Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Bromadiolone (ISO); 3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one

EC number: 249-205-9
CAS Number: 28772-56-7

CLH-O-0000005446-71-01/F

Adopted

14 March 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON BROMADIOLONE (ISO); 3-[3-(4'-BROMOBIPHENYL-4-YL)-3-HYDROXY-1-PHENYLPROPYL]-4-HYDROXY-2H-CHROMEN-2-ONE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Substance name: bromadiolone (ISO); 3-[3-(4'-bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-chromen-2-one
CAS number: 28772-56-7
EC number: 249-205-9
Dossier submitter: Sweden

RCOM summary - For a quick overview of the most commented issues in the public consultation on bromadiolone

The RCOM-document for bromadiolone is extensive and many of the comments are similar in nature. Therefore, the dossier submitter has assembled this summary to highlight the most common comments, while providing a quick overview of our responses. Most of the comments were with regard to read-across to human data for warfarin (reproductive toxicity). Six member state comments agreed with the proposal for read-across whereas most of the comments against were repeat of the company manufacturers position that read-across to human data for warfarin for reproductive classification is not scientifically justified. This comment was provided by 6 company manufacturers and one industry-trade association.

<u>Comments challeging read-across to warfarin for reproductive toxicity:</u>
Dossier Submitter's Response: The dossier submitter does not agree that read-across is scientifically unjustified. Method sensitivity of OECD 414 have not been shown with the new warfarin study and the non-warfarin AVK rodenticides are intrinsically similar, biologically and toxicologically, to warfarin. The current CLP criteria, annex 1, point 1.1.1.3 of the CLP regulation supports a weight of evidence evaluation of the available data. The available data shows that the physicochemical properties and the mammalian toxicity profile of all the 2nd generation AVK rodenticides and warfarin 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. The evidence for classification is thus the clear human data for warfarin, justified by the similarity in pharmacology and toxicology for bromadiolone, the other AVKs and Warfarin as a group. Classification regarding developmental toxicity for bromadiolone as reproductive toxicants in category 1A is thereby warranted and based on evidence.

The new warfarin study does not invalidate a read-across for bromadiolone to the human teratogenicity data for warfarin:

- The new warfarin study does not substantiate method sensitivity of the 414 protocol to capture AVK teratogenicity/embryo toxicity of warfarin. I.e. there were no clear evidence of foetal sensitivity to haemorrhage, embryotoxicity in the form of small foetuses, adequate evidence of malformations or general foetal toxicity in the form of mortality in the new warfarin study. Absence of clear effects in the bromadiolone OECD 414 data can therefore not be interpreted as absence of potential of bromadiolone to cause teratogenicity (especially since the only rat study for bromadiolone was performed with the shorter dosing protocol).
- The new warfarin study does not substantiate that there are inherent pharmacological/toxicological differences between bromadiolone and warfarin.

- The modern CLP regulation allows for weight of evidence assessment and read-across to other existing data.

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.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: bromadiolone (ISO); 3-[3-(4'-bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-chromen-2-one
CAS number: 28772-56-7
EC number: 249-205-9
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Spain	Laboratorios Agrochem S.L.	Company-Manufacturer	1
Comment received				
In our view, the read-across from Warfarin teratogenicity to Bromadiolone is not justified from a scientific point of view, based on the results of valid and good quality data.				
Dossier Submitter's Response				
Thank you for your comment. The dossier submitter does not agree that read-across is scientifically unjustified. Method sensitivity of OECD 414 have not been shown and the non-warfarin AVK rodenticides are intrinsically similar, biologically and toxicologically, to warfarin. See response to comment 11 for a detailed explanations.				
RAC's response				
Thank you for the comment. Based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (Repro Cat 1A), the reproductive toxicity of Bromadiolone has been analysed in detail. It is acknowledged that the animal developmental toxicity studies on Warfarin are weakly positive and that the animal developmental toxicity studies on Bromadiolone are negative. However, in comparison with Warfarin, Bromadiolone 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 Bromadiolone in humans, classification in cat 1A is not considered to be applicable for Bromadiolone. 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 Bromadiolone includes consideration of the total data base for the AVKs. A weight of evidence assessment resulted				

in the conclusion that Bromadiolone 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:

