**Committee for Risk Assessment**

**RAC**

# Opinion

proposing harmonised classification and labelling
at EU level of

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

**EC number: 227-424-0**
**CAS number: 5836-29-3**

CLH-O-0000003397-68-03/F

**Adopted**

**14 March 2014**

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CLH-O-0000003397-68-03/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 harmonized classification and labelling (CLH) of:

| | |
|---|---|
| **Chemicals name:** | **Coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin** |
| **EC number:** | **227-424-0** |
| **CAS number:** | **5836-29-3** |

The proposal was submitted by **Denmark** and received by the RAC on **31 October 2012**. All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonized System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

## PROCESS FOR ADOPTION OF THE OPINION

**Denmark** 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: **Bert-Ove Lund**

Co-Rapporteur, 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 harmonized 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 **Coumatetralyl (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** | 607-059-00-7 | coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin | 227-424-0 | 5836-29-3 | Acute Tox. 1<br>Acute Tox. 2 *<br>STOT RE 1<br>Aquatic Chronic 3 | H310<br>H300<br>H372 **<br>H412 | GHS06<br>GHS08<br>Dgr | H310<br>H300<br>H372 **<br>H412 | | |
| **Dossier submitters proposal** | 607-059-00-7 | coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin | 227-424-0 | 5836-29-3 | **Add:**<br>Acute Tox. 2<br>Repr. 1A<br>**Modify:**<br>Acute Tox. 3<br>Acute Tox. 2<br>STOT RE 1<br>Aquatic Chronic 1 | **Add:**<br>H330<br>H360D<br>**Modify:**<br>H311<br>H300<br>(blood coagulation) for H372<br>H410<br>**Remove:**<br>** for H372 | **Add:**<br>GHS09 | **Add:**<br>H330<br>H360D<br>**Modify:**<br>H311<br>H300<br>H372 (blood coagulation) for H372<br>H410<br>**Remove:**<br>** for H372 | | **Add:**<br>STOT RE 1; H372: C ≥ 0,2 %<br>STOT RE 2; H373 0,02 % ≤ C < 0,2 %<br>M (chronic) =10 |
| **RAC opinion** | 607-059-00-7 | coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin | 227-424-0 | 5836-29-3 | **Add:**<br>Repr. 1B<br>Acute Tox. 2<br>**Modify:**<br>Acute Tox. 3<br>Acute Tox. 2<br>STOT RE 1<br>Aquatic Chronic 1 | **Add:**<br>H360D<br>H330<br>**Modify:**<br>H311<br>H372 (blood) for H372<br>H410<br>**Remove:**<br>** for H372 | GHS09 | **Add:**<br>H360D<br>H330<br>**Modify:**<br>H311<br>H372 (blood) for H372<br>H410<br>**Remove:**<br>** for H372 | | Repr. 1B; H360D: C ≥ 0,003 %<br>STOT RE 1; H372: C ≥ 1,0 %<br>STOT RE 2; H373 0,1 % ≤ C < 1,0 %<br>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** | 607-059-00-7 | coumatetralyl (ISO); 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)coumarin | 227-424-0 | 5836-29-3 | Repr. 1B<br>Acute Tox. 2<br>Acute Tox. 2<br>Acute Tox. 3<br>STOT RE 1<br>Aquatic Chronic 1 | H360D<br>H330<br>H300<br>H311<br>H372 (blood)<br>H410 | GHS06<br>GHS08<br>GHS09<br>Dgr | H360D<br>H330<br>H300<br>H311<br>H372 (blood)<br>H410 | | Repr. 1B; H360D: C ≥ 0,003 %<br>STOT RE 1; H372: C ≥ 1,0 %<br>STOT RE 2; H373 0,1 % ≤ C < 1,0 %<br>M=10 |

# SCIENTIFIC GROUNDS FOR THE OPINION

## HUMAN HEALTH HAZARD ASSESSMENT

## RAC general comment

Coumatetralyl 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).

