Committee for Risk Assessment

RAC

Annex 1

Background document

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

Difethialone(ISO); 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one;

EC number: -
CAS number: 104653-34-1

CLH-O-0000003391-80-03/F

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:

3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one;
Difethialone

EC Number: None assigned

CAS Number: 104653-34-1

Index Number: None assigned

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Version number: **2** **Date:** 28 September 2012

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1. Substance identity

Substance name:	3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one; Difethialone
EC number:	None assigned
CAS number:	104653-34-1
Annex VI Index number:	None assigned
Degree of purity:	Specification > 97.6% Specification of purity is based on the combined concentration of both diastereoisomers (cis and trans). *(The exact ratio can be found in a confidential annex in the IUCLID dossier).*
Impurities:	No significant impurities. *(Details of impurities are presented in a confidential annex in the IUCLID dossier).*

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	Difethialone is not included in Annex VI of the CLP Regulation	Difethialone is not included in Annex I of Council Directive 67/548/EEC
Current proposal for consideration by RAC	Acute Tox. 1; H300 Acute Tox. 1; H310 Acute Tox. 1; H330 STOT RE 1; H372 Repr. 1A; H360D EUH070 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 **SCLs for repeated toxicity**, and environment. **SCL for reprotoxicity to be considered** *(see below)*	T+; R26/27/28, T; R48/23/24/25, **Repr. Cat . 1; R61**, N; R50-53 **SCLs for acute toxicity, repeated toxicity** and environment**.** **SCL for reprotoxicity to be considered** *(see below)*
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	As above	As above

Specific concentration limits (SCL) according to Directive 67/548/EEC

C ≥ 0.5 %	T+, N; R61*- 26/27/28 - 48/23/24/25 - 50/53
0.25 ≤ C <0.5%	T+, N; R26/27/28 - 48/23/24/25 - 50/53
0.025% ≤ C< 0.25%	T, N; R23/24/25 - 48/23/24/25 - 51/53
0.0025% ≤ C <0.025%	Xn; R20/21/22 - 48/20/21/22 - 52/53

*0.5% is the general concentration limit (GCL) for reprotoxicity which has been included above for completeness. The possibility for setting SCL for reprotoxicity should be considered.

Specific concentration limits according to the CLP Regulation (EC 1272/2008)

Setting SCLs for acute toxicity is not relevant under the CLP regulation as the ATEmix formula takes into account both the toxicity and the concentration of an ingredient.

STOT RE 1; H372 above 0.02% and STOT RE 2; H373 between 0.002% and 0.02%.

The possible for setting SCL for reprotoxicity (Repr. 1A; H360D) should be considered.
M factor of 100 for Environmental effects.

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**		Not included in Annex VI	Conclusive but not sufficient for classification
2.2.	Flammable gases	**n.a.**		Not included in Annex VI	
2.3.	Flammable aerosols	**n.a.**		Not included in Annex VI	
2.4.	Oxidising gases	**n.a.**		Not included in Annex VI	
2.5.	Gases under pressure	**n.a.**		Not included in Annex VI	
2.6.	Flammable liquids	**n.a.**		Not included in Annex VI	
2.7.	Flammable solids	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	**n.a.**		Not included in Annex VI	
2.10.	Pyrophoric solids	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	**n.a.**		Not included in Annex VI	
2.14.	Oxidising solids	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
2.15.	Organic peroxides	**n.a.**		Not included in Annex VI	
2.16.	Substance and mixtures corrosive to metals	**None**		Not included in Annex VI	Conclusive but not sufficient for classification *(Difethialone has been stored in a range of*

					containers (such as plastic bags in metallic containers and plastic containers). No interaction between the active ingredient and the container materials has been observed in the past 20 years of production. Based on results in use and examination of the chemical structure, there are considered to be no problems with reactivity of the active substance towards the container material).
3.1.	Acute toxicity - oral	**Acute Tox. 1; H300**	Setting SCLs for acute toxicity is not relevant under the CLP regulation as the ATEmix formula takes into account both the toxicity and the concentration of an ingredient.	Not included in Annex VI	
	Acute toxicity - dermal	**Acute Tox. 1; H310**		Not included in Annex VI	
	Acute toxicity - inhalation	**Acute Tox. 1; H330**		Not included in Annex VI	
3.2.	Skin corrosion / irritation	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	**None**		Not included in Annex VI	
3.4.	Respiratory sensitisation	**None**		Not included in Annex VI	Data lacking
3.4.	Skin sensitisation	**None**		Not included in Annex VI	Conclusive but not sufficient for classification.
3.5.	Germ cell mutagenicity	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	**None**		Not included in Annex VI	Data lacking
3.7.	Reproductive toxicity	**Repr. 1A; H360D**	**SCL to be considered**	Not included in Annex VI	
3.8.	Specific target organ toxicity –single exposure	**None**		Not included in Annex VI	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	**STOT RE 1; H372**	**STOT RE1; H372 C ≥ 0.02%** **STOT RE2; H 373 0.002% ≤ C < 0.02%**	Not included in Annex VI	
3.10.	Aspiration hazard	**n.a.**		Not included in Annex VI	

4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400** **Aquatic Chronic 1; H410**	**M factor of 100**	Not included in Annex VI	
5.1.	Hazardous to the ozone layer	**n.a**		Not included in Annex VI	
Supplemental hazard information (Annex II, Part I)					
1.2.5	Toxic by eye contact	**EUH070**		Not included in Annex VI	

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

Labelling: Signal word:
Danger

Hazard statements:
H360D: May damage the unborn child
H300: Fatal if swallowed
H310: Fatal in contact with skin
H330: Fatal if inhaled
H372: Causes damage to organs through prolonged or repeated exposure
EUH070: Toxic by eye contact
H400: Very toxic to aquatic life
H410: Very toxic to aquatic life with long lasting effects

Precautionary statements:
As precautionary statements are not included in Annex VI of Regulation EC 1272/2008, no proposal is made.

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**	-	Not included in Annex I of DSD	Conclusive but not sufficient for classification
Oxidising properties	**None**	-	Not included in Annex I of DSD	Conclusive but not sufficient for classification
Flammability	**None**	-	Not included in Annex I of DSD	Conclusive but not sufficient for classification
Other physico-chemical properties	**None**	-	Not included in Annex I of DSD	Conclusive but not sufficient for classification
Thermal stability	**None**	-	Not included in Annex I of DSD	Conclusive but not sufficient for classification
Acute toxicity	**T+; R26/27/28**	**C ≥ 0.25; T+; R26/27/28** **0.025% ≤ C< 0.25% T; R23/24/25** **0.0025% ≤ C <0.025% Xn; R20/21/22**	Not included in Annex I of DSD	
Acute toxicity – irreversible damage after single exposure	**None**		Not included in Annex I of DSD	Conclusive but not sufficient for classification
Repeated dose toxicity	**T; R48/23/24/25**	**C ≥ 0.025%; T; R48/23/24/25** **0.0025% ≤ C <0.025% Xn; R48/20/21/22**	Not included in Annex I of DSD	
Irritation / Corrosion	**None**		Not included in Annex I of DSD	Conclusive but not sufficient for classification
Sensitisation	**None**		Not included in Annex I of DSD	Conclusive but not sufficient for classification
Carcinogenicity	**None**		Not included in Annex I of DSD	Data lacking
Mutagenicity – Genetic toxicity	**None**		Not included in Annex I of DSD	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility	**None**		Not included in Annex I of DSD	Data lacking
Toxicity to reproduction – development	**Repr. Cat 1; R 61**	**SCL to be considered**	Not included in Annex I of DSD	
Toxicity to reproduction – breastfed babies. Effects on or via	**None**		Not included in Annex I of DSD	Data lacking

lactation				
Environment[3]	**N, R50-53**	**C ≥ 0.25%: N, R50-53** **0.025% ≤ C < 0.25%: N, R51-53** **0.0025% ≤ C < 0.025%: R52-53**	Not included in Annex I of DSD	

[1] Including SCLs
[2] Data lacking, inconclusive, or conclusive but not sufficient for classification
[3] Decision on use of S-phrases for Environmental Effects taken by TC C&L (April 2006):
C ≥ 0.25%: N, R50-53 (S60-61),0.025% ≤ C < 0.25%: N, R51-53 (S61), 0.0025%≤ C < 0.025%: R52-53 (S61)

Labelling: Indication of danger:
Very toxic
Dangerous for the Environment

R-phrases:
R26/27/28: Very toxic by inhalation, in contact with skin and if swallowed.
R48/23/24/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R61: May cause harm to the unborn child
R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in aquatic environments.

S-phrases:
Chemicals classified with Repr. Cat. 1 are prohibited for general public use.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S53: Avoid exposure - obtain special instructions before use
S60: This material and its container must be disposed of as hazardous waste
S61: Avoid release to the environment. Refer to special instructions/Safety data sheets.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Difethialone is an existing biocidal active substance, a second-generation, single-dose anticoagulant rodenticide (product type 14), reviewed under Directive 98/8/EC with Norway as the Rapporteur.

Difethialone is not currently included in Annex VI of the CLP Regulation, nor has the substance been included in Annex I of Council Directive 67/548/EEC.

Classification proposals were submitted to the Technical Committee on Classification and Labelling of Dangerous Substances (TC C&L) in February 2006 and discussed in the Groups on Environmental and Human Health effects, respectively.