- Bromadiolone 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, even 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 Bromadiolone, 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
18.04.2013	Hungary	Babolna Bio Ltd.	Company-Manufacturer	2
Comment received				
Submitting a position paper: Teratogenicity of AVK Rodenticides Classification by Read-Across from Warfarin is not Correct (*ECHA note: The attachment provided is copied under Comment 11)*				
Dossier Submitter's Response				
Thank you for your comment. Please see response to comment 11 for a detailed explanation to why the dossier submitter disagrees with the position provided.				
RAC's response				
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Italy	Activa s.r.l	Company-Manufacturer	3
Comment received				
we support the documents uploaded (*ECHA note: The attachments provided are copied under Comment 11 and 16)*				
Dossier Submitter's Response				
Thank you for your comment. Please, see response to comment 11 and 16 for a detailed explanation to why the dossier submitter disagrees with the positions.				
RAC's response				
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Denmark		MemberState	4
Comment received				
Danish comments to the CLP report on bromadiolone: Denmark agrees with the classifications proposed by the Swedish rapporteur for the end-points of acute and repeated dose toxicity for reproductive toxicity as well as for aquatic toxicity for bromadiolone. With respect to classification for reproductive toxicity, toxicity for development, Denmark agrees with the proposed classification for bromadiolon 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 mechanistical similarity with warfarin, which is a known human teratogen classified as Repr. Cat 1; R61 (DSD) and recognising that OECD 414 guideline has limitations as to showing the teratogenic effects seen in humans of anticoagulant rodenticides. Denmark agrees with the Swedish dossier submitter that the newer studies on warfarin and flocoumafen do not alter this position. A new study according to OECD 414 on warfarin, includes an extra high dose group added some time after the beginning of the study. The time shift makes it difficult to fully include this dose group in the assessment of the study outcome. Also the study, although showing some developmental effects in the rats, does not mirror the embryopathy-picture seen in humans. Due to the differences in development of the neonate rat and human, dosing of the rat postnatally would be required in order for one of the human effects of warfarin, nasal hypoplasia, to be detected. Therefore, the concern that the OECD 414 protocol is not adequate to show developmental effects of AvKs remains.				

New studies of plasma levels and placenta transfer in the rat with warfarin and flocoumafen indicate some placental transfer of flocoumafen and lower plasma levels in the foetus for flocoumafen than for warfarin pointing at differences in the plasma levels between the different AvK-substances. However, it is not possible to extrapolate this information to bromadiolon. Therefore, this new information does not counter the proposed read-across to warfarin. In conclusion, the Danish CA therefore still supports that read-across to the known developmental toxicant warfarin should be applied and that bromadiolon, as all AvKs should be classified as Repr cat 1; R61 (DSD)/Repro cat1A; H360D (CLP). Denmark supports the proposed specific concentration limits for acute and repeated dose toxicity both in relation to directive 67/458/EC and, for repeated dose toxicity, in relation to CLP regulation 1272/2008 and the M-factors proposed for acute and aquatic toxicity.
Dossier Submitter's Response
Thank you for your comment.
RAC's response
Thank you for the comment. The RAC is also of the opinion that Bromadiolone should be classified for developmental toxicity. For all evaluated AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" is proposed (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Spain	Laboratorios Agrochem S.L.	Company-Manufacturer	5
Comment received				
In our view, the read-across from Warfarin teratogenicity to the non-Warfarin anticoagulant rodenticides is not justified from a scientific point of view, based on the results of valid and good quality data.				
Dossier Submitter's Response				
Thank you for your comment. The dossier submitter does not agree that read-across is scientifically unjustified. Method sensitivity of OECD 414 have not been shown and the non-warfarin AVK rodenticides are intrinsically similar, biologically and toxicologically, to warfarin . See response to comment 11 for a detailed explanations.				
RAC's response				
Thank you for your comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	6
Comment received				

We agree with the classification proposal for human health and the environment.
Dossier Submitter's Response
Thank you for your comment.
RAC's response
Thank you for the comment.

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	France	Liphatech SAS	Company-Manufacturer	7
Comment received				
Our comments are about Developmental toxicity (section 4.11 of CLH report). As data owner, we do not support the CLH proposal, Bromadiolone 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 Bromadiolone is invalid.				
Dossier Submitter's Response				
Thank you for your comment. The dossier submitter does not agree that read-across is scientifically unjustified. Method sensitivity of OECD 414 have not been shown and the non-warfarin AVK rodenticides are intrinsically similar, biologically and toxicologically, to warfarin . See response to comment 11 for a detailed explanations.				
RAC's response				
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Norway		MemberState	8
Comment received				
The Norwegian CA agrees with the proposal to classify bromadiolone as Repr. 1A; H360D (CLP) /Repr. Cat. 1; R61 (DSD) based on the rationale put forward in the CLH report. We support the argument that no clear conclusions can be drawn from the performed teratogenicity studies because of limitation 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 bromadiolone has a similar chemical structure and the same mechanism of action responsible for the teratogenicity of warfarin. As potential developmental effects would be expected at very low doses, the possibility of setting specific concentration limits for reprotoxicity should be considered.				
Dossier Submitter's Response				
Thank you for your comment. The dossier submitter agrees.				
RAC's response				
Thank you for the comment. The RAC is also of the opinion that Bromadiolone should be classified for developmental				

toxicity. For all evaluated AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" is proposed (please see the justification under RAC response to Comment number 1).

Regarding Specific concentration limit for reprotoxicity, it is acknowledged that the specific data on developmental toxicity of Bromadiolone is too scarce to guide in setting the SLC. Sufficient data to set SCL for developmental toxicity is only available for Warfarin: 0.003% based on human data (with doses of 0.04-0.08 mg/kg/day that may cause developmental toxicity in women regarded as an ED10 level) and on animal data (0.125 mg/kg/day from Kubaszky et al. 2009). 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 Bromadiolone.