The present CLH proposal contains the classifications agreed in 2007 and new proposals for developmental toxicity and chronic aquatic toxicity.

## RAC evaluation of acute toxicity

### Summary of the Dossier submitter's proposal

Acute toxicity data is available for all routes of exposure (Cat. 2 for oral and inhalation exposure, and Cat. 3 for dermal exposure).

### Comments received during public consultation

One Member State supported the proposed classification and no dissenting views were expressed.

### Assessment and comparison with the classification criteria

The rat was the most sensitive species for the oral route, with $LD_{50}$ values of 15 and 30 mg/kg in females and males, respectively. As these values lie in the range of 5-50 mg/kg, Coumatetralyl should be classified as Acute Tox. 2, H300, for the oral route.

One dermal study consistent with the relevant technical guideline in rats gave $LD_{50}$ values of 258 mg/kg for females and 100-500 mg/kg for males. Three non-guideline studies (all three from the same author) gave much lower $LD_{50}$ values (5-62 mg/kg), but there were no study summaries available for the non-guideline studies in the CAR. Since no study summary was available to the RAC, it was difficult to make an independent assessment of the data. The CLH dossier based the classification proposal on the guideline study, and the RAC assumed that it is more reliable than the other studies. Thus, the $LD_{50}$ value of 258 mg/kg lies within the range of 200-1000 mg/kg, indicating that Coumatetralyl should be classified for the dermal route with Acute Tox. 3, H311.
No guideline study was available for the inhalation route, either by acute or long term exposure. The dossier refers to a study from 1982, which in IUCLID is scored 2 for reliability (valid with restrictions), but in the CLH proposal is not considered sufficiently reliable to establish precise $LC_{50}$ values. There was no study summary available for the study in the CAR. Despite this, the CLH dossier presents precise, $LC_{50}$ values of 0.063, 0.039 and 0.054 mg/L for male rats, female rats, and mice, respectively, which can only be considered indicative. The range for category 2 is 0.05-0.5 mg/L for dusts, and the above $LC_{50}$ value for female rats would place coumatetralyl in

category 1 (≤ 0.05 mg/L) instead, while the values for male rats and mice are borderline for category 2. Since no study summary was available to the RAC, it was difficult to make an independent assessment of the data. However, the proposed classification had been previously agreed by the TC C&L, and other AVK rodenticides are acutely toxic by the inhalation route. The proposed classification is therefore justified by the weight of evidence and the RAC supported classification of coumatetralyl as Acute Tox. 2 (H330) by the inhalation route.

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

### Summary of the Dossier submitter's proposal

The dossier submitter considers this endpoint to be covered by the acute toxicity and skin and eye irritation endpoints.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

The information provided in the acute toxicity studies was too limited to assess whether specific organs were affected by Coumatetralyl after single exposure. However, considering the anticoagulant mode of action, it is not likely that there are specific target organs other than blood (for which the acute toxicity classification is based) affected by single exposure to Coumatetralyl.

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier submitter's proposal

As there are no signs of irritancy in a recent guideline study in rabbits, no classification was proposed.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

There were no signs of skin irritation in a guideline rabbit study, and the RAC therefore supports the conclusion of the dossier submitter of no classification for this endpoint.

## RAC evaluation of eye corrosion/irritation

### Summary of the Dossier submitter's proposal

As there are no signs of irritancy in a recent guideline study in rabbits, no classification was proposed.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

There were no signs of eye irritation in a guideline rabbit study, and the RAC therefore supports the conclusion of the dossier submitter of no classification for this endpoint.

## RAC evaluation of skin sensitisation

### Summary of the Dossier submitter's proposal

A Buehler patch test was negative when testing a 50% suspension of Coumatetralyl for induction and a 25% formulation for challenge. A Magnusson-Kligman guinea–pig maximisation test has also been performed, but the high mortality caused by the intra-dermal application of the substance (5% suspension) hampered assessment of sensitisation potential in this study. As there were no indications of sensitisation, no classification was proposed.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

There were no signs of sensitisation potential of Coumatetralyl in a Buehler patch test, and the RAC therefore supports the conclusion of the dossier submitter of no classification for this endpoint.