Because of a common mechanism of action, anticoagulant rodenticides were discussed as a group.

At a meeting in TC C&L in April 2006, the proposed environmental classification was agreed. Specific concentration limits were set as proposed (M factor of 100), and S60-61 was assigned:

C ≥ 0.25%: N, R50-53 (S60-61)
0.025% ≤ C < 0.25%: N, R51-53 (S61)
0.0025% ≤ C < 0.025%: R52-53 (S61)

As for classification for human health the substance was discussed by the Specialised Experts (SE) for Reproductive Toxicity (September 2006) as well as at two meetings in TC C&L (November 2006 and May 2007) and in written follow up periods to these meetings.

The Specialised Experts for Reproductive Toxicity unanimously recommended that the anticoagulant rodenticides should collectively be regarded as human **teratogens** and be classified as Repr. Cat. 1; R61 (read across from warfarin) (ECBI/121/06, ECBI/51/07).

At the TC C&L Meeting in November 2006, a provisional classification with R61 was agreed, but without a final decision on the category to be used (Repr. Cat 1 or Repr. Cat 2). Member States were invited to react in writing during the follow-up period with arguments for the relevance to read across from warfarin.

In May 2007 the provisional classification for reprotoxicity was not confirmed as the TC C&L decided to await results from further studies on anticoagulant rodenticides (i.e. new rat data on developmental toxicity of warfarin (OECD 414 study) and placental transfer of warfarin and flocoumafen) before finalising the discussion on reprotoxicity. The intention was to ask the Specialised Experts on their opinion on the new data, followed by a final written procedure in the TC C&L. However, the studies were not made available in the follow up period, and no final decision on classification for reprotoxicity could be made.

The proposed classification for difethialone for **acute and repeated dose toxicity** was agreed upon at the TC C&L Meeting in November 2006, and S45 -53 was assigned.

A classification in accordance with the draft CLP Regulation was agreed, including an additional hazard statement for **toxicity through contact with the eye**, EUH070.

Setting **specific concentration limits (SCL)** for acute and repeated dose toxicity (according to Directive 67/548/EEC) was supported based on the extreme low LD_{50} and LOAEL values and reported incidences of poisonings with anticoagulant rodenticides. A proposal for such SCLs was sent to TC C&L in December 2006 (ECBI/33/06 Add. 1).

Specific concentration limits for difethialone for acute and repeated dose toxicity were agreed upon as proposed at the TC C&L Meeting in May 2007.

0.25 ≤ C T+, N; R26/27/28 - 48/23/24/25 - 50/53
0.025% ≤ C< 0.25% T, N; R23/24/25 - 48/23/24/25 - 51/53
0.0025% ≤ C <0.025% Xn; R20/21/22 - 48/20/21/22 - 52/53

Although the SCLs for difethialone were agreed at the meeting, the general discussion on setting SCLs for anticoagulant rodenticides was not finalised. Further comments were received in the follow up period, but no common understanding was reached.

The final follow up sheet from the last of the TC C&L meetings (FU V) was distributed in June 2008 (document dated 29 May 2008). Non-finalised C&L discussions were handed over to the European Chemicals Agency (ECHA) in June 2008.

No REACH registration dossiers were available for difethialone on submission of the CLH Report (ECHA database last updated 19 July 2012).

Summary:

Agreement has been made in the TC C&L on all issues except reprotoxicity and the principle of setting of Specific Concentration Limits (SCL). As these are general issues for all anticoagulant rodenticides a common discussion should take place in the Risk Assessment Committee of the European Chemicals Agency for these substances.

2.2 Short summary of the scientific justification for the CLH proposal

The classification proposal is based on information provided in a Competent Authority Report which was made in September 2005 and revised in June 2007 by the Norwegian Competent Authority for the possible inclusion of the substance in Annex I to Council Directive 98/8/EC.

Table 5. Justification for the CLH proposal

Study information	Result	Classification
Physico-chemical properties	Difethialone is thermally stable at room temperature. It is not classified as highly flammable and does not undergo self ignition below its melting point. It is not explosive nor does it have oxidising properties. There is no record that it has reacted with any storage container during many years of industrial production. Therefore, there are no hazards associated with normal use of the active substance.	None
Acute Toxicity Oral LD_{50} rat Dermal LD_{50} rat Inhalation LC_{50} rat	 0.4 to 0.8 mg/kg bw 6.5 mg/kg bw ≥ 5.0 µg/l/4h but <19.3 µg/l/4h (nose only exposure, dust) ≤ 10.7 µg/l/4h (whole body exposure, dust)	**T+; R26/27/28**: Very toxic by inhalation, in contact with skin and if swallowed. **Acute Tox. 1;** **H300**: Fatal if swallowed **H310**: Fatal in contact with skin **H330**: Fatal if inhaled
Toxicity by eye contact	Delayed death of two rabbits in an eye irritation study (50 mg difethialone technical installed), degree of ocular irritation minimal.	**EUH070**: Toxic by eye contact
Irritancy/Corrosivity	Criteria for skin or eye irritancy not met	None
Sensitisation	No evidence of skin sensitisation potential in a guinea pig maximation test, GPMT (study of low reliability)	None
Repeat dose toxicity	90 day rat: LOAEL = 4 µg/kg bw/day based on haemorrhagic changes seen at necropsy The classification is based on the oral repeated dose toxicity data plus extrapolation from the acute data for the dermal and inhalation route of exposure.	**T; R48/23/24/25**: Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed **STOT RE 1; H372**: Causes damage to organs through prolonged or repeated exposure
Genotoxicity	Not mutagenic in four guideline studies.	None
Reproductive toxicity	Fertility: Two generation reproduction test not available. Long term use of the structurally similar active substance warfarin in humans has shown no adverse effects on human fertility. Developmental toxicity: Difethialone has not caused any observed teratogenic effects in conventional OECD Guideline 414. However, due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to difethialone, no clear conclusion can be drawn from these studies Based on read across from warfarin, it is proposed to classify difethialone as a developmental toxicant with **Repr. Cat 1; R61/ Repr. 1A; H360D**	**Repr.Cat1;R61**: May cause harm to the unborn child **Repr. 1A; H360D**: May damage the unborn child

Carcinogenicity	Study not available. Long term use of the structurally similar active substance warfarin in humans has shown no carcinogenic effects.	None
Environmental effects Biodegradation Toxicity data: Fish acute LC_{50} 96 h Daphnia EC_{50} 48 h Algae E_bC_{50} 72 h Algae ErC_{50} 72 h Bioconcentration: log Pow BCF (calculated based on log Pow)	 Not readily biodegradable 51 µg/l 4.4 µg/l 65 µg/l > 180 µg/l 6.29 >39000	**N; R50-53**. Very toxic to aquatic organisms, may cause long-term adverse effects in aquatic environments **Aquatic Acute 1; H400** Very toxic to aquatic life **Aquatic Chronic 1; H410**: Very toxic to aquatic life with long lasting effects

Because of the high toxicity of the substance, setting specific lower concentration limits for the substance is proposed for both environmental and human health effects.

2.3 Current harmonised classification and labelling

Difethialone is not currently included in Annex VI of the CLP Regulation, nor has the substance been included in Annex I of Council Directive 67/548/EEC.

2.4 Current self-classification and labelling

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Harmonised classification needed as difethialone is an existing biocidal active substance reviewed under Directive 98/8/EC (cf. article 36(2) in the CLP Regulation).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

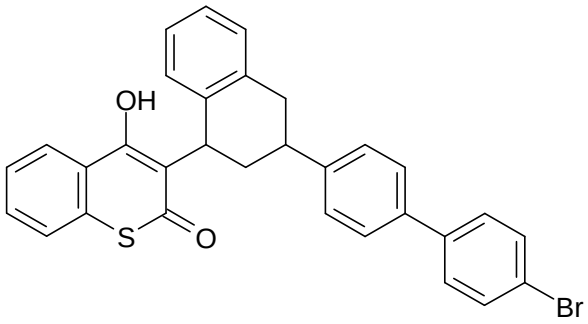
1.1 Name and other identifiers of the substance

Table 6. Substance identity

EC number:	None assigned
EC name:	None assigned
CAS number (EC inventory):	None assigned
CAS number:	104653-34-1
CAS name:	2H-1-Benzothiopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-
IUPAC name:	3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one *
Common name, synonyme	Difethialone
CLP Annex VI Index number:	None assigned
Molecular formula:	$C_{31}H_{23}BrO_2S$
Molecular weight range:	539.495 g/mol

** From the 1980s until 2007, an incorrect IUPAC name (3-((1RS,3RS;1RS,3SR)-3-(4'-bromobiphenyl-4-yl-1,2,3,4-tetrahydro-1-napthyl)-4-hydroxy-1-benzothin-2-one) was in use, but that henceforth the correct IUPAC name will be used.*

Structural formula:



1.2 Composition of the substance

Constituents (non-confidential information)

Table 7. Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
3-[3-(4'-bromo [1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxy-2H-1-benzothiopyran-2-one; Difethialone		Minimum purity 976g/kg	The purity of the active substance (> 97.6%) is the minimum degree of purity as specified by the producer for the substance production process. Specification of purity is based on the combined concentration of both diastereoisomers (cis and trans). Both diastereomers are considered as active substances (in the meaning of the Biocidal Products Directive 98/8/EC). The exact ratio is considered confidential and can be found in a confidential annex in the IUCLID dossier (as well as in the confidential Document V of the Competent Authority Report).