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Spain	Laboratorios Agrochem S.L.	Company-Manufacturer	9

Comment received

When compared with the criteria for classification, there is inadequate evidence for classification of the non-Warfarin anticoagulants for developmental toxicity.

(*ECHA note: The attachment provided is copied under Comment 11)*

Dossier Submitter's Response

Comparison to the CLP criteria

The dossier submitter does not agree with the company manufacturer's expert that classification by read-across from Warfarin is incorrect.

Even though the SE (special expert's) conclusion lacks a clear comparison to the CLP criteria, the CLP supports weight of evidence analysis and read-across to other existing data.

Comparison to CLP criteria according to modern standards:
The current CLP criteria, annex 1, point 1.1.1.3 of the CLP regulation supports a weight of evidence evaluation of the available data. The available data shows that the physicochemical properties and the mammalian toxicity profile of all the 2nd generation AVK rodenticides and warfarin 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. The evidence for classification is thus the clear human data for warfarin, justified by the similarity in pharmacology and toxicology for bromadiolone, the other AVKs and Warfarin as a group (table 1). Classification regarding developmental toxicity for bromadiolone as reproductive toxicants in category 1A is thereby warranted and based on evidence.

Comparison with CLP Criteria
Criteria Cat. 1:
Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal

studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.

The criteria for Cat. 1A is fulfilled for bromadiolone since read-across is performed to warfarin (absence of epidemiological evidence for bromadiolone itself is therefore irrelevant). Warfarin fulfils this criterion: 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[2]).

Justification for read across

- 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.
- The presumed mechanism for the warfarine syndrome in humans is similar to the pharmacological/toxicological MoA for effects on coagulation proteins i.e. inhibition of post-translational carboxylation. But in the case of effects after treatment during the first trimester, 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 haemorrhages.
- The available OECD 414 studies do not show clear teratogenicity of AVK inhibitors. For example there was absence of warfarine embryopathy expressed as effects on bone formation in studies performed according to OECD TG 414 (new and old version) on warfarin or any other AVK rodenticide. But, 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.
- In addition, the new warfarin study does not show clear effects on other foetal effects such as bleedings, small foetuses and embryotoxicity. This also indicates that the OECD 414 is not a good model for embryotoxic effects of AVK's. The only effect observed effect in the new warfarin study was cataracts after treatment with the longer dosing protocol, in rats. But since no OECD 414 study of bromadiolone, in rats, according to the longer treatment protocol are available and 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).
- Small and large AVK's pass the placenta. Warfarin and flocoumafen (with a much higher molecular weight) pass the placenta. Some differences in placental transfer and potency are observed between warfarin and flocoumafen 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.

1. Hall, J. G., Pauli, R. M., & Wilson, K. M. (1980). Maternal and fetal sequelae of anticoagulation during pregnancy. The American Journal of Medicine, 68, S. 122-140.
2. Howe AM and Webster WS (1992): The warfarin embryopathy: a rat model showing

maxillonasal hypoplasia and other skeletal disturbances. Teratology. Oct;46(4):379-90.

RAC's response

Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	PelGar International Limited	Company-Manufacturer	10

Comment received

We strongly support the positions given in the attached papers.

(*ECHA note: The attachments provided are copied under Comment 11 and 16)*

Dossier Submitter's Response

Thank you for your comment. See response to comment 11 for a detailed explanation to why the dossier submitter disagrees with the positions.

RAC's response

Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United States	Bell Laboratories, Inc.	Company-Manufacturer	11

Comment received

Please see the report (under Public Attachment)entitled "Teratology of AVK Rodenticides Classification by Read-Across from Warfarin is not Correct" prepared by Simon Warren, Exponent International Limited, representing the CEFIC Rodenticide Data Development Group. Bell Laboratories, Inc. is a member of this group and fully supports the arguments presented in this document.

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

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

The conclusion of the Specialised Experts ("SE Conclusion") that the classification of all anti-Vitamin K (AVK) rodenticides as teratogens should be read-across from warfarin is no longer valid.
- The SE Conclusion is inadequate by modern standards, since it lacks a clear comparison of the data against the classification criteria.
- New data overturn a key consideration on which the SE Conclusion was based (i.e., doubt on the ability of the OECD 414 study design to detect AVK embryopathy). A new OECD 414 study of warfarin now demonstrates method sensitivity.
- The SE Conclusion was not based on the most appropriate endpoint, being concerned with

