Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Difethialone(ISO); 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one;

EC number: -
CAS number: 104653-34-1

CLH-O-0000003391-80-03/F

Adopted

14 March 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON DIFETHIALONE (ISO); 3-[3-(4'-BROMOBIPHENYL-4-YL)-1,2,3,4-TETRAHYDRONAPHTHALEN-1-YL]-4-HYDROXY-2H-1-BENZOTHIOPYRAN-2-ONE

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: difethialone (ISO); 3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydronaphthalen-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one
CAS number: 104653-34-1
EC number:
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	1
Comment received				
☐ Environmental hazards We agree with the current proposal for consideration by rac: CLP regulation: • Aquatic acute 1 ; • Aquatic chronic 1 ; • 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. (*ECHA note: The text below was provided as a separate attachment)* Pages 5 and 17: the term "no significant impurities" used in the CLH report is not clear. Impurities present in Difethialone are significant under the biocidal regulation as their content is higher than 0.1% but there are not relevant. Under REACH and CLP regulation, the term significant impurity is not define: impurities with a concentration higher than 1% should be specified, and relevant impurities shall always be specified irrespective of the concentration. Moreover in the LOEP, it is specified that there are no relevant impurities. It would be better to specify in the CLH report "no relevant impurities". --- *End of attachment* ---				
Dossier Submitter's Response				
Thank you for your agreement with the classification for environmental hazards. We agree that "no relevant impurities" should have been used in the CLH report rather than "no significant impurities".				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON DIFETHIALONE (ISO); 3-[3-(4'-BROMOBIPHENYL-4-YL)-1,2,3,4-TETRAHYDRONAPHTHALEN-1-YL]-4-HYDROXY-2H-1-BENZOTHIOPYRAN-2-ONE

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Denmark		MemberState	2

Comment received

Danish comments to the CLP report on difethialone

Denmark agrees with the classifications proposed by the Norwegian rapporteur for the end-points of acute and repeated dose toxicity for reproductive toxicity as well as for aquatic toxicity for difethialone.

With respect to classification for reproductive toxicity, toxicity for development, Denmark agrees with the proposed classification for difethialone of Repr cat 1; R61 (DSD)/Repro cat1A; 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. It should be emphasized that human foetuses seem to be much more vulnerable to vitamin K deficiency than rodent foetuses, leading the animal model to be insufficient for this group of substances.

New data including a new study according to OECD 414 on warfarin show some developmental effects in the rats, but it not able to detect all warfarin human embryopathy effects. Dosing interval is very close in the warfarin study, and the developmental effects were seen at a dose close to the maternally toxic dose. The highest dose in the difethialone rat developmental study was not toxic to the dams. Thus, an effect on the foetus could have been missed due to inadequate dosing. Therefore concern that studies performed according to the OECD 414 protocol are not adequate to show developmental effects of AvK's remains.

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 Norwegian dossier submitter for acute and aquatic toxicity for difethialone.

Dossier Submitter's Response

Thank you for your support.

It might be that we were a bit unclear in the CLH report for difethialone when it comes to the M-factor. We propose to set an M-factor of 100 for acute hazards on the basis of the lowest acute 48 hours EC50 of 4.4 µg/L for *Daphnia magna*. No chronic data are available apart from a 72 hour NOEC of 0.032 mg/L based on growth rate from an algal growth inhibition test with *Selenastrum capricornutum*. According to table 4.1.3 of the document "Guidance on Application of the CLP criteria" an M-factor of 1 should be set for chronic toxicity if a NOEC value is between 0.01 and 0.1 mg/L. According to the same guidance document, an M-factor derived for acute aquatic hazard classification should also be applied to the long-term aquatic hazard classification only in case where chronic data are not available. Therefore, the M-factor of 100 is only proposed for acute effects.

RAC's response

Health part.
Thank you for your comments.

RAC agrees with the proposed by DS classiciation for for the end-points of acute and repeated dose toxicity.

Regarding developmental toxicity based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (Repr 1A), the reproductive toxicity of Difethialone has been analyzed in detail.
It is acknowledged that the animal developmental toxicity studies on Warfarin are weakly positive and that the animal developmental toxicity studies on Difethialone are negative. However, in comparison with Warfarin, Difethialone and other 2nd generation AVKs have higher acute and repeated dose toxicity, steeper dose-response curves, and much longer half-lives in the exposed organisms, making the evaluation of developmental effects of all 2nd generation AVK rodenticides difficult. Thus, relatively low doses in repeated exposure during gestation lead to maternal toxicity and lethality which hinders the detection of developmental toxicity at higher doses.
As there are no data on the outcome of maternal exposure to Difethialone in humans, classification in cat 1A is not considered to be applicable for Difethialone.
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 Difethialone includes consideration of the total data base for the AVKs. A weight of evidence assessment resulted in the conclusion that Difethialone 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:

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

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

thus proposes classification with category Repr. 1B, H360 May damage the unborn child, i.e. "presumed human reproductive toxicant".

Regarding specific concentration limits for repeated dose toxicity both in relation in relation to CLP regulation 1272/2008 RAC also support a DS proposal of specific concentration limits calculated according to the Guidance on the Application of the CLP Criteria. SCLs should rounded down to the nearest preferred value (1, 2 or 5), results in a SCL of 0.02% for STOT RE 1 and SCL of 0.002% for STOT RE 2 (ECHA, 2009. Guidance on the Application of the CLP Criteria, section 3.9.2.6.)

