Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 March 2014

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Coumatetralyl

EC Number: **227-424-0**

CAS Number: **5836-29-3**

Index Number: **607-059-00-7**

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Part A.

PART A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance identity

Table 1: Substance identity

Substance name:	***Coumatetralyl***
EC number:	***227-424-0***
CAS number:	***5836-29-3***
Annex VI Index number:	***607-059-00-7***
Degree of purity:	***min. 980 mg/kg***
Impurities:	***Information on impurities is presented in the Confidential part of the CA-report.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox. 1; H310 Acute Tox. 2; H300 STOT RE 1; H372 Aquatic Chronic 3; H412	T+; R27/28; T; R48/24/25 and R52/53
Current proposal for consideration by RAC[1]	Repr. 1A; H360D Acute Tox 2; H330 Acute Tox 3; H311 STOT RE 1; H372 *(blood coagulation)* Aquatic Chronic 1; H410	Repr. Cat 1; R61 T+; R26; T; R24 (instead of T+;R27); T; R48/23[/24/25] R52/53
Proposal for specific concentration limits[2]	*C ≥ 0.2%:* STOT RE 1; H372 *(blood coagulation)* *0.02% ≤ C < 0.2%*: STOT RE 2; H373 *(blood coagulation)*	*C ≥ 0.1:* T; 48/23/24/25 *0.01% ≤ C < 0.1%:* Xn; R48/20/21/22

	Setting SCL for Repr. 1A; H360D should be considered. Aquatic Chronic 1; H410 M-factor: 10	
Precautionary statements/ S-phrases	P201 P308 +P313 P320 P273 P391 P405 P501 Additional statement on the specific antidote: vitamin K, should be given.	S53 S45 S61
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1A; H360D Acute Tox 2; H300 Acute Tox 2; H330 Acute Tox 3; H311 STOT RE 1; H372 Aquatic Chronic 1; H410 *SCL*: [Discussion on SCL for reprotox H360D] *≥ 0.2%:* STOT RE 1; H372 *(blood coagulation)* and *0.02% ≤ C < 0.2%*: STOT RE 2; H373 *(blood coagulation)* M-factor: 10	Repr. Cat 1; R61 T+; R26/28; T; R24 T; R48/23/24/25 R52/53 *SCL:* *C ≥ 25%:* T+; R61-24-26/28-48/23/24/25-52-53 *7% ≤ C < 25%:* T+; R61-21-26/28-48/23/24/25 *3% ≤ C < 7%:* T; R61-21-23/25-48/23/24/25 *1% ≤ C < 3%:*T; R61-23/25-48/23/24/25 *0.5% ≤ C < 1%:* T; R61-20/22-48/23/24/25 *0.1% ≤ C < 0.5%:*T; R20/22-48/23/24/25 *0.01% ≤ C < 0.1%:* Xn; R48/20/21/22

[1] The classification proposal for all end-points except for Reproductive Toxicity, Developmental (Repr.1A, H360D and Repr. Cat1;R61 respectively) was agreed by the Member States at the TC C&L meeting in May 2007.

[2] The calculation method for the proposed specific lower concentration limits for T; R48/23/24/25 according to Directive 67/548/EC was agreed by the TC C&L in 2007. Setting specific concentration limits according to the CLP regulation was not discussed by the TC C&L.

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	**None**	-	None	Conclusive data but not sufficient for classification
2.2.	Flammable gases	**n.a.**	-	-	-
2.3.	Flammable aerosols	**n.a.**	-	-	-
2.4.	Oxidising gases	**None**	-	None	Conclusive data but not sufficient for classification
2.5.	Gases under pressure	**n.a.**	-	-	-
2.6.	Flammable liquids	**n.a.**	-	-	-
2.7.	Flammable solids	**None**	-	None	Conclusive data but not sufficient for classification
2.8.	Self-reactive substances and mixtures	**None**	-	None	-
2.9.	Pyrophoric liquids	**n.a.**	-	-	-
2.10.	Pyrophoric solids	**None**	-	None	-
2.11.	Self-heating substances and mixtures	**None**	-	None	-
2.12.	Substances and mixtures which in contact with water emit flammable gases	**None**	-	None	-
2.13.	Oxidising liquids	**n.a.**	-	-	-
2.14.	Oxidising solids	**None**	-	None	Conclusive data but not sufficient for classification
2.15.	Organic peroxides	**n.a.**	-	-	-
2.16.	Substance and mixtures corrosive to metals	**None**	-	None	-
3.1.	Acute toxicity - oral	**Acute Tox 2, H300**	**n.a.**	Acute Tox. 1 H300	-
	Acute toxicity - dermal	**Acute Tox 3; H311**	-	Acute Tox. 2 H310	-
	Acute toxicity - inhalation	**Acute Tox 2; H330**	-	none	-
3.2.	Skin corrosion / irritation	**None**	-	-	Conclusive data but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.3.	Serious eye damage / eye irritation	**None**		none	Conclusive data but not sufficient for classification
3.4.	Respiratory sensitisation	**None**		none	-
3.4.	Skin sensitisation	**None**		-	Conclusive data but not sufficient for classification
3.5.	Germ cell mutagenicity	**None**		-	Conclusive data but not sufficient for classification
3.6.	Carcinogenicity	**None**		-	Data lacking
3.7.	Reproductive toxicity	**Repr. 1A; H360D**	**To be discussed.**	**none**	-
3.8.	Specific target organ toxicity –single exposure	**None**		**none**	-
3.9.	Specific target organ toxicity – repeated exposure	**STOT RE1; H372**	**C ≥ 0.2%: STOT RE1; H372:** **0.02% ≤C < 0.2 %:** **STOT RE2; H373**	STOT RE 1	-
3.10.	Aspiration hazard	**None**		none	-
4.1.	Hazardous to the aquatic environment	**Aquatic Chronic 1; H410**	**M-factor: 10**	Aquatic Chronic 3; H412	-
5.1.	Hazardous to the ozone layer	**None**			Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word:

Danger

Hazard statements:
H360D: May damage the unborn child
H300: Fatal if swallowed
H311: Toxic in contact with skin
H330: Fatal if inhaled
H372: Causes damge to organs through prolonged or repeated exposure
H410: Very toxic to aquatic life with long lasting effects

Precautionary statements:
P201; P 273; P308 +P313; **P320;** P391; P405; P501
Additional statement on the specific antidote: vitamin K, should be given.

Proposed notes assigned to an entry: none

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	**none**	-	-	Data conclusive but not sufficient for classification
Oxidising properties	**none**	-	-	Data conclusive but not sufficient for classification
Flammability	**none**	-	-	Data conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	**none**	-	-	Data conclusive but not sufficient for classification
Thermal stability	**none**	-	-	Data conclusive but not sufficient for classification
Acute toxicity	**T+:R26/28; T:R24**	**C≥7%**	T+; R27/28;	-
Acute toxicity – irreversible damage after single exposure	**none**	-	-	Data conclusive but not sufficient for classification
Repeated dose toxicity	**T: R48/23/24/25** **Xn: R48/20/21/22**	**C≥0.1%** **0.01%≤C<0.1%**	T; R48/24/25	-
Irritation / Corrosion	**none**	-	-	Data conclusive but not sufficient for classification
Sensitisation	**none**	-	-	Data conclusive but not sufficient for classification
Carcinogenicity	**none**	-	-	Data lacking
Mutagenicity – Genetic toxicity	**none**	-	-	Data conclusive but not sufficient for classification
Toxicity to reproduction – fertility	**none**	-	-	Data conclusive but not sufficient for classification
Toxicity to reproduction – development	**Repr 1; R61**	-	-	-
Toxicity to reproduction – breastfed babies. Effects on or via lactation	**none**	-	-	Data lacking
Environment	**R52/53**	-	R52/53	-

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: **T+**

R-phrases: **R61-24-26/28-48/23/24/25-52/53**
S-phrases: **S53-45-61**

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Coumatetralyl was classified in 24th ATP to dir 67/548/EC.

2.2 Short summary of the scientific justification for the CLH proposal.

Coumatetralyl is an anticoagulant rodenticide. The CLH proposal is based on the information of the CA report under directive 98/8/EC, and the discussion and conclusions of the TC C&L group in 2006 and 2007. All end-points of the proposal except the classification for developmental toxicity and the setting of specific concentration limits were agreed by the TC C&L as referred in the Follow-up sheet V (FUV) from the meeting of May 2007. However, additional criteria introduced with the 2nd ATP to the CLP regulation relating to aquatic toxicity classification trigger reevaluation of this end-point.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation:

Hazard Class and Category Code(s):
Acute Tox. 1
Acute Tox. 2
STOT RE 1
Aquatic
Chronic 3

Pictogram, Signal Word Code(s):
GHS06
GHS08

Hazard statement code(s):
H310
H300
H372
H412

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification (Hazard class and R-phrases):
T+; R27/28
T; R48/24/25
R52-53

Labelling (Symbols of Danger, R- and S phrases):
T+
R: 27/28-48/24/25-52/53
S: (1/2-)28-36/37-45-61

2.4 Current self-classification and labelling

Not relevant, as substance was included in Annex I to directive 67/548/EC and is thus included in Annex VI, tables 3.1 and 3.2 of CLP Regulation.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is a biocide, regulated under dir 98/8/EC, and must therefore be classified at Community Level (cf. art. 36(3) of CLP Regulation. The existing classification is proposed to be amended with respect to acute toxicity, repeated dose toxicity, reproductive toxicity (development) and chronic aquatic toxicity. No changes are proposed to the existing classification on physico-chemical properties.

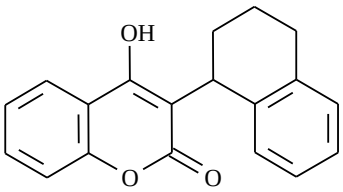
PART B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity and purity

EINECS-No.	227-424-0
Chemical name	3-(alpha-tetralyl)-4-hydroxycoumarin
CAS-No.	5836-29-3
CAS name:	2H-1-Benzopyran-2-one, 4-hydroxy-3-(1, 2, 3, 4-tetrahydro-1-naphthalenyl)-
Other No. (CIPAC, ELINCS)	CIPAC No.: 189
IUPAC Name	IUPAC name: 4-hydroxy-3-(1, 2, 3, 4-tetrahydronaphtalen-1-yl)-2*H*-chromen-2-one
Common name, Synonym	Coumatetralyl ENE 11183 b (Manufacturer's development code number), Racumin®
Molecular formula	C_{19} H_{16} O_3
Structural formula	
Molecular weight (g/mol)	292.3
Purity	≥98,8%
Impurities, additives in the active substance	The specification and details of impurities of the coumatetralyl as placed on the market are presented in the attached confidential annex on impurities.. The impurities are not considered toxicologically significant. The purities of the batches tested in the toxicological and environmental studies are of 98.8-99.8%, with is the specification.

4.10 Physico-chemical properties

In this section summaries and evaluation of data for which robust study summaries are presented in the annex: “CLH report coumatetralyl RSS CAR identity-phys chem” which is reproduced from the final CAR report under the review programme under the biocides directive (98/8/EC are reported, as far as possible in summary tables. Due to an interim period agreement, the robust study summaries are not available in IUCLID.

A summary of the physico-chemical properties of coumatetralyl is given in table 1.3.

Table 1.3 Physico-chemical properties of coumatetralyl

Study	**Method**	**Purity/ Specification**	**Result**	**Reference**	**Section in CA-report**
Physical state	Data based on visual or other sensual assessment at room temperature	99.96 % / ≥ 98 %	Solid, crystalline powder	Stöcker, 2001	A3.3.1
Colour	Data based on visual or other sensual assessment at room temperature	99.96 % / ≥ 98 %	White to yellow-grey	Stöcker, 2001	A3.3.2
Odour	Data based on visual or other sensual assessment at room temperature	99.96 % / ≥ 98 %	Characteristic, slight	Stöcker, 2001	A3.3.3
Melting point	Directive 92/69/EC, A.1 (DTA-system)	98.8 % / ≥ 98 %	168.8 °C	Jungheim, 2000	A3.1.1
Boiling point	Directive 92/69/EC, A.2 (DTA-system)	98.8 % / ≥ 98 %	No boiling point up to the composition of the test substance	Jungheim, 2000	A3.1.2

Study	**Method**	**Purity/ Specification**	**Result**	**Reference**	**Section in CA-report**
Relative density	Directive 92/69/EC, A.3 (displacement method)	98.8 % / $\geq$ 98 %	1.328 at 20 °C	Jungheim, 2000	A3.1.3
Bulk density			Approx. 1 kg/l	Bayer AG, 1999 (MSDS)	
Vapour pressure	Directive 92/69/EC, A.4 (vapour pressure balance method)	98.8 % / $\geq$ 98 %	< 1.0E-05 hPa (20 °C)	Olf, 2000	A3.2
Surface tension	OECD guideline 115, Directive 92/69/EC, A.5 (ring method)	98.8 % / $\geq$ 98 %	73.08 mN/m at 20.1 °C and 4.383 mg/L (90% saturation) The test substance is not surface active.	Olf, 2001	A3.13
Water solubility	Directive 92/69/EC, A.6 (flask method)	98.9 % / $\geq$ 98 %	at 20 °C:4.40E-03 g/l pH 5.1 (pH 5:4.78E-03 g/l, pH 7: 4.60E-01 g/l,pH 9: 4.65 g/l)	Erstling and Jungheim, 2002a	A3.5
Partition coefficient n-octanol/water	Directive 92/69/EC, A.8 (shaking method)	98.9 % / $\geq$ 98 %	Results at 23 °C demineralised water: Log P_{ow}(pH 5.8) = 2.9 (buffer solutions: log P_{ow} (pH 5) = 3.4, log P_{ow} (pH 7) = 1.5, log P_{ow} (pH 9) = -0.1)	Erstling and Jungheim, 2002b	A3.9
Henry's Law constant	Calculation		At 20°C : $K < 6.64.10^{-2}$ $Pa.m^3.mol^{-1}$	Stöcker, 2004	A3.2.1

Study	**Method**	**Purity/ Specification**	**Result**	**Reference**	**Section in CA-report**
Flash-point	Directive 92/69/EC, A.9	98.8 % / ≥ 98 %	The test substance is a solid. The requested test according to EC method A.9 was not necessary. This test guideline is only applicable to liquid materials.	Heitkamp, 2001	A3.12
Flammability	Directive 92/69/EC, A.10	98.8 % / ≥ 98 %	The test substance is not highly flammable.	Heitkamp, 2001	A3.11
Evolution of flammable gases when contact with water	Directive 92/69/EC, A.12	98.8 % / ≥ 98 %	The test substance does not liberate gases in hazardous amounts upon contact with water.	Heitkamp, 2001	A3.11
Pyrophoric properties	Directive 92/69/EC, A.13	98.8 % / ≥ 98 %	The EC method A.13 was omitted as the test substance did not deliver indications of pyrophoric properties during the realisation of tests as defined in EC methods A.10 and A.12.	Heitkamp, 2001	A3.11
Explosive properties	Directive 92/69/EC, A.14	98.8 % / ≥ 98 %	The test substance has no explosive properties	Heitkamp, 2001	A3.15
Self-ignition temperature (Auto-flammability)	Directive 92/69/EC, A.16	98.8 % / ≥ 98 %	No self-ignition up to the melting point at 168.8°C	Heitkamp, 2001	A3.11
Oxidizing properties	Directive 92/69/EC, A.17	98.8 % / ≥ 98 %	The test substance has no oxidising properties	Smeykal, 2006	A3.16
Granulometry	No data submitted				

Study	Method	Purity/ Specification	Result	Reference	Section in CA-report
Solubility in organic solvents	Comparable to OECD-Guideline 105	98.8 % / ≥ 98 %	Results at 20 °C: 2-Propanol: 16.4 g/l, Dichloromethane: 44.3 g/l Xylene: 2.7 g/l, Polyethylenglycol: 37.9 g/l Acetone: 25.4 g/l, Ethylacetate: 11.8 g/l, Acetonitrile: 6.8 g/l, Dimethylsulfoxide: >250 g/l Cyclohexanone:80.9 g/l Toluene: 3.9 g/l	Jungheim, 2001	A3.7
Thermal stability	Directive 92/69/EC, A.1 (Test was conformed using Differential-and Isothermal Step-Thermal Analysis (DTA and ISTA)	98.8 % / ≥ 98 %	Exothermal decomposition starts at 195 °C	Jungheim, 2000	A3.10
Dissociation constant	In accordance with OECD 112	98.8 % / ≥ 98 %	pK-value in water/acetone = 5.3	Jungheim, 2001	A3.6
Viscosity			Not applicable, active substance is a solid		A3.14
Reactivity towards container material	Coumatetralyl has been produced by Bayer since 1957. For at least 25 years the active substance has been packaged in an LDPE sack enclosed in a steel drum. Based upon this experience, recommended container materials are plastic materials e.g. PE or high-grade steel. Aluminium, unprotected steel or iron are not suitable for container material.			Böcker, 2004	A3.17

2 MANUFACTURE AND USES

2.11 Manufacture

No data available in the CA-report under 98/8/EC review programme

2.12 Identified uses

Coumatetralyl is a biocidal active substance used in products to control rodents, especially rats. It is included on Annex I to the biocides directive for use in PT 14, rodenticide.