teratogenicity when more recent epidemiological data show foetotoxicity in human pregnancies to be of greater incidence.
The CEFIC teratogenicity study of warfarin demonstrates developmental and foetotoxicity, and therefore confirms sensitivity of the OECD 414 study design. There is clear evidence of specific foetal sensitivity to haemorrhage; borderline evidence of an increase of small foetuses (10-day group only) in the absence of maternal toxicity, and adequate evidence of malformation. The incidences of foetal haemorrhage at the low dose demonstrates the ability of the OECD 414 study design to detect specific foetal sensitivity to warfarin, and therefore the same ability to detect specific foetal sensitivity to the AVKs.
The basis for read-across for developmental toxicity from warfarin to the non-warfarin AVK rodenticides, is therefore invalid.
Careful comparison of the guideline developmental toxicity data for each of the non-warfarin AVKs against the classification criteria therefore show:
- Criteria for classification as CLP Cat 1A are not met. There is no evidence that any of the non-warfarin AVK rodenticides are associated with adverse pregnancy outcomes in humans.
- Criteria for classification as CLP Cat 1B are not met. There is no "clear evidence", from valid GLP- and guideline- compliant studies, that any of the non-warfarin AVK rodenticides cause an adverse effect on development in animals. Indeed, with the multiplicity of good and reliable studies (for which validity of the model is demonstrated) there is strong evidence that they do not.
- Criteria for classification as CLP Cat 2 ("some evidence") are not met. There is no evidence from GLP- and guideline- compliant studies, that any of the non-warfarin AVK rodenticides cause an adverse effect on development in animals. Indeed, with the multiplicity of acceptable and reliable studies (for which validity of the model is demonstrated) there is strong evidence that they do not.
- No classification for developmental toxicity is therefore appropriate.

Introduction:

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

1. Review of the Specialised Experts Conclusion

a) The SE Conclusion is no longer adequate for modern purposes since it lacks a clear comparison with modern (DSD or CLP) criteria.
b) In addition, recent data amend some of the assumptions from which the conclusion is derived; in particular:
c) The OECD 414 study of warfarin demonstrates sensitivity of the method; it is therefore appropriate to base classification on the actual results achieved in OECD 414 teratogenicity studies with each of the AVKs.
d) Teratogenicity is not the most appropriate human or animal endpoint. It is unusual for teratology to occur in the complete absence of other toxicity. A more usual picture is that teratology occurs as a particularly notable feature, among a spectrum of other foetotoxic change. This would appear to be the clinical picture among the therapeutic AVKs including warfarin. A multicentre prospective clinical trial (Schaefer et al, 2006[3]) examined 666 pregnancies to mothers receiving anticoagulant treatment (with warfarin, phenprocoumon, acenocoumarol, fluindione, or phenindione); birth defects were rare but the more numerous findings were of foetotoxicity – prematurity, miscarriage, decreased mean gestational age at delivery, decreased mean birth weight of term infants. Embryotoxicity (of which the teratology would be only one factor) is more meaningful for protection of the foetus; and is identified in the CEFIC warfarin study. The epidemiology of therapeutic AVKs shows that among human pregnancies foetotoxicity is of higher incidence than teratogenicity; the OECD 414 study of warfarin predominantly shows foetotoxicity. The warfarin-related incidence of foetotoxicity in human pregnancies (as stillbirth, prematurity, small at term) is mentioned in a number of the CLH reports, without drawing appropriate parallels to the warfarin study.

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

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

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

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

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

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

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

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

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

At the level of conceptus warfarin treatment induced:

- an increase of foetal mortality with a NOAEL of 0.150 mg/kg bw/day;
- a dose related increase of foetal haemorrhages even at the lowest dose tested of 0.125 mg/kg bw/day;
- central ocular cataract (typical malformation of warfarin embryopathy) even at the lowest dose tested of 0.125 mg/kg bw/day.

Warfarin is seen to be embryotoxic and teratogenic in the rat.

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

studies in rabbit also demonstrate absence of developmental toxicity.

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

Most CLH proposals (March 2013) consider the results of the new OECD 414 study of warfarin, and available placental transfer data.

For all of the non-warfarin AVK rodenticides (with the possible exception of bromadiolone), the animal data are concluded to show no evidence of teratogenicity. In cases where classification is recommended, proposals therefore remain entirely based on the common position of read-across from warfarin.

Current proposals for reproductive classification from the seven non-warfarin AVK CLH proposals range from CLP 1A (4 substances), 1B (one), 2 (one) and no classification (one).

In the CLH report for brodifacoum, comparison with criteria is not considered (no entry).

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

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

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

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

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

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

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

3. Comparison with Criteria

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

Classification should be based on evidence, not hypothesis.

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

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

There is clear epidemiologic evidence that warfarin causes developmental toxicity in humans; and that other AVK anticoagulants used as therapeutics (which do not include the non-warfarin AVK rodenticides) also cause developmental toxicity in humans. However, the criterion for "sufficient epidemiologic evidence" is not met for the non-warfarin AVK rodenticides.

There is evidence to support that, due to absence of effect in appropriately-sensitive teratogenicity studies, the non-warfarin AVK rodenticides are intrinsically different to warfarin.

Because the criterion for "sufficient epidemiologic evidence" is not met for the non-warfarin AVK rodenticides, classification into DSD Cat 1/ CLP Cat 1A is not appropriate.

With respect to DSD Cat 2/CLP Cat 1B:

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

There is a concern, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans. However, there is evidence that the non-warfarin AVK rodenticides are intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits.