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	3
Comment received				
Toxicokinetics : On the basis of presented data we agree with conclusions drawn by the Dossier Submitter regarding: fast and extensive absorption of difethialone after oral administration with liver being the main organ of substance accumulation and faecal excretion being an exclusive route of elimination of the substance in not metabolized form. We also think that sufficient proof has been presented on the similar hepatic kinetics of two diastereoisomers of difethialone.				
Dossier Submitter's Response				
Thank you for your agreement.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	France	LIPHATECH SAS	Company-Manufacturer	4
Comment received				
Our comments are about Developmental toxicity (section 4.11 of CLH report). As data owner, we do not support the CLH proposal, Difethialone should not be classified for developmental toxicity. We provide two statements from an Expert toxicologist to demonstrate that the basis for read-across for developmental toxicity from warfarin to Difethialone is invalid.				
Dossier Submitter's Response				
The weight of evidence justifies that a classification for difethialone and the other AVK rodenticides should be based on read across to the human teratogen Warfarin. Therefore, difethialone should be classified in regards to developmental toxicity as a reproductive toxicant in category 1 (DSD)/category 1A (CLP)(for details, see responses to comments number 7 and 8).				
RAC's response				
Thank you for comment. Regarding classification for developmental toxicity please see above under Comment number 2, because response regaring developmental toxicity classification that has been provided jointy under Comment number 2.				

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

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	5
Comment received				
In the presented studies (both in vitro and in vivo) no signs of the mutagenicity were found. Therefore, no classification for germ cell mutagenicity is required as suggested by the Dossier Submitter.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Agree. Thank you for cemments.				

TOXICITY TO REPRODUCTION

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 difethialone. 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 for your support.				
RAC's response				
Thank you for comments. RAC also is of the opinion that Difethialone should not be classified for fertility				

Regarding classification for developmental toxicity please see above under Comment number 2, because response regaring developmental toxicity classification that has been provided jointy under Comment number 2.

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

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	Exponent international, on behalf of CEFIC RDDG	Industry or trade association	7

Comment received

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

(ECHA note: The text below was provided as a separate attachment)

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

The conclusion of the Specialised Experts ("SE Conclusion") that the classification of all anti-Vitamin K (AVK) rodenticides as teratogens should be read-across from warfarin is no longer valid.
- The SE Conclusion is inadequate by modern standards, since it lacks a clear comparison of the data against the classification criteria.
- New data overturn a key consideration on which the SE Conclusion was based (i.e., doubt on the ability of the OECD 414 study design to detect AVK embryopathy). A new OECD 414 study of warfarin now demonstrates method sensitivity.
- The SE Conclusion was not based on the most appropriate endpoint, being concerned with teratogenicity when more recent epidemiological data show foetotoxicity in human pregnancies to be of greater incidence.
The CEFIC teratogenicity study of warfarin demonstrates developmental and foetotoxicity, and therefore confirms sensitivity of the OECD 414 study design. There is clear evidence of specific foetal sensitivity to haemorrhage; borderline evidence of an increase of small foetuses (10-day group only) in the absence of maternal toxicity, and adequate evidence of malformation. The incidences of foetal haemorrhage at the low dose demonstrates the ability of the OECD 414 study design to detect specific foetal sensitivity to warfarin, and therefore the same ability to detect specific foetal sensitivity to the AVKs.
The basis for read-across for developmental toxicity from warfarin to the non-warfarin AVK rodenticides, is therefore invalid.
Careful comparison of the guideline developmental toxicity data for each of the non-warfarin AVKs against the classification criteria therefore show:

- Criteria for classification as CLP Cat 1A are not met. There is no evidence that any of the non-warfarin AVK rodenticides are associated with adverse pregnancy outcomes in humans.
- Criteria for classification as CLP Cat 1B are not met. There is no "clear evidence", from valid GLP- and guideline- compliant studies, that any of the non-warfarin AVK rodenticides cause an adverse effect on development in animals. Indeed, with the multiplicity of good and reliable studies (for which validity of the model is demonstrated) there is strong evidence that they do not.
- Criteria for classification as CLP Cat 2 ("some evidence") are not met. There is no evidence from GLP- and guideline- compliant studies, that any of the non-warfarin AVK rodenticides cause an adverse effect on development in animals. Indeed, with the multiplicity of acceptable and reliable studies (for which validity of the model is demonstrated) there is strong evidence that they do not.
- No classification for developmental toxicity is therefore appropriate.

Introduction:

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

1. Review of the Specialised Experts Conclusion

a) The SE Conclusion is no longer adequate for modern purposes since it lacks a clear comparison with modern (DSD or CLP) criteria.
b) In addition, recent data amend some of the assumptions from which the conclusion is derived; in particular:
c) The OECD 414 study of warfarin demonstrates sensitivity of the method; it is therefore appropriate to base classification on the actual results achieved in OECD 414 teratogenicity studies with each of the AVKs.
d) Teratogenicity is not the most appropriate human or animal endpoint. It is unusual for teratology to occur in the complete absence of other toxicity. A more usual picture is that teratology occurs as a particularly notable feature, among a spectrum of other foetotoxic change. This would appear to be the clinical picture among the therapeutic AVKs including warfarin. A multicentre prospective clinical trial (Schaefer et al, 2006[3]) examined 666 pregnancies to mothers receiving anticoagulant treatment (with warfarin, phenprocoumon, acenocoumarol, fluindione, or phenindione); birth defects were rare but the more numerous findings were of foetotoxicity – prematurity, miscarriage, decreased mean gestational age at delivery, decreased mean birth weight of term infants. Embryotoxicity (of which the teratology would be only one factor) is more meaningful for protection of the foetus; and is identified in the CEFIC warfarin study. The epidemiology of therapeutic AVKs shows that among human pregnancies foetotoxicity is of higher incidence than teratogenicity; the OECD 414 study of warfarin predominantly shows foetotoxicity. The warfarin-related incidence of foetotoxicity in human pregnancies (as stillbirth, prematurity, small at term) is mentioned in a number of the CLH reports, without drawing appropriate parallels to the warfarin study.
e) The essential evaluation of animal developmental toxicity studies is to assess whether a chemical is able to produce adverse effects in the foetus of experimental animals and whether the foetus is directly affected and/or is more susceptible than the mother. It is not generally expected that the same effects occur across species. It is however generally accepted that if a chemical is able to produce adverse effects on embryos of experimental animals, it could be a hazard also for human embryos, independently of the specific features of the effect. In the case of the CEFIC study of warfarin, results show that the test was able to identify warfarin as a substance toxic for the conceptus, inducing embryofetal mortality, haemorrhages, and malformations i.e. cataract. It appears to be a reliable test to identify a risk for human foetuses.
f) A placental transfer study demonstrated that there was foetal exposure to both warfarin and flocoumafen (which may also be the case for the other AVKs). These data identify foetal

exposure in this study yet there is still a significant difference in the foetotoxic effects observed with warfarin compared to those observed with the other AVKs. For all of the nonwarfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies despite clear exposure.
g) It is unclear how maternal toxicity is taken into account in the classification process for the AVKs. From the Regulation, classification should address the foetus as an especially sensitive target for toxicity. All evidence of warfarin teratogenicity and foetotoxicity in humans is at levels of maternal 'toxicity' (i.e., therapeutic anticoagulation). Further, comments from at least one MS appear to use a potential concern of maternal Vitamin K depletion leading to the embryopathy, as a reason to discount arguments of the AVKs reaching the foetus. A mechanism dependant entirely on maternal toxicity is however justification to not classify.