Impurities (non-confidential information)

No significant impurities.
Details of impurities are presented in a confidential annex in the IUCLID dossier on difethialone (as well as in the confidential Document V of the Competent Authority Report).

Additives (non-confidential information)

No additives contained in the substance

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 8. Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	yellow powder with no discernible odour	Loken, 1988	
Melting point	233 - 236 ºC (purity 99%)	Hoffman, 1988a	Block method/OECD 102
Boiling point	No boiling point has been determined	-	
Relative density	Density[1]: 1.36 g/ml at 25°C (purity 99%)	Hoffman, 1988b	Pycnometer method/ OECD 109/CIPAC MT 3
Vapour pressure	The vapour pressure could not be determined experimentally because the amount of difethialone recovered in the study was below the analytical detection limit of the method used. The vapour pressure was therefore set as twice as much as the detection limit, resulting in an estimated value of $< 1.33 \times 10^{-5}$ Pa. (22.6°C)	Hoffman, 1988c	Gas saturation method/ OECD 104
Surface tension	-	-	Testing not required because the water solubility of the active substance is below 1 mg/L.
Water solubility	0.39 mg/l at 25°C	Hoffman, 1988d	Column elution method/ OECD 105
Partition coefficient n-octanol/water	log Pow = 6.29 at pH 7.3 (ambient temperature) The test item is not considered ionisable. Investigation of the pH effect on the partition coefficient has of that reason not been done.	Ramsay, 2003b	HPLC method/OECD 117
Flash point	Not required for a solid substance.	-	
Flammability	Not highly flammable (according to EU	Jackson, 2002	EEC A10

	method A.10)		
Explosive properties	Not explosive. Tested for explosivity due to heat, mechanical shock and friction.	Jackson, 2002	EEC A14
Self-ignition temperature	Does not undergo self ignition below its melting point	Jackson, 2002	EEC A16 (auto-ignition)
Oxidising properties	Not oxidising	Jackson, 2002	EEC A17
Granulometry	-	-	
Stability in organic solvents and identity of relevant degradation products	-	-	Not applicable because the active substance as manufactured does not include an organic solvent and is not formulated in organic solution in the biocidal product.
Dissociation constant	-	-	Due to the low water solubility of difethialone, the test item is not considered ionisable and attempts to determine this are impractical.
Viscosity	-	-	Not applicable because the active substance is a solid.

[1] *The given value refers to the density and not to the relative density.*

For further details on physico-chemical properties (e.g. methods/guidelines) see the study summary included in the IUCLID dossier on difethialone (Document IIIA, Section 3).

2 MANUFACTURE AND USES

2.1 Manufacture

Information on the manufacturing process(es) is provided in a confidential annex in the IUCLID dossier on difethialone.

2.2 Identified uses

Difethialone is a second-generation single-dose anticoagulant rodenticide used for the urban and agricultural control of rodents indoors (i.e. in grain silos, warehouses), in and around farms buildings and in sewers.

The substance is used in a range of cereal-based baits (i.e. pellets, wax blocks and paste) at a concentration of 25 mg/kg (0.0025% w/w).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

For information on physico-chemical properties see Table 8. No classification is proposed for physico-chemical properties as criteria for classification for physico-chemical properties are not met.

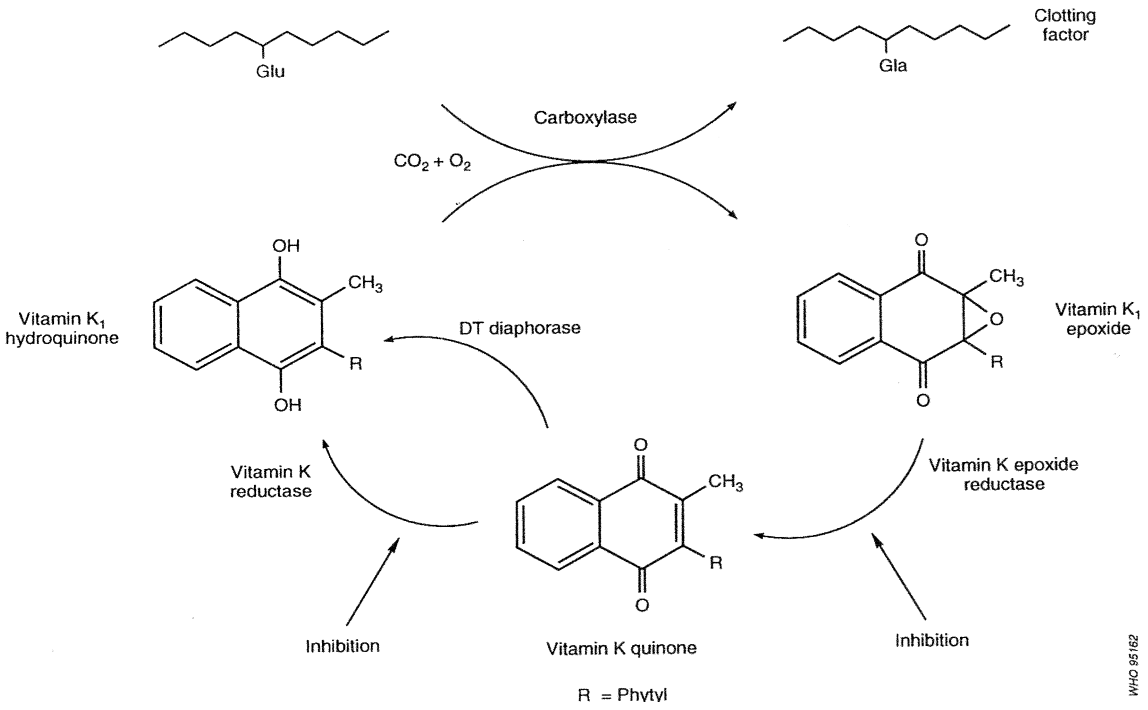
Difethialone is thermally stable at room temperature. It is not classified as highly flammable and does not undergo self ignition below its melting point. It is not explosive nor does it have oxidising properties. There is no record that it has reacted with any storage container during many years of industrial production. Therefore, there are no hazards associated with normal use of the active substance.

4 HUMAN HEALTH HAZARD ASSESSMENT

Anticoagulant rodenticides are vitamin K antagonists. They disrupt the normal blood clotting mechanisms resulting in increased bleeding tendency and, eventually, profuse haemorrhage and death.

Blood forms a clot at the site of injury by virtue of a complicated 'clotting cascade' involving numerous clotting factors. The clotting factors are made in the liver as inactive precursors, converted to active form and allowed to circulate in the bloodstream. Vitamin K is employed in the liver in the activation process, and is used in a continuous cyclic process involving several enzymes (see Figure 1). The anticoagulant rodenticides block these enzymes, preventing regeneration of the vitamin K and preventing activation of the clotting factors.

Figure 1 Epoxide cycle: The mechanism of clotting inhibition caused by hydroxycoumarin-related anticoagulants



Vitamin K hydroquinone is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide provides the energy required for the carboxylation reaction where glutamate (Glu) in the precursor is converted to γ-carboxyglutamate (Gla) to make the activated clotting factor (IPCS, 1995. Environmental Health Criteria 175).

The amount of vitamin K in the body is finite, and progressive blocking of the regeneration of vitamin K will lead to an increasing probability of a fatal haemorrhage. In general terms, progressive intake of anticoagulants results in death.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

A single oral dose of 0.5 mg ^{14}C-difethialone/kg bw resulted in no signs of toxicity among the 30 treated rats (Belleville, 1986). Radioactivity was traced in blood, tissues, organs and the residual carcass in samples from animals killed at interim terminations between one day and six months after dosing. Radioactivity was first detected in blood 30 minutes after dosing, reaching the maximum level, 0.09 µg eq. LM 2219/ml, approximately 24 hours after dosing and decreasing to undetectable levels within 14 days with a (plasma) half-life of 2.3 days. The AUC as a function of time, extrapolated to infinity, was 7.9 mg/l hr. Samples were collected for venous and arterial whole blood and plasma, but there were no notable differences in results from the different sample types. Radioactivity levels in the organs were always higher than in plasma samples, and the ratio of concentration in tissue to concentration in plasma increased over time indicating elimination from tissues was slower than from blood. The liver contained the highest levels of radioactivity at 24 hours after administration (22.8 to 42.5% of administered dose in males and females) and at the end of the six month observation period (10.5 and 9.16% in males and females respectively). The half-life in the liver was in the region of 18 weeks for both males and females.
Elimination was exclusively in the faeces, and this was reflected in the radioactivity levels found in the digestive tract after 24 hours (29.4 to 42.1%). After six months there were only small residual levels in the GI tract walls (1 to 2% of administered dose). Faecal excretion was substantial for the first three days after dosing, accounting for up to 37.07% of the administered dose (up to 56.6% was eliminated within 14 days). There was no radioactivity found in expired carbon dioxide and none in the urine samples from treated rats.