4.11 Mode of action

Coumatetralyl is classified as a first-generation anticoagulant rodenticide. Anticoagulant rodenticides are vitamin K antagonists, thereby inhibiting coagulation of the blood and making the walls of the blood vessels permeable. The mode of action of coumatetralyl was described by Andrews (1999):

The main site of action is the liver and the main effect consists in inhibition of blood clotting by interference with the hepatic synthesis of the vitamin K-dependent clotting factors II, VII, IX and X which effectively inhibits de-novo synthesis of vitamin K_1, thereby interrupting cellular recycling of vitamin K_1. Vitamin K_1 in its hydroquinone form is an essential co-factor for the synthesis of functional clotting factors. The function of vitamin K_1 is the post translational transformation of the precursor protein to respective functional clotting factors by γ-carboxylation of their glutamic acid moieties, which in turn, enhances the binding of Ca^{++} by chelating phosphate on the phospholipids, thus accelerating, and providing a template for, the blood clotting mechanism. Concomitant with the γ-carboxylation of glutamic acid residues to form clotting factors, an epoxidation reaction occurs, converting the active form of vitamin K_1, the hydroquinone, to vitamin K_1 2, 3-epoxide, which in turn is returned to vitamin K_1 quinone by vitamin K_1 2, 3-epoxide reductase, which in turn has to be reduced to vitamin K_1 hydroquinone, thus forming the vitamin K_1 cycle. The regeneration of vitamin K_1 quinone from vitamin K_1 2, 3-epoxide is the step inhibited by coumatetralyl.

First-generation anticoagulant rodenticides as coumatetralyl require a few days until the onset of clinical symptoms. Typical symptoms are general weakness, anorexia, blood in faeces or urine, nasal bleeding.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1.1 Conclusions on classification and labelling on physico-chemical properties

Based on the results of the available data, no classification for physico-chemical properties is proposed.
No classification for physico-chemical properties was agreed by the TC CL 2007.
No classification according to the CLP regulation is necessary.

4 HUMAN HEALTH HAZARD ASSESSMENT

In this section summaries and evaluation of data for which robust study summaries are presented in the annex: "CLH report coumatetralyl RSS CAR toxicology" which is reproduced from the final CAR report under the review programme under the biocides directive (98/8/EC are reported, as far as possible in summary tables. Due to an interim period agreement, the robust study summaries are not available in IUCLID.

The data highlighted by the use of a **grey background in the tables** are so-called **key studies**. Results from such studies are the basis for risk assessment. Supplementary studies give additional information for the risk assessment. Key studies have a high reliability, while other studies can be used as supporting evidence and in view of an overall weight of evidence approach. Unless otherwise stated, all studies were conducted according to internationally accepted guidelines and principles for good laboratory practice (GLP). The text related to the table highlights the data used for the risk assessment. These data will in most cases also be the ones relevant for classification.

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). Coumatetralyl was classified for acute and repeated dose toxicity, as well as for environmental hazards in the 24th ATP to Dir 67/548/EC. As a consequence of the assessment of Coumatetralyl under the biocides Directive, a classification proposal was discussed and agreed in 2007 by the former classification and labelling group, but it was not implemented. This agreement included all endpoints except developmental toxicity and a potential SCL. The present CLH proposal contains the classifications agreed in 2007

and new proposals for developmental toxicity and chronic aquatic toxicity.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 4.1 gives an overview of the available studies on toxicokinetics of coumatetralyl in the rat.

Table 4.1: Toxicokinetic and metabolism studies with coumatetralyl in rats

Route	Method, Guideline	Test system	Label, Exposure	Dose level	Analysed parameters	Reference
Oral	Methods in accordance with the OECD 417 and the US-EPA, OPPTS 870.7485	Rat, Wistar, Single dose: m+f (1:1), 4/sex/group Repeated dose: m, 5/group	^{14}C ring labelled coumatetralyl, Single and repeated exposure	Single application: 0.1 mg/kg bw labelled active substance Repeated application: 0.1 mg/kg bw non-labelled substance on 14 consecutive days and a final dose at day 15	Absorption, distribution, elimination, metabolism	Anderson (1999)
In vitro	Determination of metabolite profile and quantitation of parent compounds in the microsomal fraction and cyto-plasmatic fraction of liver cells, No guideline available.	Rat, Wistar, Subcellular fraction of liver cells	^{14}C ring labelled coumatetralyl	1. Study on dependency of metabolism on various parameters: 0.8 – 6.4 µg/ml 2. Preparative incubation: 31.4 µg/ml	Metabolism	Anderson & Bornatsch (1998)
Oral	Investigation of the persistence of coumatetralyl in sub-lethally poisoned rats, No guideline available.	Rat, f, 4 - 6/group Examined target organ: liver	Unlabelled coumatetralyl	Single dose of 4 mg/kg bw (sub-lethal)	Persistence	O'Connor et al. (2001)
Oral	Examination of the potency of coumatetralyl to bioaccumulate, No guideline available.	Rat, f, 6/group Examined target organ: liver	Unlabelled coumatetralyl	Repeated dose of 4 mg/kg bw (approx. LD15); three doses with intervals of 12 weeks	Accumulation	Eason et al. (2003)

The toxicokinetic properties of coumatetralyl have been investigated after single and repeated oral dosing in the rat; metabolism was additionally examined in vitro in rat liver cells.

4.1.2 Human information

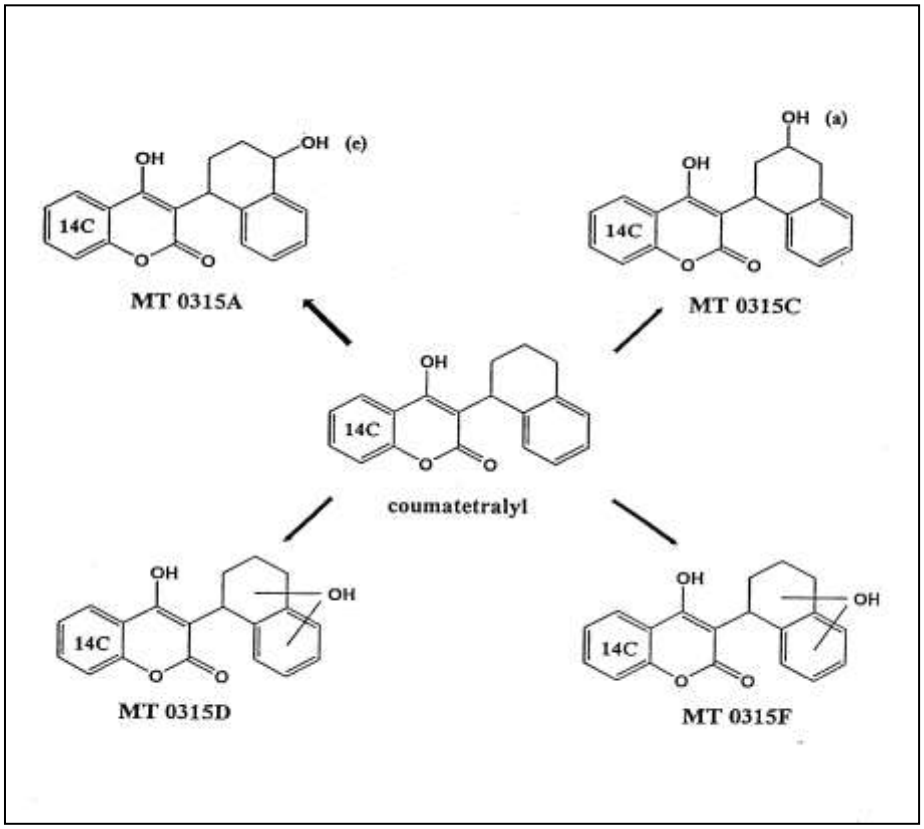
No information was available.

4.1.3 Summary and discussion on toxicokinetics

Absorption:
The maximum relative concentration in blood plasma after a single oral dose was found 3 hours after administration in males and after 8 hours in females. An absorbed fraction of at least 75% and 86% of administered dose was calculated for males and females, respectively.
Distribution:
After single dosage to rats, 49 – 56% of applied radioactivity was retained in the body (gastrointestinal tract exclusive). After repeated dosage, 18% was retained. Coumatetralyl reaches relatively high concentrations in the liver and the skin. The largest fraction was found in liver (21–25% after single treatment and 7% after repeated dosing), followed by the skin (7-16% after a single dose and 4% of the dose after multiple dosing). All other organs retained less than 1% of the dose at sacrifice.
Excretion:
Excretion was slow in all tests and dependent on sex and on the number of applications (single dose or repeated dose). The primary route of excretion is via the urine and to a smaller extend via faeces. Single-dosed males excreted about 20% of the administered dose with the urine and about 20% with the faeces until sacrifice (7 days post administration). After repeated dosing the ratio shifted towards 44% renal and 33% faecal excretion until sacrifice, which could be an indication of enzyme induction during the 14-day pre-treatment. Single-dosed females excreted about 37% with the urine and about 12% with the faeces until sacrifice.
Excretion of coumatetralyl in the exhaled air was negligible, i.e. less than 0.60% of the administered dose.
Plasma curve analysis revealed distinct effects of sex and dose on the elimination process. The toxicokinetic behaviour of radioactivity in the plasma could be characterized by a quick rise followed by a broad maximum and a steady and fairly slow decline. Male rats reached maximum plasma concentration about 3 hours post administration (t_{max}), and t_{max} for females was between 8 and 24 hours post administration. The half-lives of the terminal phase after a single dose application were approximately 71 and 46 hours for male and female rats, respectively. After multiple dosing of males the half-live of the terminal phase was 36 hours. The total clearance (CL_{total}) was very low with approximately 0.02 ml/min/rat, the renal clearance (CL_R) ranged from 0.004 to 0.007 ml/min/rat. The theoretical volume of distribution under steady state conditions (V_{SS}) was also sex dependent: 54% of the body volume for male rats and 28% for females. The computed V_{SS} for pre-treated males was 18% of the body volume. The mean residence time (MRT), which is indicative for the "body burden", was approximately 98 and 70 hours after a single dose to male and female rats and 44 hours after multiple dosing, respectively.

In-vivo metabolism:
The molecule is extensively metabolised within the organism. Sex and dose had only minor effects on the metabolite profiles. The main pathway of biotransformation proceeded via hydroxylation of the tetrahydronaphtyl moiety. Four metabolites of coumatetralyl were identified in urine and faeces (Figure 1). The main metabolite MT 0315A accounted for up to 27% of the applied dose. Additionally, three isomers of the main metabolite (MT 0315C, MT 0315D, MT 0315F) were detected, all far below 10% of the dose. In urine two further metabolites (MT 0315B and MT 0315E) could be verified (both approx. 2%). Only traces of the dose in urine and faeces were unchanged coumatetralyl. The total rate of identification ranged from 23% (42% total radioactivity excreted) to 37% (77% total excreted) of administered dose.
An in-vitro metabolism study was conducted to determine the metabolite profile and to isolate and identify the main metabolites of coumatetralyl. All six metabolites from the rat in-vivo study were also found in-vitro.

Figure 1: Proposed metabolism of coumatetralyl



Mode of action:

Anticoagulant rodenticides are vitamin K antagonists. The mode of action of coumatetralyl was described as follows:

The main site of action is the liver and the main effect consists in inhibition of blood clotting by interference with the hepatic synthesis of the vitamin K-dependent clotting factors II, VII, IX and X which effectively inhibits de-novo synthesis of vitamin K_1, thereby interrupting cellular recycling of vitamin K_1. Vitamin K_1 in its hydroquinone form is an essential co-factor for the synthesis of functional clotting factors. The function of vitamin K_1 is the post translational transformation of the precursor protein to respective functional clotting factors by γ-carboxylation of their glutamic acid moieties, which in turn, enhances the binding of Ca^{++} by chelating phosphate on the phospholipids, thus accelerating, and providing a template for, the blood clotting mechanism. Concomitant with the γ-carboxylation of glutamic acid residues to form clotting factors, an epoxidation reaction occurs, converting the active form of vitamin K_1, the hydroquinone, to vitamin K_1 2, 3-epoxide, which in turn is returned to vitamin K_1 quinone by vitamin K_1 2, 3-epoxide reductase, which in turn has to be reduced to vitamin K_1 hydroquinone, thus forming the vitamin K_1 cycle. The regeneration of vitamin K_1 quinone from vitamin K_1 2, 3-epoxide is the step inhibited by coumatetralyl.

First-generation anticoagulant rodenticides as coumatetralyl require a few days until the onset of clinical symptoms. Typical symptoms are general weakness, anorexia, blood in faeces or urine, nasal bleeding.

There are no convulsions, squealing or other sounds connected with the poisoning process. The target animal seems to die with no apparently painful symptoms.

