed out by the UK a true chronic NOEC or EC10 will in all probability be lower than 0.005 mg/l, because the given NOEC value is from a prolonged acute test and not a true chronic test. Therefore the choice in the Danish CLH report of an M-factor of 10 based on the prolonged acute test NOEC in fish is more likely to under-estimate the M-factor than the opposite.<br>On basis of the arguments above Denmark sticks to the original proposal of an M-factor of 10. |
| RAC's response |
| Normally the prolonged test cannot be used as a chronic test, however in this case, the 21d-NOEC (fish-mortality) is the most sensitive endpoints and although it is not a true chronic value, it indicates that the real chronic value may well be lower than this.<br>RAC agrees with the DS's proposal that classification for long-term aquatic hazards should be based on the NOEC of 0.005 mg/l, provided by the prolonged fish toxicity test (OECD TG 204) in Oncorhynchus mykiss. This study should be used as a decisive study since a true chronic toxicity study in fish is not available and since it shows highest toxicity among the reported NOEC values. Therefore, a classification as Aquatic Chronic 1 (H410) with an M-factor of 10 according to CLP.<br>However, if reliable chronic data for fish were to become available, it is possible that the classification might need to be reviewed. |

**ATTACHMENTS RECEIVED:**

Coumatetralyl, Comment on the CLH proposal, 5 March 2013 (File name: Coumatetralyl classification - developmental EWC0009), submitted on 19 April by Exponent International for CEFIC RDDG. *(ECHA's comment: additional information provided in the document copied under Toxicity to reproduction)*

Teratogenicity of AVK Rodenticides Classification by Read-Across from Warfarin is not Correct (File name: Read-across rebuttal EWC0008), submitted on 19 April by Exponent International for CEFIC RDDG. *(ECHA's comment: additional information provided in the document copied under Toxicity to reproduction)*

Comments on Annex XV dossiers proposing harmonised Classification & Labelling (File name: COM_CLH_PC_Coumatetralyl_SE), submitted on 19 April 2013 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)*

**CONFIDENTIAL ATTACHMENTS RECEIVED:**

Teratogenicity of AVK Rodenticides Classification by Read-Across from Warfarin is not Correct (File name: Read-across rebuttal EWC0008), submitted on 19 April 2013 by a company-manufacturer from France

Position paper on Coumatetralyl, Rebuttal of classification proposal in Reprotoxicity Category 1 (File name: Coumatetralyl_ ECHA_rebuttal_Final_18_04_2013), submitted on 19 April 2013 by a company-manufacturer from France

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1- naphthyl)coumarin**