Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

EC number: 259-978-4
CAS number: 56073-07-5

CLH-O-0000003392-78-03/F

Adopted

14 March 2014

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14 March 2014

CLH-O-0000003392-78-03/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: **Difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin**

EC number: **259-978-4**

CAS number: **56073-07-5**

The proposal was submitted by **Finland** and received by the RAC on **23 November 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

Finland 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 **05 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: **Sonja Kapelari**

Co-rapporteurs, appointed by the 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 Difenacoum(ISO) 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	607-157-00-X	difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin	259-978-4	56073-07-5	Acute Tox. 2* STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H300 H372** H400 H410	GHS06 GHS08 GHS09 Dgr	H300 H372** H410		
Dossier submitters proposal	607-157-00-X	difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin	259-978-4	56073-07-5	**Add:** Acute Tox. 1 Acute Tox. 1 Repr. 1A **Modify:** Acute Tox. 1	**Add:** H310 H330 H360D **Retain:** H300 **Modify:** H372 (blood)		**Add:** H310 H330 H360D **Retain:** H300 **Modify:** H372 (blood)		**Add:** STOT RE 1; H372: C ≥ 0,1 % STOT RE 2; H373: 0,01 % ≤ C < 0,1 % M (acute) = 10 M (chronic) = 10
RAC opinion	607-157-00-X	difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin	259-978-4	56073-07-5	**Add:** Acute Tox. 1 Acute Tox. 1 Repr. 1B **Modify:** Acute Tox. 1	**Add:** H310 H330 H360D **Retain:** H300 **Modify:** (blood) for H372 **Remove:** ** for H372		**Add:** H310 H330 H360D **Retain:** H300 **Modify:** (blood) for H372 **Remove:** ** for H372		Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,02 % STOT RE 2; H373: 0,002 % ≤ C < 0,02 % M = 10 M = 10

	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	607-157-00-X	difenacoum (ISO); 3-(3-biphenyl-4-yl-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin	259-978-4	56073-07-5	Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H330 H310 H300 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H330 H310 H300 H372 (blood) H410		Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,02 % STOT RE 2; H373: 0,002 % ≤ C < 0,02 % M = 10 M = 10

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

Difenacoum belongs to a group of compounds known as anticoagulant rodenticides, i.e. those with an anti-vitamin K mode of action (sometimes abbreviated to AVK) 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, only Warfarin is currently classified for toxicity to reproduction in category 1A.

The eight substances 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 referred to be continued at 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).

Endpoints for which no classification was proposed by the dossier submitter have not been assessed by RAC.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Difenacoum is currently classified for acute oral toxicity as Acute Tox. 2* (H300) according to CLP. Due to the different classification criteria between DSD and CLP and based on the available data on Difenacoum, it is now proposed to remove the minimum classification (*) and update the CLP classification for acute oral toxicity 1; H300.

The dossier submitter (DS) also proposed additional classifications for acute toxicity via other routes of exposure i.e. acute inhalation toxicity 1; H330 and acute dermal toxicity 1; H310.

Comments received during public consultation

Three Member States supported the proposed classification.

Assessment and comparison with the classification criteria

Acute toxicity: oral
There are five studies in rats and two in mice performed according to OECD Test Guideline (TG) 401 except for one of the two studies in mice.

The LD_{50} values ranged from 1.8 mg/kg bw to < 50 mg/kg bw in rats and are around 1 mg/kg bw for mice. Deaths resulted from haemorrhages due to anticoagulation occurring at 3-14 days after ingestion of the dose. A study with separate *cis* and *trans* isomers of Difenacoum revealed that the *cis* isomer is somewhat more toxic (approx. 2-5 times depending on species) than the *trans* isomer.

In two studies that were performed according to OECD TG 401 the LD_{50} values in rats were 1.8 mg/kg bw and 2.6 mg/kg bw. These values fall within the criteria for classification for acute toxicity 1; H300 (CLP criterion; $LD_{50} \leq 5$ mg/kg bw).

RAC agreed with the DS that Difenacoum should be classified via oral route as acute toxicity 1, H300.

Acute toxicity: inhalation
In two studies performed according to OECD TG 403, the acute inhalation LC_{50} values in rats ranged between 4-6 µg/L/4hr and 16-21 µg/L/4hr. The limit for classification for acute toxicity 1 via inhalation route is ≤ 0.05 mg/L/4hr for dusts and mists, therefore RAC agreed with the DS that Difenacoum should be classified for acute toxicity 1; H330.