Both warfarin and flocoumafen are seen to cross the placenta. Only warfarin induces clear anticoagulant and developmental effects in the foetus. In contrast, flocoumafen clearly does not.

Therefore, for all of the non-warfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies.

In the absence of relevant effect in animal studies, and with the demonstration of method sensitivity to warfarin, read-across of warfarin developmental toxicity to the other rodenticidal AVKs becomes a scientifically unjustified extrapolation.

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

With respect to DSD Cat 3/ CLP Cat 2:

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

There is a concern, based on warfarin and the therapeutic AVKs that AVKs may cause developmental toxicity in humans. However, there is evidence that the non-warfarin AVK rodenticides are intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits.

Both warfarin and flocoumafen are seen to cross the placenta. Only warfarin induces clear anticoagulant and developmental effects in the foetus. In contrast, flocoumafen clearly does not.

Therefore, for all of the non-warfarin AVK rodenticides, the key determinant of classification is the absence of effects specific to the foetus in the respective teratogenicity studies.

In the absence of relevant effects in animal studies, and with the demonstration of method sensitivity to warfarin, read-across of warfarin developmental toxicity to the other rodenticidal AVKs becomes a scientifically unjustified extrapolation.

Negative results in adequate studies of the non-warfarin AVK rodenticides are meaningful.

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

Placement in DSD Category 3/ CLP Category 2 is not appropriate.

By comparison of evidence with the criteria, no classification for developmental toxicity is appropriate.

In conclusion, ample evidence is provided that a read-across from warfarin teratogenicity to the nonwarfarin AVK rodenticides is not justified from a scientific point of view, based on the results of valid and good quality data. When compared with the criteria for classification, there is inadequate evidence for classification of the non-warfarin AVKs for developmental toxicity.

Simon Warren
18 April 2013

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

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

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

4 Kubaszky R (2009) Teratology study of Test Item Warfarin Sodium with Rats. Unpublished report 07/396-105P, LAB Research Ltd. CEFIC RDDG.

5 Schardein J (2000) Chemically induced birth defects. Third edition revised and expanded. Marcel Dekker: New York. ISBN: 0-8247-0265-4

6 Hall *et al*. (1980). Maternal and fetal sequelae of anticoagulation during pregnancy. *Am J. Med.* 68: 122-140.

7 Howe AM & Webster WS (1994): Vitamin K – its essential role in craniofacial development. Australian Dental Journal, **39**(2) 88-92.

8 Howe AM & Webster WS (1992): The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances, Teratology, **46**(4) 379-90

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

Dossier Submitter's Response

Method sensitivity of the OECD 414 guideline has not been shown for Warfarin:
The dossier submitter disagrees with the statement that method sensitivity has been shown in the new warfarin OECD 414 study in rats.

a) ***There is no clear evidence of foetal sensitivity to haemorrhage***. The quantitation of foetal haemorrhage was unclear, which was also pointed out by the Warfarin dossier submitter (for example in the TP2 study 6 haemorrhages was reported in seven litters, see table). In addition there was no apparent increase with increasing dose, doing time or increasing effects on the mothers. In addition the frequency of observed pin-prick sized haemorrhages was generally low (see table 2). But in any case, the studies are not directly comparable. It is explicitly mentioned in the study design of the warfarin study, that special attention were paid to haemorrhages and that photographs was taken of them when observed. Considering the relatively low numbers observed, the sensitivity to capture these effects seems low even when special attention to these effects was paid. In addition control animals also had incidences of haemorrhages although to a low extent, whereas no similar findings were reported for the control animals in the three bromadiolone studies. The potential of observing similar effects under the standard OECD 414 protocol therefore seem very low.
b) ***There is no clear evidence of embryo toxicity in the form of small foetuses in the new warfarin study.*** One would have expected more pronounced or at least that the effect is repeated to state that this is a sign of embryo toxicity of warfarin. An increase of small foetuses was only seen when the short dosing protocol was used. The mean foetal weight was also unaffected and the incidence of small foetuses did not increase with prolonged dosing.
c) ***Adequate evidence of malformations was not shown in the new warfarin study.*** The only effect observed were an increased incidence of cataracts in the prolonged dosing scheme. The only rat study performed was in accordance with the shorter dosing protocol of warfarin in rat, where there was no apparent significant increase of cataracts. The only study in accordance with the longer protocol was performed with bromadiolone in rabbits. The results are therefore not directly comparable and it can therefore not be concluded that bromadiolone do not cause cataracts in OECD 414 teratogenicity studies.
d) ***There is no clear evidence of general embryo toxicity in the form of foetal***

mortality in the new warfarin study. The statistically significant effect was not repeated with the longer dosing scheme and did not increase with increased dosing time. Furthermore, similar effects are also seen in the bromadiolone rabbit study (the longer treatment protocol) and is therefore not unique for the new warfarin study in rats (see table 2).

