Committee for Risk Assessment

RAC

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

14 March 2014

CLH-O-0000003391-80-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: 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

The proposal was submitted by **Norway** and received by the RAC on **28 September 2012.** All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

Norway has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 March 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 April 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Bogusław Barański**

Co-rapporteur, appointed by RAC: **José Luis Tadeo**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **14 March 2014** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on **Difethialone** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	No current Annex VI entry									
Dossier submitters proposal	607-717-00-3	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	-	104653-34-1	Repr. 1A; Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE.1 Aquatic Acute 1 Aquatic Chronic1	H360D H300 H310 H330 H372 H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 H317 H410	EUH070	STOT RE 1; H372: C ≥ 0,02 % STOT RE 2; H373: 0,002 % ≤ C < 0,02 % M-factor =100 M-factor =100
RAC opinion					Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE.1 Aquatic Acute 1 Aquatic Chronic1	H360D H300 H310 H330 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 (blood) H317 H410	EUH070	Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,02 % STOT RE 2; H373: 0,002 % ≤ C < 0,02 % M =100 M =100

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Resulting Annex VI entry if agreed by COM	607-717-00-3	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	-	104653-34-1	Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE1 Aquatic Acute 1 Aquatic Chronic1	H360D H300 H310 H330 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 (blood) H317 H410	EUH070	Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,02 % STOT RE 2; H373: 0,002 % ≤ C < 0,02 % Repr. 1B; H360D: C ≥ 0,003 % M =100 M =100

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

Difethialone belongs to a group of compounds known as the anticoagulant rodenticides, i.e. those with an anti-vitamin K (AVK) mode of action (MoA) which are used mainly as active substances in biocidal products for pest control of rats, mice and other rodents. Some of the substances had an existing harmonised classification. However, at the time of writing, only Warfarin is currently classified for toxicity to reproduction in category 1A.

The eight AVK rodenticides were previously discussed by the Technical Committee on Classification and Labelling of Dangerous Substances (TC C&L) of the European Chemicals Bureau (ECB) (2006 – 2008). However, the work was transferred to ECHA and to that end Member State Competent Authorities (MSCAs) were requested to prepare CLH proposals.

CLH proposals for eight AVK rodenticides, Coumatetralyl (Denmark), Difenacoum (Finland), Warfarin (Ireland), Brodifacoum (Italy), Flocoumafen (The Netherlands), Difethialone (Norway), Chlorophacinone (Spain) and Bromodialone (Sweden), were submitted by eight different Dossier Submitters (DS). The dossiers were handled as a group but the Committee for Risk Assessment (RAC) proceeded to evaluate the proposals on a substance by substance basis comparing the human data available for Warfarin (and other AVKs) and relying on a weight-of–evidence approach as required by Regulation 1272/2008 (CLP).

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Oral acute toxicity

Difethialone was very toxic to rats and mice with LD50 (male, females and both sexes combined) between 0.4 and 0.8 mg/kg bw in rats (the lowest oral LD50 in male rats was 0.55 mg/kg bw in the key study) and 1.29 mg/kg bw in mice. Difethialone is less toxic to dogs (estimated LD50 of approximately 12 mg/kg bw). An acute oral cat study of low quality showed no substance related mortalities at doses up to 16 mg/kg bw. Pigs showed a greater sensitivity (LD50 values 2.0 to 3.0 mg/kg bw). In chicken, the LD50 was calculated to be 0.87 mg/kg bw. The dose-response curve appeared to be very steep in rodents. Most deaths occurred between days 4 and 16 after dosing.

In the key rat study, which is the basis for the proposed classification, (Mally and Porret-Blanc, 1985b), there were no deaths observed after dosing at 0.4 mg/kg bw. Deaths occurred among animals dosed at 0.48 mg/kg bw or above. All rats died within 4 to 12 days of exposure. There were no significant differences in bodyweight gains for treated and control surviving rats.