Acute toxicity: dermal
Three studies in rats are available, performed according to OECD TG 402. In one of these studies the LD_{50} values are well below the threshold value for classification for acute toxicity 1, H310 under CLP Regulation. Besides all animals died on days 5-14.

In the other two studies the values are around the threshold value which is ≤ 50 mg/kg bw. In one of these studies Difenacoum was applied on moistened skin and in the other one the substance was used undiluted with no vehicle. Corresponding to OECD TG 402 the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. RAC is of the opinion that the difference between the obtained LD_{50} values can be explained definitely by the fact that Difenacoum was applied in a sesame oil matrix in the study that yielded the lowest LD_{50} values whereas pure powder was used in the other studies.

Consequently RAC agreed with the DS that Difenacoum should be classified as acute toxicity 1; H310 via the dermal route.

In conclusion, RAC supported the classification for acute toxicity 1 for all three routes of exposure.

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

Summary of the Dossier submitter's proposal

Difenacoum is currently classified as STOT RE 1; H372**. In Annex VI of CLP, the existing entry contains two asterisks with a general hazard statement not specifying the route of exposure as the necessary information was not available when the entry was translated from DSD to CLP. The DS proposed to remove the asterisks from the hazard statement H372 because no route of exposure can be excluded.

The DS proposed to derive SCLs using the rabbit LOAEL of 0.01 mg/kg bw/day based on maternal toxicity in a reproductive toxicity study and the dog LOAEL of 0.01 mg/kg bw/day based on anticoagulation effects after repeated exposure.

Comments received during public consultation

Three Member States supported the proposed classification. One of them supported specifically the proposed SCLs by the DS.

Assessment and comparison with the classification criteria

Repeated dose toxicity: oral
There are three studies conducted in rats (two 90 day and one 28 day) according to Guideline OECD No. 408. Each of them shows that repeated oral administration of Difenacoum resulted in marked increase in clotting time and haemorrhages in a wide range of tissues, with treatment related death due to the massive haemorrhages.

The lowest dose used causing treatment-related death was 0.1 mg/kg bw/day. This value is 100 times lower than the limit of 10 mg/kg bw/day for classification for STOT RE 1 according to the CLP Regulation.

Besides there is one 90 day dog study following principles of OECD TG 409 that only lasted for 42 days due to premature sacrifice of the animals when prothrombin time (PT) exceeded the CLP criteria (PT increased to 40/100 seconds). In this study, a clear dose-dependency was seen in the prolonged PT and kaolin-cephalin time (KCT) values in both male and female dogs. Furthermore prolonged PT and KCT were time-dependent effects, and were observed at the lowest administered dose 0.01 mg/kg bw/day which caused prolongation of PT and KCT from day 30. This time-dependence of the increase in PT and KCT is probably a sign of Difenacoum accumulation. A LOAEL of 0.01 mg/kg bw/day was obtained based on prolonged PT and KCT in dogs. The only shortcoming of this study is the low number of experimental animals (2 dogs/dose).

In addition, in two reproductive toxicity studies in rabbits maternal LOAELs were derived based on increased clotting time: a LOAEL of 0.01 mg/kg bw/day was derived based on increased PT and PTT (partial thromboplastin time) in a 22 day study and a LOAEL of 0.015 mg/kg bw/day was derived based on increased PT and KCT in a similar 13 day study.

RAC agreed on the classification for STOT RE 1 according to CLP.

Repeated dose toxicity: inhalation and dermal
Repeated dose studies are available via the oral route only. However, due to similar effects seen in acute oral, dermal and inhalation toxicity studies, a route-to-route extrapolation is reasoned and classification via all three routes for repeated dose toxicity is justified.

RAC therefore supports not specifying exposure routes in the hazard statement. The effect levels are well below the guidance value of 10 mg/kg bw/day warranting classification with STOT RE category 1; H372 (Causes damage to the blood through prolonged or repeated exposure).

Setting specific concentration limits (SCLs):
SCLs based on the rat LOAEL of 0.1 mg/kg bw/day were agreed by TC C&L (Technical Committee on Classification and Labelling) in May 2007 (Follow-up V, May 2008) but were not inserted into Annex VI of CLP.

However, it seems that dogs are more sensitive in terms of change in blood clotting parameters in the studies reviewed in the CLH report. Therefore RAC proposed to derive SCLs based on a dog LOAEL of 0.01 mg/kg bw/day from the repeated dose toxicity study.