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

### Summary of the Dossier submitter's proposal

Three oral repeated dose toxicity studies in rats were available, with a 112 day guideline study as the key study. Extensive mortality was seen at 0.1 mg/kg/day, and blood clotting time was adversely prolonged from doses of 0.02 mg/kg/day. The mode of action was independent of the route of exposure, and the classification should therefore apply to all routes. The effect levels are well below the guidance value of 10 mg/kg/day for a 90 days study, warranting classification as STOT RE 1; H372 (Causes damage to organs (blood) through prolonged or repeated exposure).

### Comments received during public consultation

One Member State supported the proposed classification and no dissenting views were expressed. One Member State pointed out the need for consistent setting of the specific concentration limit among the anti-vitamin K rodenticides.

### Assessment and comparison with the classification criteria

The RAC supported the DS assessment of the repeated dose toxicity data, and agreed that the effect level was < 0.1 mg/kg/day after repeated oral exposure of rats. The blood system is clearly the primary target organ system, with haemorrhages causing mortality. There were histopathological findings in the liver that potentially could be relevant (single cell necrosis and centrilobular fatty changes), but they only occurred at lethal exposure levels and no quantitative information was provided in the CAR. The RAC therefore supported the conclusion that the blood system is the primary target organ, with adverse effects on blood coagulation. Regarding the routes of exposure, repeated dose toxicity studies were only available for the oral route. However, the acute toxicity studies indicated that the toxicity by the inhalation and dermal routes are also significant. The RAC therefore supported not specifying exposure routes in the hazard statement. The effect levels, including that for mortality, are well below the guidance value (GV) of 10 mg/kg/day for a 90 days study, warranting classification as STOT RE 1; H372 (Causes damage to the blood through prolonged or repeated exposure).

#### Specific Concentration Limits (SCL)

A conservative effect level (extensive mortality) of 0.1 mg/kg/day from the 112 day rat study indicates that a SCL should be set for Coumatetralyl, since the effect level is more than one order of magnitude lower than the guidance value (GV). Using Haber's law, the effect level at day 112 was recalculated into an equivalent 90 days effect level of 0.124 mg/kg/day (0.1 mg/kg/day x 112 days / 90 days).

RAC considered, based on the guidance for setting SCLs for repeated dose toxicity, that an effect level of 0.124 mg/kg/day would result in a SCL of 1.24% for STOT RE 1 (0.124/10 x 100%). The SCL value should, according to the guidance, be rounded down to the nearest preferred value of 1, 2, or 5, resulting in a SCL of 1% for STOT RE 1, and 0.1% for STOT RE 2.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier submitter's proposal

The mutagenicity of coumatetralyl has been extensively studied both *in vitro* and *in vivo*, and the data indicate that it does not cause permanent transmissible changes in the amount or structure of a single gene or gene segment, a block of genes or chromosomes. Overall, Coumatetralyl is unlikely to pose a genotoxic hazard to humans, and should not be classified for mutagenicity.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

There are no indications of any genotoxicity potential of Coumatetralyl, and the RAC therefore supported no classification for mutagenicity.

## RAC evaluation of carcinogenicity

### Summary of the Dossier submitter's proposal

Although there was no carcinogenicity study for Coumatetralyl, carcinogenicity is not expected based on lack of mutagenic/genotoxic effects and the absence of effects in the 112 day study that could indicate non-genotoxic carcinogenesis. Thus, no classification for carcinogenicity is warranted.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

The RAC agreed with the dossier submitter that there were no indications for carcinogenicity of Coumatetralyl, and that classification was not warranted.