4.2 Acute toxicity

Table 4.2: Acute toxicity of coumatetralyl

Route	Method, Guideline	Species, Strain, Sex, No/group	Dose levels, duration of exposure	Value LD50/LC50	Reference

Route	**Method, Guideline**	**Species, Strain, Sex, No/group**	**Dose levels, duration of exposure**	**Value LD50/LC50**	**Reference**
Oral	OECD 401, US-EPA, Subdivision F, 81-1	Rat, Wistar, m+f (1:1), 5/sex/group	1 to 500 mg/kg bw (m+f),single oral dose	30/15 mg/kg bw (m/f)	Bomann (1992a)
Oral	No guideline, No GLP	Rat		< 20 mg/kg bw (m+f)	Herrmann (1973)
Oral	No guideline, No GLP	Rat		5 - 25 mg/kg bw (m+f)	Kimmerle (1958)
Oral	No guideline, No GLP	Mouse		2000 – 4000 mg/kg bw (m)	Herrmann (1973)
Oral	No guideline, No GLP	Guinea pig		approx. 250 mg/kg bw	Kimmerle (1958)
Oral	OECD 401, US-EPA, Subdivision F, 81-1	Rabbit, New Zealand, m+f (1:1), 5/sex/group	50 to 500 mg/kg bw (m), 50 to 750 mg/kg bw (f), single oral dose	> 500 mg/kg bw (m), > 750 mg/kg bw (f)	Bomann (1992b)
Oral	No guideline, No GLP	Rabbit		Death occurred at 10 mg/kg bw and above	Kimmerle (1958)
Oral	No guideline, No GLP	Cat		Death occurred at 50 mg/kg bw and above	Kimmerle (1958)
Oral	No guideline, No GLP	Dog		approx. 35 mg/kg bw	Herrmann (1960)
Dermal	OECD 402, US-EPA, Subdivision F, 81-2	Rats, Wistar, m+f (1:1), 5/sex/group	50 - 2000 mg/kg bw, 24 hours, one single dermal application	258 mg/kg bw (f) 100 mg/kg bw <LD_{50} (m)< 500 mg/kg bw	Bomann (1992c)
Dermal	No guideline, No GLP	Rat, m	4 hours	25 – 50 mg/kg bw	Kimmerle (1970a)
Dermal	No guideline, No GLP	Rat, m	4 hours	61.9 mg/kg bw	Kimmerle (1970b)
Dermal	No guideline, No GLP	Rat, f	7 days	approx. 5 mg/kg bw	Kimmerle (1970a)
Inhalation	Comparable to OECD 403 No GLP	Rat, Wistar, m+f (1:1), 10/sex/group, additionally groups with 20 f dosed with 145.0 or 184.0 mg/m³	**30 - 202 mg/m³** 4 hours, (head and nose) MMAD not specified.	0.063 mg/l/4h) (m), (0.039 mg/l/4h) (f),	Pauluhn (1982)
Inhalation	Comparable to OECD 403 No GLP	Mouse, NMRI, m 20/group	53 - 117 mg/m³ 4 hours	approx. 54 mg/m^3/4h (equiv. to 0.054 mg/l/4h) (m)	Pauluhn (1982)
Waiver for acute toxicity studies in rat by inhalation					

From Doc. III-A6.1.1, 6.1.2 and 6.1.3

Acute toxicity: oral

Coumatetralyl is highly toxic by acute oral exposure. The rat appears to be the most sensitive species with LD_{50} values for coumatetralyl of 30 and 15 mg/kg bw for males and females, respectively in a guideline study (Bomann, 1992a).
: The oral LD_{50} values in rabbits is of > 500 mg/kg bw for males and >750 mg/kg for females based on a guideline study (Bomann, 1992b). Other mammalian species (mouse, guinea pig, dog and cat) are less susceptible to single oral doses of coumatetralyl.
Based on the available IUCLID data and none guideline compliant studies, the sensitivity of different species to the oral toxicity of coumatetralyl seems to be in declining order: rat/dog/cat/rabbit/mouse/guinea pig. However it is not known if the data are fully comparable.

Acute toxicity: dermal

The LD_{50} values from a guideline study in rats were 100 - 500 mg/kg bw for males and 258 mg/kg bw for females (Bomann, 1992c). The lowest value is supported by the results from three non-guideline studies.

Acute toxicity: inhalation

No acute and short-term inhalation guideline-studies are available. The acute toxicity by inhalation was investigated in rats and in the mouse in an unpublished test reported in IUCLID (Pauluhn, 1982). The study was not considered as reliable to establish a precise LC_{50} value because of major methodological and reporting deficiencies with the current guideline. However, despite its deficiencies, the study can be used as supportive for classification/labelling of coumatetralyl. The study indicates an LC_{50} in rats of 0.063 mg/l/4h (males) and 0.039 mg/l/4h (females) while the mouse LC_{50} is reported to be 0.054mg/l/4h. The applicant proposed classification according the Directive 67/548/EEC as T; R25 and submitted a justification for non-performance of the study for acute and short-term studies by inhalation for coumatetralyl.

4.2.1 Conclusion on classification and labelling

Based on the data supporting the dossier for coumatetralyl, in relation to the criteria on Annex VI to directive 67/548, the following classification for acute toxicity according to Directive 67/548/EEC is proposed: T+; - R26/28 very toxic by inhalation and if swallowed T; R24: Toxic in contact with skin. The corresponding labelling is T+; R24-26/28. This classification was agreed by the TC CL group in November 2006.
According to the available data and the criteria of Regulation no. 1272/2008, coumatetralyl should be classified as Acute Tox 2, H300: Fatal if swallowed, Acute Tox 3; H311 Toxic in contact with skin and Acute Tox 2; H330: Fatal if inhaled. Labelling will be Signal Word: Danger and Hazard Statements H300, H330 and H311.
This classification was agreed by the TC CL group in May 2007.

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 ($\leq$ 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.

4.3 Specific target organ toxicity – single exposure (STOT SE)

This end-point target organ toxicity after single exposure is considered covered by the section on acute toxicity (see point 4.2 above) and irritation (see point 4.4 below)

Conclusion: no classification is proposed for STOT SE.

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.

4.4 Irritation

4.4.1 Skin irritation

Table 4.3: Skin irritation of coumatetralyl

Species	Method	Average score 24, 48, 72 h		Reversibility yes/no	Result	Reference
		Erythema	**Oedema**			
Rabbit	OECD 404, US-EPA (TSCA§798.4470,§798.4500 and Sudivision F, §81-4, § 81-5)	0.0	0.0	No	Not irritating	Renhof (2003a)
Rabbit	No Guideline No GLP	0.0 (intact skin) 0.4 (scarified skin)	0.0 (intact skin) 0.4 (scarified skin)		Not irritating	Bhide (1984a)

Skin irritation: Coumatetralyl was not irritating to the rabbit's skin in a guideline study (Renhof, 2003a).

Conclusion on classification for irritative effect to the skin:
No classification or labelling for skin irritation according to Regulation 1272/2008 or Directive 67/548/EEC is warranted**.** This conclusion was endorsed by the TC CL group in November 2006.

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.

4.4.2 Eye irritation

Table 4.4: Eye irritation of coumatetralyl

Species	Method	Average score 24, 48, 72 h				Reversibility yes/no	Result	Reference
		Cornea	**Iris**	**Conjunctiva**				
				Redness	**Chemosis**			
Rabbit	OECD Guideline 405 GLP	0.0	0.0	0.0	0.0	yes	Not irritating	Renhof, (2003b)
Rabbit	No Guideline No GLP	0.0	0.0	0.0	0.0		Not irritating	Bhide (1984b)

Eye irritation: Coumatetralyl was not irritating to the rabbit's eye in study compliant to OECD guidelines (Renhof, 2003b) .

Conclusion on classification for irritative effect to the eyes:
No classification or labelling for eye irritation according to Regulation 1272/2008 or Directive 67/548/EEC is warranted**.** This conclusion was endorsed by the TC CL group in November 2006.

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.

4.4.3 Respiratory tract irritation

No information on irritative effects to the respiratory tract was reported.

Conclusion on classification for respiratory tract irritation

No classification or labelling for respiratory tract irritation is warranted for coumatetralyl. This conclusion was endorsed by the TC CL group in November 2006.

4.5 Corrosivity

This end-point is described under point 4.4.1 Skin irritation.

Conclusion on classification for corrosivity

No classification is proposed.

4.6 Sensitisation

4.6.1 Skin sensitistaion

Table 4.5: Skin sensitisation of coumatetralyl

Species	Method	Number of animals sensitized/total number of animals	Result	Reference
Guinea pig	Buehler Patch Test according to OECD 406 and US-EPA, Sudivision F, § 81-6	0/20 (24 h after challenge), 0/20 (48 h after challenge)	Negative; no skin-sensitising potential	Stropp (1998)

Skin sensitisation: not sensitising.
Due to coumatetralyl intrinsic anticoagulant properties and its toxicity, it was not possible to conduct a guinea-pig maximisation test under the current guidelines with application of irritating concentrations of the test substance.
A Magnusson and Kligman guinea-pig maximisation test was initiated in order to assess the sensitising potential of coumatetralyl. Five days following the intradermal injection of 5% of coumatetralyl in polyethylene glycol (PEG) 400 some animals showed clinical signs of toxicity. However, the test had to be terminated on day 6, as three animals out of ten died and the remaining animals were killed in a moribund state on that same day.
Therefore coumatetralyl potential to induce skin sensitisation was tested using the Buehler method (Stropp G., 1998). In this study a 50% coumatetralyl suspension in PEG 400 was used for the first induction. The challenge with a 25% coumatetralyl formulation did not induce any skin effect. It was concluded that under the conditions of the Buehler patch test and with respect to the evaluation criteria coumatetralyl exhibits no skin sensitisation potential.
The conductance of the Buehler test instead of an adjuvant test was scientifically explained by the toxicity of coumatetralyl in intradermal injections and the explanation is acceptable.

Conclusion on classification and labelling for skin sensitising potential

No classification/labelling of coumatetralyl for skin sensitisation according to Regulation 1272/2008 or Directive 67/548/EEC is warranted based on the result of a Buehler test by Stropp (1998). This conclusion was endorsed by the TC CL group in November 2006.

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.

4.6.2 Respiratory sensitisation

No data were available indicating a potential of coumatetralyl to be a respiratory sensitizer.

Conclusion of classification and labelling

No classification for respiratory sensitisation is proposed for coumatetralyl.

The conclusions that no classification is warranted for sensitising effects were endorsed by the TC C&L group.

4.7 Repeated dose toxicity

4.7.1 Non-human information

Key studies shaded in grey in the table. Summaries of these studies can be found in the appendixed CAReport, section 6. Some studies were only filed by the applicant as IUCLID summaries. This is indicated in column 7 of the table. Reliability indicators are included in column 2.

Table 4.6 Short-term repeated dose, subchronic and chronic toxicity of coumatetralyl

Route	Duration of study, Method Study reliability.	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LOAEL /NOAEL	Reference RSS or only IUCLID summary available
Oral/ diet	3 weeks, Pilot feeding study according to OECD 407, No GLP. Valid with restrictions	Rat, Wistar, m+f 3 per sex/group	130 and 270 ppm Racumin® Tracking Powder 0.769% containing 1 or 2 ppm coumatetralyl	All animals died after administration of 2 ppm coumatetralyl and one male after feeding of 1 ppm. The clinical signs observed (paleness), prolongation of blood clotting time and internal haemorrhages are consistent with the pharmacological mechanism of coumatetralyl.	LOAEL ≈1 ppm. No NOAEL could be determined.	Andrews (1996) IUCLID only
Oral/ gavage	9 weeks, Pilot gavage study, No GLP. Valid with	Rat, Wistar, m+f, 3 -5 per sex/group	0, 0.01, 0.03, 0.05, 0.1, 0.12, 0.16 or 0.2 mg/kg bw, daily	Deaths occurred in males at 0.16 mg/kg bw/day and above and in females at 0.2 mg/kg bw/day. Blood clotting time in male rats were increased at 0.05 mg/kg	LOAEL: 0.05 mg/kg bw/day in males NOAEL: 0.03	Andrews (1996) IUCLID onl

Route	Duration of study, Method Study reliability.	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LOAEL /NOAEL	Reference RSS or only IUCLID summary available
	restrictions			bw/day (23 days of treatment) and in females at 0.1 mg/kg bw day (42 days of treatment).	mg/kg bw/day	
Oral/ diet	112 days, OECD 408, US-EPA, Sub-division F, § 82-1 Valid,	Rat, Wistar, m+f (1:1), 10 per sex/ group	0, 13, 40, 85, 170 ppm Racumin® Tracking Powder daily, containing 0.769 % (These dose levels correspond to 0, 0.0068, 0.0210, 0.0458, 0.0994 mg/kg coumatetralyl bw/day for males and 0, 0.0083, 0.0270, 0.0563, 0.1223 mg coumatetralyl/kg bw/day for females) purity: 0.769% a.s.	Mortality: 14/20 males and 5/20 female died at 170 ppm. Haematology: significantly decreased erythrocyte count in males at 170 ppm. Slight, but statistically significant increase in blood clotting time in males from 40 ppm and above. In females the clotting time values were significantly increased from 85 ppm and above, with at sporadic occurence of a statistically significantly increased value at 40 ppm. Clinical chemistry: increased cholesterol values, decreased triglyceride values, decrease in total bilirubin from 85 ppm and above in both sexes were seen. Pathology: haemorrhages in many tissues and organs at 170 ppm Histopathology: Stimulated extramedullary haemopoiesis in the liver and the spleen as well as haemopoiesis in the bone marrow at 170 ppm. Hepatic centrolobular cytoplasmatic vacuolation, single cell necrosis and centrolobular fatty changes in the high-dose animals, primarily in the males.	LOAEL: 40 ppm (equivalent to 0,0210/0.0270 mg/kg bw/day in males and females, respectively. NOAEL: 13 ppm equiv. to 0.0068/ 0.0083 mg coumatetralyl/kg bw/day (m/f), based on significantly increased blood clotting time and haemorrhage in males at 40 ppm.	Andrews & Romeike (1997)
Oral/ diet	1 or 3 days, Secondary poisoning study in dogs; poisoned rats served as chow (according to OEPP protocol) Valid with restrictions	Dog, Beagle, m+f, 5/group	Rats were fed for three days with 375 mg/kg coumatetralyl and were then prepared to chow for the dogs. One rat/day, as single dose or on three consecutive days.	Single application: Moderate increase of prothrombin time (2 of 5 animals), the value peaked at day 3 after exposure. Repeated dose: All dogs exhibited an increased prothrombin time; the value peaked at day 5 after study initiation. Prothrombin times returned to normal values rapidly thereafter.	No LOAEL/NOAEL set due to study protocol (unclear dosing)	Berny (1999 IUCLID onl

Route	Duration of study, Method Study reliability.	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LOAEL /NOAEL	Reference RSS or only IUCLID summary available
Oral	Sub-chronic	dog	Study not performed. Waiver submitted.			Lautraite (2003b)
Oral	chronic	rodent	Study not performed. Waiver submitted.			Lautraite (2003b)

Sub-chronic oral toxicity study in rats:

A guideline feeding study over 16 weeks was performed in rats with a formulation of coumatetralyl, Racumin® 0.75%Tracking Powder (Andrews & Romeike, 1997). The study is acceptable as a subsequent submission of the composition of the formulation was provided by the applicant showing that none of the non-active components are expected to interact toxicologically with coumatetralyl.

Mortality in the high dose group (approx. 0.1 mg/kg bw/day) was 70% in males and 25% in females. No mortality was seen at any lower dose level. Animals that died on study showed direct or indirect signs of haemorrhages, i.e. either dark discoloration of stomach and/or intestinal contents, dark red discoloration of testes, lungs or the abdominal cavity, or pale liver, kidneys, lungs and spleen.

The effects seen in the subchronic study were directly or indirectly related to the well-known pharmacological effects of coumatetralyl on blood clotting. Effects observed were generally haemorrhages and pallor and reduced activity before the death of the animal. Blood clotting time was consistently (and statistically significantly) increased in both sexes at 85 ppm and above. A slight (5-10%) but statistically significant increase in blood clotting time was observed consistently in males fed 40 ppm at all time points except the last one. In females of the 40 ppm group, only one time point showed statistical significant prolonged clotting time. The effect on blood clotting time was dose dependent in both sexes.