A clear estimate of the percentage of oral absorption cannot be given. Neither can such information be derived from the study referred below (Belleville and Picano, 1987). Data concerning blood levels and organ levels indicate an extensive absorption. However, it is uncertain how much of the substance found in the gastrointestinal tracts that represents the unabsorbed fraction. Absorption is difficult to quantify because of the strong binding in the liver: biliary excretion studies (normally used to assess absorption) are too short to give a meaningful result. A minimal quantification would be the 43% found in the liver at 24 hours. An oral absorption of approximately 80% is probably a more realistic estimate than the 100% absorption value that is used in the risk assessment.

Following exposure to a high dose of difethialone (5.0 mg ^{14}C difethialone/kg bw), all of the animals showed signs of haemorrhage, and these effects were often fatal (five of the ten rats allocated to the study died within five days of treatment). Decedent rats were replaced, but the observation period was reduced to six days rather than remaining at up to 14 days. Blood radioactivity was greater than 3 µg eq LM 2219/ml within 2 hours of dosing, reaching a maximum at 8 hours (3.27 µg eq. LM 2219/ml) and then decreasing to 1.21 µg eq.LM 2219/ml within six days. The half-life in blood was calculated to be approximately 2.8 days.The AUC as a function of time, extrapolated to infinity, was 127.3 mg/l hr, some 30 fold higher than at 0.5 mg/kg bw.

Following an exposure of 5 mg/kg bw, the levels of difethialone in lung and heart were similar to the plasma levels, whereas the levels in suprarenals, kidneys and liver was higher than in plasma. In general, the percentages of administered diefthialone found in the organs were lower following a dose of 5.0 mg/kg bw than a dose of 0.5 mg/kg bw, indicating a phenomenon of saturation of the sites of fixation. The concentration in the liver was again the highest at 25.7 to 29.4% of the administered dose at 24 hours and 3.6 to 6.8% at 6 days after dosing. The digestive tract contained high levels of radioactivity 24 hours after dosing (circa 17 to 22%) which fell to approximately 4% after six days, consistent with high levels of faecal elimination. Expired CO_2 contained no radioactivity, and the levels in urine samples were less than 0.1% over three days indicating these were not significant routes of elimination. Faecal elimination accounted for 83% of the administered dose over the initial four days.

Acetone extracts of radioactivity from liver and faeces at both dose levels followed by thin layer chromatography indicated that the dominant analyte was parent compound and did not reveal any significant metabolites.

A second study (Belleville and Picano, 1987) investigated liver levels after low (0.5 mg/kg) and high (1.0 mg/kg) oral doses to rats. After dosing at 0.5 mg/kg bw, the plasma concentrations were only measurable for approximately two days before falling below the limit of quantification. The concentrations varied between 50 and 90 ng/ml. Liver concentrations were approximately 2 µg/g for three days, but then fell to 1.2 µg/g by the week 8 sacrifice. One day after dosing the percentage of dose retained in the liver was 13.3%, falling to 10.8% by week 8.

The highest dose, 1 mg/kg, resulted in death by haemorrhage within 5 to 6 days. Plasma concentrations were markedly higher than at 0.5 mg/kg – 515 ng/ml after 4 hours and still at 156 ng/ml 96 hours after dosing.

Liver concentrations were maximal four hours after dosing at 5.97 µg/g (equivalent to 19.4% of administered dose present in the liver) and remaining at 3.97 µg/g (equivalent to 14% of administered dose) at the 96 hour sampling point. The 1 mg/kg dose of difethialone was a lethal dose with all rats dying within 6 days of administration.

Based on the molecule weight of 539.495 g/mol and the partition coefficient (log Pow) of 6.29 at pH 7.3, a default dermal absorption value of 10 % can be derived for difethialone (Guidance document on Dermal Absorption, Sanco/222/2000 rev. 7, European Commission, 2004).

Difethialone is acutely toxic by dermal route; the dermal LD_{50} in rats being 6.5 mg/kg bw; i.e. one order of magnitude higher than the oral LD_{50} in rats (0.55 mg/kg, male rats). However, an estimate of dermal absorption cannot be deduced from the results of acute toxicity studies.

No repeat dose dermal toxicity study is available for difethialone.

An *in vivo* human dermal absorption of 4% may be calculated by combining rat *in vivo* data (Roper and Gedik, 2003) and rat:human *in vitro* data (human and rat split thickness skin

samples, Roper, 2003). This value was derived from the dermal delivery (absorbed dose and amounts retained in the epidermis and dermis layers of the skin, but excluding amounts in stratum corneum) of difethialone in glycol solvent (25 g/l) 24h after application.

Difethialone is present as two diastereoisomers. Both diastereomers are active. A rat study (Cohet, 1995) has been performed to compare the hepatic kinetics of the diastereoisomers following a single oral dose of 0.5 mg/kg bw. The half life of difethialone in liver was 41 days for LM 2472 and 55 days for LM 2473. Residue levels in livers 42 days after dosing were 11% of the administered dose for LM 2472 and 14% for LM 2473. Residue levels for ^{14}C-labelled parent, LM 2219, measured in an earlier study were 17%. It was concluded that the half-life, elimination rate and residue levels for the two diastereoisomers were similar.
In an additional rat study (Belleville, 1991), the comparative hepatic kinetics of brodifacoum and difethialone were investigated. The rats were fed a dose of 0.06 mg/kg bw of test substance at day 0, 7, 14 and 21 and hepatic levels examined for up to 6 months. Hepatic levels were generally higher for brodifacoum, and liver half-life was significantly longer for brodifacoum. Residual liver levels after six months were also higher for brodifacoum than difethialone. The liver half life of difethialone was found to be approximately 80 days, and about 7% of the applied dose was present after 6 months.

4.1.2 Human information

No data.

4.1.3 Summary and discussion on toxicokinetics

In conclusion, the key metabolism study in rats showed that difethialone is rapidly and extensively absorbed with a short plasma half-life (2.3 days), but a longer liver half-life (up to 18 weeks). The liver was the main organ of accumulation, with 23 to 43% of administered dose present in the liver at 24 hours, and approximately 10% still present after 6 months. Elimination was exclusively in the faeces, with 37% excreted in the first 3 days, 57% within 14 days following a dose of 0.5 mg/kg bw. There was no excretion via expired air or urine. The higher dose levels of 1 and 5 mg/kg bw were fatal. Difethialone is present as two diastereoisomers, with similar kinetics.

Human *in vivo* dermal absorption was estimated to 4% based on data from an in vitro dermal absorption study (human and rat split thickness skin samples) and an *in vivo* rat study. The value was derived from the dermal delivery (absorbed dose and amounts retained in the epidermis and dermis layers of the skin, but excluding amounts in stratum corneum) of difethialone in glycol solvent (25 g/l) 24h after application.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 9. Summary table of relevant acute oral toxicity studies

Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}/LC_{50}	Remarks	Reference
EPA 81-1	Rat OFA IOPS Sprague-Dawley 10 male/group 10 female/group	Single dose at 0, 0.1, 0.2, 0.4, 0.8, 1.6 mg/kg bw. Post exposure period, 21 days	LD_{50} (male, female and combined sex) between 0.4 and 0.8 mg/kg bw		(Mally and Porret-Blanc, 1985a)
EPA 81-1	Rat OFA IOPS Sprague-Dawley 10 male/group 10 female/group	Single dose at 0, 0.4, 0.48, 0.58, 0.69, 0.83 mg/kg bw. Post exposure period 21 days	LD_{50}: Male: 0.55 mg/kg bw. Female: 0.58 mg/kg bw.	***Key Study*** *(basis for classi-fication)*	(Mally and Porret-Blanc, 1985b)
EPA 81-1	Mouse CD-1 5 male/group 5 female/group	Single dose at 0, 1.1, 1.3 and 1.6 mg/kg bw. Post exposure period 21 days	LD_{50} (male, female and combined sex): 1.29 ± 0.056 mg/kg bw		(Kynoch, 1986)
EPA 81-1	Cat European common breed domestic cat 3 male/group 3 female/group	Single dose at 2, 4, 8 or 16 mg/kg bw. Post exposure period 21 days	Minimum lethal dose in excess of 16 mg/kg bw	Single mortality associated with gut haemorrhage at site of tapeworm attachment Study of low reliability	(Lorgue, 1986c)
EPA 81-1	Dog Various breeds 4 dogs per group either or both sex	Single dose at 2, 4, 8 or 16 mg/kg bw. Post exposure period 23 days	Mortality observed at all doses	Inadequate study	(Lorgue, 1985b)
EPA 86-1	Pig Belguim Landrace 3 of either sex per group	Single doses of 0.3, 0.9, and 2.7 mg/kg bw in preliminary study and 1, 2, 3, 4.5, 6, and 24 mg/kg bw in final study. Post exposure period up to 16 days.	Estimated acute median lethal dose between 2 and 3 mg/kg bw		(Lorgue, 1984)
EPA 86-1	Dog Beagle	Single dose at 100, 20, 10 and 5 mg/kg	LD_{50} (combined sex) estimated to be	***Key study***	(Mally and Porret-