## RAC evaluation of reproductive toxicity

### Summary of the Dossier submitter's proposal

The available guideline animal studies did not show any developmental toxicity effects. However, due to the difficulties in the design of an optimal study protocol for the detection of potential teratogenic effects following exposure to Coumatetralyl without mortality, these studies are not regarded as suitable to evaluate the developmental toxicity potential of anticoagulants. Since Coumatetralyl belongs to the same chemical group and has the same well-known mode of action by which Warfarin causes teratogenicity in humans and in experimental animals (through vitamin K inhibition), Coumatetralyl should be classified for developmental toxicity as Repr. 1A (H360D) based on its similarities with Warfarin.

### Comments received during public consultation

Comments were received from four Member States which all supported classification as Cat. 1A for reproductive toxicity, mainly based on read across from the human data on Warfarin (having the same anti-vitamin K anticoagulation mode of action (MoA) as Coumatetralyl) and since animal

studies on AVK rodenticides were found to be inconclusive. Three industry organisations opposed any classification for effects on development, mainly because none of the non-warfarin AVK rodenticides caused adverse effects on development in animals.

## Assessment and comparison with the classification criteria

Coumatetralyl and Warfarin share the same MoA, i.e., they inhibit vitamin K epoxide reductase, an enzyme involved with blood coagulation and bone formation. Several other AVK rodenticides have also been developed with the aim of retaining the same MoA but with more potent rodenticide activity than warfarin. However, Coumatetralyl is regarded as a first generation AVK rodenticide, and has physico-chemical properties that are very similar to those of Warfarin. The AVK rodenticides have similar functional groups (hydroxycoumarin) and all inhibit vitamin K epoxide reductase and vitamin K reductase. Vitamin K is necessary for proper functioning of carboxylases needed for both blood coagulation and bone development.

In humans, Warfarin is known to cause death of embryos or foetuses and malformations, mainly nasal hypoplasia. Since deformation of the naso-maxial part of the face is very specific, it is also referred to as human "warfarin embryopathy", and Warfarin is consequently classified as a known human developmental toxicant in category Repr. 1A (H360D).

Two other coumarins, i.e., Acenocoumarol and Phenprocoumon, are also used as AVK-drugs in medicine because of their anticoagulant properties. They also are human teratogens, with five and eight cases of congenital anomalies (85% involving the nose) reported until 2002 for Acenocoumarol and Phenprocoumon, respectively (van Driel, 2002). It has been argued that the second generation rodenticides have different elimination half-lives compared to Warfarin and therefore are less likely to be teratogens. Therefore, it is noteworthy that Acenocoumarol and Phenprocoumon exhibit teratogenicity despite having different pharmacokinetics (half-lives) compared to Warfarin. Thus, half-lives of 2-8 hours are reported for Acenocoumarol, 30-45 hours for Warfarin, and 156-172 hours for Phenprocoumon (Rane and Lindh, 2010). It seems that the MoA is more important than half-life as determinant for developmental toxicity as expressed as e.g. deformation of the face.

There are also 2 human cases described for the second generation AVK rodenticide Brodifacoum, indicating similar effects of Brodifacoum and Warfarin in humans, and more severe effects in the foetus than in the mother.

Although the experimental animal studies with Coumatetralyl do not indicate any developmental toxicity, there are uncertainties as to the predictability of these studies for humans, and there is also some theoretical basis for assuming that humans and experimental animals may respond differently to the AVK rodenticides, including Coumatetralyl.

### 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 Coumatetralyl has been analysed in detail. It is acknowledged that the animal developmental toxicity studies with Warfarin were weakly positive and that the animal developmental toxicity studies with Coumatetralyl were negative.