Therefore, the statistically significant dose dependent effect on blood clotting in males at 40 ppm (0.021 mg/kg bw/day) and in both sexes from 85 ppm is regarded as adverse.. No measurement of the prothrombin levels (possibly more sensitive parameter) were performed, and no other study is available on subchronic or chronic toxicity of coumatetralyl. Therefore a conservative interpretation of the result of this study has been chosen.

The NOAEL is therefore set at 13 ppm equivalent to 0.0068 mg coumatetralyl/kg bw/day in males.

Prolonged blood clotting time occurs at and above the dose level of 40 ppm, corresponding to 0.021 and 0.027 mg/kg bw/day in males and females, respectively. At this effect level, blood clotting function is impaired and this is considered a serious functional adverse effect on the blood.

4.7.2 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

The effects on blood clotting and haemorrhages seen in males in the oral subchronic study in the rat (Andrews & Romeike, 1997) at a dose level 0.021 mg/kg bw/day are regarded as serious functional impairment.

The criteria for classification with T; R48 include a guidance value of 50:10 = 5 mg/kg bw/day based on an oral rat subchronic study. Therefore, classification as T; R48/25 is relevant for coumatetralyl.

As the mechanism of toxicity of coumatetralyl (and other anticoagulant rodenticides), i.e. vitamin K1 antagonism, is independent of the route of exposure, classification with T; R48 is also relevant for the

other routes the following classification/labelling for coumatetralyl is proposed for repeated dose toxicity according to Directive 67/548/EEC.

Specific concentration limits
No guidelines for setting specific concentration limits under the DSD for repeated dose toxicity are available. The proposed method for setting SCL was agreed by the TC C&L group in May 2007. The effect level (impaired blood clotting and haemorrhages) was 0.021 mg coumatetralyl/kg bw/day in male rats, based on a 16 week oral study. The ratio between this effect level and the cut-off level for T; R48/25 of 50 : 10 = 5 mg/kg bw/day is 238. The generic concentration limits for preparations containing a substance classified as T; 48 according to the DPD are 10% for T; R48 and 1% for Xn; R48.
Specific concentration limits can be calculated as 10% : 238 = 0.04% for T; R48 and 1% : 238 = 0.004% for Xn; R48. However, in order to avoid too many different levels specific concentration limits, it is proposed to use the orders of magnitude, in this case 2, in calculating a specific concentration limit. Subsequently, specific concentration limits of 10 %:100 = 0.1% for T; R48/25 and 0.01% for Xn; R48/22 are proposed. The same concentration limits are proposed for the dermal and inhalation routes, as no specific data are available for these routes.

4.7.3 Conclusion on the classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

The following proposal for repeated exposure toxicity is proposed for coumatetralyl: T, R48/23/24/25: Toxic, danger of serious damage to health by prolonged exposure by inhalation, in contact with skin and if swallowed.
This classification proposal was endorsed by the TC C&L group in November 2006.

The proposed specific concentration limits for the end-point of repeated dose toxicity under the DSD are:
C> 0.1%: T; R48/24/25/26
0.01%< C < 0.1%: Xn; R48/20/21/22.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and dicussion of repeated dose toxicity finding relevant for classification as STOT RE according to CLP Regulation

As described above under point 4.7 the effect level for the statistically significant increased blood clotting time and haemorrhages the repeated dose toxicity, is 0.021 mg/kg bw/day in male rat in a 16-week oral study (Andrews & Romeike, 1997), which is in accordance with current guidelines The effect on blood coagulation time is regarded as a serious functional impairment of the coagulation process.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The effects seen in the oral rat repeated dose study are regarded to show the potential to produce a significant toxicity (blood clotting impairment) in humans following repeated exposure. The effect

level of 0.021 mg/kg bw/day in the 16 week oral study in rats is significantly lower than the guidance value for classification as STOT RE Cat 1 of 10 mg/kg bw/day based on a 90 day study. Therefore classification of coumatetralyl as STOT RE Cat 1 is proposed.

As the mechanism of toxicity of coumatetralyl (and other anticoagulant rodenticides), i.e. vitamin K1 antagonism, is independent of the route of exposure, classification for specific target organ toxicity repeated exposure (STOT RE) will be relevant for the dermal and inhalation routes as well.

Specific concentration limits
The critical effect of coumatetralyl (impaired blood clotting and haemorrhages) occurs at a very low level 0.021 mg coumatetralyl/kg bw/day (male rats, 16 week oral study). The ratio between this effect level and the guidance value (GV) for category 1 STOT RE 10 mg/kg bw/day is 476. Therefore, specific concentration limits are proposed for coumatetralyl. Based on the ECHA Guidance on the Application of the CLP Criteria (2009), these should be calculated as

$$SCL\text{-}_{STOT\ cat1} = \frac{ED}{GV_{cat1}} = \frac{0.021\ mg/kg\ bw/day \times 100\%}{10\ mg/kg\ bw/day} = 0.2\%$$

$$SCL\text{-}_{STOT\ cat2} = \frac{ED}{GV_{cat2}} = \frac{0.021\ mg/kg\ bw/day \times 100\%}{100\ mg/kg\ bw/day} = 0.02\%$$

No adjustment for preferred values is needed.

4.8.3 Conclusions on classification and labelling for repeated dose toxicity (STOT RE)

Classification of coumatetralyl as STOT RE Cat 1; H372: "Causes damage organs (blood coagulation) though prolonged or repeated exposure" is proposed. No specification of route of exposure is warranted.

Proposed specific concentration limits:

C ≥ 0.2%: STOT RE cat 1: H372
0.02% ≤ C < 0.2%: STOT cat 2 H373

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.

4.9 Germ cell mutagenicity (Mutagenicity)

Coumatetralyl has been tested for genotoxic potential *in-vitro* and *in-vivo* with various end points.

4.9.1 In-vitro genotoxicity

The *in-vitro* screening program included an Ames test, an *in vitro* recombination assay in yeast and an *in vitro* test for gene mutations in mammalian cells.

Coumatetralyl was not found to be a point mutagen in studies on bacteria (Herbold,1986a). The bacterial reverse mutation assay from 1986 was conducted in accordance with generally accepted scientific principles and is found acceptable with respect to the four strains used. However, the test does not follow the updated OECD guideline from 1997 in which the inclusion of a fifth strain is recommended (Salmonella typhimurium strain TA 102 or E. coli WP2) in order to detect certain oxidising mutagens, cross-linking agents and hydrazines that may not be detected by the other Salmonella typhimurium strains. However, as there is no indication from kinetic studies that these effect types could occur, the negative result of the study is considered valid.

In an *in vitro* test for gene mutations in eukaryotic cells (Chinese hamster V79 cells), coumatetralyl was non-mutagenic in the HPRT forward mutation assay, both with and without metabolic activation (Herbold, 2004).

In addition, no mitotic recombinant effects occurred in the yeast strain Saccharomyces cerevisiae D7 (Herbold,1986b)..

No *in vitro* test on chromosome aberrations was performed. However the relevant endpoint (damage to the chromosomes) is covered by the *in vivo* micronucleus assay, which would have been the recommended choice for a follow-up procedure, if the *in vitro* cytogenicity study in mammalian cells had been performed and showed to be positive.

Table 4.8: In-vitro genotoxicity of coumatetralyl

Test system, Method, Guideline	**Organism/ strain(s)**	**Concentrations tested**	**Result**		**Remark**	**Reference**
			+ S9	**- S9**		
In-vitro gene mutation study in bacteria, Methods comparable to OECD 471, No GLP	Salmonella typhimurium, TA 1535, 1537, 98, 100	First test: + S9/-S9: 0 – 12500 µg/plate Repeat test: + S9/-S9: 0 – 2000 µg/plate (TA 1535) 0 – 8000 µg/plate (TA 100, 1537, 98)	Neg.	Neg.	Doses from 250 µg/plate onward cause bacteriotoxic effects	Herbold (1986a)
In-vitro mitotic recombination assay in yeast, Methods comparable to OECD 481, No GLP	Saccharomyces cerevisiae D7	First and repeat test: + S9/-S9: 0 –10000 µg/ml	Neg.	Neg.	Cytotoxicity was observed over the entire range of concentrations used. Substance precipitation at 5000 µg/ml and above.	Herbold (1986b)
In-vitro gene mutation assay in mammalian cells (HPRT assay) OECD 476	V79 cells	First and repeat test: + S9/-S9: 75-900 µg/ml	Neg.	Neg.	No precipitation occurred in the medium with or without S9 mix. Significant concentration-related cytotoxicity.	Herbold (2004)

4.9.2 In vivo genotoxicity

In the *in vivo* micronucleus test (Herbold 1987), which is compliant with the OECD guideline at the time but not performed according to GLP, the administration of single oral doses of coumatetralyl at doses up to 1000 mg/kg induced the same clinical signs as observed in the acute toxicity studies (apathy, reduced motility, digging and grooming movements, bristling coats, staggering gaits, prostration on stomach, salivation, jumping spasms and dyspnoea) and a reduced erythrocyte formation. In the second trial of the test, at the 48-hour sample time, there was a statistically significant increase ($p \leq 0.05$) in micronucleated polychromatic erythrocytes (MPE) per 1000 polychromatic erythrocytes (PCE) at the highest dose level (1000 mg/kg). However, the effect is not considered biologically meaningful because:

1) there was no evidence of a dose-related increase in MPE,

2) the incidence of MPE in the negative control group was relatively low (1.0 ± 0.8) compared to the negative control group in test 1 (1.2 ± 1.1) and in test 2, sample time 72 h (1.7 ± 1.4), and
3) there was no increase in MPE per 1000 PCE in the first test (750 mg/kg).

Current guidelines recommend the scoring of more than 2000 PCE. The potential effect on the chromosomes is not adequately clarified, because the slide assessment was not extended such that more than 2000 PCE were scored for MPE incidence per animal. However, despite the lower number of PCE, and the study is considered acceptable, because a clear positive response of the positive control in the first test demonstrated sufficient sensitivity of the test system. Thus, it is considered unlikely that the performance of a repeat test would detect a statistically significant, dose-related response at a 1.5-fold increase level over control using 7-10 animals/group with a background incidence of 1-1.5 MPE/1000, when the first test did not show any increase.

A negative in vivo dominant lethal test was reported in IUCLID. However, the study and report qualities are deficient. The result may have been biased by too low doses (no toxicity seen), and the low number of animals used (max. 15 instead of 30-50 females per group).

Table 4.8: In vivo genotoxicity of coumatetralyl

Type of test, Method/ Guideline	Species, Strain, Sex, no/group	Frequency of application	Sampling times	Dose levels	Results	Remarks	Reference
In-vivo micronu-cleus assay, Comparable to OECD 474, No GLP	Mouse, NMRI, 5/sex/group	One oral application	Bone marrow, First test: 24, 48, 72h post-application Second test: 48 and 72h post-application	First test: 0 or 750 mg/kg bw Second test: 0, 500, 750 or 1000 mg/kg bw	No clastogenic effects. Same clinical signs as seen in acute toxicity studies. Reduced erythrocyte formation.	Maximum tolerable dose: 500 mg/kg bw	Herbold (1987)
In-vivo Dominant lethal test OECD 478	Mouse, Swiss, 5 m/ group	5 daily oral applica-tions	Treated males treated over 8 weeks with 3 females/week. Females sacrificed day 14 of gestation	0.015; 0.03 x 0.06 mg/kg bw/day	Negative. No substance-related induction of lethal mutations.		Balakrishna Murthy (1989)

From Doc. III –A6.6

4.9.3 Human information

No information from humans is available on the mutagenicity of coumatetralyl.

4.9.4 Other relevant information

QSAR analysis of the results of a structural alert test performed using a computer program – DEREK showed no indication of a potential genotoxicity.

4.9.5 Summary and discussion of mutagenicity

The available data indicate that coumatetralyl does not cause permanent transmissible changes in the amount or structure of a single gene or gene segments, a block of genes or chromosomes. Overall coumatetralyl is unlikely to pose a genotoxic hazard to man.

4.9.6 Conclusion on classification and labelling

No Classification/labelling for genotoxicity according to Directive 67/548/EEC is warranted.
No Classification/labelling for germ cell mutagenicity according to Regulation 1272/2008 is warranted. The TC C&L group concluded in November 2006 that classification of coumatetralyl for mutagenicity was not required.

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.

Carcinogenicity

4.9.7 Non-human information

No long-term study was conducted for the evaluation of the carcinogenicity of coumatetralyl. A waiver (Lautraite, 2003b) was submitted for the non-conduction of such a study. The scientific justification for the non-conduction of a carcinogenicity study with coumatetralyl is based on:
- the lack of mutagenic/genotoxic effects of coumatetralyl,
- the absence of any other effects in the subchronic study in rats that may lead to non-genotoxic carcinogenesis,
- the absence of any carcinogenic effects following long-term administration of warfarin, a coumarin compound, in humans. The argument of structural relationship with warfarin, is acceptable with respect to the mechanism of action of the two compounds. However, coumatetralyl is not metabolised to any compound related to warfarin in chemical structure. Carcinogenic effects of metabolites can thus not be completely excluded based on the SAR argument. However, due to the restrictions on human exposure required in the proposal for Annex I inclusion, non-genotoxic carcinogenicity is of low concern. Potential for genotoxic carcinogenicity of metabolites is expected to be low because of the non-mutagenic effect of coumatetralyl itself.
- the low risk of long-term exposure during manufacturing and use and of long-term exposure of the public population as well as indirect exposure via the environment, due to the physico-chemical properties of the substance and the formulation and their use pattern.

It is foreseen that the practical difficulties in finding suitable, non-lethal doses for long-term administration of coumatetralyl would be almost impossible to overcome and the conduct of such a study would possibly by unethical and contrary to Directive 86/609/EEC on animal welfare.

4.9.8 Human information

No data are available.

4.9.9 Other relevant information

In addition to the elements brought forward in the waiver, the applicant has submitted the results of a structural alert test performed using a computer program – DEREK. In that QSAR analysis coumatetralyl has no indication of a potential genotoxicity or carcinogenicity.

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.

4.9.10 CONCLUSION ON CLASSIFICATION AND LABELLING

According to the criteria of directive 67/548/EEC and those of Regulation 1272/2008, no classification and labelling for carcinogenicity is warranted for coumatetralyl.

4.10 Reproductive toxicity

Key studies shaded in grey in the table. Summaries of these studies can be found in the appendixed CAReport, section 6. Reliability indicators are included in column 2.