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

The study is reviewed in the CLH proposal for warfarin, and for that reason a detailed description is not given here. The following observations are however offered:
The study carefully examines dose levels around the limit of maternal toxicity. This is important, since the dose-response curve for teratogenicity can be steep (Schardein, 2000[5]). This might be particularly so with the AVKs, since the dose-response for maternal toxicity is also particularly steep. The study also examines two different periods of exposure: days 6-15 of pregnancy ("TP1", corresponding to the pre-2001 OECD 414 guideline) and days 6-19 of pregnancy ("TP2", corresponding to the revised 2001 OECD 414 guideline).
The warfarin study provides clear evidence (for classification purposes) of specific foetal sensitivity to haemorrhage (i.e., foetal haemorrhage is a dose-related finding, found at the lowest dose level which was not maternally toxic, thus demonstrating detection of specific foetal sensitivity). Both exposure periods (10- and 14-day) were adequate to demonstrate foetotoxicity.
In the opinion of this reviewer, the study also showed: borderline evidence of an increase of small foetuses (10-day treatment group only) in the absence of maternal toxicity; and adequate evidence of malformation (cataract, which has been noted in human foetuses from mothers administered warfarin during pregnancy [Hall *et al*., 1980[6]]). Although this study examines dose levels very closely spaced in the maternally toxic range, the incidence of foetal haemorrhage at the low dose is clear demonstration of the ability of the standard "OECD 414" design to detect specific foetal sensitivity to warfarin and the AVKs.
In summary: the study showed maternotoxic effects primarily due to haemorrhages in different organs and mortality. The No Adverse Effect Level (NOAEL) for maternal toxicity was 0.125 mg/kg bw/day.
At the level of conceptus warfarin treatment induced:
- an increase of foetal mortality with a NOAEL of 0.150 mg/kg bw/day;
- a dose related increase of foetal haemorrhages even at the lowest dose tested of 0.125 mg/kg bw/day;
- central ocular cataract (typical malformation of warfarin embryopathy) even at the lowest dose tested of 0.125 mg/kg bw/day.

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

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

Most CLH proposals (March 2013) consider the results of the new OECD 414 study of warfarin, and available placental transfer data.
For all of the non-warfarin AVK rodenticides (with the possible exception of bromadiolone), the animal data are concluded to show no evidence of teratogenicity. In cases where classification is recommended, proposals therefore remain entirely based on the common position of read-across from warfarin.
Current proposals for reproductive classification from the seven non-warfarin AVK CLH proposals range from CLP 1A (4 substances), 1B (one), 2 (one) and no classification (one).
In the CLH report for brodifacoum, comparison with criteria is not considered (no entry).

For bromadiolone, the CLH report concludes teratogenicity in the rabbit, based on dissimilar findings in 3 foetuses at two dose levels. The evaluation however appears inconsistent within the CLH report (evaluated as "may constitute a possible risk" on p48, or "some effects" on p51, or "inconclusive" then "teratogenic" on p 53) and there is no evaluation of "strength" (the reader cannot determine if the evaluation constitutes "clear" or "some" animal evidence). This review notes that the findings fall within the range of spontaneous incidence and show no syndrome. There is no evident consideration of warfarin effects other than teratogenicity (i.e. foetotoxicity) or consideration of human foetotoxicity.

The CLH recommendation for chlorophacinone accepts the new data as adequate to not classify.

For coumatetralyl, the CLH report offers a comparison with criteria. The comparison states "*However, due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to coumatetralyl, no clear conclusion can be drawn from the standard guideline studies.*" This statement is inconsistent with the CEFIC warfarin study results; no explanation is offered as to how the studies of coumatetralyl might significantly differ from the warfarin study design. There is no discussion as to the relevance of foetoxicity in the warfarin study with respect to the human epidemiology. The CLH report postulates that a study including Vitamin K supplementation might be meaningful, and that post-natal exposure (after Howe & Webster, 1994[7]) might also be necessary; neither of which were features of the warfarin study design. It must be noted that the design of Howe & Webster (1992)[8], examining bone growth post-natally in rats, probably differs fundamentally from the process of embryonic cell death and remodeling that occurs during the period of major organogenesis and that is the target of teratogenicity studies. Further, in the teratogenicity studies with coumatetralyl, to overcome the fact that developing rodent fetus is typically evaluated at a time when ossification of the skeleton is incomplete (at gestation day 20 in the rat), the skeletons are double-stained (Alizarin red S and Alcian blue) for a thorough assessment of skeletal development including both ossified and cartilaginous structures.

The CLH report for difenacoum offers no comparison with criteria. The warfarin study is assessed as not having shown malformation using the typical TP1 dosing regimen. There is no consideration of the relevance of embryotoxicity in the warfarin study or in humans. Teratogenicity studies of difenacoum were considered not suitable for determination of teratogenicity, citing a need for postnatal exposure (after Howe & Webster, 1992).

The CLH report for difethialone offers a comparison with criteria. The comparison states: "*Due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to difethialone, no clear conclusion can be drawn from these studies*". This statement is inconsistent with the warfarin study results; no explanation is offered as to how the studies of difethialone might significantly differ from the warfarin study design. The difethialone rat study is also criticized for absence of maternal toxicity at the highest dose (50 µg/kg bw/day), with mortality having been observed only in a pilot study (at 70 µg/kg bw/day); this review notes the dose spacing to be within the range of the (effective) warfarin study. There is no discussion of the relevance of foetotoxicity as seen in the warfarin study and in humans.