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

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

The reasons for this conclusion are:

- Coumatetralyl shares the same MoA as that expressed by other anticoagulant AVK rodenticides and coumarin pharmaceuticals (inhibition of vitamin K epoxide reductase, an

enzyme involved with blood coagulation and foetal tissues development, including bone formation, CNS development and angiogenesis)

- Warfarin and 2 other coumarin pharmaceuticals (acenocoumarol, phenprocoumon) have been shown to cause developmental toxicity in humans.
- One of the 2nd generation AVK rodenticides (Brodifacoum) has been shown to cause foetal effects in humans, possibly after one or a few exposures.
- The standard animal studies will not pick up all developmental toxicity effects of the AVK rodenticides, most notably the face and CNS malformations that are characteristic for Warfarin and other AVK coumarin pharmaceuticals.
- The most sensitive window for face malformations in humans is the first trimester. Thus, 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 only a Cat. 2 classification.

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

**Specific Concentration Limit (SCL)**

Regarding a specific concentration limit (SCL) for Coumatetralyl, it is acknowledged that the specific data on developmental toxicity of Coumatetralyl are too limited to be used to set the SCL.

However, for Warfarin there are sufficient data to set a SCL for developmental toxicity. Thus, based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg/day) may cause developmental toxicity and could be regarded as an $ED_{10}$ level. This human $ED_{10}$ value would, if using the guidance for setting SCLs based on animal data place coumatetralyl in the high potency group (< 4 mg/kg/day). The CLP 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 has 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 is not considered appropriate to apply the generic concentration limit for these substances (0.3%), but instead 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 Coumatetralyl.

# ENVIRONMENTAL HAZARD ASSESSMENT

# RAC evaluation of environmental hazards

## Summary of Dossier submitter's proposal

There is a current entry in Annex VI of CLP Regulation for Coumatetralyl with an environmental classification as Aquatic Chronic 3 (H412) under CLP. According to the data included in the CLH report, the DS proposed to harmonise the classification for Coumatetralyl as Aquatic Chronic 1, H410 (M = 10) according to CLP.

*Degradation*
Degradation was studied in one hydrolysis test, one photolysis test in water, one ready biodegradability test, one inherent biodegradation test and two aerobic and anaerobic biodegradation tests in soil.

The DS considered Coumatetralyl as hydrolytically stable. It is also rapidly photodegradable with an experimental half-life less than 1 day. It was degraded rapidly in the atmosphere by reaction with ozone and OH radicals.

Coumatetralyl is not readily or inherently biodegradable under test conditions. In the ready biodegradability tests according to OECD TG 301D, the level of degradation was 0-1% after 28 days, being therefore below the ready biodegradability pass levels of 60%. In the inherent biodegradation test conducted according to OECD draft guideline 302D, the degradation was 8%.

Coumatetralyl showed very slow degradation under anaerobic conditions in soil with degradation lower than 1 % during 60 days. Under aerobic conditions, the $DT_{50}$ was < 30 days based on primary degradation and after 6 months more than 50% of the originally applied radioactivity was degraded to $CO_2$. The proportion of bound residues was about 30%.

Based on the available data a non-rapid/ready degradation was proposed for Coumatetralyl.

*Bioaccumulation*
The bioconcentration of [$^{14}$C]-coumatetralyl in bluegill sunfish was conducted according to OECD guideline 305E.

The calculated bioconcentration factors for edible parts and whole fish were 3.32 and 11.4 L/Kg based on total residues level, and therefore the values may have been overestimated. However, these BCFs are lower than the cut-off value of BCF > 500 L/Kg, and the overestimation of the experimental BCFs is not relevant for the classification.

Furthermore, the experimental log $K_{ow}$ of Coumatetralyl is 1.50 at pH 7. This value is below the cut-off value of log $K_{ow}$ ≥4.

In conclusion, based on the available data, the DS concluded that Coumatetralyl has no potential for bioaccumulation.

*Aquatic toxicity*
Acute and chronic toxicity studies in fish (*Oncorhynchus mykiss,* OECD TG 203 and 204), invertebrates (*Daphnia magna,* OECD TG 202 and OECD TG 202 part II) and algae (*Pseudokirchneriella subcapitata,* OECD TG 201) were reported by the DS.