Using Haber's law, the effect level derived at day 30 is recalculated into an equivalent 90-day effect level of 0.003 mg/kg bw/day (0.01 mg/kg/day x 30 days / 90 days). Based on the guidance for setting SCL for repeated dose toxicity, an effect level of 0.003 mg/kg/day results in a SCL of 0.03% for STOT RE 1. The SCL value should, according to the guidance, be rounded down to nearest preferred value of 1, 2, or 5, resulting in a SCL of 0.02% for STOT RE 1, and 0.002% for STOT RE 2.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Difenacoum is an anticoagulant that is structurally related to warfarin and other antivitamin K anticoagulants rodenticides (AVKs). Classification for reproductive toxicity is proposed by the DS because of teratogenicity.

A rat multi-generation study high toxicity and premature death were observed and dose levels administered during the study had to be lowered. Sign of changes in oestrus cycle and decreased total sperm count were observed from two very low dose levels but they did not

affect the fertility. However, effects on reproduction may have been masked due to the excessive mortality

The DS concluded that based on the current knowledge of absence of fertility effects of analogous compounds and vitamin K deficiency, Difenacoum should not be classified as toxic to fertility.

Developmental toxicity data on Difenacoum are equivocal. Clear developmental toxicity was not observed. However, Difenacoum is a coumarin derivative like warfarin which is classified as Repr. 1A according to the CLP Regulation. Since also the mode of action causing vitamin K deficiency is the same, and the maternal vitamin K deficiency is the underlying reason for teratogenicity, it is proposed to classify Difenacoum as a reproductive toxicant in category 1A; H360D.

The DS argued that SCLs should be set together with the other AVKs. No numerical value was proposed but the DS supported setting SCLs for Difenacoum at least equal to these proposed for Warfarin.

Comments received during public consultation

Four Member States agreed with the DS proposal to classify Difenacoum as Repr. 1A; H360D based on the human evidence of developmental toxicity of Warfarin.

One Member State pointed out that SCLs for reprotoxicity are necessary for Difenacoum. Furthermore the same Member State suggested to harmonise the SCLs between the other AVK anticoagulants (Warfarin, Flocoumafen, Difethialone, Coumatetralyl, Brodifacoum, Bromadiolone, Chlorophacinon).

Six industry organisations disagreed with the proposed classification for Repr. 1A. They provided two statements from an expert toxicologist to demonstrate that the basis for read-across for developmental toxicity from Warfarin to Difenacoum is invalid.

Assessment and comparison with the classification criteria

Fertility:
A rat multi-generation study conducted according to OECD TG 416 showed excessive mortality. Dose levels had to be lowered twice during the course of the study. Deaths occurring at 0.020 mg/kg bw/day and above were caused by general haemorrhagic diathesis.

There were irregular oestrous cycles in treated animals in both generations and ovarian cyst at maternally toxic dose of 0.06 mg/kg bw/day in F0 females perhaps due to ovarian hormonal disturbance. The fertility index was not affected and no severe increase in post-implantation loss was observed. In addition, there are no indications of adverse fertility effects associated to vitamin K deficiency in the literature.

RAC agreed with the DS that Difenacoum should not be classified as toxic to fertility based on the current knowledge of absence of fertility effects of analogous compounds and vitamin K deficiency.

Developmental toxicity:
Two rat and two rabbit teratogenicity studies performed according to the OECD TG 414 are available.

In the rat studies the NOEL/NOAEL for maternal toxicity was 0.03 mg/kg bw/day. There was no evidence of embryotoxic or teratogenic potential following oral exposure of pregnant rats at 0.09 mg/kg bw/day (= NOEL/NOAEL for developmental toxicity).

In a 22-day rabbit study the LOAEL value for maternal toxicity was 0.001 mg/kg bw/day based on increased haemorrhages in the kidneys. In this study no NOAEL could be set. In the second rabbit study (13 day study) the LOAEL value for maternal toxicity based on prolongation of

prothrombin time was 0.015 mg/kg bw/day. The maternal NOEL/NOAEL value was 0.005 mg/kg bw/day. The longer exposure period lead to typical adverse effects at lower dose. This could be due to accumulation of Difenacoum. Also the slightly different toxicokinetics and different acute toxicity potencies of the cis- and tans-isomers may have contributed to the small difference in the results.

In both rabbit studies foetal effects (mainly skeletal) were not dose but time-dependent. Concerning the 13=day rabbit study it has to be pointed out that many of the vertebral and rib defects were atypical, i.e. not recorded previously in the testing laboratory. However, no clear developmental toxicity was observed in rabbits. The NOEL/NOAEL value for developmental toxicity was 0.015 mg/kg bw/day after 13 days and 0.01 mg/kg bw/day after 22 days of exposure.