In all, the new warfarin study does not invalidate a read-across for bromadiolone to the human teratogenicity data for warfarin:

- The new warfarin study does not substantiate method sensitivity of the 414 protocol to capture AVK teratogenicity/embryo toxicity.
- The new warfarin study does not substantiate that there are inherent pharmacological/toxicological differences between bromadiolone and warfarin.
- The modern CLP regulation allows for weight of evidence assessment and read-across to other existing data (see also comment 9).

RAC's response

Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013			Individual	12

Comment received

(*ECHA note: Information was provided in an attachment, and the document is copied under Comment number 11)*

Dossier Submitter's Response

Please see the dossier submitters detailed response to comment no 11.

RAC's response

Thank you for the comment. Please see the RAC response to Comment number 1.

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

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.

We support the need of the read-across for the developmental toxicity and the classification Repr. 1A based on the structural similarity and the same mode of action (vitamin K deficiency).

Dossier Submitter's Response

Thank you for your comment.

RAC's response

Thank you for the comment. The RAC is also of the opinion that Bromadiolone should be classified for developmental toxicity. For all evaluated AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the

weight of evidence assessment, and classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" is proposed (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	Exponent, International on behalf of CEFIC RDDG	Industry or trade association	14
Comment received				
4.11, Toxicity for reproduction. The proposal to classsify for developmental toxicity is not supported. Data are conclusive but not sufficient for classification. See attachement Exponent doc ID 1109091.UK0 EWC0008. (*ECHA note: The attachment provided is copied under Comment 11)*				
Dossier Submitter's Response				
Thank you for your comment, please see comment 11 and 9 for a further explanation to why the dossier submitter disagrees.				
RAC's response				
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Germany		MemberState	15
Comment received				
Proposal: Please include the following study in the CLP report, if possible: Johnson, TL (2009): A placental transfer study of warfarin and flocoumafen in rats. Confidential report of BASF: report no. 2009/7000085, dated 16 July 2009. Study number WIL-234006. Justification: On page 50 of the CLH Report is written: On the basis of currently available data, there are no convincing arguments that other AVKs including the second generation compounds could not pass the placenta. The study on placental transfer of warfarin and flocoumafen (for further information on the study see CLH report on flocoumafen) was evaluated by the Netherlands and is included in the CLH report on flocoumafen. The study demonstrates that flocoumafen, like warfarin, is able to pass the placenta. It is not possible however to quantitatively extrapolate data on foetal exposure between the AVK rodenticides. Therefore, we agree to the proposal of SE to maintain the classification for bromadiolone with Repr. Cat.1; R61 / Repr. 1A H360D. Proposal: Please include a reference list.				

Justification:
A reference list is missing.

Respiratory Sensitisation:

Skin Sensitisation:

Any other hazard classes or endpoints:
p.61 Growth inhibition of algae (OECD 201) Ref.Lipha Tech) (Scenedesmus subspicatus)
In the combined Assessment Report it is written:
'recalculated 72 h ErC50 = 1mg/L' instead of '> 1 mg/L' as given in the CLH Report.

p.61
Growth inhibition of algae (OECD 201) Pseudokirchneriella subcapitata (Task Force)
In the combined Assessment Report –LoEP it is written:
'72 h ErC50 = 0.38 mg/L given as the geometric mean of the initial measured conc. (TF)'.
Instead in the CLH Report an ErC50 value of 1.14 mg/L is reported. This value is obviously the actual ErC50 value and not the geometric mean which then would be 0.38 mg/L.

Dossier Submitter's Response

Thank you for your comments. The new flocumafen study does indeed show that also larger molecular weight molecules can pass the placenta. A summary of the flocoumafen placental study is available in the flocoumafen CLH dossier.
Aquatic data (algae):
The LiphaTech value should correctly be given as ErC50 > 1 mg/L, which is the same as given in the text in the Assessment Report (in the LoEP it is written ErC50 = 1 mg/L, which is a mistake).
The Task Force value is correctly given in the CLH report and corresponds with the Assessment Report, both with the value given in the LoEP and in the text, so we disagree with this comment.
The correct version of the combined Assessment Report for bromadiolone is the one dated "30 May 2008, revised 16 December 2010".

RAC's response

Thank you for the comment. The RAC is also of the opinion that Bromadiolone should be classified for developmental toxicity. For all evaluated AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" is proposed (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	United Kingdom	Exponent International on behalf of CEFIC RDDG	Industry or trade association	16

Comment received

4.11 Toxicity for reproduction
Bromadiolone should not be classified for developmental toxicity. Data are conclusive and not sufficient for classification. Please see attached document (Exponent docID 1109091.uk0 EWC0009 - bromadiolone)

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

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

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

Reasoning

1. Rabbit Teratogenicity is not real.

The CLH proposal for bromadiolone interprets the result of one rabbit study as 'teratogenic'. 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 p53) and there is no evaluation of "strength" (the reader cannot determine if the evaluation constitutes "clear" or "some" animal evidence).
This interpretation is not agreed; the findings in question occur in the CNS of a total of three pups in two separate dose groups, are different abnormalities for each pup, with a different pathogenesis; occur within the background incidence of findings in this strain, do not represent a syndrome, and occur at an incidence too low (single cases) to be interpreted as a positive result. Two expert reports[1,2] are available, that unequivocally conclude the absence of any evidence of developmental toxicity in this study.