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

It would therefore appear that none of the CLH reports address the significance of foetotoxicity, as seen in humans and in the rat study of warfarin; and therefore they all fail to address the most appropriate endpoint.

3. Comparison with Criteria

This review offers a detailed comparison with criteria, under the assumption that all of the nonwarfarin AVKs show a clear absence of developmental toxicity in animal studies (i.e. dismissing the bromadiolone interpretation as discussed earlier).

Classification should be based on evidence, not hypothesis.

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

There is no epidemiological evidence that the non-warfarin AVK rodenticides cause developmental toxicity in humans.

There is clear epidemiologic evidence that warfarin causes developmental toxicity in humans; and that other AVK anticoagulants used as therapeutics (which do not include the non-warfarin AVK

rodenticides) also cause developmental toxicity in humans. However, the criterion for "sufficient epidemiologic evidence" is not met for the non-warfarin AVK rodenticides.
There is evidence to support that, due to absence of effect in appropriately-sensitive teratogenicity studies, the non-warfarin AVK rodenticides are intrinsically different to warfarin.
Because the criterion for "sufficient epidemiologic evidence" is not met for the non-warfarin AVK rodenticides, classification into DSD Cat 1/ CLP Cat 1A is not appropriate.
With respect to DSD Cat 2/CLP Cat 1B:
There is no evidence that the non-warfarin AVK rodenticides cause developmental toxicity in animals.
There is a concern, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans. However, there is evidence that the non-warfarin AVK rodenticides are intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits.
Both warfarin and flocoumafen are seen to cross the placenta. Only warfarin induces clear anticoagulant and developmental effects in the foetus. In contrast, flocoumafen clearly does not.
Therefore, for all of the non-warfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies.
In the absence of relevant effect in animal studies, and with the demonstration of method sensitivity to warfarin, read-across of warfarin developmental toxicity to the other rodenticidal AVKs becomes a scientifically unjustified extrapolation.
Negative results in adequate studies of the AVK rodenticides are meaningful, and placement in DSD Category 2/ CLP Category 1B is not appropriate.
With respect to DSD Cat 3/ CLP Cat 2:
There is no evidence that the non-warfarin AVK rodenticides cause developmental toxicity in animals.
There is a concern, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans. However, there is evidence that the non-warfarin AVK rodenticides are intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits.
Both warfarin and flocoumafen are seen to cross the placenta. Only warfarin induces clear anticoagulant and developmental effects in the foetus. In contrast, flocoumafen clearly does not.
Therefore, for all of the non-warfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies.
In the absence of relevant effects in animal studies, and with the demonstration of method sensitivity to warfarin, read-across of warfarin developmental toxicity to the other rodenticidal AVKs becomes a scientifically unjustified extrapolation.
Negative results in adequate studies of the non-warfarin AVK rodenticides are meaningful.
Concern is reduced in that warfarin as a therapeutic is administered to humans orally; operator exposure to rodenticidal biocidal products is dermal; and the skin presents a considerable and effective barrier to the AVK rodenticides.
Placement in DSD Category 3/ CLP Category 2 is not appropriate.
By comparison of evidence with the criteria, no classification for developmental toxicity is appropriate.
In conclusion, ample evidence is provided that a read-across from warfarin teratogenicity to the nonwarfarin AVK rodenticides is not justified from a scientific point of view, based on the results of valid and good quality data. When compared with the criteria for classification, there is inadequate evidence for classification of the non-warfarin AVKs for developmental toxicity.
Simon Warren
18 April 2013

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[1] The CEFIC RDDG is comprised of the following companies: Activa, Babolna-Bio, BASF, Bayer, Bell Laboratories, Hentschke & Sawatzki KG, Laboratorios Agrochem, Liphatech, PelGar and Syngenta who each have joint ownership of this document

[2] Commission Working Group of Specialised Experts on Reproductive Toxicity. ECBI/121/06. Ispra, 19-20 September 2006

[3] Schaefer C, Hannemann D *et al* (2006) Vitamin K antagonists and pregnancy outcome. A multi-centre prospective study. Thromb.Haemost. 95(6) 949-57.

[4] Kubaszky R (2009) Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
[5] Schardein J (2000) Chemically induced birth defects. Third edition revised and expanded. Marcel Dekker: New York. ISBN: 0-8247-0265-4
[6] Hall *et al*. (1980). Maternal and fetal sequelae of anticoagulation during pregnancy. *Am J. Med.* 68: 122-140.
[7] Howe AM & Webster WS (1994): Vitamin K – its essential role in craniofacial development. Australian Dental Journal, **39**(2) 88-92.
[8] Howe AM & Webster WS (1992): The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances, Teratology, **46**(4) 379-90

----- *End of attachment* -----

Dossier Submitter's Response

Anticoagulant rodenticides of the coumarin-family share structural and mechanistic similarity with warfarin, which is a known human teratogen classified as Repr. Cat. 1; R61 (DSD)/Repr.1A; H360D (CLP). The most common consistent feature of the warfarin syndrome is a hypoplastic nose with a depressed or narrowed nasal bridge causing respiratory distress. In the new OECD 414 guideline study on warfarin (Kubaszky, 2009), cataracts and haemorrhages were found. In the TP1 study no clear dose-response relationship for cataracts were found (one finding at 0.200 mg/kg) whereas there was a dose related increase in cataracts in the TP2 study. Central cataract was according to the author, diagnosed in all affected eyes, except the single 0.125 mg/kg TP2 foetus. Hence, the statement "central ocular cataract (typical malformation of warfarin embryopathy) even at the lowest dose tested of 0.125 mg/kg bw/day" is not entirely correct. Other teratogenic effects which are observed in humans, like skull malformations, were not convincingly demonstrated in this rat study.