A single acute inhalation toxicity study, conducted according to Technical Guideline (TG) US EPA 81-3, was reported (Hardy and Jackson, 1986). In this study, the LC50 (males and females combined) for Wistar rats (5/sex/dose group, 4 h exposure period) was ≤ 10.7 µg/l following whole body exposure and between 5 and 19.3 µg/l following nose only exposure.
A single acute dermal toxicity study, conducted according to TG US EPA 81-2, was reported (Gardner, 1986). In this study, the LD50 (males and females combined) for SD rats (5/sex/dose group) was 6.5 mg/kg bw (test material was applied to 10 % body surface at 1% w/v in PEG 300)

Classification proposed by the dossier submitter

Acute oral toxicity: Based on the oral LD_{50} for rats (0.4 to 0.8 mg/kg bw), the DS proposed to classify Difethialone as **Acute Tox. 1 H300** (classification criterion: LD_{50}, oral, rat ≤ 5 mg/kg).

Acute dermal toxicity: Based on the dermal LD_{50} for rats (6.5 mg/kg bw), the DS proposed to classify Difethialone as **Acute Tox. 1 H310** (classification criterion: LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg).

Acute inhalation toxicity: Based on the inhalatory LC_{50} value between 5.0 µg/l and 19.3 µg/L/4h (nose only exposure to dust) and $LC_{50} \leq 10.7$ µg/l/4h (whole body exposure to dust) for the rat, the DS proposed to classify Difethialone as **Acute Tox. 1 H330** (classification criterion: LD_{50}, inhalation, rat, for dusts and mists ≤ 50 µg/l/4h).

Comments received during public consultation

One MS agreed with the classifications proposed by the DS for acute toxicity.

Assessment and comparison with the classification criteria

Following a comparison of the available acute oral, dermal and inhalation LD_{50} and LC_{50} values with the classification criteria, RAC supported the conclusion of the DS that, according to CLP Regulation, Difethialone should be classified in Category 1 for acute oral, dermal and inhalation toxicity as follows:

- Acute Tox. 1; H300 (criterion: LD_{50}, oral, rat ≤ 5 mg/kg) based on the oral LD_{50} for rats (range from 0.4-0.8 mg/kg bw in key studies: Mally and Porret-Blanc, 1985a and 1985b).
- Acute Tox. 1; H310 (criterion: LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg) based on the dermal LD_{50} for rats (range 5.2 to 10.4 mg/kg bw in the key study: Gardner, 1985).
- Acute Tox. 1; H330 (criterion: LD_{50}, inhalation, rat, for dusts and mists ≤ 0.05 mg/l/4h) based on the inhalatory LD_{50} values of ≤ 0.0107mg/l/4h for the rat (both sexes combined) (Hardy and Jackson, 1986).

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

No classification was proposed for STOT-SE.

Comments received during public consultation

One MS agreed that since no data from specific target organ toxicity investigations following a single exposure were presented, it could be concluded that classification as STOT SE is not possible.

Assessment and comparison with the classification criteria

In the opinion of RAC, after single exposure to Difethialone the blood coagulation system is adversely affected, and this is the main cause of mortality. However, this does not warrant classification of Difethialone for specific target organ toxicity – single exposure, because it is already covered by the classification as Acute Tox. 1.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

No skin irritation was observed in rabbits (strain not specified) in the single study reported (Gonnet and Guillot, 1985a), which was conducted according to TG US EPA 81-51. Therefore, Difethialone does not fulfil the EU criteria for classification as a skin irritant.

Comments received during public consultation

One MS supported the conclusion of non-classification for Difethialone as a skin irritant.

Assessment and comparison with the classification criteria

In the opinion of RAC there are no data reported which would warrant classification of Difethialone for skin corrosion/irritation. The proposal of the DS was therefore supported.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Difethialone was found to be a weak eye irritant in one study in rabbits (Gonnet and Guillot, 1985b). The study was terminated prior to determination of reversibility of the ocular changes. Reversibility of ocular lesions (transient iritis and slight conjunctival redness) was demonstrated in two other studies in rabbits (Myers and Christopher, 1992 and Gonnet and Guillot, 1985c).
In one of these studies (Myers and Christopher, 1992), delayed death of two rabbits (on days 7 and 11, respectively) occurred after instillation of 50 mg of Difethialone into the lower conjunctival sac of the right eye. From necropsy findings it was apparent that the deaths occurred as a result of the known mode of action of this substance (ie internal haemorrhages). The degree of ocular irritation was minimal.
The results of the eye irritation studies indicate that Difethialone is a weak irritant, but that systemic toxicity and death can occur following instillation of a small quantity of material in close proximity to mucous membranes.
Difethialone is considered not irritating to eyes according to the CLP criteria.
The DS noted that the supplemental hazard statement EUH070 should apply for substances where "*…an eye irritation test has resulted in overt signs of toxicity or mortality among the animals tested, which is likely to be attributed to absorption of the substance through the mucous membranes of the eye. The statement shall also be applied if there is evidence in humans for systemic toxicity after eye contact*."
The criterion for includingEUH070 is fulfilled based on the observations in one of the eye irritation studies (Myers and Christopher, 1992). While the degree of ocular irritation was minimal, the test substance caused the delayed death of two rabbits. Both animals showed signs of treatment-induced haemorrhage at necropsy.