2. Relevance of the Specialised Experts Conclusion[3]

The CLH proposal to classify bromadiolone for developmental toxicity follows the SE Conclusion, which can no longer be considered valid. 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) 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.

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

4. Comparison with Criteria

The CLH report provides a comparison with criteria, concluding bromadiolone to be teratogenic based on CNS effects in offspring; and that classification is made by read-across from warfarin. As has been shown in earlier discussion, both of these bases are scientifically invalid.
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 bromadiolone 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 bromadiolone.
Because the criterion for "sufficient epidemiologic evidence" is not met for bromadiolone, 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 bromadiolone 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 bromadiolone is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies of bromadiolone in both rats and rabbits. The method used to test bromadiolone is appropriate and sufficient to detect developmental toxicity of warfarin.
Negative results in adequate studies of bromadiolone 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 bromadiolone 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 bromadiolone is intrinsically different to warfarin, based on absence of foetotoxicity in teratogenicity studies in both rats and rabbits. The method used to test bromadiolone is sufficient to detect developmental toxicity of warfarin.
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; 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 bromadiolone is not valid. When compared with the criteria for classification, there is inadequate evidence for any classification of bromadiolone for developmental toxicity.

Simon Warren *DABT DIBT DipRCPath*
18 April 2013

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[1] Druga A, Esdaile D, Hirka G (2006) Clarification on the Interpretation of Study 03/735-105N (teratology study of the test Item bromadiolone technical in Rabbits. Unnumbered report, LAB International Research Centre Hungary Ltd.
[2] Wood E (2013) Study Review of teratology study of the test item bromadiolone technical in rabbits. Letter reference L26-05-06 from SafePharm Laboratories to to Skarman D, Sundbyberg.
[3] ECBI/121/06, 20 September 2006. ECB, Ispra.
[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

--- End of attachment ---

Dossier Submitter's Response
Thank you for your comment. It is acknowledged that he CNS eefcts observed in rabbits were only in a limited number of animals. However the dossier submitter did include these as possible effects since CNS effects are alo one of the major features of warfarin toxicity after exposure in utero in humans and no such effects were seen in the concurrent control animals in either of the rabbit studies. This however has no major impact on the conclusion to classify as a category 1A for teratogenicity in accordance to the CLP criteria since the conclusion is mainly based on read-across to warfarin teratogenicity data in humans. The dossier submitter does not agree that the non-warfarin AVK rodenticides are intrinsically different to warfarin based on absence of foetotoxicity in the teratogenicity studies for bromadiolone in rats and rabbits. The new warfarin study is not directly comparable to the bromadiolone studies and method sensitivity of the OECD 414 study has not been shown with the new warfarin study in rats. See response to comment 11 for a detailed explanation to why the dossier submitter still considers read-across to warfarin human data valid and also comment 9 for a comparison to CLH criteria.
RAC's response
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Finland		MemberState	17
Comment received				
We support the proposed classification for developmental effects as Repr. 1A; H360D for bromadiolone. 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 comment we largely agree with your comment but would like to clarify that the presumed mechanism for the disturbance in ossification in warfarine syndrome 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 Gla protein. Exposure during the second and third trimesters is mainly associated with anatomical abnormalities of CNS that are thought to be secondary to hemorrhages.				
RAC's response				
Thank you for the comment. The RAC is also of the opinion that Bromadiolone should be classified for developmental toxicity. For all evaluated AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the				

weight of evidence assessment, and classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" is proposed (please see the justification under RAC response to Comment number 1).

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Hungary	Babolna Bio Ltd.	Company-Manufacturer	
Comment received				
(*ECHA note: The attachment provided is copied under Comment 16).*				
Dossier Submitter's Response				
Please see the dossier submitters response detailed in the answer to comment 11 and 16.				
RAC's response				
Thank you for the comment. Please see the RAC response to Comment number 1.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	France	Liphatech SAS	Company-Manufacturer	17
Comment received				
In the statements from an Expert toxicologist provided, ample evidence that a read-across from warfarin teratogenicity to Bromadiolone is not justified from a scientific point of view, based on the results of valid and good quality data. By comparison of evidence with the criteria, no classification for developmental toxicity is appropriate for Bromadiolone. (*ECHA note: The attachments provided are copied under Comment 11 and 16)*				
Dossier Submitter's Response				
Thank you for your comment. The dossier submitter does not agree that read-across is scientifically unjustified. See response to comment 11 and 9 for detailed explanations.				
RAC's response				
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Spain	Laboratorios Agrochem S.L.	Company-Manufacturer	18
Comment received				
Ample evidence is provided that the basis for a read-across from Warfarin teratogenicity to Bromadiolone is not valid. When compared with the criteria for classification, there is inadequate evidence and it should not be classified for developmental toxicity. (*ECHA note: The attachment provided is copied under Comment 16)*				
Dossier Submitter's Response				
Thank you for your comment. The dossier submitter does not agree that read-across is				

scientifically unjustified. See response to comment 11 and 9 for detailed explanations.
RAC's response
Thank you for the comment. The RAC is of the opinion that for AVK rodenticides, including Bromadiolone, a potential for human developmental toxicity is presumed based on the weight of evidence assessment, and proposes classification with Repro Cat 1B, i.e. "presumed human reproductive toxicant" (please see the justification under RAC response to Comment number 1).