The OECD 414 guideline study (old and new) have limitations in detecting teratogenic effects observed in humans after exposure to warfarin;

i) due to the differences in development of the neonate rat and human; in the rat, mineralization of the skeleton starts about 5 days before birth, on embryonic day 17, with most bones showing ossification centers by birth. Ossification is largely complete by postnatal day 21. In the human, the developing skeleton starts to mineralize at about 6-9 weeks after gestation; so unlike the rat a major part of skeletal development takes place prenatally. Therefore, a postnatal dosing in the rat would be required in order to detect typical findings seen in human warfarin syndrome (nasal hypoplasia, which is observed in humans following exposure during 1st trimester of pregnancy).The observed hemorhages in the OECD 414 guideline warfarin study (Kubaszky, 2009) are probably similar to the effects observed during human exposure in the 2nd or 3rd trimesters;
ii) human foetuses seem to be much more vulnerable to vitamin K deficiency than rodent foetuses (Howe and Webster, 1994). In humans there is a 13 times difference in vitamin K level between mother and foetus, and this may explain the teratogenic effects observed in foetuses at dose levels without maternal toxicity. In contrast, the difference in vitamin K levels is only 2.5 between mother and foetus in the rat. Hence, the dose causing adverse effects in the foetus are most likely closer to the maternal lethal dose in rats than in humans, indicating that rats are not an appropriate model for AVK rodenticides;
iii) dosing interval is very narrow in the TP1 and TP2 warfarin study (Kubaszky, 2009) (4 dose levels within a factor of 2), and dose-response relationships and study findings are therefore difficult to evaluate/interprete;
iv) the absence of bleedings in the foetuses treated with the AVK rodenticides (with the exception of warfarin) may indicate that it is a very narrow margin between the effect dose for the conceptus and the maternally lethal dose. This should not

be used as an argument to state that effect on bone formation process is unlikely. The concern that the OECD 414 guideline is not suitable to detect human relevant developmental effects of AVK rodenticides remains;

Some effects on the foetus have been seen in OECD 414 studies performed for other AVK rodenticides. In a OECD 414 study on bromadiolone (Reference: A6.8.1, CLH report on bromadiolone), two rabbit foetuses with severe malformations and increased incidence of skeletal variations were reported (4 µg/kg) and one with hydrocephalus (8 µg/kg).

There is no evidence indicating that there are differences in the placenta barrier passage between the other AVK rodenticides and warfarin. A case reports on brodifacoum (Munday and Thomson, 2003) as well as a placental transfer study on flocoumafen (Johnson, 2009, CLH report on flocoumafen) demonstrate that AVK rodenticides cross the placenta barrier. Brodifacoum was found in the liver of two puppies with severe hemorrhages, without maternal effects.

In general all findings on developmental toxicity should be considered for classification purposes irrespective of the level of maternal toxicity. The observed developmental effects in foetuses exposed to warfarin are severe and are due to specific effects; i.e. inhibition of the vitamin K (epoxide) reductase complex; blocking the regeneration of vitamin K (vitamin K hydroquinone). Vitamin K seems to cross placenta, and the maternal and foetal levels follow each other.

According to Annex 1, point 3.7.2.4 of the CLP regulation, developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated that the effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring e.g. irreversible effects as structural malformations. As for AVK rodenticides (see ii), the dose which might cause adverse effects in the foetus are most likely closer to the maternal lethal dose in rats than in humans. Hence, if effects relevant for man are to be detected in animal studies maternal toxicity is probably unavoidable.

Annex 1, point 1.1.1.3 of the CLP regulation supports a weight of evidence evaluation approach, and the available data shows that the MoA and the toxicity profile of the AVK rodenticides (incl. warfarin) are very similar.

AVK rodenticides show absence of clear developmental toxicity in (most) animal studies, and there is no epidemiological evidence that the non-warfarin AVK rodenticides cause developmental toxicity in humans. One important difference between warfarin and the other AVK rodenticides is that only warfarin is used therapeutically. Thus, the lack of epidemiological evidence for the AVK rodenticides (compared to warfarin) should not be overinterpreted. Furthermore, as shown above, the OECD 414 protocol has limitations in detecting relevant developmental effects.

Therefore, since the AVK rodenticides have the same chemically active groups, shows structural similarities, have the same well-known mode of action by which warfarin causes teratogenicity in humans and in experimental animals, and since there is no evidence that the AVK rodenticides do not cross the placenta barrier, classification of all AVK rodenticides for developmental toxicity with Repr. Cat. 1; R61 (Directive 67/548/EEC) and Repr. 1A H360D (Regulation EC 1272/2008) is warranted based on read across to the human teratogen warfarin.

Howe AM & Webster WS (1994).Vitamin K – its essential role in craniofacial development. AustralianDental Journal, 39(2) 88-92.
Kubaszky R (2009). Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
Munday, J. S. and Thompson, L. J. (2003). Brodifacoum toxicosis in two neonatal puppies. Vet. Pathol. 40:216-219.

RAC's response

Thank you for comments.

Regarding classification for developmental toxicity please see above under Comment number 2, because response regaring developmental toxicity classification that has been provided jointy under Comment number 2.

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

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	Exponent International on behalf of CEFIC RDDG	Industry or trade association	8

Comment received

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

(ECHA note: The text below was provided as a separate attachment)

Difethialone
Comment on the CLH proposal, 5 March 2013
Developmental toxicity:
Difethialone should ***not be classified*** for developmental toxicity.
Careful comparison of the guideline developmental toxicity data for difethialone against the classification criteria show:
- Criteria for classification for developmental toxicity are not met.
o There is no evidence of difethialone being causally associated with developmental toxicity in humans.
o There is no evidence from acceptable GLP- and guideline-compliant studies, that difethialone causes an adverse effect on development in animals.
o 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 difethialone 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 is proven 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 is reviewed in the CLH proposal for warfarin, and for that reason a detailed description is not given here. The following observations are however offered:
The study carefully examines dose levels around the limit of maternal toxicity. This is important, since the dose-response curve for teratogenicity can be steep (Schardein, 2000[3]). This might be particularly so with the AVKs, since the dose-response for maternal toxicity is also particularly steep. The study also examines two different periods of exposure: days 6-15 of pregnancy ("TP1", corresponding to the pre-2001 OECD 414 guideline) and days 6-19 of pregnancy ("TP2", corresponding to the revised 2001 OECD 414 guideline).
The warfarin study provides clear evidence (for classification purposes) of specific foetal sensitivity to haemorrhage (i.e., foetal haemorrhage is a dose-related finding, 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 difethialone, the pilot teratogenicity study in rats examined developmental toxicity at a clearly maternally toxic dose based on mortality; the main study was conducted at a dose 30% lower, within the range of sensitivity shown by the warfarin study. Neither study showed evidence of foetotoxicity. Further adequate studies in rabbit also demonstrate absence of developmental toxicity. There was therefore no evidence of foetotoxicity, in studies closely comparable in design to the effective study of warfarin.