Method Guideline	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Value LD_{50}/LC_{50}**	**Remarks**	**Reference**
	10 males 9 females Phase I: 100 (1 M) and 20 mg/kg (3 M, 3F), postexposure periode 35 days Phase II: 20 (3M, 3F), 10 (2M, 1F) and 5 mg/kg (1M, 2F), postexposure period 21 days	bw. Post exposure period either 21 or 35 days	11.81 mg/kg bw (confidence limits 6.60 and 21.16 mg/kg) A 50% reduction in plasma prothrombin levels, only a minor increase in coagulation time detected in Quick time and no mortalities observed at 5 mg/kg.	$LOAEL_{acute}$ for clinical effects: 5 mg/kg bw	Blanc, 1985c)
Not stated, but based on EC method B.1	Chicken Star bro whites Phase I: 10 (each sex) in five groups Phase II: 5 (each sex) in three groups	Phase I: Single dose at 5, 10, 20, 40 and 80 mg/kg bw. Post exposure period 5 to 8 days. Phase II: Single dose at 0.63, 1.25 and 2.5 mg/kg bw. Post exposure period 39 days	Median lethal dose 0.87 mg/kg bw with fiducial limits of 0.48 to 1.26 mg/kg bw.		(Lorgue, G. 1986d)
No	Mice Swiss albino Five groups: 6 male/group 6 female/group	Single dose of 1.206 or 2.24 mg/kg bw of difethialone (predetermined LD_{50} and LD_{90} doses respectively) Post exposure period 1, 3 or 5 days	The predetermined LD_{50} and LD_{90} doses of difethialone induced severe hepatic damage	Study of hepato-toxicity following acute high dose exposures	Sahni and Saxena, 1998)

Difethialone was very toxic to rats and mice with LD_{50} (male, female and combined sex) between 0.4 and 0.8 mg/kg bw (lowest oral LD_{50} to the male rat of 0.55 mg/kg bw in the key study) and to the mouse 1.29 mg/kg bw. Difethialone is less toxic to dogs (estimated LD_{50} of approximately 12 mg/kg bw). An acute oral cat study of low quality showed no substance related mortalities at doses up to 16 mg/kg bw. Pigs showed a greater sensitivity (LD_{50} of 2.0 to 3.0 mg/kg bw). In chicken the LD_{50} was calculated to be 0.87 mg/kg bw. The dose-response curve seems to be very steep, at least in rodents. Most deaths appear between day 4 and 16 after dosing.

In the rat key study, which is the basis for the proposed classification, (Mally and Porret-Blanc, 1985b), there were no deaths observed after dosing at 0.4 mg/kg bw. Death occurred among animals dosed at 0.48 mg/kg bw or above. All rats died within 4 to 12 days of exposure. There were no significant differences in bodyweight gains for treated and control surviving rats. Clinical observations were limited to animals dosed at 0.48 mg/kg bw and above. The observed signs were consistent with the known mode of action i.e. induction of internal haemorrhage noted grossly as blood on nostrils, pale mucous membranes and difficulty breathing, together with general weakness and the presence of haematomas. Autopsy confirmed the presence of

haemorrhages especially in the thoracic, and to a lesser degree in cranial and abdominal cavities.

In the dog key study (Mally and Porret-Blanc, 1985c) death occurred among animals dosed at 10 mg/kg bw or above. At 5 mg/kg bw, the lowest dose used, the plasma prothrombin level was reduced by up to 50% indicating a certain effect of difethialone on vitamin K. Only a minor increase in coagulation time was observed. At higher doses a dose dependent increase in coagulation time was noted that seemed to reflect the severity of haemorrhage. Deaths generally occurred within 7 to 10 days of dosing. Clinical observations were typical of a haemorrhagic syndrome – slower haemostasis at puncture points, haematomas at various locations, pale mucous membranes, respiratory difficulties, melaena and generally poor condition. Coagulation times increased markedly from day 3 in animals dosed at 10 mg/kg or greater. Increased Quick time and reduced prothrombin levels (falling to less than 5% of baseline) were good indicators of imminent death. Necropsy revealed haemorrhages particularly in the thorax (haemothorax was considered the major cause of death). Microscopic pathology included extra medullary haematopoiesis in the liver and spleens of decedent dogs and hepatocellular and Kupffer cell pigmentation arising from haemoglobin catabolism. The microscopic changes were slight or non-existent in animals surviving treatment.

A non-guideline study in mice (Sahni and Saxena, 1998) has been performed to examine liver toxicity following single exposure to difethialone. 60 Swiss albino mice were exposed to 1.206 or 2.24 mg/kg bw of difethialone (predetermined LD_{50} and LD_{90} doses respectively). The post exposure period was 1, 3 or 5 days. The hepatic changes observed were more severe with increasing post exposure times and with increasing dose. Both doses produced severe and irreversible hepatic damage. The hepatic changes reported included necrosis of hepatocytes, dilation of sinusoids, fatty degeneration with vacuolisation, lymphocytic infiltration in and around portal and central veins and in sinusoids, as well as mild to moderate fibrosis. Similar severe hepatic toxicity has not been described in the other acute toxicity studies. The report did not comment on possible differences in hepatotoxicity between moribound animals and animals likely to survive the exposure to difethialone. Thus it is not possible to evaluate the hepatotoxicity induced by acute, sublethal doses of difethialone based on this study.

4.2.1.2 Acute toxicity: inhalation

Table 10. Summary table of relevant acute inhalational toxicity studies

Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD50/LC50	Remarks	Reference
EPA 81-3	Rat Wistar 5 male/group 5 female/group	Whole body: 0, 20.0, 44.7 and 10.7 mg/m³. Nose only: 0, 46.8, 52.5, 5.0 and 19.3 mg/m³. Exposure period 4 hours	Whole body: $LC_{50} \leq$ 10.7 µg/l Nose only: $LC_{50} \geq$ 5.0 µg/l but < 19.3 µg/l	***Key study***	(Hardy and Jackson, 1986)

4.2.1.3 Acute toxicity: dermal

Table 11. Summary table of relevant acute inhalational toxicity studies

Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD50/LC50	Remarks	Reference
EPA 81-2	Rat Sprague-Dawley CD 5 male/group 5 female/group	Single dose of 1% w/v test compound in PEG 300, applied to 10% body surface (at 0.40, 0.60, 0.90 or 1.35 ml/kg bw for 24 hours) to obtain exposures of 4.0, 6.0, 9.0 and 13.5 mg/kg bw.	LD_{50} (95% confidence limits) Combined: 6.5 (5.2 to 7.9) mg /kg bw Male: 7.9 (6.1 to 10.4) mg /kg bw Female: 5.3 (4.0 to 6.8) mg /kg bw	***Key study***	(Gardner, 1986)

4.2.1.4 Acute toxicity: other routes

No data

4.2.2 Human information

Many incidents of human poisoning, both accidental and intentional, of anticoagulant rodenticides have been reported in literature. Fatalities and severe clinical syndromes are in general due to the second generation anticoagulants. These substances have longer retention time in the body and consequently a more prolonged effect than warfarin (IPCS, 1995. Environmental Health Criteria 175).

Difethialone is manufactured in small quantities worldwide and only one published case report of difethialone intoxication has been found (see 4.7.1.5 for further information on the case report).

Symptoms of acute intoxication to anticoagulant rodenticides range from increased bleeding tendency in minor or moderate poisoning (haematoma, gum bleeding, haematuria, blood in faeces and excessive bleeding from minor cuts or abrasions) to massive haemorrhage in more severe cases. The onset of the signs of poisoning may not be evident until several days after ingestion.

The specific antidote is vitamin K_1(phytomenandione). Because of the long body retention of the second generation anticoagulants, antidotal treatment may be required for weeks or even months. Prothrombin time should be monitored for some time after cessation of the treatment to ensure there is no regression (IPCS, 1995. Environmental Health Criteria 175).

4.2.3 Summary and discussion of acute toxicity

Difethialone is acutely toxic by the oral, dermal and inhalation routes. Death was a result of internal haemorrhage, which is the mechanism of action of the active substance.

4.2.4 Comparison with criteria

Classification proposal according to the Dangerous Substance Directive (Directive 67/548/EEC):

Table 12: Summary table of results from acute toxicity studies relevant for classification

Oral LD_{50} rat	Dermal LD_{50} rat	Inhalation LC_{50} rat
0.4 to 0.8 mg/kg bw Key study: 0.55 mg/kg bw (male rat) 0.58 mg/kg bw (female rat) *(Mally and Porret-Blanc, 1985b)*	6.5 mg/kg bw *(Gardner, 1986)*	≥ 5.0 µg/L/4h but < 19.3 µg/L/4h (nose only exposure, dust). ≤ 10.7 µg/L/4h (whole body exposure, dust) *(Hardy and Jackson, 1986)*

Oral: Classification with **T+; R28**; 'Very toxic if swallowed' is warranted based on the acute oral toxicity study in rats. The oral LD_{50} is far below the cut off value for classification for acute toxicity ($LD_{50,}$ oral, rat ≤ 25 mg/kg bw).

Dermal: Classification with **T+; R27**; 'Very toxic in contact with skin' is warranted based on the dermal LD_{50} of 6.5 mg/kg bw for rats. Criterion for classification: $LD_{50,}$ dermal, rat or rabbit 50 ≤ mg/kg.

Inhalation: Classification with **T+; R26**; 'Very toxic by inhalation'is warranted based on the result of the acute inhalation study in rats. Criterion for classification: $LC_{50,}$ inhalation, rat for aerosols or particulates ≤ 0.25 mg/l/4h.

Specific lower concentration limits
Setting specific lower concentration limits for classification of preparations containing difethialone for acute toxicity is proposed based on the extreme low LD_{50}/LC_{50} values and reported incidences of poisonings with anticoagulant rodenticides.