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	19
Comment received				
We support the classification Acute Toxicity Cat. 1 based on the following results - Oral route:LD50 < 5mg/kg, - Inhalation route LC50: < 0.05 mg/kg, - Dermal route LD50: < 50 mg/kg. Based on these findings, the criteria for Acute Toxicity Cat1 are fulfilled for each endpoint.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
Thank you for the comment.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Belgium		MemberState	20
Comment received				
We agree with the classification STOT RE Cat.1, indeed the studies show significant and severe toxic effects which are relevant for the human health and which are produced at low exposure concentration (≤ 10 mg/kg). We also agree with the extrapolation of oral toxicity data to dermal toxicity due to the acute toxicities after oral and dermal, comparable indicating comparable absorptions.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
Thank you for the comment.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

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 1(most sensitive species Algae with 72hErC50>1 mg/l, but due to the rapid hydrolysis considered <1mg/l) and toxicity band between 0.1 mg/l and 1 mg/l).				

Based on the most stringent outcome for Aquatic Chronic toxicity (on the basis of the Algae NOEC and the LC50 for the other trophic levels) an M-factor for chronic toxicity of 1 could be assigned .
Dossier Submitter's Response
Thank you for your comment.
RAC's response

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	22
Comment received				
We agree with the current proposal for consideration by rac: CLP regulation: • Aquatic acute 1 (M=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.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2013	Finland		MemberState	23
Comment received				
Degradation and bioaccumulation potential: We agree with the conclusions that bromadiolone is not rapidly degradable and that it fulfills the criteria for bioaccumulation potential based on log Kow.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	Germany		MemberState	24
Comment received				
p.61 Growth inhibition of algae ((OECD 201) Ref.Lipha Tech) (Scenedesmus subspicatus) In the combined Assessment Report it is written: 'recalculated 72 h ErC50 = 1mg/L' instead of '> 1 mg/L' as given in the CLH Report.				

p.61 Growth inhibition of algae (OECD 201) Pseudokirchneriella subcapitata (Task Force) In the combined Assessment Report –LoEP it is written: '72 h ErC50 = 0.38 mg/L given as the geometric mean of the initial measured conc. (TF)'. Instead in the CLH-Report an ErC50 value of 1.14 mg/L is reported. This value is obviously the actual ErC50 value and not the geometric mean which then would be 0.38 mg/L.
Dossier Submitter's Response
Thank you for your comments. The LiphaTech value should correctly be given as ErC50 > 1 mg/L, which is the same as given in the text in the Assessment Report (in the LoEP it is written ErC50 = 1 mg/L, which is a mistake). The Task Force value is correctly given in the CLH report and corresponds with the Assessment Report, both with the value given in the LoEP and in the text, so we disagree with this comment. The correct version of the combined Assessment Report for bromadiolone is the one dated "30 May 2008, revised 16 December 2010".
RAC's response

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2013	France		MemberState	25
Comment received				
p 17, Density: In the CLH report, "1.45g/cm3 at 20-21°C" is reported whereas in the combined AR, "1.45-1.46g/cm3 at 20-21°C" is reported. Please clarify. p 17, Vapour pressure: In the CLH report, "0.05 x 10-3 Pa at 45°C" is reported whereas in the combined AR, "<0.05 x 10-3 Pa at 45°C" is reported. Please clarify.				
Dossier Submitter's Response				
Thank you for noticing, the correct figures are those quoted from the combined AR.				
RAC's response				
Thank you for the comment.				

ATTACHMENTS RECEIVED:

1. **Teratogenicity of AVK Rodenticides - Classification by Read-Across from Warfarin is not Correct** (Filename: Read-across rebuttal EWC0008).
   Submitted on 19.04.2013 by:
   Bell Laboratories, Inc.
   Activa s.r.l
   PelGar International Limited
   Liphatech SAS
   Laboratorios Agrochem S.L.
   Exponent, International on behalf of CEFIC RDDG
   Individual

Submitted on 18.04.2013 by:
Babolna Bio Ltd

(*ECHA note: This attachment has been copied under the section Toxicity to Reproduction)*

2. **Bromadiolone - Comment on the CLH proposal, 5 March 2013** (Filename: Bromadiolone classification - developmental EWC0009).
Submitted on 19.04.2013 by:
Exponent International on behalf of CEFIC RDDG
Activa s.r.l
PelGar International Limited
Liphatech SAS
Laboratorios Agrochem S.L.
Babolna Bio Ltd

(*ECHA note: This attachment has been copied under the section Toxicity to Reproduction*)