Comments received during public consultation

One MS agreed that classification of Difethialone as an eye irritant is not warranted. The proposed supplemental hazard information EUH070 'Toxic by eye contact' was nevertheless supported.

Assessment and comparison with the classification criteria

In the opinion of RAC, the results of three studies in rabbits (Gonnet and Guillot, 1985b; Myers and Christopher, 1992; Gonnet and Guillot, 1985c) which were conducted according to method US EPA 81-4 do not warrant classification of Difethialone for eye corrosion/irritation, because the observed effects did not meet the CLP classification criteria. The proposed additional labelling with Supplemental Hazard statement *EUH070; Toxic by eye contact* is supported due to the death of two rabbits after instillation of 50 mg of Difethialone to the conjunctival sac in one study (Myers and Christopher, 1992).

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

In a guinea pig maximisation test (GPMT) of low reliability (Parker, 1993) there was no indication of delayed contact hypersensitivity among guinea pigs subject to an induction and challenge regimen that involved exposure to Difethialone up to lethal levels According to the DS, classification for sensitisation is not warranted based on the available data.
Comments received during public consultation

No comments were received addressing this endpoint.

Assessment and comparison with the classification criteria

In the opinion of RAC the results of the guinea pig maximisation test (Parker, 1993) do not warrant classification of Difethialone for skin sensitisation, because the observed effects do not meet the CLP classification criteria.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

Difethialone was shown to have anticoagulant effects in the rat at doses of 4 µg/kg bw/day in a 90-day oral repeated dose toxicity study (Mally, 1986). No lethality was observed following doses of 2 and 4 µg/kg bw/day. All males fed 8 µg/kg bw/day were moribund at week 13. Doses of 16 µg/kg bw/day and above resulted in the death of all animals. The deaths occurred between weeks 1 and 2 at 128 µg/kg bw/day, weeks 4 and 5 at 32 µg/kg bw/day and weeks 6 and 8 at 16 µg/kg bw/day.
In the oral repeated toxicity study on beagle dogs (Harling *et al*. 1986), no toxicologically significant effects were observed at dose levels of 5 or 10 µg/kg bw/day. The high dose of 20 µg/kg bw/day elicited some reactions after 13 weeks which were consistent with the anticoagulant mode of action, with non-lethal haemorrhagic events (pale gums, reduced haemoglobin levels).
No repeated dose inhalation or dermal toxicity studies were available.
Classification as STOT RE 1; H372 is warranted based on the 90-day oral repeat dose toxicity data and on an extrapolation from the acute toxicity data for the dermal and inhalation routes of exposure.
Specific concentration limits
The oral LOAEL established in a 90 day repeated dose study is as low as 4 µg/kg bw/day in rats (based on haemorrhagic changes seen at necropsy) with a steep dose response curve. All males fed 8 µg/kg bw/day were moribund at week 13, and doses of 16 µg/kg bw/day and above resulted in the death of all animals. Using this information, the SCL was calculated as follows (ECHA, 2009: Guidance on the Application of the CLP Criteria, section 3.9.2.6.):

$$SCLCat1 = \frac{ED}{GV1} \cdot 100\% = \frac{0.004\ mg/kg\ bw/day}{10\ mg/kg\ bw/day} \cdot 100\% = 0.04\%$$

$$SCLCat2 = \frac{ED}{GV2} \cdot 100\% = \frac{0.004\ mg/kg\ bw/day}{100\ mg/kg\ bw/day} \cdot 100\% = 0.004\%$$

ED - Effective Dose: LOAEL 0.004 mg/kg bw/day
GV1 - Guidance Value for category 1 according to CLP Annex I, Table 3.9.2: 10 mg/kg bw/day
GV2 - Guidance Value for category 2 according to CLP Annex I, Table 3.9.3: 100 mg/kg bw/day

According to the Guidance on the Application of the CLP Criteria, the SCL obtained should be rounded down to the nearest preferred value (1, 2 or 5), resulting in the following SCLs for Difethialone:
STOT RE 1; H372 above 0.02% and STOT RE 2; H373 between 0.002% and 0.02%.