An approach resembling (but not identical) to the method for setting SCLs for environmental effects was chosen. The basis for both approaches is a comparison of cut off values for classification and effect level with a resulting reduction of the general concentration limits (GCLs) defined in the Dangerous Preparation Directive (Directive 99/45/EC). The GCLs for a very toxic substance are 0.1% (Xn), 1% (T) and 7% (T+). To avoid too many and narrow SCLs the number of SCLs was reduced by clustering narrow SCLs (e.g. by using the existing SCLs for environmental effects also for health effects instead of introducing additional concentration limits of comparable size).

0.25 ≤ C < 0.5%	**T+; R26/27/28**
0.025% ≤ C < 0.25%	**T; R23/24/25**
0.0025% ≤ C <0.025%	**Xn; R20/21/22**

The actual values for the ratio (cut off value for classification/effect level) for difethialone are:

45 (oral exposure), 8 (dermal exposure), 13-50 (inhalation). In the proposal the GCLs have been reduced by 1/40 (T, Xn level) and 1/28 (T+ level).

Classification proposal according to Regulation EC 1272/2008

Oral: Based on the oral LD_{50} for rats (0.4 to 0.8 mg/kg bw, with 0.55 mg/kg bw for the male rat in the key study), it is proposed to classify difethialone with **Acute Tox. 1 H300** (classification criterion: LD_{50}, oral, rat ≤ 5 mg/kg).

Dermal: Based on the dermal LD_{50} for rats (6.5 mg/kg bw), it is proposed to classify difethialone with **Acute Tox. 1 H310** (classification criterion: LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg).

Inhalation: Based on the inhalatory LC_{50} value (LC_{50} between 5.0 µg/L and 19.3 µg/L/4h (nose only exposure to dust) and LC_{50} ≤ 10.7 µg/L/4h (whole body exposure to dust) for the rat, it is proposed to classify defethialone with **Acute Tox. 1 H330** (classification criterion: LD_{50}, inhalation, rat, for dusts and mists ≤ 0.05 mg/l/4h).

Specific lower concentration limits
Setting SCLs for acute toxicity is not relevant under the CLP regulation as the ATEmix formula takes into account both the toxicity and the concentration of an ingredient.

Only "relevant ingredients", substances with higher concentrations than 1%, should be included in the calculations using the ATEmix formula. However, this limit should not apply if "there is a reason to suspect that an ingredient present at a concentration of less than 1% is still relevant for classification the mixture for acute toxicity" (3.1.3.3 in Annex I of the CLP Regulation, cf also 3.1.3.4 Guidance on the Application of the CLP Regulation).

4.2.5 Conclusions on classification and labeling

Classification proposal according to the Dangerous Substance Directive (Directive 67/548/EEC):

Classification with **T+; R26/27/28**; 'Very toxic by inhalation, in contact with skin and if swallowed' is warranted based on the extreme low LD_{50}/LC_{50} values which is far below the cut off values for classification for acute toxicity.

Classification proposal according to Regulation EC 1272/2008

Classification with **Acute Tox 1 and H300**: Fatal if swallowed, **H310**: Fatal in contact with skin and **H330**: Fatal if inhaled is warranted based on the extreme low LD_{50}/LC_{50} values which is far below the cut off values for classification for acute toxicity.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal *Oral acute toxicity* Difethialone was very toxic to rats and mice with LD50 (male, females and both sexes combined) between 0.4 and 0.8 mg/kg bw in rats (the lowest oral LD50 in male rats was 0.55 mg/kg bw in the key study) and 1.29 mg/kg bw in mice. Difethialone is less toxic to dogs (estimated LD50 of approximately 12 mg/kg bw). An acute oral cat study of low quality showed no substance related mortalities at doses up to 16 mg/kg bw. Pigs

showed a greater sensitivity (LD50 values 2.0 to 3.0 mg/kg bw). In chicken, the LD50 was calculated to be 0.87 mg/kg bw. The dose-response curve appeared to be very steep in rodents. Most deaths occurred between days 4 and 16 after dosing.

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

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

- *Classification proposed by the dossier submitter*

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

TC-C&L conclusion on acute toxicity

T+; R26/27/28 was agreed by the TC C&L in November 2006 (ECBI/20/27, Rev. 1).

A classification in accordance with the draft CLP Regulation (Regulation EC 1272/2008) was agreed at the TC C&L Meeting in May 2007 (Acute Tox 1 and H300, H310 and H330)

Specific concentration limits (SCL) for acute toxicity for difethialone (according to the Dangerous Substance Directive (Directive 67/548/EEC)) were agreed upon as proposed at the TC C&L Meeting in May 2007. Although the SCLs for difethialone were agreed at the meeting, the general discussion on setting SCLs for anticoagulant rodenticides is not finalised. Further comments were received in the Follow up period and no common understanding was reached (cf. the final follow up sheet from the last of these meetings (FU V), distributed in June 2008 (dated 29 May 2008)).

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

See section 4.2. on Acute toxicity. No classification proposed.

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

Summary of the Dossier submitter's proposal

No classification was proposed for STOT-SE.

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

4.4 Irritation

4.4.1 Skin irritation

Table 13. Summary table of relevant skin irritation studies

Species	Method	Average score 24, 48, 72 h		Reversibility yes/no	Result	Reference
		Erythema	Oedema			
Rabbit	EPA 81-51 [2]	0.00	0.00	Not applicable	No irritation observed	(Gonnet and Guillot, 1985a)

[2] *comparable to EC Method B.4. Directive 92/69/EEC*

4.4.1.1 Non-human information

Transient very slight erythema was observed in two of six rabbits after a 4 hour dermal application of difethialone (Gonnet and Guillot, 1985a). The animals were assessed for dermal reactions 1, 24, 48 and 72 hours after dressing removal. The reaction was observed at the one hour assessment only (average score for six rabbits: 0.33), resolving within 24 hours. The material is considered to be non-irritating.

4.4.1.2 Human information

No data.

4.4.1.3 Summary and discussion of skin irritation

Transient erythema was observed in two animals at the one hour assessment only, resolving within 24 hours.

4.4.1.4 Comparison with criteria

Classification proposal according to the Dangerous Substance Directive (Directive 67/548/EEC)-R38 Irritating to skin
Classification proposal according to Regulation EC 1272/2008- Skin irritant category 2

Transient erythema was observed in two animals at the one hour assessment only, resolving within 24 hours. The mean values of the scores for both erythema and eschar formation and oedema formation calculated over all the animals tested (24, 48 and 72 hour) were 0.
The criteria for classification with R38; Irritating to skin and Skin irritant category 2 are not fulfilled (substances and preparations which cause significant inflammation of the skin which persists for at least 24 hours after an exposure period of up to four hours determined on the rabbit according to the cutaneous irritation test method cited in Annex V).

4.4.1.5 Conclusions on classification and labelling

Difethialone does not fulfil the EU criteria for classification as a skin irritant.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

4.4.2 Eye irritation

Table 14. Summary table of relevant eye irritation studies

Species	Method	Average Score 24, 48, 72 h				Reversibility yes/no	Result	Reference
		Cornea	Iris	Conjunctiva				
				Redness	Chemosis			
Rabbit	EPA 81-4	0.00	0.89	0.94	0.83	No. Observation period too short to assess full reversibility. Reactions persisted at termination on Day 3.	Slightly irritating. The study was terminated prior to determining reversibility of ocular changes	(Gonnet and Guillot, 1985b)
Rabbit	EPA 81-4	0.00	0.00	0.39	0.00	Yes, no signs of eye irritation from day 10.	Slightly irritant (transient iritis and slight conjunctivitis). Reduced dose used (50 mg) due to systemic toxicity and death following 100 mg. Toxicity consistent with the compound mode of action.	(Myers and Christopher, 1992)
Rabbit	EPA 81-4	0.00 (R) 0.00 (U)	0.44 (R) 0.22 (U)	0.22 (R) 0.33 (U)	0.00 (R) 0.22 (U)	Yes. Postexposure observation time 72h. No eye irritation reported at the 72h timepoint.	Only slightly irritant (transient iritis and slight conjunctivitis)	(Gonnet and Guillot, 1985c)

2 comparable to EC Method B.5. Directive 92/69/EEC

(R) = Rinsed. (U) = Unrinsed.

4.4.2.1 Non-human information

Difethialone was found to be a weak irritant in one study in rabbits (Gonnet and Guillot, 1985b). The study was terminated prior to determination of reversibility of ocular changes. Reversibility of ocular lesions (transient iritis and slight conjunctival redness) was demonstrated in two studies in rabbits (Myers and Christopher, 1992 and Gonnet and Guillot, 1985c).

In one of these studies (Myers and Christopher, 1992) delayed death (day 7 and 11) occurred for two rabbits after instillation of 50 mg of difethialone into the lower conjunctival sac of the right eye. From necropsy findings it was apparent that death occurred as a result of the known mode of action of this substance. The degree of ocular irritation was minimal.

4.4.2.2 Human information

No data.

4.4.2.3 Summary and discussion of eye irritation

The results of the eye irritation studies indicate that difethialone is a weak irritant, but that systemic toxicity and death can occur following instillation of a small quantity of material in close proximity to mucous membranes.