3. Comparison with Criteria

The CLH report for difethialone offers a comparison with criteria which 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 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. The CLH report also suggests (p50) that greater potency than warfarin indicates a steeper dose-response curve, which is not correct: greater potency implies only that the dose-response curve is shifted to lower concentrations with no inference on slope. There is no discussion of the relevance of foetotoxicity as seen in the warfarin study and in humans.
Since the CLH discussion does not adequately address the implications of foetotoxicity seen in the warfarin study, 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 difethialone 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 difethialone.
Because the criterion for "sufficient epidemiologic evidence" is not met for difethialone, 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 difethialone 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 difethialone is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies of difethialone in both rats and rabbits. The method used to test difethialone is appropriate and sufficient to detect developmental toxicity of warfarin.

Negative results in adequate studies of difethialone 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 difethialone 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 difethialone is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits. The method used to test difethialone is appropriate and sufficient to detect developmental toxicity of warfarin.
Negative results in adequate studies of the non-warfarin AVK rodenticide difethialone are meaningful.
Concern is reduced in that warfarin as a therapeutic is administered to humans orally; biocidal exposure to rodenticides is dermal; and the skin presents a considerable and effective barrier to the AVK rodenticides.
Placement in DSD Category 3/ CLP Category 2 is not appropriate. No classification for developmental toxicity is appropriate.

Conclusion

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

Simon Warren *DABT DIBT DipRCPath*
18 April 2013

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

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Dossier Submitter's Response

Difethialone and warfarin have a similar chemical structure resembling vitamin K. Both substances inhibit the vitamin K (epoxide) reductase complex which mainly results in effects on coagulation and bone formation. The same mechanism of action is also considered relevant for the developmental effects of warfarin in humans and rats. Based on this, it is likely that also difethialone would induce similar developmental effects as warfarin if the foetuses were exposed at relevant concentrations and appropriate exposure windows.

The most common consistent feature of the warfarin syndrome is a hypoplastic nose with a depressed or narrowed nasal bridge causing respiratory distress. In the new OECD 414 guideline study on warfarin (Kubaszky, 2009) cataracts and haemorrhages were found. In the TP1 study no clear dose-response relationship for cataracts were found (one finding at 0.200 mg/kg), whereas there was a dose related increase in cataracts in the TP2 study. Other teratogenic effects which are observed in humans, like skull malformations, were not convincingly demonstrated in this rat study.

The OECD 414 guideline study (old and new) have limitations in detecting teratogenic effects observed in humans after exposure to warfarin;

i) due to the differences in development of the neonate rat and human; in the rat,

mineralization of the skeleton starts about 5 days before birth, on embryonic day 17, with most bones showing ossification centers by birth. Ossification is largely complete by postnatal day 21. In the human, the developing skeleton starts to mineralize at about 6-9 weeks after gestation; so unlike the rat, a major part of skeletal development takes place prenatally. Therefore, a postnatal dosing in the rat would be required in order to detect typical findings seen in human warfarin syndrome (nasal hypoplasia, which is observed in humans following exposure during 1st trimester of pregnancy).The observed hemorhages in the OECD 414 guideline warfarin study (Kubaszky, 2009) are probably similar to the effects observed during human exposure in the 2nd or 3rd trimesters;

ii) the human foetus is more vulnerable towards vitamin-K deficiency dependent toxicity than the rat foetus. This may explain why the toxicity in human foetus is observed at doses not toxic to the mother, while in the animal studies it is difficult to observe the developmental effects in the foetus because of maternal lethality. The doses of coumarins possibly causing effect in the rodent foetus are most likely close to the maternal lethal dose, and to detect the relevant dose seems to be a challenge. This difference between rats and humans is important to have in mind when evaluating developmental effects of coumarins in animal studies;

iii) dosing interval is very narrow in the TP1 and TP2 warfarin study (4 dose levels within a factor of 2) and dose-response relationships and study findings are therefore difficult to evaluate/interprete;

The highest dose administrated (50 µg/kg bw/day) in the difethialone rat study did not show any maternal toxicity. Mortality was observed in a pilot study at 70 µg/kg bw/day. The main difethialone study is therefore inadequate due to lack of a relevant dose range; the study should have included dose intervals between 50 and 70 µg/kg bw/day. This is the reason why the study has been critizised. We can not exclude the possibility of observing developmental effects if the dosing of difethialone was closer to doses resulting in maternal toxicity.

Some effects on the foetus were seen in OECD 414 studies performed for the other AVK rodenticides. In a OECD 414 study on bromadiolone (Ref. A6.8.1, CLH report on bromadiolone), two rabbit foetuses with severe malformations and increased incidence of skeletal variations were reported (4 µg/kg) and one with hydrocephalus (high dose group, 8 µg/kg).

There is no evidence indicating that there are differences in the placenta barrier passage between the other AVK rodenticides and warfarin. A case reports on brodifacoum (Munday and Thomson, 2003) as well as a placental transfer study on flocoumafen (Johnson, 2009, CLH report on flocoumafen) demonstrate that AVKs cross the placenta barrier. Brodifacoum was found in the liver of two puppies with severe hemorrhages, without maternal effects.