All the acute endpoints (L(E)$C_{50}$) reported in the CLH dossier for the three trophic levels were higher than 1 mg/L: $LC_{50}$ (96h, fish) = 53 mg/L; $EC_{50}$ (48h, invertebrate) > 14 mg/L and $E_rC_{50}$ (72h, algae) > 18 mg/L and therefore, no aquatic acute classification was proposed. In chronic tests the most sensitive trophic level was fish (*Oncorhynchus mykiss,* OECD TG 204) with a NOErC value of 0.005 mg/L and this was used as a decisive study for the chronic classification. NOEC values for invertebrates and algae were 0.1 mg/L (*Daphnia magna*) and 5.6 mg/L (*Pseudokirchneriella subcapitata*), respectively.

## Comments received during public consultation

Two Member States supported the classification proposed by the DS. One member state did not agree because the prolonged acute NOEC for fish (0.005 mg/L) was seen as not appropriate for chronic classification and that a true chronic value may well be lower than this and that reliable chronic endpoints for algae and Daphnia (0.1 mg/L) are also available. They considered, therefore, that for a non-readily degradable substance the lowest Daphnia endpoint should be used - resulting in a CLP classification of 'Chronic 1' (M-factor = 1).

In their post public consultation response, the DS did not agree with this comment because the substance is an anticoagulant and fish are expected to be the most sensitive group and algae and crustacean less sensitive. Basing the M-factor on the crustacean chronic NOEC as proposed by the MS would clearly underestimate the M-factor.

As pointed out by the MS a true chronic NOEC or $EC_{10}$ will in all probability be lower than 0.005 mg/L, because the given NOEC value is from a prolonged acute test and not a true chronic test.

On the basis of the arguments above the DS continued to support the original proposal of an M-factor of 10.

## RAC assessment and comparison with criteria

*Degradation*
RAC agreed that Coumatetralyl can be considered hydrolytically stable and rapidly photodegradable based on the information provided in the CLH report, but was not readily or inherently biodegradable under test conditions (OECD TG 301D and OECD TG 302B), with a level of degradation lower than 8% after 28 days. Furthermore, in an aerobic soil study, Coumatetralyl showed a rapid primary degradation ($DT_{50}$ < 30 days), however 50% of the originally applied radioactivity was degraded to $CO_2$ only after 6 months.

Based on this data, RAC agreed with the DS that Coumatetralyl is **not rapidly degradable** according to CLP.

*Bioaccumulation*
The experimental bioconcentration factors based on tests consistent with OECD TG 305 for edible parts and whole fish are 3.32 and 11.4 kg/L, respectively, based on total residues. These values are lower than the cut-off values of BCF > 500, and although the experimental BCFs are overestimated because they were based on total residues, the levels were so low that they are not relevant for classification. Furthermore, the experimental log $K_{ow}$ for Coumatetralyl is 1.50 at pH 7 (pH dependent) which is also below the cut-off value of log $K_{ow}$ ≥ 4 (CLP), therefore the RAC agreed with the DS that Coumatetralyl has **low potential for bioaccumulation**.

*Aquatic toxicity*
Under CLP, classification for acute toxicity should be based on the lowest L(E)$C_{50}$, and in this case all the acute endpoints reported in the CLH dossier for the three trophic levels were higher than 1 mg/L: fish $LC_{50}$ (96h) = 53 mg/L; invertebrate $EC_{50}$ (48h) > 14 mg/L and algae $E_rC_{50}$ (72h) > 18 mg/L. For fish, the defined $LC_{50}$ was based on nominal concentrations, since the concentration of Coumatetralyl was not measured in the test media. An acute toxicity test with invertebrates did not induce sufficient toxicity to calculate a proper $EC_{50}$ value and therefore the reported $EC_{50}$ value (based on measured concentrations) can only be used to indicate that no toxicity was observed in the concentration range applied. Similarly, insignificant acute toxicity on the growth rate of algae was observed and the result indicates only that the $E_rC_{50}$ is higher than the concentration range applied. For algae, the concentration of the substance was measured only at the beginning of the study and the concentrations varied from 78% to 94% and led to use of nominal concentrations. However, an additional control with algae was carried out in the chronic Daphnia study (OECD TG 202 part II) and the recoveries after 72 h incubation were higher than 80%. This together with the high recovery of the nominal concentration in the beginning of the algae test suggested that the nominal concentrations in the algae test are reliable and the $E_rC_{50}$ value is not expected to be lower than 1 mg/L. RAC concluded that the reported acute toxicities of the three trophic levels do not justify classification for aquatic acute hazards.