Comments received during public consultation

Two MS agreed with the classifications proposed by the DS for STOT RE.
One MS was of the view that the SCLs for acute and chronic toxicity should be harmonised with other anticoagulant rodenticides. The approach used to set SCLs for Difenacoum could be used.
One MS supported the proposed setting of specific concentration limits for STOT RE.

Assessment and comparison with the classification criteria

In the opinion of RAC, the existing data warrant classification of Difethialone as proposed by the DS as STOT RE 1 without specifying a specific route but with blood as the main affected organ as follows: "Causes damage to the blood through prolonged or repeated exposure".
Death of all exposed animals due to anticoagulation effect of Difethialone was observed in the 90-day rat study at levels greater than or equal to 0.016 mg/kg bw/day, with a LOAEL of 0.004 mg/kg bw/day. Deaths were attributable to haemorrhages seen at necropsy (Mally, 1986). The LOAEL is well below the CLP criterion of ≤ 10 mg/kg bw/day following 90-days oral dosing in the rat, which is used for classification as STOT RE 1 (H372) for the oral route. In the 90-day dog study an LOAEL of 0.020 mg/kg/day was established.
Taking into account the high absorption of Difethialone through skin and via the respiratory system as indicated by comparison of oral LD_{50} with dermal and inhalation LD_{50} in rats, the classification based on results of 90-day oral exposure should also extend to the other routes.

SCL for STOT RE

Death of all exposed animals due to the anticoagulation effect of Difethialone was observed in the 90-day rat study at levels greater than or equal to 0.016 mg/kg bw/day, with an LOAEL of 0.004 mg/kg/day (Mally, 1986).. In the 90-day dog study an LOAEL of 0.020mg/kg bw/day was established.
RAC supported the DS proposal for specific concentration limits calculated according to the Guidance on the Application of the CLP Criteria. SCLs should be rounded down to the nearest preferred value (1, 2 or 5), which results in a SCL of 0.02% for STOT RE 1 and a SCL between 0.002% and 0.02% for STOT RE 2.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The results from *in vitro* bacterial gene mutation, *in vitro* cytogenicity in mammalian cells and *in vitro* mammalian cell gene mutation tests (Weill, 1988a, Murli, 1992a, Weill 1988b) were negative. The mouse micronucleus test (Murli 1992b and 1992c) was also negative.
Difethialone does not fulfil the CLP criteria for harmonised classification and labelling as a mutagenic substance.

Comments received during public consultation

One MS supported no classification for germ cell mutagenicity because no signs of the mutagenicity were found in the presented studies (both *in vitro* and *in vivo*).

Assessment and comparison with the classification criteria

In the opinion of RAC, classification of Difethialone for germ cell mutagenicity is not warranted, because no genotoxic effects were observed in mutagenicity studies.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Carcinogenicity and long-term toxicity studies are not available.
Based on the available data, no classification for carcinogenicity is warranted for Difethialone.

Comments received during public consultation

No comments were received addressing this endpoint.

Assessment and comparison with the classification criteria

No human or animal evidence suggesting that Difethialone has carcinogenic properties was reported. Taking into account the high repeated dose toxicity of Difethialone in rats, a carcinogenicity study might be very difficult to carry out due to high mortality of animals exposed even to very low doses.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Effects on fertility
There were no indications of any adverse effects on human fertility (i.e. mating performance) of either sex undergoing treatment with anticoagulants (IPCS, 1995. Environmental Health Criteria, No. 175).
One or two-generation animal studies are not available for Difethialone.

Testing anticoagulant substances in multi-generation studies is associated with practical difficulties related to the fact that several events in the reproductive cycle are associated with incidental or inevitable haemorrhages.