Difethialone (and AVK rodenticides) exposure leads to specific effects (as described above). Vitamin K seems to cross placenta, and the maternal and foetal levels follow each other. According to Annex 1, point 3.7.2.4 of the CLP regulation, developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated that the effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring e.g. irreversible effects as structural malformations. As for AVK rodenticides the dose which might cause adverse effects in the foetus are most likely closer to the maternal lethal dose in rats than in humans. Hence, if effects relevant for man are to be detected in animal studies maternal toxicity is probably unavoidable.

Annex 1, point 1.1.1.3 of the CLP regulation supports a weight of evidence evaluation approach, and the available data shows that the MoA and the toxicity profile of the AVK rodenticides (incl. warfarin) are very similar.

Difethialone did not cause any observed developmental effects in the experimental animal studies. However, as described above, the OECD 414 protocol has limitations in detecting relevant developmental effects. There is no epidemiological evidence for developmental toxicity in humans for difethialone. One important difference between warfarin and difethialone is that difethailone is not used therapeutically. Thus, the lack of epidemiological evidence for difethialone should not be overinterpreted.

Since difethialone has the same chemically active group, shows structural similarity and has the same well-known mode of action by which warfarin causes teratogenicity in humans and in experimental animals and there is no evidence indicating that difethialone does not cross the placenta barrier, classification of difethialone for developmental toxicity with Repr. Cat. 1; R61 (Directive 67/548/EEC) and Repr. 1A H360D (Regulation EC 1272/2008) based on read across to the human teratogen, warfarin is warranted.

Kubaszky R (2009). Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.
Munday, J. S. and Thompson, L. J. (2003). Brodifacoum toxicosis in two neonatal puppies. Vet. Pathol. 40:216-219.

RAC's response

Thank you for comments.

Regarding classification for developmental toxicity please see above under Comment number 2, because response regaring developmental toxicity classification that has been provided jointy under Comment number 2.

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

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

Comment received

In the experimental animal studies presented in the Dossier no clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects was shown.

This could be related with 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. Moreover, 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. Since difethialone has the same chemically active group and the same well-known mode of action by which warfarin causes teratogenicity in humans and in experimental animals (through vitamin K hydroquinone deficiency) and 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, should be considered. Potential developmental effects of difethialone would be expected at very low doses, and the possibility of setting specific concentration limits for reprotoxicity should therefore be explored.

Dossier Submitter's Response

Thank you for your support.

RAC's response

Thank you for comments.

Regarding classification for developmental toxicity please see above under Comment number 2, because response regaring developmental toxicity classification that has been provided jointy under Comment number 2.

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

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	France	LIPHATECH SAS	Company-Manufacturer	10

Comment received

The section concerned is 4.11 in the CHL report.
The classification is based on a read-across from warfarin teratogenicity. But as demonstrated in the enclosed statements from the Expert toxicologist, the basis for a read-across from warfarin teratogenicity to Difethialone is not valid.
When compared with the criteria for classification, Difethialone should not be classified for developmental toxicity.

(*ECHA note: Two attachments were provided and they are copied under comment 7 and 8)*

Dossier Submitter's Response

The weight of evidence justifies that a classification for difethialone and the other AVK rodenticides should be based on read across to the human teratogen Warfarin. Therefore, difethialone should be classified in regards to its developmental toxicity as a reproductive toxicant in category 1 (DSD)/category 1A (CLP), (for details, see responses to comment

number 7 and 8).

RAC's response

Thank you for comments.

Regarding classification for developmental toxicity please see above a response under Comment number 2, because response regaring developmental toxicity classification has been provided jointy under Comment number 2.

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

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	11

Comment received

SCL for reprotoxicity should be harmonized with warfarin.

Dossier Submitter's Response

Potential developmental effects of difethialone would be expected at very low doses, and the possibility of setting specific concentration limits for reprotoxicity should therefore be considered. In the CLH report for difethialone, no definite proposal was made. A common approach should be taken for all AVK rodenticides when a decision has been made on the classification for reprotoxicty.

RAC's response

Classification to category Repr. 1B for developmental toxicity for Difethialone is supported by the RAC.
However, only for warfarin is there sufficient data to set a SCL for developmental toxicity. Thus, based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg/day) may cause developmental toxicity and could perhaps be regarded as an ED10 level. This human ED10 value would, if using the guidance for setting SCLs based on animal data, belong to the high potency group (<4 mg/kg/day). The guidance states that for an ED10 <4 mg/kg/day, the SCL is 0.03%, and for ED10 below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from an ED10 value obtained from animal studies (0.125 mg/kg/day; Kubaszky et al 2009), it would qualify warfarin for the high potency group and result in a SCL of 0.003%. Thus, the RAC is concluding on a SCL on 0.003% for the developmental toxicity of warfarin.
As the other AVK rodenticides are equally or more toxic than warfarin, it is not considered appropriate to apply the generic concentration limit for these substances (0.3%), but rather to base the SCLs on the SCL proposed for Warfarin. Thus, the RAC is of the opinion that the SCL for Warfarin can be used as a surrogate SCL for the other AVK rodenticides, resulting in a SCL of 0.003% for all AVK rodenticides, including Difethialone.

Date	Country	Organisation	Type of Organisation	Comment number
19/04/2013	Sweden		MemberState	12

Comment received

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

The Swedish CA supports the classification proposal for difethialone regarding reproductive toxicity. We support that the classification for difethialone (as well as for the other AVK rodenticides) should be based on read across to human data for Warfarin (i.e warfarin embryopathy). Therefore, difethialone 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 hypoplasia1). 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 Webster2 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 mother3. 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 is provided as a separate attachment to this comments table

Dossier Submitter's Response

Thank you for your support.

RAC's response

Thank you for comments.

Regarding classification for developmental toxicity please see above a response under Comment number 2, because response regaring developmental toxicity classification has been provided jointy under Comment number 2.