Relevance of the OECD TG 414 test for AVKs:
The OECD guideline study on Warfarin (Kubazky, 2009) performed by the CEFIC Rodenticide Data Development Group indicates that Warfarin caused haemorrhages and cataract in the foetus. The incidence of cataract was considered as a manifestation of teratogenicity of Warfarin (Driel van et al., 2002). The skeletal malformations typical for humans were not convincingly observed in the study. However, most of the skeletal and facial defects typical for Warfarin in humans have been demonstrated in rats in studies where Warfarin has been given postnatally since the nasal and skeletal development in rat takes place during late foetal and early postnatal life (Howe and Webster, 1990; Howe and Webster, 1992).

In summary the Kubazky study showed that some of the developmental effects induced in humans by Warfarin were also detectable in rats, but others were not. There is no study on Difenacoum that could be compared to the above mentioned Kubazky study. Therefore it is not possible to draw any conclusions whether Difenacoum would be able to induce cataract in rat if equivalent treatment protocol to Warfarin was used.

It remains doubtful whether a standard OECD TG 414 test can detect coumarin-specific developmental effects.

Overall conclusion on classification for developmental toxicity

Based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (Repr. 1A), the reproductive toxicity of Difenacoum has been analysed in detail. It is acknowledged that the animal developmental toxicity studies on Warfarin were weakly positive and that the animal developmental toxicity studies on Difenacoum were negative. However, in comparison with Warfarin, Difenacoum 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 to avoid maternal toxicity and lethality, relatively low doses in repeated exposure during gestation were used which hindered the detection of developmental toxicity effects.

As there are no data on the outcome of maternal exposure to Difenacoum in humans, classification in category 1A is not considered to be applicable for Difenacoum.

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

The reasons for this presumption were:

- Difenacoum 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 TG 414 test where 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 of pregnancy. Thus, also if some AVK rodenticides may have a lower degree of placental transfer than Warfarin, this will not affect the face malformation hazard as the placenta is not yet fully developed during the first trimester.

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 was of the opinion that the uncertainty is not sufficiently big to warrant a category 2 classification.

Reliable evidence of an adverse effect on reproduction in humans, which is required for Repr. 1A, was not available for Difenacoum, but a potential for human developmental toxicity is presumed based on the above stated weight of evidence assessment. Thus RAC proposed to classify Difenacoum as Repr. 1B; H360D, i.e. "presumed human reproductive toxicant", instead as Repr. 1A; H360D as proposed by the DS.

Setting specific concentration limits (SCLs):
Regarding SCLs for Difenacoum, it is acknowledged that the specific data on developmental toxicity of Difenacoum is too scarce to guide in setting the SCLs.

However, for Warfarin there is sufficient data to set a SCL for developmental toxicity. Thus, based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg bw/day) may cause developmental toxicity and could perhaps 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 bw/day). The guidance states that for an ED_{10} <4 mg/kg bw/day, the SCL is 0.03%, and for ED_{10} below 0.4 mg/kg bw/day the SCL becomes 0.003%. Also if starting from an ED_{10} value obtained from animal studies (0.125 mg/kg bw/day; Kubaszky et al., 2009), it would qualify Warfarin for the high potency group and result in a SCL of 0.003%. Thus, RAC concluded on a SCL of 0.003% for the developmental toxicity of Warfarin.

As the other AVK rodenticides are equally or more toxic than Warfarin, it was not considered appropriate to apply the generic concentration limit (GCL) for these substances (0.3%), but rather to base the SCLs on the SCL proposed for Warfarin. Thus, RAC was of the opinion that the SCL for Warfarin can be used as a surrogate SCL for other AVK rodenticides resulting in a SCL of 0.003% for Difenacoum and the AVK rodenticides Flocoumafen, Defethialone, Coumatetralyl, Brodifacoum, Bromadiolone and Chlorophacinon.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

There is a current entry in Annex VI for Difenacoum with an environmental classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) with no M factors. The DS proposed to add to the current entry M-factors of 10 for both Aquatic Acute 1 and Aquatic Chronic 1.

Degradation
Degradation was studied in four hydrolysis tests, two photolysis tests in water, four ready biodegradability tests, one inherent biodegradation test and one degradation test in soil.

The DS considered Difenacoum as hydrolytically stable (DT_{50} > 1 year, pH =7, 25ºC) and rapidly photodegradable with an experimental half-life about 8 hours at pH 7. It was degraded rapidly in the atmosphere by reaction with OH radicals, although the presence of this compound in air is not expected due to its low vapour pressure.