Regarding chronic toxicity, only the tests on invertebrates and algae are technically recognised as chronic tests with NOEC values of 0.10 and 5.6 mg/L, respectively. In the semi-static Daphnia test, the concentration of Coumatetralyl analysed in the freshly prepared test medium were between 72 and 97% of the corresponding nominal concentrations (on average 83.4%). The concentrations in the old medium were not measured, however, according to the acute toxicity test in Daphnia, the substance showed stability after exposure period of 48 hours. A similar 48 hours renewal of the medium was applied in the chronic semi-static test and the use of nominal concentrations can be considered acceptable.

In addition to the chronic daphnia and algae tests, prolonged toxicity of Coumatetralyl to rainbow trout (*Oncorhynchus mykiss*) following the OECD TG 204 was reported. The study was extended to 21 days instead of the usual 14 days in the guideline. The DS considered the prolonged fish toxicity test as a long-term toxicity test but the Guidance on the Application of the CLP Criteria does not recognise this study as a chronic test. The test was also assessed under the Biocides Directive and it was considered reliable and the 21d NOEC was used, although not as a chronic value.

A 21d NOEC (mortality) was determined to be 5.0 µg/L. The value is based on nominal concentrations, since the measured concentrations were between 81% and 120% of nominal. In the test, there were no statistically significant differences in body weight, length and condition factor between the control and the treated groups. The study (the total number of fish at each

concentration/control was 10) showed over 50% mortality at concentrations 15.8 µg/L (70% mortality), 50 µg/l (60%), 158 µg/l (77.8%) and 500 µg/l (44.4%); no mortality was observed at other concentrations (0.158 µg/L, 0.500 µg/L, 1.58 µg/L and 5 µg/L) or controls. However, there was no clear dose-response relationship (see in depth analysis section below) in the observed mortality. Also, other effects were reported, including exophthalmos at 15.8 µg/L (day 20) and inactive fish that lay at the bottom of the tanks and irregular swimming at 500 µg/l (day 21). In spite of the observed deficiencies, the test indicated that a true chronic value may well be lower than the reported NOEC value. Further, the mechanism of action of anticoagulant rodenticides suggests that fish would be expected to be the most sensitive trophic level; for none of the other seven anticoagulants under discussion is chronic data available and in five cases out of seven the fish was the most sensitive trophic level for acute toxicity. However, the structure, log $K_{ow}$, molecular weight and size of Coumatetralyl show closest similarity to warfarin, the only anticoagulant out of seven with an Aquatic Chronic 2 classification and no Aquatic Acute classification. The other anticoagulants have a higher log $K_{ow}$ than Coumatetralyl and warfarin, which is possibly one of the factors explaining their higher toxicity and classification as Aquatic Acute 1 and Chronic 1.

Based on the evidence summarized above and the weight of evidence and expert judgment, RAC agreed with the DS's proposal that classification for long-term aquatic hazards should be based on the NOEC of 0.005 mg/L, provided by the prolonged fish toxicity test (OECD TG 204) in *Oncorhynchus mykiss*. This study should be used as a decisive study since a true chronic toxicity study in fish is not available and since it shows highest toxicity among the reported NOEC values. Therefore, a classification as **Aquatic Chronic 1 (H410)** with an **M-factor** of **10** according to CLP is warranted.

However, if reliable chronic data for fish were to become available, it is possible that the classification might need to be reviewed.

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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).