The short-term studies (up to 90-days duration) in rats and dogs showed no adverse effects on the reproductive organs (based on macroscopic observation, organ weight analysis and histology). However, the doses used were low and the function of the reproductive organs was not examined. Thus, based on short-term studies, it cannot be concluded that there are no effects on fertility.

According to the DS, there is insufficient evidence for a potential effect of Difethialone on fertility, thus no classification was proposed.

Effects on Developmental toxicity

Difethialone did not cause any teratogenic effects in the experimental animal studies. Due to the difficulties in designing an optimal study protocol for the detection of potentially teratogenic effects following exposure to Difethialone, no clear conclusion can be drawn from these studies.

Warfarin, a well-documented human teratogen, is classified as a reproductive toxicant (Repr. 1A; H360D). 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, classification of Difethialone for developmental toxicity similar to that for Warfarin should be considered.

Effects on or via lactation

No conclusion can be drawn from the available information, and no classification is proposed.

Specific concentration limits

Potential developmental effects of Difethialone would be expected at very low doses, and the possibility of setting specific concentration limits for toxicity to reproduction should therefore be considered. It should be noted that the DS did not propose how the SCL should be calculated but stated that it should be considered.

However, it is recognized that a potency evaluation is very difficult where the classification for toxicity to reproduction is based on read across from other substances, and no direct estimate of the reproductive toxicity potency is possible.

Comments received during public consultation

Four MS agreed with the proposed classification for Difethialone as Repr. 1A; H360D. Three of these MS suggested that read-across from the human and animal data for warfarin should be considered. One of the MS specifically noted that it agreed that Difethialone should not be classified for fertility. A further MS suggested that the SCL for toxicity to reproduction should be harmonised with those for Warfarin.

Comments from the industry did not support the CLH proposal for classification for developmental toxicity. They provided two statements from an expert toxicologist to demonstrate that the basis for read-across for developmental toxicity from Warfarin to Difethialone is invalid.

Assessment and comparison with the classification criteria

Fertility /Lactation
In the opinion of RAC, classification of Difethialone is not warranted for adverse effects on sexual function and fertility or for effects on or via lactation due to lack of relevant data.
Developmental Toxicity
Based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (classified as Repr. 1A), the reproductive toxicity of Difethialone 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 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, repeated exposure to relatively low doses during gestation lead to maternal toxicity and lethality which hinders the detection of developmental toxicity at higher doses.
As there were no data available on the outcome of maternal exposure to Difethialone in humans, classification as Repr. 1A was not considered to be applicable for Difethialone.

Based on the assumption that all AVK rodenticides, including Warfarin and other anticoagulant coumarin-based pharmaceuticals (see below) share the same MoA, namely inhibition of vitamin K epoxide reductase, 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 as Repr. 1B is proposed with the reasoning given below.

The reasons for this conclusion are:

- Difethialone shares the same MoA as expressed by other anticoagulant AVK rodenticides and coumarin-based 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 instead the repeated exposures may lead to maternal mortality with steep dose-response.
- The standard animal studies do 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 into the assessment. However, the RAC is of the opinion that the uncertainty is not sufficient to warrant a Repr. 2 classification.

Reliable evidence of an adverse effect on reproduction in humans, which is required for Repr. 1A, was not available for Difethialone, but potential for human developmental toxicity is presumed based on the weight of evidence assessment above, and RAC thus proposes classification as Repr. 1B (H360): May damage the unborn child, i.e. "presumed human reproductive toxicant".

Specific Concentration Limit

Classification as Repr. 1B for developmental toxicity for Difethialone is supported by the RAC. However, there is only sufficient data for Warfarin 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 be regarded as an ED_{10} level. This human ED_{10} 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 ED_{10} <4 mg/kg/day, the SCL is 0.03%, and for ED_{10} below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from an ED_{10} 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 concluded 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 8 AVK rodenticides concurrently evaluated by RAC at this time, including Difethialone.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

Difethialone is currently not included in Annex VI of the CLP Regulation. The DS proposed to classify Difethialone as Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=1) according to CLP.

Degradation
Degradation was studied in a hydrolysis test, a photolysis test in water, a ready biodegradability test, an anaerobic degradation test and finally one biodegradation test in soil.