Difenacoum is not readily or inherently biodegradable under test conditions. In the ready biodegradability tests according to OECD TG 301B, OECD TG 301D, and OECD TG 301F, the level of degradation was between 0-31%, being therefore below the ready biodegradability pass levels of 60 or 70%. In the inherent biodegradation test according to OECD TG 302D draft guideline, the degradation was 3%.

Difenacoum showed a very slow degradation under aerobic conditions in soil with a DT_{50} of 439 days.

The DS concluded based on the available data that Difenacoum is not rapidly degradable.

Bioaccumulation
The estimated log K_{ow} of Difenacoum is 7.62, which is above the cut-off value of log K_{ow} ≥ 4 in CLP. Furthermore, a bioaccumulation test on *Oncorhynchus mykiss* is available, and although it is not considered as a valid study due to the lack of measured concentrations in water, absence of steady-state and high mortality at the higher Difenacoum concentration, the test indicated accumulation of Difenacoum in fish.

In conclusion, since the log K_{ow} indicated high potential for bioaccumulation, the DS concluded that Difenacoum has potential for bioaccumulation.

Aquatic toxicity
Four acute toxicity studies in fish (*Oncorhynchus mykiss* and *Lepomis macrochirus*) with LC_{50} values between 0.064 and 0.557 mg/L, four tests in invertebrates (*Daphnia magna*) with EC_{50} = 0.52-0.91 mg/L and three studies in algae (*Pseudokirkneriella subcapitata* and *Desmodesmus subspicatus)* with E_rC_{50} = 0.51-4.73 and NOE_rCs = 0.13-1.3 mg/L were reported by the DS. No long-term tests in fish and invertebrate are available but the three algae tests can be also considered chronic tests. All the toxicity values for these tests were based on mean measured concentrations.

Fish (*Oncorhynchus mykiss*) was the most sensitive taxonomic group in acute tests, with LC_{50} value of 0.064 mg/l, while in chronic tests the most sensitive algae species was *Pseudokirkneriella subcapitata,* with a NOE_rC value of 0.13 mg/l. However, no adequate chronic data is available for all trophic levels, and in this case the surrogate approach from fish shall be chosen as the most stringent outcome to propose the aquatic chronic classification, taking into account that the substance is no rapidly biodegradable, the log K_{ow} ≥ 4 and the LC_{50} (for fish) ≤ 1 mg/l (EC_{50} = 0.064 mg/L).

Comments received during public consultation

Three Member States supported the environmental classification proposed by the DS. One Member State agreed with the aquatic acute classification and the M-factor of 10 but asked if this M-factor was also for aquatic chronic classification.

In their post public consultation response, the DS confirmed that the M-factor of 10 was proposed for both, aquatic acute and aquatic chronic toxicity.

RAC assessment and comparison with criteria

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

RAC also agreed that Difenacoum is not readily or inherently biodegradable under test conditions. Furthermore, in an aerobic soil study Difenacoum showed a very slow degradation rate (DT_{50} = 439 days), therefore, based on these data, RAC agreed with the DS that Difenacoum should be considered **not rapidly degradable** according to CLP.

Bioaccumulation
The estimated log K_{ow} for Difenacoum was 7.62 which is above the cut-off values of log $K_{ow} \geq 4$ (CLP), therefore RAC agreed with the DS that Difenacoum has **high potential for bioaccumulation**.

Aquatic toxicity
The acute hazard classification should be based on the lowest acute toxicity value, i.e. LC_{50} of 0.069 mg/l (*Oncorhynchus mykiss,* OECD TG 203). Since this value is ≤ 1 mg/l, RAC agreed with the DS to classify Difenacoum as Aquatic Acute category 1 (H400) with an M-Factor of 10.

Regarding chronic toxicity, no adequate chronic data was available for all three trophic levels. Only chronic information from algae were submitted in the CLH report and according to the lowest NOEC of 0.13 mg/L a classification as Aquatic Chronic 2 (H411) could be applied for Difenacoum. However, the surrogate approach must also be applied for chronic toxicity due to the lack of chronic data for fish and invertebrates. Taking into account that the substance is not rapidly degradable, the log $K_{ow} \geq 4$ and the LC_{50} (fish) ≤ 0.1mg/L (0.069 mg/L), which was the highest acute toxicity between invertebrates and fish, classification as Aquatic Chronic 1 (H410) with an M- factor of 10 is justified.

In conclusion, RAC agreed with the DS's proposal to classify Difenacoum according to CLP criteria as Aquatic Acute 1 (H400) with an M-factor of 10 and Aquatic Chronic 1 (H410) with an M-factor of 10.

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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 Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

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