Table 4.10: Summary table of reproductive studies with coumatetralyl

Route of exposure	Test type, Method, Guideline	Species, Strain, no/group	Exposure Period	Doses	Critical effects 1) dams 2) foetuses	Maternal toxicity NOAEL / LOAEL	Teratogenicity Embryo-toxicity NOAEL / LOAEL	Reference
	Study of fertility	Not performed. Justification for non-submission available						Lautraite (2004)
Oral / gavage Reliability 2	Develop-mental toxicity test, OECD 414, US-EPA, Subdivision F, § 83-3 and 712-C-94-207 Valid	Rat, HanIbm: WIST, 25 f/dose group and 29 f/control	Day 6-20 post mating	0, 0.035, 0.07 or 0.14 mg/kg bw	1) profuse bleedings, symptoms of anaemia and mortalities at 0.07 mg/kg and above. 2) no evidence for teratogenic potential	NOAEL = 0.035 mg/kg bw; LOAEL = 0.070 mg/kg bw	Teratogenicity NOAEL: 0.14 / LOAEL >0.14 mg/kg bw; Embryotoxicty NOAEL: 0.14 / LOAEL: >0.14 mg/kg bw	Becker & Biedermann (1996a)
Oral / gavage Reliability 2	Develop-mental toxicity test, OECD 414, US-EPA, Subdivision F, § 83-3 and 712-C-94-207 Valid	Rabbit, Chbb:CH 24 f/group	Day 6-27 post mating	0, 0.0125, 0.025 or 0.05 mg/kg bw/day	1) internal and external bleedings and mortalities at 0.025 mg/kg and above 2) at 0.05 mg/kg bw total post implantation loss in one dam.	NOAEL 0.0125 mg/kg bw /; LOAEL 0.025 mg/kg bw	Teratogenicity: NOAEL: 0.05 / LOAEL > 0.05 mg/kg bw; Embryotoxicity: NOAEL: 0.025 mg/kg bw / ; LOAEL: 0.05 mg/kg bw	Becker & Biedermann (1996b)

From Doc. III A-6.8.1 and A-6.8.2

4.10.1 Effects on fertility

4.10.1.1 Non-human information

No single of multiple generation studies are available for coumatetralyl.

A scientifically justified waiver for the required multigeneration study with coumatetralyl based on the following argument was accepted by the RMS:

- the long half-life of the active compound would result in high body levels following the pre-mating period thus rendering the animals susceptible to death by haemorrhage from the natural events of reproduction and parturition,
- technical difficulties in achieving of a sub-MTD level at which a potential reproductive effect could be seen. The practical difficulties of long-term administration of coumatetralyl are such that an attempt at a study would be certain to fail and would be unethical and contrary to Directive 86/609/EEC.
- the absence of effect on reproduction in the developmental toxicity studies in rats and rabbits.

- residue levels in plant foodstuffs and water are expected to be negligible due to the physical-chemical properties and the use pattern of the product.
- the absence of residues in plant foodstuffs and water.

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.

Additional key elements

The CLH report mentions two developmental toxicity studies in rats and rabbits on Coumatetralyl conducted according to OECD test guideline 414 (Becker & Biedermann, 1996). These studies did not reveal any teratogenic or other developmental effect (doses up to 0.14 mg/kg bw/day in the rat and 0.035 mg/kg bw/day in the rabbits). Maternal toxicity included deaths and internal bleeding and occurred at low doses (0.07 mg/kg bw/day in the rat, and 0.025 mg/kg bw/day in the rabbits).

Another developmental toxicity study has been published more recently (Morgan, 2006), and was not mentioned the CLH proposal. The study deviates from the guideline by having a shorter exposure period (days 6-15), fewer rats per group (10), having only 2 exposed groups (0.83 and 1.65 mg/kg/day; the purity of the test material was not stated), and lacking information on the administration route and choice of vehicle. The reporting also had serious short-comings and this study cannot be used in a quantitative manner. However, it is noted that dose-dependent effects on, for example, resorptions (↑), live foetuses per dam (↓) and foetal body weights (↓) were observed in the absence of maternal effects.

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.

Supplemental information - In depth analyses by RAC

Coumatetralyl is a first generation AVK-rodenticide, having the same MoA as Warfarin (EHC, 1995). Warfarin is known to cause death of embryos or foetuses and malformations, mainly nasal hypoplasia in humans. 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).

In addition to skeletal malformation, Warfarin may cause spontaneous abortion, stillbirth, neonatal death, premature delivery, and ocular atrophy, among which spontaneous abortion and stillbirth are the most frequent (ca. 27% of pregnancies), and naso-maxial hypoplasia the most frequent among live births (ca. 5% of pregnancies). Substitution of Warfarin by Heparin during first trimester of pregnancy removes the risk of naso-maxial hypoplasia. Differences in human sensitivity to AVK-agents mainly relate to metabolic polymorphisms in the enzymes CYP2C9 and VKORC1 (Verhoef et al., 2013), but may also depend on e.g. vitamin K intake via the food, and differences in parameters related to hepatic accumulation, protein binding, and placental transfer.

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 them having the same mode of action (MoA) but with more potent rodenticide activity than warfarin. They 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. Effects on blood coagulation are shared between all AVK, and as vitamin K also is involved in bone formation, effects on bone formation is expected but not proven for other AVK rodenticides. Effects on the foetal bone formation can theoretically either be direct via inhibited enzymes in the foetus or indirectly via inhibition in the dam resulting in low circulating concentrations of vitamin K.

Considering the likely similar/identical MoA and similar physico-chemical properties for Coumatetralyl and Warfarin, a question is whether they will have similar developmental toxicological effects in humans. There are no human data for Coumatetralyl, but it is noted that there is human evidence of developmental toxicity for 4 different AVK coumarins (Warfarin, Brodifacoum, Acenocoumarol and Phenprocoumon) making it plausible that Coumatetralyl may also be a human teratogen.

Another question is whether the apparently negative rat developmental studies for Coumatetralyl have a predictive value for effects on humans, and how much weight the negative rat data should be given in a weight of evidence analysis which also includes human evidence.

Human warfarin embryopathy may involve foetotoxicity (e.g., spontaneous abortion and stillbirth), ocular atrophy, and skeletal malformations. The animal developmental toxicity studies on Coumatetralyl did not show any foetal toxicity, and this could either be because of no such inherent toxicity or that animal studies are not sufficiently predictive for effects in humans. A comparison of animal and human effects of Warfarin is therefore performed.

In some rat studies, Warfarin is indicated to cause foetotoxicity, foetal haemorraghes, and ocular effects. With very specific design of the studies, bone-related malformations also appear in rat studies (Howe and Webster, 1992). The rat foetal effects will be discussed further below, in order to assess to what extent rat studies on AVK rodenticides are predictive for effects in humans.

<u>Developmental toxicity - haemorrhage</u>

In the recent warfarin study, increased incidence (without clear dose-response) of foetal haemorrhages, external or visceral, were observed in a reliable study on rats (Kubaszky, 2009; see CLH report on Warfarin). However, it should be noted that small foetal haemorrhages are not easily detected, and in the reporting of the Kubaszky study (2009) it is stated specifically that clinical observations were made "*with special attention to external signs of haemorrhages*". Considering the lack of dose-response, it can be questioned if the haemorrhages are substance-related. On the other hand, one may not expect a very clear dose-response relationship considering the small dose spacing in this study (0.125-0.25 mg/kg bw/day).

AVK rodenticides act via inhibiting the formation of vitamin K, which in the subsequent step acts by regulating carboxylases, and the AVK rodenticides are therefore having effects on the processes (e.g., coagulation, bone formation) regulated by these carboxylases. It is noted that the expression of carboxylases in the foetal liver, which is responsible for the coagulation system, starts at day 16 (Romero et al., 1998), so it is unexpected that haemorrhages are found at similar incidences in foetuses exposed until day 15 as in foetuses exposed until day 19. In both cases foetuses were dissected on day 20. However, a (poorly reported) study on Warfarin by Mirkova and Antonov (1983; see CLH report on Warfarin) also reported foetal haemorrhages, and James et al. (1989; see CLH report on Flocoumafen) reported a low incidence of haemorrhage in controls that did not increase with increasing exposure to another AVK rodenticide, Flocoumafen.

It seems that haemorrhages sometimes can be picked up in an OECD TG 414 study, but it is not clear how severe they need to be or if special attention is needed to detect them.

Coumatetralyl data: No foetal haemorrhages were reported in the rat and rabbit guideline studies on Coumatetralyl, neither in the treated nor in the control groups.

Developmental toxicity – bone effects

Human warfarin embryopathy includes effects on bone formation, typically in the nose region. There were equivocal indications of disturbed ossification in skull bones (in foetuses from one mid-dose litter) in the Kubaszky study (2009). The finding of malformed skulls only concerned a single litter given the mid-dose, with malformations in 2 of 7 pups, indicating that a relationship with treatment is not likely. The critical period for nasal and skeletal development is not the same for humans (during the first trimester) and rats (late foetal/early postnatal period), and it is concluded that this malformation can therefore not be detected by a standard rat/rabbit OECD TG 414 study.

Developmental toxicity – ocular effects

In the recent rat study on Warfarin, a low incidence of an extremely rare foetal ocular effect was observed (Kubaszky, 2009), potentially supporting the possibility that prenatal animal toxicity studies can detect this effect of Warfarin. However, the ocular effects were only noted at the high dose and at such a low incidence (in 1 of 17 TP1-litters and 3 of 21 TP2-litters at the dose of 0.2 mg/kg bw/day) such that, if they were to be caused by other rodenticides, they would only occasionally occur in normal sized studies (n=20). No such effects were noted in other warfarin studies (e.g., Mirkova and Antonov, 1983).

Developmental toxicity – general foetal toxicity

Foetal toxicity has been indicated in the warfarin study by Kubaszky (2009), but only in one of the subgroups and in the presence of severe maternal toxicity (mortality). Foetal toxicity was also indicated in a poorly reported study by Mirkova and Antonov (1983).

Coumatetralyl data: No foetal toxicity was observed in the guideline developmental toxicity studies.

Dose-effect relation between haemorrhages and nose/bone defects

It is not known from the human coumarin data if there are differences in the dose-effect relationships between haemorrhages and nose/bone defects in humans. If, for instance, it would be the case that in humans (and animals) haemorrhages always occur before nose/bone defects (because of marked inhibition of vitamin K epoxide reductase leading to reduced carboxylation of the critical bone proteins), then one could use the absence of haemorrhages in animal studies to conclude that nose/bone defects also will not be induced. But since this information is not available, that conclusion cannot be drawn for the AVKs.

Based on available literature, one may rather speculate that the opposite may be true in humans, i.e., that bone effects may precede the haemorrhagic effects of AVKs. Cases of warfarin-induced teratogenicity with no reported haemorrhagic event have been reported. For example, Baillie described "a term infant with a hypoplastic nose due to failure of development of the nasal septum. No other abnormality was detected on routine clinical examination. X-rays of pelvis and femora showed stippling in the greater trochanters and left pubis and also abnormal vertebral bodies at S4/5" (Baillie, 1980). A similar case with slightly enlarged head and flattened face with a depressed nasal bridge and small nose, stippling of the vertebrae and femoral epiphyses was noted in a stillborn neonate in the 26th week of gestation where no abnormalities other than mild hydrocephalus, nasal hypoplasia, foetal growth restriction were revealed (by autopsy) (Tongsong et al., 1999). Van Driel and co-workers (2002) summarised the foetal outcome in cohort studies on the use of coumarins (mainly Warfarin but also acenocoumarol and phenprocoumon) during pregnancy and reported a two-times higher prevalence of embryopathy (22 cases of skeletal anomalies seen after in utero exposure

to coumarins) than bleeding (11 cases) if coumarins were given from the beginning of and throughout the pregnancy. For the cases reported for acenocoumarol and phenprocoumon, 85% involved the nose and only one case mentions bleedings (7%) (van Driel, 2002).

A possible explanation for the presence of bone effects with no haemorrhagic effects could be related to the specificity of haemostatic mechanisms in the developing foetus. During in utero development, vitamin K levels are low in the foetus, even close to deficiency levels (Howe and Webster, 1994). Coagulation factors do not cross the placenta, and vitamin K crosses the placenta at a very low rate, with concentrations in the cord plasma at 0.2-0.3% of maternal plasma concentration (Shearer, 1982). Therefore, concentrations of the vitamin K dependent clotting factors (II, VII, IX, and X), as well as of the proteins C and S, are reduced at birth to about 50% of normal adult values. Nevertheless, due to other, vitamin K non-dependent mechanisms (e.g. higher plasma concentration of von Willebrand factor and higher haematocrit level), healthy neonates have normal haemostasis and are no more prone to bleeding diathesis than adults (Revel-Vilk, 2012).

A biochemical basis for the higher sensitivity of the skeletal system than of the hepatic coagulation system in humans has also been suggested in the literature. Thus, the recycling of vitamin K 2,3 epoxide to vitamin K hydroquinone, which is essential for modification of glutamic acid residues to gamma-carboxyglutamate in vitamin K–dependent proteins (including coagulation factors, protein C, S, and Z, Matrix Gla protein – MGP, and osteocalcin), requires two steps. In the first step, the vitamin K 2,3-epoxide is reduced to vitamin K, and in the second step vitamin K is further reduced to the hydroquinone. The first step is catalysed by vitamin K epoxide reductase (VKOR) both in hepatic and extra-hepatic tissues, while in the second step VKOR is essential only in extra-hepatic tissues. In hepatic tissue other enzymes, such as DT diaphorase (a NADH-dependent reductase, which is not inhibited by Warfarin), are also involved (Teichert et al., 2008). Wallin and co-authors showed, for example, that in vascular smooth muscle cells the activity of DT-diaphorase is 100 times lower compared to liver tissue, whereas the activity of VKOR is 3 times higher (Wallin et al., 1999).

It could be expected, therefore, that extra-hepatic tissues are more sensitive to vitamin K deficiency or inhibition (such as that induced by warfarin-) than hepatic tissue. Undercarboxylated osteocalcin (ucOC) has been found in healthy adults with normal coagulation (prothrombin time within the normal range), and its level decreased by approximately 50% after one-week vitamin K supplementation (1000 micrograms of vitamin K1 per day) (Binkley et al., 2000). Because of the high accumulation of vitamin K in the liver, the liver will take up vitamin K from the blood at the expense of other tissues also needing vitamin K (Vermeer, 2001). The dose of vitamin K that inhibited the effect of Warfarin on blood coagulation could not prevent warfarin-induced inhibition of gamma-carboxylation of osteocalcin in rats ("liver-bone dichotomy" model) (Price and Kaneda, 1987). Similarly, Warfarin induced bone and cartilage changes in the absence of haemorrhages in developing rats treated concomitantly with Warfarin and vitamin K1 during the first 12 weeks of life (Howe and Webster, 1992).

Human experiences of vitamin K deficiencies also support the conclusion that the skeletal system is very sensitive, even more sensitive than the coagulation system. Thus, facial malformations identical to those caused by Warfarin have been shown to be caused in humans by many agents that decrease the concentrations of vitamin K, such as the anticonvulsant phenytoin (Howe et al., 1995), other coumarin drugs such as Acenocoumarol and Phenprocoumoun (Hetzel et al., 2006), liver dysfunction (Xie et al., 2013), and genetic vitamin K epoxide reductase deficiency (Keppler-Noreuil and Wenzel, 2012).

Overall, it is concluded that there might be differences between how humans and experimental animals respond to the AVK rodenticides, and also individual differences

between humans. It is therefore difficult to exclude human developmental toxicity based on negative animal studies, particularly considering that there are cases of developmental toxicity seen in humans exposed to four different AVK coumarins.

Toxicokinetics and transplacental transfer

The AVK rodenticides have different physico-chemical characteristics (e.g., a range of 0.7-6.3 for the log Pow and 292-542 for the molecular weight) which lead to differences in kinetics, mainly expressed as different half-lives of elimination. However, Coumatetralyl and Warfarin are both first generation AVK rodenticides, and have very similar physico-chemical characteristics. They can therefore be expected to behave rather similarly in the body, for instance with respect to transplacental transfer, although there are no data available for Coumatetralyl. Other AVK anticoagulants have been shown to cross the placenta in humans, e.g., Flocoumafen, Brodifacoum, Acenocoumarol and Phenindione (Hoyer, 2010).