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

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	13

Comment received

p6: 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

The same approach as used for difenacoum, was in fact used.
When setting specific concentration limits (SCLs) according to Directive 67/548/EEC, a comparison was made of cut off values for classification and effect levels, with a resulting reduction of the general concentration limits (GCLs) defined in the Dangerous Preparation Directive (Directive 99/45/EC). To avoid too many and narrow SCLs, the number of SCLs was reduced by clustering narrow SCLs (e.g. by using the existing SCLs for environmental

effects also for health effects instead of introducing additional concentration limits of comparable size). Specific concentration limits for difethialone for acute and repeated dose toxicity were agreed upon as proposed at the TC C&L Meeting in May 2007. As for classification according to CLP, the methodology specified in the CLP guidance for setting SCLs for STOT-RE was used (section 3.9.2.6).
RAC's response
Thank you for comments. SCLs for acute toxicity is not applicable under CLP. SCLs derivation for STOT RE for various AVKs has be harmonised based on the Guidance on the Application of the CLP Criteria.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	14
Comment received				
We support following classification of imidazole: Acute Tox. 1: H300: Fatal if swallowed, H330: Fatal if inhaled and H310: Fatal in contact with skin				
Dossier Submitter's Response				
Thank you for your support for the classification (of difethialone).				
RAC's response				
Agreed. Thank you for comment.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	15
Comment received				
Considering presented results and the CLP ECHA Guideline criteria, we support conclusion of non-classification of difethialone as Skin Irritant 2				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Agreed. Thank you for comment.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	16
Comment received				
We agree on the non-classification of difethialone as Eye irritant 2. Proposed supplemental hazard information EUH070: Toxic by eye contact is nevertheless supported.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Agreed. Thank you for comment.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	17
Comment received				
Taking into account that no data on the specific target organ toxicity investigation were presented, it can be concluded that the classification as STOT SE is not possible.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Agreed. Thank you for comment.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	18
Comment received				
Difethialone classification as STOT RE 1 as well as set specific concentration limits for STOT RE (STOT RE 1 H372 above 0.02% and STOT RE 2 H373 between 0.002% and 0.02%) are supported by us.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Agreed. Thank you for comment. RAC support proposal of specific concentration limits calculated by the DS according to the Guidance on the Application of the CLP Criteria. SCLs should rounded down to the nearest preferred value (1, 2 or 5), results in a SCL of 0.02% for STOT RE 1 and SCL of 0.002% for STOT RE 2 (ECHA, 2009. Guidance on the Application of the CLP Criteria, section 3.9.2.6.)				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Finland		MemberState	19
Comment received				
We support the proposed classification: Aquatic Acute 1; H400, M-factor of 100 and Aquatic Chronic 1; H410, M-factor 100 for difethialone. Degradation and bioaccumulation potential: We agree with the conclusions that difethialone is not rapidly degradable and that it is assumed to bioaccumulate in aquatic organisms. Aquatic toxicity: Page 56: Reference is made to another fish test with analytical measurements and recovery rates. However, the reference details of this study are missing.				
Dossier Submitter's Response				
Please, see our answer to comment 2 from DK.				

The reference is Kelly, C.R. and Paterson, K. (2004). [^{14}C] difethialone: Determination of acute toxicity (LC50) to rainbow trout (96 h, semi-static). Inveresk Research, laboratory report number 23461, 12 March 2004 (unpublished). [Doc III A7.4.1.1-01 in the CA-report on difethialone].
RAC's response

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	20
Comment received				
We agree with the current proposal for consideration by RAC. CLP regulation: • Aquatic acute 1 ; • Aquatic chronic 1 ; • H400 – very toxic to aquatic life; • H410 – very toxic to aquatic life with long lasting effects. DSD: N; R50-53 – very toxic to organisms, may cause long-term adverse effects in the aquatic environment. Nevertheless, we need some clarification: In section 5.7, it is indicated that "Under the CLP regulation, considering the 2nd ATP criteria, this classification is accordingly - "Aquatic Acute 1; H400, Aquatic Chronic 1; H410" with a M factor of 100" This M factor corresponds to acute classification. No indication about the derivation of the same M factor for chronic classification is mentioned. Therefore, could you please add a precision about the M factor value and also add more information about its derivation.				
Dossier Submitter's Response				
Please, see our answer to comment 2 from DK.				
RAC's response				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	21
Comment received				
We support the proposed M-factor for acute toxicity of 100 (most sensitive species Daphnia magna with 48hEC50 = 0.0044mg/l) with toxicity band between 0.001 mg/l and 0.01 mg/l), as well as with the proposed SCLs : N, R50/53 C≥0.25% N, R51/53 0.025%≤C<0.25% R52/53 0.0025%≤C<0.025% Based on the most stringent outcome for Aquatic Chronic toxicity , on the basis of the Algae 72hNOErC=0.0321 mg/l and the LC50 for the other trophic levels with most sensitive species Daphnia magna : 48hEC50 = 0.0044mg/l) an M-factor for chronic toxicity of 100 could be assigned.				

Dossier Submitter's Response
Please, see our answer to comment 2 from DK.
RAC's response

ATTACHMENTS RECEIVED:

1. **Classification and labelling of dangerous substances - French comments on Difethialone (CAS 104653-34-1)** (File name: Com_Difethialone_CONF_PC), submitted on 18/04/2013 by France. (*ECHA note: This attachment has been copied under the section General Comments)*
2. **Comments on Annex XV dossiers proposing harmonised Classification & Labelling** (File name: COM_CLH_PC_Difethialone_SE), submitted on 19/04/2013 by Sweden (*ECHA note: This attachment has been copied under Toxicity to Reproduction, with the exception of Table 1. Physicochemical properties and mammalian toxicity summarized from the hydroxyl coumarin AVK dossiers, substances organized according to molecular weight*)
3. **Difethialone - Comment on the CLH proposal, 5 March 2013** (File name: Difethialone classification - developmental EWC0009), submitted on 19/04/2013 by Exponent International on behalf of CEFIC RDDG and by LIPHATECH SAS. (*ECHA note: This attachment has been copied under the section Toxicity to Reproduction*)
4. **Teratogenicity of AVK Rodenticides - Classification by Read-Across from Warfarin is not Correct** (File name: Read-across rebuttal EWC0008), submitted on 19/04/2013 by Exponent international, on behalf of CEFIC RDDG and by LIPHATECH SAS. (*ECHA note: This attachment has been copied under the section Toxicity to Reproduction*)