The DS considered Difethialone as hydrolytically stable (DT_{50} = 175 days, pH 7 at 25 ºC) and rapidly photodegradable with an experimental half-life between 20 and 60 minutes.

Difethialone is not readily biodegradable under test conditions (OECD 301B), with a degradation of less than 6% after 28 days and it is not degraded under anaerobic conditions. Less than 5% degradation was observed after 60 days.

In a simulation test in soil, Difethialone showed a very slow degradation with a mean dissipation half-life (DT_{50}) of 635 days at 12 ºC.

Based on the available data Difethialone as. was proposed to be non-rapid/ready degradation

Bioaccumulation
The experimental log K_{ow} of Difethialone is 6.29 at pH 7, this value is above the cut-off value of log K_{ow}≥4 (CLP). Experimental bioconcentration tests are not available.

In conclusion, based on the high log K_{ow}, the DS concluded that Difethialone has potential for bioaccumulation.

Aquatic toxicity
One acute toxicity study in fish (*Oncorhynchus mykiss;* LC_{50}(96h)=0.051 mg/l*)*, one in invertebrates (*Daphnia magna;* EC_{50}(48h)=0.0044 mg/l) and one in algae (*Pseudokirchneriella subcapitata*; E_rC_{50}(72h) >0.18 mg/l, NOE_rC(72h)=0.032 mg/l) were reported by the DS. Long-term tests in fish and invertebrates are not available, but the algae test submitted in the CLH report can be considered as an acute (EC_{50}) and chronic (NOEC) test.

The tests summarized in the CLH report for Difethialone were performed under static conditions and concentrations were not measured during the test. Due to the relatively low water solubility of Difethialone, 0.39 mg/L, and the high K_{ow}, test concentrations may have decreased during the study due to adsorption of the test substance on the surfaces of the exposure containers. It is possible that lower EC_{50} values would have been achieved in semi-static or flow-through tests. However, in an additional fish test, where concentrations were measured and recovery rates between 74 and 90% after 24 hours were shown, a LC_{50} of < 0.15 mg/l was obtained.

The effect of Difethialone on *Daphnia magna*, which is the most sensitive species in the reported acute tests, occurs after 24 hours, and a further possible decline in test substance concentration from 24 to 48 hours is not considered significant enough to have influenced the outcome of the test. As the *Daphnia* test has been used as the key study, the losses in the concentrations for fish and algae have no influence on the environmental classification.

Invertebrates (*Daphnia magna*) were the most sensitive taxonomic group in acute tests, with an EC_{50} value of 0.0044 mg/l, while in chronic tests the most sensitive species was *Pseudokirchneriella subcapitata,* with a NOErC value of 0.032 mg/l. Both values were based on nominal concentrations. These two values were used as key end-points to establish the proposed classification and labeling by the DS.

Comments received during public consultation

The acute aquatic classification as Aquatic Acute 1 with an M-factor of 100 was supported by three MS.

Assessment and comparison with the classification criteria

Degradation
RAC agreed that Difethialone can be considered hydrolytically stable and rapidly photodegradable based on the information provided in the CLH report.

RAC also agreed that Difethialone is not readily biodegradable under test conditions. Furthermore, in an aerobic soil study Difethialone showed only a very slow degradation (DT_{50}=635 days). Therefore, based on these data, RAC agreed with the DS that Difethialone should be considered **not rapidly degradable** according to CLP.

Bioaccumulation
The experimental log K_{ow} for Difethialone is 6.29 at pH=7. This value is above the cut-off value of log kow≥4 (CLP), therefore RAC agreed with the DS that Difethialone has a **high potential for bioaccumulation**.

Aquatic toxicity
Classification for acute aquatic toxicity should be based on the lowest toxicity value, i.e. in this case EC_{50} = 0.0044 mg/l (*Daphnia magna*, OECD 203). Since the value is ≤ 1 mg/l, Difethialone should be classified as Aquatic Acute 1 (H400) with an M-factor of 100.