4.4.2.4 Comparison with criteria

Classification proposal according to the Dangerous Substance Directive (Directive 67/548/EEC)--R36 Irritating to eyes

Classification proposal according to Regulation EC 1272/200- Eye irritant category 2

Difethialone is considered not irritating to eyes according to the criteria in the Dangerous Substance Directive (Directive 67/548/EEC) and the Regulation EC 1272/2008

EUH070; Toxic by eye contact (CLP Regulation, Annex II, 1.2.5)

EUH070 should apply for substances where an eye irritation test has resulted in overt signs of toxicity or mortality among the animals tested, which is likely to be attributed to absorption of the substance through the mucous membranes of the eye. The statement shall be also be applied if there is evidence in humans for systemic toxicity after eye contact.

The criterium for classification with EUH070 is fulfilled based on the observations in one of the eye irritation studies (Myers and Christopher, 1992). While the degree of ocular irritation was minimal, the test substance caused the delayed death of two rabbits. Both animals showed signs of treatment-induced haemorrhage at necropsy.

4.4.2.5 Conclusions on classification and labelling

Difethialone does not fulfil the EU criteria for classification as an eye irritant.

Classification with **EUH070**; Toxic by eye contact is warranted

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No study available.

In the acute inhalation test, it was stated that there was no evidence of respiratory tract irritation following a 4 hour nose-only exposure.

4.4.3.2 Human information

No data

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

No classification proposed.

4.5 Corrosivity

See section 4.4. Irritation.

No classification for corrosivity warranted.

TC-C&L conclusion on corrosivity and irritation

TC C&L concluded in November 2006 (ECBI/20/07, Rev 1) that classification for corrosivity, skin irritation and serious eye damage/eye irritation was not required.

EUH070 agreed for difethialone at TC C&L in May 2007.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 15. Summary table of relevant skin sensitisation studies

Species	Method	Number of animals sensitized/total number of animals	Result	Reference
Guinea Pig	EPA 81-6	Controls: 10/sex Test group: 12/sex Positive controls: 5/sex	No indications of delayed contact hypersensitivity among guinea pigs subject to an induction and challenge regimen that included dose concentrations up to lethal levels.	(Parker, 1993)

			However the study was of low reliability.	

In a guinea pig maximation test (GPMT) of low reliability (Parker, 1993) there was no indication of delayed contact hypersensitivity among guinea pigs subject to an induction and challenge regimen that included dose concentrations up to lethal levels of difethialone.

4.6.1.1 Human information

No data

4.6.1.2 Summary and discussion of skin sensitisation

There was no indication of delayed contact hypersensitivity in a Guinea pig maximization test (study of low reliability).

4.6.1.3 Comparison with criteria

Difethialone does not fulfil the EU criteria for classification as a skin sensitiser.

4.6.1.4 Conclusions on classification and labelling

Classification for sensitisation is not warranted based on the available data.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal In a guinea pig maximisation test (GPMT) of low reliability (Parker, 1993) there was no indication of delayed contact hypersensitivity among guinea pigs subject to an induction and challenge regimen that involved exposure to Difethialone up to lethal levels According to the DS, classification for sensitisation is not warranted based on the available data. **Comments received during public consultation** No comments were received addressing this endpoint. **Assessment and comparison with the classification criteria** In the opinion of RAC the results of the guinea pig maximisation test (Parker, 1993) do not warrant classification of Difethialone for skin sensitisation, because the observed effects do not meet the CLP classification criteria.

4.6.2 Respiratory sensitisation

No data
No classification proposed.

TC-C&L conclusion on sensitisation

TC C&L concluded in November 2006 (ECBI/20/07, Rev 1) that classification for sensitisation was not required.

4.7 Repeated dose toxicity

Table 16. Summary table of relevant repeated dose toxicity studies

Route	Duration of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Oral	30 days A 14 day study was extended to 30 days. Subsequently, the control animals were exposed to Difethialone for 14 days.	Pig Belgium Landrace Male and female 3 animals/group (2 males and one female in the treated groups)	1 mg/pig daily for 30 days. The doses were administered as a premix in capsules. At the end of the study, the three control pigs (3 males) were given 2.5, 5 or 10 mg Difethialone/pig/day for up to 14 days	At 1 mg/pig/day difethialone there were no mortalities after 30 days administration. Clear hemorrhagic lesions observed at sacrifice in one animal. In the second phase, the highest exposed animal died at day 9 with clear hemorrhagic syndrome. In the middle dose animal clinical signs of stress were evident at day 13 and hemorrhagic lesions were present at sacrifice. Some sub-endocardiac haemorrhage was observed at sacrifice in the animal exposed to 2.5 mg/day.		Inade-quate study.	(Lorgue, 1985c)
Oral EPA 82-1 ***Key study*** *(basis for classification)*	90 days	Rat ICO OFA SD (IOPS CAW) Main study: 20 male/group 20 female/group Additional study: 10 male/group 10 female/group	Main study: 0, 2, 4 or 8 µg/kg bw per day Additional study: 0, 16, 32 and 0, 64 or 128 µg/kg bw per day	Difethialone was shown to have anticoagulant activity in the rat at doses of 4 µg/kg bw/day. No lethality was observed following doses of 2 and 4 µg/kg bw. All males fed 8µg/kg were moribund at week 13. Doses of 16 µg/kg and above resulted in the death of all animals,	4 µg/kg bw per day (based on haemorrhagic changes seen at necropsy)	2 µg/kg bw per day	(Mally, 1986)

Route	Duration of study	Species Strain Sex no/group	Dose levels frequency of application	Results	LOAEL	NOAEL	Reference
				between weeks 1 and 2 at 128 µg/kg, weeks 4 and 5 at 32 µg/kg and weeks 6 and 8 at 16 µg/kg.			
Oral EPA 82-1 ***Key study***	90 days	Dog Beagle 4 male/group 4 female/group	0, 5, 10 or 20 µg/kg bw per day	No toxicologically significant effects at dose levels of 5 or 10 µg/kg bw per day. High dose elicited some reactions after 13 weeks which were consistent with anticoagulant mode of action, with non-lethal haemorrhagic events (pale gums, reduced haemoglobin levels).	20 µg/kg bw per day	10 µg/kg bw per day	(Harling et al. 1986)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Repeat-dose oral studies show that even at doses as low as 4 µg/kg bw/day in the rat and 20 µg/kg bw/day in the dog, hemorrhagic effects begin to be seen after around 90 days administration. The clinical signs, haematological and post mortem data were consistent with the known pharmacological action of the active substance; impairment of the clotting cascade and increased prevalence of haemorrhage leading to death. There were no indications of other secondary toxicities: histopathology revealed no hypertrophy or hyperplasia of the target organ, the liver.

A study in rats (Markewicz, 1991a) where difethialone pellets (25 ppm end use product) were given to rats as sole food for 24, 48 or 72 hours has been performed. Half of the rats in each group were given antidotal vitamin K administration by subcutaneous injection followed by 13 days oral administration, whereas the other half of the rats were not given vitamin K. All of the rats that were not given vitamin K died. Observations prior to death and post mortem findings were consistent with death by haemorrhage. All of the rats treated with Vitamin K after 24 hours exposure to difethialone survived, but only one rat treated with Vitamin K after respectively 48 and 72 hours exposure survived.

4.7.1.2 Repeated dose toxicity: inhalation

A repeat dose inhalation study is not available. An acute inhalation study (Hardy. and Jackson, 1986) showed that difethialone is acutely toxic by inhalation. The LD_{50} for male and female

rats was between 5 and 20 µg/l. Appropriate protection measures must ensure no exposure to the (powdered) technical material or to the products during the production process. The active ingredient is not volatile, and the products are either wax blocks, wax based pellets or paste. The pellets have been shown to be dust free. As such, none of the end-products have the potential to generate a toxic inhalable atmosphere. Repeated exposure by inhalation will probably result in death by induction of a haemorrhagic syndrome (see studies on acute toxicity (section 4.2) for indications of haemorrhagic syndrome). The mechanism of clotting inhibition caused by hydroxy coumarin-type anticoagulant rodenticides is dependent on inhibition of vitamin K epoxide or vitamin K reductases and is unaffected by route of application. Therefore specific repeat dose inhalation studies would not provide any additional important information. Furthermore, performing additional repeated dose studies would contravene Directive 86/609/EC which militates against unnecessary testing using animals.

4.7.1.3 Repeated dose toxicity: dermal

A repeat dose dermal toxicity study is not available. The acute study (Gardner, 1986) showed high dermal toxicity with a LD_{50} value of 6.5 mg/kg bw for male and female rats. The highly toxic nature of the material is such that repeated dermal administration is likely to cause severe toxic effects at doses lower than those resulting in death following a single dose. The highly cumulative nature of the material means that lower doses, administered over several days, can also be predicted to cause death. In all cases death was caused by the specific pharmacological action of the molecule, inducing fatal haemorrhage. The mechanism of clotting inhibition caused by hydroxy coumarin-type anticoagulant rodenticides is dependent on inhibition of vitamin K epoxide or vitamin K reductases and is unaffected by route of application. Therefore specific repeat dose dermal studies would not provide any additional important information to that obtained in repeated dose studies by the oral route. Furthermore, performing additional repeated dose studies would contravene Directive 86/609/EC which militates against unnecessary testing using animals.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

Difethialone is manufactured in small quantities worldwide and only one published case report of difethialone intoxication has been found. The physicians Alejandro Vásquez G. and María de los Angeles Rodríguez S., reported an incident where a 22 year old man was admitted to a hospital in Chile due to poisoning with a biocide product named Rodilon (Vásquez and Rodríguez, 2000). The patient had on three occasions within a month spread Rodilon powder in an attic without use of protective equipment. After use he did not wash his hands. When arriving in the hospital, his condition was life threatening due to coagulation disorder. Treatment with fresh plasma (until antidote was available) and the antidote Vitamin K was successful. The concentration of difethialone in the product was not reported. However, according to Liphatech, the company applying for authorisation of the compound/ manufacturing difethialone products (personal communication, 10 August 2004), the formulation in the incident was a tracking powder containing 2g/kg difethialone (or 0.2% w/w).The concentration of active substance in this product is much higher than the concentration of active substance in products available in the EU (25 ppm difethialone or 0.0025% w/w).