It is concluded that all AVK rodenticides are expected to cross the placenta, and although there might be some quantitative differences, the toxicokinetic data support the conclusion that the effects of Warfarin and Coumatetralyl are similar in humans.

4.11.1.2 Human information

No human data are available on effects on fertility of coumatetralyl.

Warfarin, which is structurally and functionally comparable to coumatetralyl, was not classified for fertility.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

No teratogenic effects on the developing foetus were seen in the animal species (rat and rabbit) tested.

In the **rat,** the NOAEL for teratogenicity and embryotoxicity is 0.14 mg/kg bw, the highest dose tested. Administration of coumatetralyl caused dose-related maternal mortalities at 0.07 mg/kg bw and above. Thus NOAEL of 0.035 mg/kg bw and effect level (LOAEL) of 0.07 mg/kg bw was established for maternel toxicity (Becker and Biedermann, 1996a).

In the **rabbit** the NOAEL for teratogenic effects is 0.05 mg/kg bw, the highest dose tested. For embryotoxicity, the NOAEL was 0.025 mg/kg bw based on the effect on one dam at the dose level of 0.05 mg/kg bw, near total post-implantation loss (probably secondary due to intrauterine bleeding) The NOAEL for maternal toxicity is 0.0125 mg/kg bw, and the LOAEL 0.0250 mg/kg bw/day. Administration from 0.025 mg/kg bw and above caused serious substance-related deaths and external and internal bleedings in the dams. (Becker and Biedermann, 1996b).

The NOAELs for developmental toxicity in the two developmental studies in rats and rabbits may suggest a species difference with NOAEL for rabbits of 0.025 mg/kg bw/day whereas the NOAEL for rats is 0.14 mg/kg bw/day. However, the NOAELs for teratogenicity for both species are the highest doses tested in the respective studies, and the suggested difference related to choice of dosage. Maternal toxicity occurs at dose levels in both rats and rabbits lower than development

NOAELs, and the possible embryotoxicity effect in rabbits is suggested to be secondary to the maternal toxicity.

4.11.2.2 Human data

No data are available from human on coumatetralyl for this endpoint. However, information on the structurally and functionally related substance warfarin is available (se point 4.11.2.3 below).

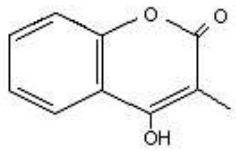
4.11.2.3 Other relevant information

Read across analysis

Coumatetralyl is a hydroxycoumarin derivative. The hydroxycoumarin derived anticoagulants are often referredt to as "first" and "second generation", where first generation hydroxycoumarin derivatives anticoagulants were developed in the 40's, while the more potent second generation anticoagulant were developed later, when resistance was noted to first generation anticoagulants. The structures of hydroxycoumarin and different derivatives are shown below in figure 1. (WHO, 1995)

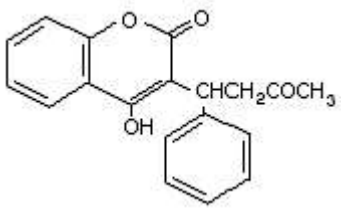
Figure 1. Structural formulas for hydroxycoumarin and derivatives.

Hydroxycoumarin

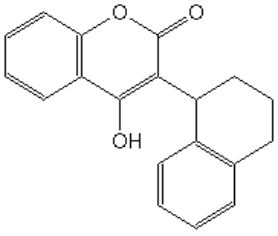


First generation hydroxycoumarin derivatives**:**

Warfarin

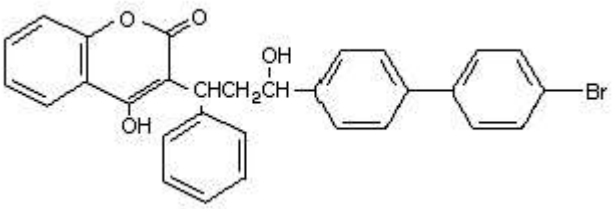


Coumatetralyl

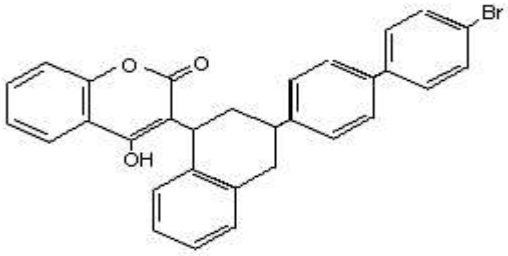


Second generation hydroxycoumarin derivatives:

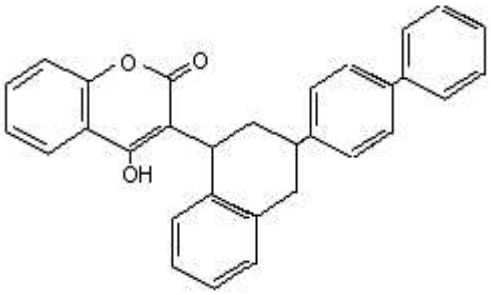
Bromadiolone



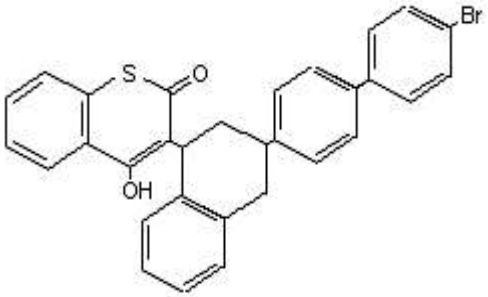
Brodifacoum



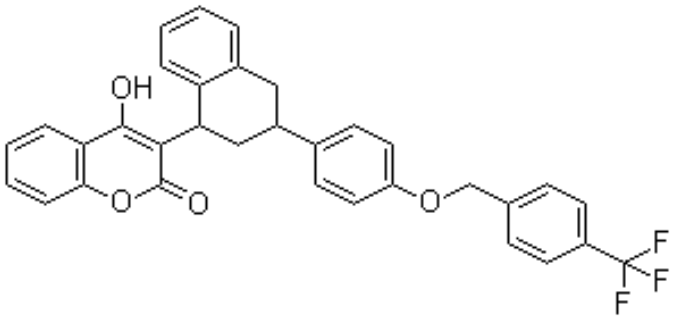
Difenacoum



Difethialone



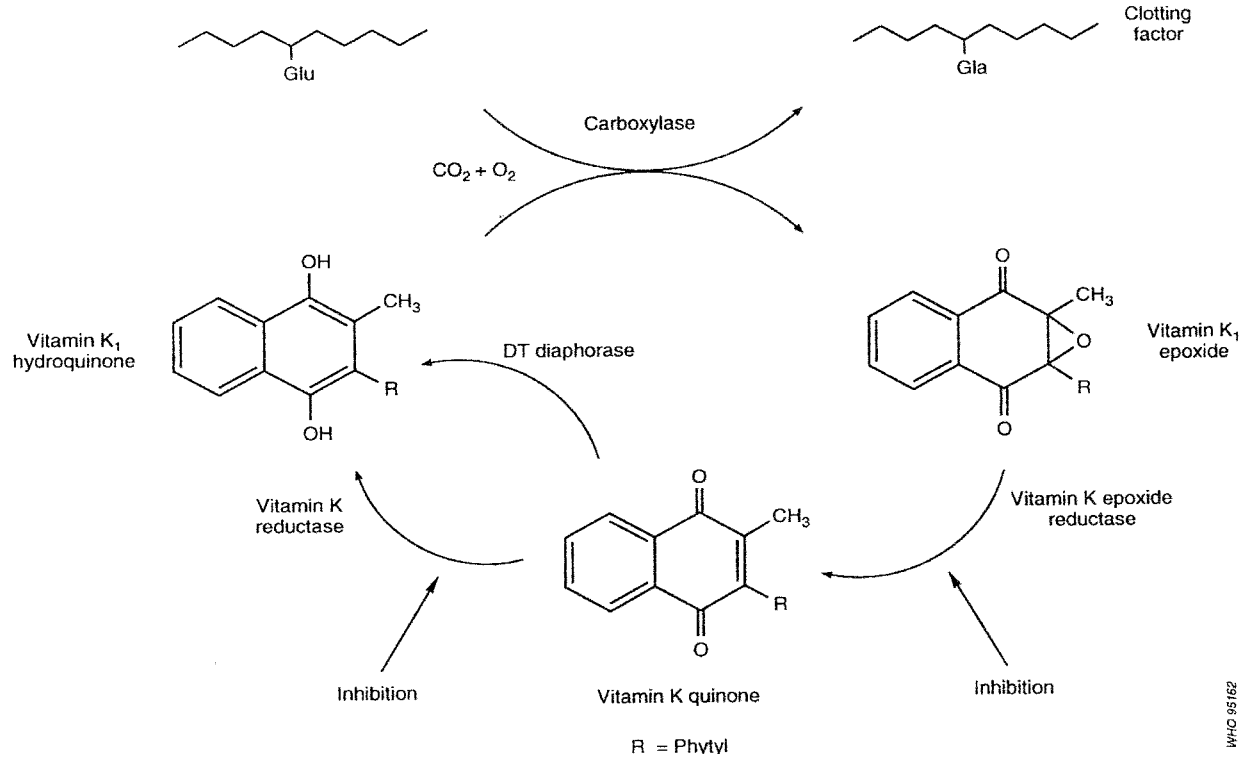
Flocoumafen



Mechanism of action

Hydroxycoumarin derivatives, including coumatetralyl and also warfarin, are vitamin K antagonists. Their use as rodenticides is based on the inhibition of the vitamin K-dependent step in the synthesis of a number of blood coagulation factors. Vitamin K is a co-enzym, in its active form Vitamin K hydroquinone, which oxidation to vitamin K 2,3-epoxide provides energy for the carboxylation reaction. Coumarin derivatives block the microsomal vitamin K epoxide reductase enzyme, thereby leading to an accumulation of non carboxylated coagulation factor precursors in the liver (WHO, 1995). Figure 2 show the epoxy-cycle, indicating the points of action of hydroxyl-coumarins derivatives

Fig 2: Epoxide cycle: the mechanism of clotting inhibition caused by hydrocycoumarin-related anticoagulation (reproduced from EHC 175: Anticoagulant Rodenticides, IPCS, WHO, 1995.



It has been demonstrated in the rat that daily administration of vitamin K can counteract the effect of warfarin on the carboxylation of coagulation proteins in the liver. However, the effect of hydroxycoumarins on bone Gla protein synthesis by osteoblasts are not affected by additional vitamin K (Howe & Webster, 1994). This is due to the fact that extrahepatic coagulation proteins do not carboxylate. Thus, extrahepatic vitamin K deficiency cannot be compensated.

Animal study on related substance

A recently performed developmental study on warfarin according to OECD 414 was filed to the Irish CA and a study summary of it would be included in its dossier for warfarin. The study reports subcutaneous haemorrhage at all dose levels (0.125; 0.150; 0.200 and an added group of 0.250 mg/kg bw/day) in the offspring, as well as visceral haemorrhage, central ocular cataract and skull malformations from 0.150 mg/kg bw/day. Maternal toxicity (deaths, vaginal bleeding and blood in the uterus at necropsy) was reported from 0.150 mg/kg bw/day. Thus, the NOAEL for maternal toxicity is set at 0.125 mg/kg bw/day ($LOAEL_{maternal}$: 0.150 mg/kg bw/day). Due to the bleeding in the offspring, seen at all doses, no NOEL could be set for developmental toxicity in this study.

Human data on related subtance

Human exposure to warfarin to therapeutic use during the first trimester of pregnancy caused “warfarin embryopathy” or “foetal warfarin syndrome”, including foetal anomalies such as nasal hypoplasia and growth retardation due to bone anomalies. Exposure during second and third trimester of pregnancy may also lead to foetal loss, stillbirth and abortion due to foetal haemorrhage, microcephaly and/or hydrocephaly.

4.11.3 Summary and discussion of reproductive toxicity

4.11.3.1 Fertility

One and two generation studies are not available for coumatetralyl. No effects on the reproductive organs were reported in repeated dose toxicity studies. Warfarin, another well known coumarin derivate with the same chemically active group as coumatetralyl is not classified as toxic to fertility. Based on the available data, no classification for effects on fertility for is proposed.

4.11.3.2 Developmental toxicity

Coumatetralyl was tested for its developmental toxicity in rats and rabbits in studies conducted in accordance with accepted guidelines (unpublished studies). These studies did not reveal any teratogenic or other developmental effect (doses up to 0.14 mg/kg bw/day in the rat and 0.035 mg/kg bw/day in the rabbits). Maternal toxicity included deaths and internal bleeding and occurred at low doses (0.07 mg/kg bw/day in the rat, and 0.025 mg/kg bw/day in the rabbits).

However, due to the high toxicity of coumatetralyl due to its effect on vitamin K depletion, standard guideline studies are regarded as insufficient to establish whether coumatetralyl has a developmental effect as developmental toxicity may be masked by the high maternal toxicity.

In order to reach potential developmental toxic dose levels of coumatetralyl without high mortality and toxicity to the dams, studies with the addition of vitamin K to maintain blood coagulation in the dams would be needed.

Also, differences in the time line in development of the rat and human foetuses should be taken into consideration in the dosing period chosen in developmental studies with coumatetralyl. For example, mineralisation of the bones is mainly postnatal in the rat, while it occurs already during weeks 5-7 of pregnancy in humans (Howe & Webster, 1994). Such specifically designed studies are not available.

No data are available from humans on developmental effects of coumatetralyl.

The chemical structure of coumatetralyl and its mechanism of toxicity – as it is the case for other hydroxycoumarin substances - is the same as for warfarin. It has been demonstrated that this group of chemically related substances affect the coagulation cascade (through vitamin K hydroquinone deficiency) in humans and in laboratory animals.

Warfarin is classified as repr 1, R61 based on the occurrence of warfarin embryopathy following exposure of humans to therapeutic anticoagulating doses.

It appears from the data on warfarin that humans are less sensitive to the bleeding effect than rodents, but more sensitive to the teratogenic effect. However, the data do not permit to perform a quantitative calculation of the potency of the different anticoagulants.

The data indicate that human foetus is more sensitive to vitamin K deficiency than the mother. Measurements in humans show that vitamin K-levels in the foetus are very low (21-30 pg/ml) compared to those in the mother (395 – 565 pg/ml) (Howe & Webster, 1994).

No study is available on the possible placental passage of coumatetralyl. However, taking the molecular weight and the partition coefficient of coumatetralyl into consideration, placental passage of the substance is found likely to occur. In that situation, the induced vitamin K deficiency in the foetus may be enhanced by a direct action of coumatetralyl on the foetus.

A developmental study on warfarin (for details, please refer tto the dossier by Ireland on warfarin) shows severe maternal toxicity from 0.150 mg/kg bw/day, and visceral and skeletal effects at the same levels. In addition, the offspring show subcutaneous haemorrhage at all dose levels, including 0.125 mg/kg bw/day. Thus the effect level (LOAEL) in dams is 0.150 mg/kg bw/day, the NOAEL being 0.125 mg/kg bw/day. For the developmental effect, the LOAEL is 0.125 mg/kg bw/day, and no NOAEL could be set.

The study on warfarin shows that the primary mechanism of action of warfarin is the impairment of the blood coagulation in the dams as well as in the offspring. Indication of a teratogenic potential of the compound is present at maternally toxic doses. However, it is not possible to extrapolate the information to the ability of coumatetralyl to induce teratogenic effects in humans.