No adequate chronic data was available for all three trophic levels and the only chronic toxicity value available was the algal NOE_rC of 0.032 mg/l (*Pseudokirchneriella subcapitata;* OECD 201*)*. Since the substance is not rapidly degradable, classification as Aquatic Chronic 1 (H410) with an M-factor of 1 would be justified. However, due to the lack of chronic data for fish and invertebrates, the classification according the surrogate approach should to be compared to the classification according to the chronic data. Taking into account the fact that the substance is not rapidly degradable, the log K_{ow} ≥ 4 and the EC_{50} = 0.0044 mg/L (*Daphnia magna*), which is the highest acute toxicity reported, classification as Aquatic Chronic 1 (H410) with an M-factor of 100 is justified, since $0.001 < E(L)C_{50} \leq 0.01$. This classification is the most stringent one and it should be applied to Difethialone.

The main problem with the available tests is that they are based on nominal concentrations and due to the water low solubility and high Log K_{ow} of the substance, test concentrations may have

declined during the study due to adsorption to test vessels and therefore the toxicity could be underestimated. The DS mentioned an additional semi-static study in fish where the recovery rates were between 74 and 90 % after 24 hours (see the section "in depth analyses by the RAC" for details). This justification may not be relevant for the fish, daphnid and algae tests because the static test durations were 96, 48 and 72 h, respectively, and therefore, the toxicity of Difethialone might be underestimated in the reported studies. Further, it is not possible to justify the recoveries of a Daphnia study with the recoveries in a fish study since because the medium is different, the decline of the test concentration from 24h to 48h could be relevant (see "in depth analyses by the RAC" section).

In conclusion, considering these deficiencies in experimental design and the available information, RAC agreed with the DS's proposal to classify Difethialone according to CLP criteria as Aquatic Acute 1 (H400) with an M-Factor of 100. RAC also agreed that the substance should be classified as Aquatic Chronic 1 but disagreed with the proposed M-factor of 1 proposed by the DS. Instead, RAC concluded that the surrogate approach should be applied as the most stringent outcome and therefore, an M-factor of 100 is justified. Therefore, a classification as **Aquatic Acute 1 (H400)** with an **M-Factor 100** and **Aquatic Chronic 1** with an **M-Factor 100** is proposed**.** However, if reliable data based on mean measured concentrations for the three trophic levels were to become available, it is possible that the M-factors might need to be reviewed.

In depth analyses by the RAC

From Doc III A-biocides CAR, Difethialone: Acute toxicity to Invertebrates.

Acute toxicity to *Daphnia magna* (48h, static).

Immobilisation data

Nominal Test-Substance Concentration [µg/L]	**Immobile *Daphnia* (%)**	
	24 hours	**48 hours**
Control	0	0
Solvent control	0	0
0.40	0	0
0.65	0	0
1.1	0	0
1.8	0	0
3.0	0	0
5.0	45	70

Effect data

Endpoint	**EC_{50} [1]**	**95 % c.l.**	**EC_0 [1,2]**
24 h [µg/L]	> 5.0	> 3 (lower limit)	3.0
48 h [µg/L]	4.4	> 3 (lower limit)	3.0

From Doc III A-biocides CAR, Difethialone: Acute toxicity to fish.

Determination of acute toxicity (LC_{50}) to rainbow trout (96 h, semi-static)

Concentrations of [^{14}C]-Difethialone measured in fresh and expired (24 hour) media samples

Table A7.4.1.1-6: Concentrations of [^{14}C]-Difethialone measured in fresh and expired (24 hour) media samples Test Substance Concentration [mg/L]	Measured concentration[a]				Recovery
	0 hours (fresh)	24 hours (expired)	Mean	Initial as % of nominal	24 hour as % of initial
Control	< lod	< lod	< lod	na	na
Solvent control	< lod	< lod	< lod	na	na
0.17	0.16	0.13	0.15	94	76
0.38	0.38	0.28	0.33	100	74
0.83	0.78	0.66	0.72	94	80
1.8	1.75	1.62	1.69	97	90
4.0	3.96	3.54	3.75	99	89

[a] mg equivalent/L; < lod: below limit of detection by LSC (30 dpm); na: not applicable.

The present study cannot be used for classification and labelling as mortality exceeded 50 % at all tested concentrations and the exposure period was only 48 hours. The test should have been performed at lower concentrations. The test has not been performed according to standard guideline which requires that a range-finding test should be performed in order to ensure a proper range of test concentrations. The test period was <96 hours.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the DS; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the DS and rapporteurs' comments (excl. confidential information).