4.11.3.3 Effects on or via lactation

The amount of coumatetralyl transferred to milk is unknown and there are no studies of effects via lactation during postnatal development. Consequently, no conclusion can be drawn from the available information and no classification is proposed.

4.11.4 4.11.5 Comparison with criteria

4.11.4.2 Fertility

Based on the available data, no classification for effects on fertility for coumatetralyl is proposed.

4.11.4.3 Development

Coumatetralyl did not cause any observed teratogenic effects in the experimental animal studies. However, due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to coumatetralyl, no clear conclusion can be drawn from the standard guideline studies.

Coumatetralyl is part of the same group of chemicals, the hydroxycoumarin derivatives, as warfarin (WHO, 1995). Coumatetralyl has also the same mode of action as warfarin, which is a well documented human teratogen classified as a reproductive toxicant (Repr. Cat 1; R61 - Repr. 1A H360D). Warfarin has been shown to cause teratogenicity in humans and in experimental animals. Based on analogy consideration to warfarin classification of coumatetralyl for developmental toxicity by, is relevant.

Warfarin and other rodenticides have been discussed at Technical meetings on Biocides. During these discussions it was realised that conventional developmental toxicity tests on rodenticide anticoagulants were difficult to perform and interpret, and it was suggested by the Rapporteurs to perform a read-across of developmental toxicity data from warfarin, already classified as a human developmental toxicant in Repr. Cat 1; R61. In 2006 the Specialised Experts group for classification for reproductive toxicity under directive 67/548/EC discussed the issue of read-across from warfarin for developmental toxicity and came to the following conclusion (ECBI/51/07):
"Warfarin is an established human teratogen classified as Repr. Cat. 1; R61. It is uncertain whether teratogenicity of warfarin can be detected in pre-natal developmental toxicity studies (including OECD guideline 414). The teratogenic mechanism of warfarin is likely to involve maternal Vitamin K depletion and/or direct effects on embryo/foetus via transplacental exposure. Given the vitamin K inhibition, there is concern that other anti-vitamin K (AVK) compounds could cause similar teratogenic effects as warfarin in humans.
The other AVK rodenticides have not shown teratogenic effects in conventional rat and rabbit developmental studies and there is no data in humans. Given the uncertainties surrounding the ability of standard pre-natal developmental toxicity studies to detect warfarin teratogenicity the predictive values to humans of these studies is uncertain.
On the basis of currently available data, there are no convincing arguments that other AVKs including the second generation compounds could not pass the placenta. Both the mechanism of action and the possible placental passage give reason for concern of possible teratogenicity in humans.
Considering the available information the Specialised Experts unanimously agreed that the AVK rodenticides should collectively be regarded as human teratogens. Therefore the other AVK rodenticides should be classified as Repr. Cat. 1; R61."

New animals studies have been performed on warfarin and on flocoumafen. Draft summaries of the new data will be included in the CLH-reports for the respective substances
However, The Danish EPA does not believe that the new data can be used to change the conclusion on the classification on coumatetralyl. The new data cannot be used to negate the evidence that the structurally and functionally analogue compound warfarin is a human teratogen, which is classified in Repr. Cat 1A; R360D in Annex VI to CLP.

Specific concentration limits

Potential developmental effects of coumatetralyl would be expected at very low doses, and the possibility of setting specific concentration limits for developmental toxicity should therefore be considered - using the newly developed guidance document for setting specific concentration limits (SCL) for reproductive toxicants within the CLP regulation. However, it is recognized that a potency evaluation is very difficult where the classification is based on read across from other substances, and no direct estimate of the developmental toxicity potency is possible.

4.11.4.4 Effects on or via lactation

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

4.11.5 Conclusions on classification and labelling

The available guideline animal studies on 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 regarded not to be 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), classification of coumatetralyl for developmental toxicity as
Repr. Cat. 1; R61 (Directive 67/548/EEC), respectively
Repr. 1A H360D (Regulation EC 1272/2008),
by analogy consideration with warfarin, is proposed.

Developmental effects of coumatetralyl would be expected at very low doses, and the possibility of setting specific concentration limits for developmental toxicity should therefore be explored.

No classification for effects on fertility or effects on or via lactation is proposed.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Acute, sub-chronic and developmental toxicity studies conducted with coumatetralyl active ingredient (a.i.) or formulations in rats and rabbits show typical clinical signs of anticoagulant effect mainly including bleeding, pallor and signs of haemorrhages.
Considering all these studies, no particular finding can be attributed to a neurotoxic origin. Moreover, a review of the data available for anticoagulant rodenticides shows that no neurotoxicity has been evidenced for this class of compounds.
Thus, there is no need to further investigate coumatetralyl effects by conducting neurotoxicity studies as these would be unethical for animal welfare reasons.

4.12.1.2 Immunotoxicity

No indication of immunotoxicity of coumatetralyl was seen in the available studies. No studies were performed on this end-point.

4.12.1.3 Specific investigations: other studies

No further animal studies on human health end-points were available with coumatetralyl.

4.12.2 Conclusions on classification and labelling

No further human health end-points lead to classification proposals.

5 ENVIRONMENTAL HAZARD ASSESSMENT

In this section summaries and evaluation of data for which robust study summaries are presented in the annex: "CLH report coumatetralyl RSS CAR environment" which is reproduced from the final CAR report under the review programme under the biocides directive (98/8/EC are reported, as far as possible in summary tables. Due to an interim period agreement, the robust study summaries are not available in IUCLID.

5.1 Degradation

Table 5.1 Hydrolysis of coumatetralyl

Guideline / Test method	**pH**	**Temperature [°C]**	**Initial TS concentration, C_0[µg/ml]**	**Reaction rate constant, K_h [1/s x 10^5]**	**Half-life, DT_{50}[h]**	**Coefficient of correlation, r^2**	**Reference**
OECD guideline "Hydrolysis as a function of pH"	4, 7, 9	5 and 55	*2 - 2.5*	n.a.	Long	n.a.	Wilmes, 1983;

From Doc III A7.1.1.1.1

Table 4.1.2 Photodecomposition of coumatetralyl

Guideline / Test method	**Initial molar TS concen-tration**	**Total recovery of test substance [% of appl. a.s.]**	**Photolysis rate constant (k^c_p)**	**Half-life ($t_{1/2E}$)**	**Extrapo-lated Half-life ($t_{1/2}$) in sunlight**	**Reference**
Exposition to artificial light and pilot study in sunlight	1.4 mg/l	-	-			Wilmes, 1982;

5.1.1 Stability

Hydrolysis

The hydrolysis of coumatetralyl was studied in accordance with the OECD guideline 111 "Hydrolysis as a function of pH" at pH 4, 7 and 9 in citrate, phosphate and borate buffer solutions.

The active ingredient content of the samples incubated at 55 °C was not observed to have changed, as compared with the refrigerated samples (degradation after 5 days at 50 °C < 10 %).

Conclusion:

Coumatetralyl is stable to hydrolysis.

Photolysis in water

From Doc III A7.1.1.1.2

The photolysis in water was studied in a saturated solution of coumatetralyl in distilled water containing 1.4 mg a.i./l. The samples were exposed to one each of two types of artificial light. In addition a range-finding experiment in sunlight was performed. Photolysis transformation products were isolated and identified in a test in which solutions of 100 mg coumatetralyl/l in distilled water/acetonitrile (2:1) were irradiated for 3 hours.

Conclusion:

Coumatetralyl was degraded rapidly by light under artificial not environmentally relevant conditions to a number of degradation products, of which salicylic acid was identified as a major product. Due to lack of information, no exact conclusion regarding possible degradation rate (half life) can be drawn. However, the results indicate that coumatetralyl is unstable to photolysis in water with an indicative DT < 1 day.

This result is in agreement with expected photolysis profile of coumatetralyl. Due to the UV absorption of the hydroxycoumarin chromophore in the range of 300 to 350 nm, photolytic degradation can be expected. For the structurally similar rodenticide difenacoum which also has the hydroxycoumarin chromophor photolytic half lives were 3.26, 8.05 and 7.32 h at pH 5, 7 and 9. (WHO environmental Health Criteria 175, Anticoagulant Rodenticides, WHO Geneva, 1995, Internet: http://www.inchem.org/documents/ehc/ehc/ehc175.htm)

Phototransformation in air

Calculation using AOPWIN (Atmospheric Oxidation Program) Version 1.91. The program is an adoption of the estimation methodology from Atkinson developed by Syracus Research Corporation. A chemical lifetime of coumatetralyl in air of utmost 3 hours (corresponding to a half-life of utmost 2 hrs) is to be expected. The atmospheric photochemical half-life was 2 hours based on ozone reaction and 2.4 hours (using 12-hour day) based on hydroxyl radicals.

5.1.2 Biodegradation

Biotic degradation

Biodegradation

Table 4.1.3 Biodegradation of coumatetralyl

Guideline	Test	Test	Inoculum	Addition	Test	Degradation	Reference

/ Test method	type[1]	para-meter	Type	Concentration	Adaptation	al substrate	substance concentr.	Incuba-tion period	Degree [%]	
OECD 301 D (Closed Bottle Test)	*Ready*	*O_2*	*Secon-dary efflue nt*	*4 ml/l of final volume*	*yes [2]*	*no*	*2 mg/l; 5 mg/l*	*28 d*	*1 % (2 mg/l); 0 % (5 mg/l)*	*Desmares-Koopmans, 2001a;*
OECD 302 B (Zahn-Wellens/ EMPA Test)	*Inheren t*	*DOC*	*Acti-vated sludg e*	*0.25 g/l dry matter in final mixture*	*yes [3]*	*no*	*100.5 mg/l*	*28 d*	*8 %*	*Desmares-Koopmans, 2001b;*

[1] Test on *inherent* or *ready* biodegradability according to OECD criteria

[2] secondary effluent was filtrated and aerated until inoculation

[3] activated sludge was coarsely sieved, washed and aerated until required

From Doc III A7.1.1.2.1 and Doc III A7.1.1.2.2

Coumatetralyl was tested for its ready biodegradability by using the Closed Bottle Test according to OECD guideline No. 301 D. A second study was performed to investigate the inherent biodegradability of coumatetralyl (OECD guideline No. 302 B, Zahn-Wellens/EMPA Test). See table 4.1.3 for test parameters and results. For both tests, the reference substance was readily degraded, whereas coumatetralyl was found to be stable.

Aerobic degradation in soil (Doc III A7.2.1 (1) and A7.2.1 (2))

The soil metabolism of radioactive labelled Coumatetralyl under aerobic conditions was investigated in two studies. The half-life in soil is less than 30 days based on primary degradation and after 6 month more than 50% of the originally applied radioactivity was degraded to CO_2. The proportion of bound residues was about 30% under aerobic conditions. The detected metabolites indicate various degradation pathways via oxidation of the active ingredient, which lead to salicylic acid. Salicylic acid is rapidly mineralized to CO_2 by soil-micro organisms. None of the metabolites occurred at a level above 10%.

Anaerobic degradation in soil (Doc III A 7.2.2.4)

Under anaerobic conditions, no degradation (< 1 %) of coumatetralyl took place during 60 days. No metabolites and no [^{14}C]CO_2 were formed in a purely anaerobic system.

5.1.3 Summary and discussion of degradation

Coumatetralyl was not readily biodegradable under the conditions of the Closed Bottle Test and not inherently biodegradable under the conditions of the Zahn-Wellens/EMPA Test.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 4.1.8 Adsorption/desorption of coumatetralyl on soil

Guideline / Test method / Soil type	Adsorbed a.s. [mean %]	K_d [1] [cm^3/g]	K_{oc} [2] [cm^3/g]	K_{des} [3] [cm^3/g]	K_{om} [4] [cm^3/g]	K_d / K_{des} [5]	Degradation products		Reference
							Name	[%] of a.s.	
OECD 106									Slangen, 2002; (Doc III A7.1.3)
Sand	53.1	2.27	403	6.67	234	0.34	n.a.	n.a.	
Clay loam	59.5	3.10	185	241	107	0.013			
Silt loam	50.1	2.14	71	7.02	41	0.30			
Sandy loam	80.0	8.10	735	27	426	0.30			
Clay	55.4	2.67	115	41	67	0.065			

[1] K_d = adsorption coefficient
[2] K_{oc} = organic carbon normalised adsorption coefficient
[3] K_{des} = desorption coefficient
[4] K_{om} = organic matter normalised adsorption coefficient
[5] K_d / K_{des} = adsorption / desorption distribution coefficient

From Doc III A7.1.3

The adsorption/desorption behaviour of coumatetralyl on soil was studied in five soils which represent major agricultural areas in Europe and North America, using the batch equilibrium method according to the OECD guideline No. 106.

The amount of test substance adsorbed to soil after 48 hours ranged from approximately 50 % (silt loam) to 80 % (sandy loam). The amount of material desorbed from the soil after 30.5 hours ranged from approximately 1 % (clay loam) to approximately 23 % (silt loam and sand).

The kinetics of adsorption and desorption showed that adsorption equilibrium and desorption equilibrium were reached within 24 hours in the soil types sand, silt loam and sandy loam, but not in clay loam and clay. Results of adsorption and desorption parameters are described in detail in table 4.1.8.

Conclusion:

Based on the K_{om} values and the mobility classification scheme according to Mensink, coumatetralyl can be considered to be immobile in sand, clay loam and sandy loam. It is considered to be slightly mobile in silt loam and clay. (Mensink B., M. Monforts, L. Wijkhuizen-Maslankiewicz, H. Tibosch and J. Linders, Manual for-summarising and evaluating the

environmental aspects of pesticides, National Institute of Public Health and Environmental Protection, the Netherlands, Report No. 679101022 (1995)).

5.2.2 Volatilisation

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

Table 4.1.10 Measurements of aquatic bioconcentration

Guideline / Test method	**Exposure**	**Log P_{OW} of a.s.**	**Initial concentr. of a.s.**	**Steady-state BCF**	**Uptake rate constant [h^{-1}]**	**Depuration rate constant [h^{-1}]**	**Depuration time (DT_{50}) [h]**	**Metabo-lites**	**Reference**
OECD 305E	120 h	3.5 [1]	52 µg/l	11.4 (± 2.8) (whole fish)	0.56 (± 0.08)	0.05 (± 0.01)	14.1 (± 2.7)	-	Grau, 1992a;
				3.32 (± 1.2) (edible)	0.26 (± 0.06)	0.08 (± 0.02)	8.81 (± 2.49)		
				20.8 (± 6.0) (viscera)	1.02 (± 0.19)	0.05 (± 0.01)	14.1 (± 3.2)		

[1] value mentioned in the study

From Doc III A7.4.2

5.3.1 Aquatic bioaccumulation

A dynamic 456-hour study was conducted according to OECD guideline No. 305 E (1984) to evaluate the bioconcentration of [14C]-coumatetralyl in bluegill sunfish (Lepomis macrochirus).

The calculated bioconcentration factors for edible parts and whole fish are 3.32 (± 1.20) and 11.4 (± 2.8), respectively. They corresponded well with the respective observed bioconcentration factors of 3.0 X and 10.4 X for [14C]-coumatetralyl at 120 hours. These values corresponded to calculated steady-state total residue levels of 0.17 and 0.59 mg [14C]-coumatetralyl equivalents/kg for edible parts and whole fish, respectively.

24 hours after cessation of exposure 63, 69 and 67 % of the maximum measured plateau residues were depurated from edible portions, non-edible portions and whole fish, respectively. After 14

days in uncontaminated water more than 99 % of the maximum plateau radio-activity was depurated from edible portions, non-edible portions and whole fish, respectively.

The uptake rate and depuration rate constants as well as the depuration time (DT50) for whole fish, edible parts and viscera/non-edible parts are given in table 4.1.10.

Table 4.1.11 Estimations on aquatic bioconcentration

Basis for estimation	**log P_{OW} (measured)**	**Estimated BCF for fish (freshwater)**	**Reference**
	1.50	*2.851 (log BCF = 0.455)*	*EPI-Win, v3.11, 2003*

The bioconcentration factor for the test substance may be overestimated in the study because all calculations refer to radioactivity (sum of parent compound, metabolites and mineralization products).

5.3.2 Summary and discussion of aquatic bioaccumulation

Coumatetralyl is accumulated very rapidly by bluegill sunfish with a total residue bioconcentration factor of 11 X for whole fish. When exposure ceases, the residues are depurated quickly with a half-life of approximately 14.5 hours. Accumulation in edible parts is less (3 X) than in whole fish (11 X).

The Log P_{ow} is pH dependant as shown in Table 1.3; however, the result of the bioaccumulation study in fish demonstrates that accumulation of coumatetralyl is not to be expected.

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Table 4.2.1 Acute toxicity to fish

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results [mg a.s./l]**			**Remarks**	**Reference**
			Design	Duration	LC_0	LC_{50}	LC_{100}		
Draft DIN 38 412	*Salmo gairdneri*	mortality	Static	96 h	32	48 (42-55)	76	nominal conc.	Hermann, 1983;
OECD 203	*Salmo gairdneri*	mortality	Static	96 h	32	53	100	nominal conc.	Sewell & McKenzie, 2003
Draft DIN 38 412	*Leuciscus idus melanotus*	mortality	Static	96 h	51	67 (61-73)	90	nominal conc.	Hermann, 1982;

From doc III A7.4.1.1. There are 3 studies described in the IUCLID data set (2 non key studies and therefore there is no study summary of these).

The acute toxicity on fish was investigated on rainbow trout (*Salmo gairdneri*) by Sewell & McKenzie, 2003 according to the OECD guideline 203 (2003). While all tests gave comparable results, the test performed according to OECD Guideline 203 was selected as the key study due to its higher reliability factor.

Following a preliminary range-finding test, juvenile fish were exposed in groups of ten, to an aqueous solution of coumatetralyl over a range of concentrations of 10, 18, 32, 56 and 100 mg/l for a period of 96 hours at a temperature of 12,5°C to 14,0°C under static conditions.

The number of mortalities and sub-lethal effects of exposure in test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until 96 hours.

Conclusion:

The 96-hour LC_{50} value based on nominal test concentration was 53 mg/l.

5.4.1.2 Long-term toxicity to fish

Table 4.2.5 Prolonged acute toxicity to fish

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results [µg/l]**		**Remarks**	**Reference**
			Design	Duration	NOEC	LOEC		
OECD 204	*Oncorhynchus mykiss*	mortality	semi-static	21 d	5.0	15.8	nominal conc.	Grau, R., 1992;

From Doc III A7.4.3.1

The prolonged toxicity of coumatetralyl to rainbow trout (Oncorhynchus mykiss) was determined in a 21-day semi-static test according to OECD guideline No. 204 (1984). In the first part of the study four groups of ten fish were exposed to nominal concentrations of 15.8, 50.0, 158.0 and 500.0 µg/l and in the second part four groups of ten fish were exposed to nominal concentrations of 0.158, 0.50, 1.58 and 5.00 µg/l Fish were examined each working day for the mortalities and symptoms of intoxication in the control, solvent control and test concentrations. Coumatetralyl concentrations were analysed during the study by means of HPLC.

The NOEC was determined to be 5.0 µg/l, the LOEC and the lowest lethal concentration (LLC) were 15.8 µg/l. A 21-day LC_{50} value was not calculated. Observable symptoms were noted among the fish in the 15.8 (Exopthalmus, day 20) and 500.0 µg/l (fish mainly at the bottom and swimming behaviour slightly irregular (slight symptom), day 21) test levels. Neither mortalities nor symptoms of intoxication occurred in the control and the solvent control groups. There were no statistically significant difference in body weight, length and condition factor between the control and the treated groups. The analytical results were between 81 and 120 % and showed that the concentrations were in accordance with the nominal values and that the test substance was stable for the duration of the test. The prolonged acute study on fish is considered valid and acceptable.

Conclusion:

For Oncorhynchus mykiss a 21-d NOEC of 5.0 µg/l was determined, the LOEC and the lowest lethal concentration (LLC) were 15.8 µg/l. The prolonged acute NOEC of 5.0 µg/l is used in the risk assessment for the aquatic compartment.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

5.4.2.2 Acute toxicity to invertebrates

Table 4.2.2 Acute toxicity to *Daphnia magna*

Guideline / Test method	**Endpoint / Type of test**	**Exposure**		**Results [mg a.s./l]**			**Remarks**	**Reference**
		Design	**Duration**	**EC_0**	**EC_{50}**	**EC_{100}**		
OECD 202	Immobility	static	48 h	≥ 14	> 14	> 14	measured conc.	Heimbach, F., 1991a;

From Doc III A7.4.1.2

Coumatetralyl was investigated in a 48-hour static test for acute toxicity to water fleas (*Daphnia magna*) according to OECD guideline No. 202 (1984). The test animals were exposed to the nominal test substance concentrations 1.0, 1.8, 3.2, 5.6, 10.0 and 18 mg/l.. Immobility of animals and symptoms were determined after 24 and 48 hours in the control, solvent control and the test concentrations. Concentrations tested were analysed at the beginning of the test and after the exposure period of 48 hours in the test vessels containing 1.0, 5.6 and 18 mg test substance/l.

After 48 hours the EC_{50} value and the LOEC were > 14 mg/l. The NOEC was determined to be 14 mg/l. The results are based on measured test concentrations. No immobility of animals or symptoms of toxicity were observed in any of the groups. The measured concentrations were 75 to 83 % of the nominal concentrations (for an average 79.5 %). A determination of the active ingredient content at the end of the exposure period showed stability of the test concentrations under the conditions of the test.

Conclusion:
The acute toxicity test for Daphnia magna resulted in a 48 h-EC_{50} of > 14 mg/l (maximum measured tested concentration). The NOEC was determined to be 14 mg/l.

5.4.2.3 Long-term toxicity to aquatic invertebrates

Table 4.2.6 Chronic toxicity to invertebrates

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results [mg/l]**		**Remarks**	**Reference**
			Design	Duration	NOEC	LOEC		
OECD 202, Part II	*Daphnia magna*	Repro-duction	semi-static	21 d	0.10	0.32	nominal conc.	Heimbach, F., 1992;

From Doc III A7.4.3.4

Coumatetralyl was tested for inhibition of reproduction of water fleas according to the OECD guideline No. 202, Part II (1984). Water fleas (*Daphnia magna*) were exposed in a 21-day semi-static test to aqueous test medium containing the test substance at the nominal concentrations 0.10, 0.32, 1.0, 3.2, 10 and 18 mg/l. Stock solutions were prepared with dimethylformamid. On day 2, 5, 7, 9, 12, 14, 16 and 19 after the start of the study and additionally on day 21, the number of offspring which had been born during the previous two or three days in each test container were counted. At the same time the surviving parents were recorded. The coumatetralyl concentrations in the test water were determined during the course of the study by means of HPLC.

The NOEC was determined to be 0.10 mg/l, the LOEC was 0.32 mg/l. There was no mortality in the control or the solvent control higher than 20 %, the level which is regarded as the natural rate of mortality. Three broods were clearly recognisable. A delay in brood times could not be observed. The mean number of newborn water fleas per adult was 141 in the control and 142 in the solvent control. Compared to control organisms, there was a biological and statistical significant reduction of reproduction at all test concentrations higher than 0.10 mg/l. At the end of the study, the body lengths of the parent control animals and those of the solvent control animals indicated well developed females. Compared to control organisms, at all concentrations higher than 0.10 mg/l a significant decrease in body length was found.

Conclusion:

The chronic NOEC for Daphnia magna was determined to be 0.10 mg/l, the LOEC was 0.32 mg/l.

5.4.3 Algae and aquatic plants

5.4.3.1 Growth inhibition on algae

Table 4.2.3 Acute toxicity on algae

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results [mg a.s./l]**			**Remarks**	**Reference**
			Design	**Duration**	**NOEC**	**E_bC_{50}[1]**	**E_rC_{50}[2]**		
OECD 201, ISO 8692	*Scenedesmus subspicatus*	growth inhibition	static	72 h	5.6	15.2	> 18	Nominal conc.	Heimbach, F., 1991b;
				96 h	5.6	14.8	> 18		

[1] calculated from the area under the growth curve; [2] calculated from growth rate

From Doc III A7.4.1.3

The effect on growth inhibition of algae was studied on the green alga Scenedesmus subspicatus in a 96-hour static test with the nominal concentrations 1.0, 1.8, 3.2, 5.6, 10 and 18 mg/l. The test was performed according to the OECD guideline No. 201 (1984) and ISO guideline ISO 8692 (1989). After 24, 48, 72 and 96 hours, the cell counts were determined photometrically in the control, solvent control and the test concentrations. At the beginning of the test, concentrations tested were analysed after preparing the test solutions by means of HPLC. The analyses indicated that starting measured concentrations were similar to nominal values and, therefore, the results of the test were based on nominal concentrations As the test substance was deemed to be stable in water, the exposure concentrations were not determined at the end of the study.

The effect of coumatetralyl on the growth of biomass was characterised with an E_bC_{50} value of 15.2 mg/l after 72 h and 14.8 mg/l after 96 h. The influence of coumatetralyl on the algae growth rate resulted in an E_rC_{50} of > 18 mg/l after 72 h and 96 h, respectively. The LOEC was determined to be 10 mg/l, the NOEC 5.6 mg/l. The results are based on nominal concentrations. No abnormalities, e.g. morphological changes were observed. The mean measured concentration levels ranged from 78 to 94 % of nominal concentrations (for an average: 90.8 %).

The growth in the control containers after 72 hours showed a rate of multiplication that is greater than a factor of 16 and thus the quality criterion of the OECD and/or ISO guideline is fulfilled. The test concentrations prepared in this test corresponded well to the nominal concentrations. However, according to the rapid degradation by light of the test substance, a degradation of the substance during the test is possible. This study is not decisive for the RA.

Conclusion:
The effect of coumatetralyl on the growth of biomass was characterised with an E_bC_{50} value of 15.2/14.8 mg/l after 72/96 h, respectively. The influence of coumatetralyl on the algae growth rate resulted in an E_rC_{50} of > 18 mg/l after both 72 h and 96 h.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Inhibition of microbial activity (aquatic)

Table 4.2.4 Inhibition of microbial activity (aquatic)

Guideline / Test method	Species / Inoculum	Endpoint / Type of test	Exposure		Results [mg a.s./l]			Remarks	Reference
			Design	Duration	EC_{25}	EC_{50}	EC_{75}		
ISO 8192	activated sludge	inhibition of respiratory rate	analytical parameter: oxygen consumption	duration of the study: 24 h	538	4210	32,900		Müller, G., 1991;

From Doc III A7.4.1.4

To assess the toxicity of coumatetralyl to bacteria a test was investigated according to the ISO 8192, "Test for inhibition of oxygen consumption by activated sludge" (1986). A defined quantity of activated sludge from laboratory sewage treatment plant was exposed to coumatetralyl at the nominal concentrations 560, 1000, 1800, 3200 and 5600 mg/l. The respiratory rate of each mixture was determined and compared to that measured in a mixture without test substance. At nominal test substance concentrations of 560 – 5600 mg/l, inhibition of respiratory rate was observed between 23.8 % and 55.6 %. The EC_{50} value of coumatetralyl was determined to be 4210 mg/l. The reference substance 3, 5-Dichlorophenol was used to control the sensitivity of the activated sludge. It should be noted that the test concentration is above the water solubility.

Conclusion:

The EC_{50} value of coumatetralyl was determined to be 4210 mg/l.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Comparison with classification criteria included in the paragraph below.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

The harmonized classification on Annex I to Directive 67/548/EC included in Annex VI of Regulation 1272/2008 is R52-53 (Harmful to aquatic organisms may cause long-term adverse effects in the aquatic environment) respectively Aquatic Chronic, category 3, H412 (Harmful to aquatic life with long lasting effects) according to DSD respectively CLP criteria.

In Directive 67/548/EC the classification is based on the acute toxicity combined with fate properties.

The EC_{50} and LC_{50} values available for coumatetralyl are in the range of 10 mg/l to 100 mg/l (Sewell & McKenzie, 2003; Heimbach, F., 1991a and b), and the substance is not rapidly degradable (*Desmares-Koopmans, 2001a and b*). Thus, according to Directive 67/548/EC, the substance is classified R52-53.

With respect to classification according to the CLP criteria, implementing elementsGHS, the criteria are divided into acute (short term) and chronic (long-term hazard) effects, the latter being based on long-term (chronic) data. With the second ATP, the criteria include attribution of M-factors.

In the data set for coumatetralyl the lowest $NOEC_{(fish)}$ was 5 µg/l (0.005 mg/l) (Grau, R., 1992). For non rapidly degrading substances the following table for classification and attribution of M-factor in relation to chronic effect ranges applies:

EC_{10} or NOEC (mg/l)	Chronic category
0.1-1	2
0.01-0.1	1
0.001-0.01	**1, M-factor = 10**
0.0001-0.001	1, M-factor = 100
Etc.	Etc.

With the lowest NOEC being in the toxicity band 0.001 mg/l – 0.01 mg/l the resulting classification under CLP is *Chronic category 1 with M-factor = 10, H410 (Warning. Very toxic to aquatic life with long lasting effects).* No classification for acute aquatic toxicity is applied.

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.

In depth analyses by the RAC (if needed - not included in the opinion).

Fish-Prolonged toxicity test (OECD TG 204).

Table A7.4.3.1-6: Mortality data

Day No.	Mortality (number of fish) / Mortality (percent)							
	Test substance: nominal concentration [µg/l]							
	0.158	0.500	1.58	5.00	15.8	50.0	158.0	500.0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
13	0	0	0	0	1 / 10	0	0	0
14	0	0	0	0	1 / 10	2 / 20	1 / 10	0
15	0	0	0	0	2 / 20	3 / 30	3 /30	0
16	0	0	0	0	2 / 20	3 / 30	3 / 30	0
17	0	0	0	0	2 / 20	3 / 30	5 / 50	0
20	0	0	0	0	6 / 60	5 / 50	7 / 70	3 / 30
21	0	0	0	0	7 / 70	6 / 60	7 / 70	4 / 40
Temperature [°C]	12 ± 1							
pH	6.9 – 8.1							
Oxygen [mg/L]	8.2 – 11.8							

In the controls as well as in the solvent controls neither symptoms nor mortalities occurred.

There was no clear dose - response relationship, which reduces the reliability of the study.

6 OTHER INFORMATION

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8 ANNEXES

Study summaries document IIIA from the final CA-report for coumatetralyl, for end-points relevant for this report, are attached to this report in IUCLID-database.

The Annexes include

Annex 1: Confidential information on identity of the substance,

Annex 2: Identity and Physico-Chemical properties of the substance.

Annex 3: Study summaries relted to Human Health effects of the substance.

Annex 4: Study summaries related to Environmental effects and fate of the substance

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