A few cases of intoxications from occupational exposure to anticoagulants have been reported.

The working physicians responsible for the personnel at the manufacturing plant (producing premix and end use products of difethialone) since 1987 did not encounter any signs of toxicity in routine medical monitoring of staff (Anon, 2005). However, a previous practitioner has reported one case of intoxication due to nail biting. No further information is available about this case.

The closely related active substance warfarin has been in use for many years as a rodenticide and for treatment of thromboembolic diseases in humans. It is used in stroke prevention, in treatment of vascular heart disease, atrial valve replacement and deep vein thrombosis. For stroke, valve replacement and heart disease, duration is 'lifelong' i.e. the patient takes the drug for the rest of their life. This may be several decades (Horton and Bushwick, 1999).

Dosing is individual with large interindividual variations. Adequate monitoring of the level of anticoagulation is essential during the course of anticoagulant therapy because the anticoagulant response to fixed dosages varies among individuals, and the safety and efficacy of the drugs are dependent on maintaining the anticoagulant effect within a defined therapeutic range.

Treatment is associated with increased risk of bleeding episodes as the most common reported side effect as well as e.g. skin necrosis and haematomas in various organ . Bone protein depletion is observed in female humans after long-term anticoagulant treatment. Warfarin is associated with the induction of developmental malformations when taken as a therapeutic agent during pregnancy (IPCS, 1995. Environmental Health Criteria No. 175). Use during pregnancy is consequently contraindicated.

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

The oral LOAELs established in 90 day repeat dose studies were 4 µg/kg bw/day in rats and 20 µg/kg bw/day in dogs. The mechanism of clotting inhibition caused by hydroxy coumarin-type anticoagulant rodenticides is dependent on inhibition of vitamin K epoxide or vitamin K reductases and is unaffected by route of application. Classification with T; R48/23/24/25 is warranted based on the 90 day oral repeat dose toxicity data plus extrapolation from the acute data for the dermal and inhalation route of exposure.

Setting specific lower concentration limits for classification of preparations containing difethialone for repeated dose toxicity is proposed based on the accumulative nature (long half life in the liver) of the substance and the low LOAEL value.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Difethialone accumulates in the body and has a long half-life in the liver (approximately 18 weeks). The oral LOAEL established in a 90 day repeated dose study is as low as 4 µg/kg bw/day in rats (based on haemorrhagic changes seen at necropsy) with a steep dose response curve. All males fed 8 µg/kg bw/day were moribund at week 13, and doses of 16 µg/kg bw/day and above resulted in the death of all animals.

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

The oral LOAELs established in the 90 day repeat dose studies in rats (4 µg/kg bw/day) is approximately thousand times lower than the dose limit for classification with T; R48/25, 5 mg/kg bw/day (Annex VI of the Dangerous Substance Directive).

The mechanism of toxicity of the anticoagulant rodenticides (blocking the regeneration of vitamin K) is unaffected by route of application. Hence, classification for the dermal and inhalation route of exposure was based on the 90 day oral repeat dose toxicity data plus extrapolation from the acute dermal and inhalational studies.

Specific concentration limits:

Setting specific lower concentration limits for classification of preparations containing difethialone for repeated dose toxicity is proposed based on the accumulative nature of the substance (long half life in the liver) and the low LOAEL value and steep dose response curve.

An approach resembling (but not identical) to the method for setting SCLs for environmental effects was chosen. The basis for both approaches is a comparison of the cut off values for classification with the effect levels with a resulting reduction of the general concentration limits (GCL) defined in the Dangerous Preparation Directive (Directive 99/45/EC). The general concentration limit is 10% for T, R48 and 1% for Xn R48/.

To avoid too many and narrow SCLs the number of SCLs was reduced by clustering narrow SCLs (using a "preferred value approach", the SCL set for Environmental effects). Hence, the proposed specific concentration limits were:

$C \geq 0.025\%$;	T; R48/23/24/25
$0.0025\% \leq C < 0.025\%$	Xn; R48/20/21/22

The actual value for the ratio (cut off value for classification/effect level) is 1250.
In the proposal the GCLs have been reduced by 1/400.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Classification with T; R48/23/24/25 is warranted based on the 90 day oral repeat dose toxicity data plus extrapolation from the acute data for the dermal and inhalation route of exposure.

Setting specific lower concentration limits for classification of preparations containing difethialone for repeated dose toxicity is proposed based on the accumulative nature (long half life in the liver) of the substance and the low LOAEL value.

$C \geq 0.025\%$;	T; R48/23/24/25
$0.0025\% \leq C < 0.025\%$	Xn; R48/20/21/22

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

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

See discussion in section 4.7

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

Classification with STOT RE 1; H372 warranted based on the findings in the 90 day oral repeated dose study in rats.

The oral LOAELs established in the 90 day repeat dose studies in rats (4 µg/kg bw/day) is approximately thousand times lower than the dose limit for classification with STOT RE 1; H372, i.e. 10 mg/kg bw/day.

The mechanism of toxicity of the anticoagulant rodenticides (blocking the regeneration of vitamin K) is unaffected by route of application. Hence, classification for the dermal and inhalation route of exposure was based on the 90 day oral repeat dose toxicity data plus extrapolation from the acute dermal and inhalational studies.

Specific concentration limits:
The oral $LOAEL_{RDT}$ established in a 90 day repeated dose study is as low as 4 µg/kg bw/day in rats (based on haemorrhagic changes seen at necropsy) with a steep dose response curve. All males fed 8 µg/kg bw/day were moribund at week 13, and doses of 16 µg/kg bw/day and above resulted in the death of all animals.

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

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

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

According to the Guidance on the Application of the CLP Criteria the resulting SCL should be rounded down to the nearest preferred value (1, 2 or 5), results in a SCLcat1 of 0.02% and SCLcat2 of 0.002% (ECHA, 2009. Guidance on the Application of the CLP Criteria, section 3.9.2.6.)

STOT RE 1 H372 above 0.02% and STOT RE 2 H373 between 0.002% and 0.02%.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification with STOT RE 1; H372 is warranted based on the findings in the 90 day oral repeated dose study in rats and extrapolation from the acute dermal and inhalational studies.

Specific concentration limits is proposed:

STOT RE 1 H372 above 0.02% and STOT RE 2 H373 between 0.002% and 0.02%.

TC-C&L conclusion on repeated dose toxicity

T; R48/23/24/25 was agreed by the TC C&L in November 2006 (ECBI/20/27, Rev. 1).

A classification in accordance with the draft CLP Regulation (Regulation EC 1272/2008) was agreed at the TC C&L Meeting in May 2007 (STOT RE 1; H372)

Specific concentration limits (SCL) for repeated dose toxicity for difethialone (according to the Dangerous Substance Directive (Directive 67/548/EEC)) were agreed upon as proposed at the TC C&L Meeting in May 2007. Although the SCLs for difethialone were agreed at the meeting, the general discussion on setting SCLs for anticoagulant rodenticides is not finalised. Further comments were received in the Follow up period and no common understanding was reached (cf. the final follow up sheet from the last of these meetings (FU V), distributed in June 2008 (dated 29 May 2008)).

SCLs according to the CLP regulation Regulation EC 1272/2008 were not proposed.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 17. *In vitro* genotoxicity

Test system Method Guideline	Organism/ strain(s)	Concentrations tested	Result		Remark	Reference
			+ S9	- S9		
Bacterial reverse mutation test EPA 84-2	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100, TA 1538	0.01, 0.05, 0.1, 0.5, 1 and 5 mg per plate	Negative	Negative	No evidence of a significant increase in the number of spontaneous mutations, either with or without metabolic activation.	(Weill, 1988a)
Mammalian chromosome aberration test EPA 84-2a	Whole blood human lymphocytes	20 hr without activation: 0.5; 1.25; 2.5; 3.75 and 5 µg/ml 30 hr without activation: 5.05; 7.58 and 10.1 µg/ml 20 hr with activation: 45; 60; 150; 299; 449 and 598 µg/ml 30 hr with activation: 302; 453 and 604 µg/ml	Negative	Negative	No induced chromosomal aberrations under conditions of metabolic activation or non-activation. Difethialone considered negative for mutagenicity. Colony death evident at doses in excess of 299 µg/ml in the presence of S9 (20 hr assay). No results obtained in the 30 hr assay due to cell death.	(Murli, 1992a)
Mammalian cell gene mutation test EPA 84-4	Chinese hamster ovary (CHO)	Prelim study: 0, 0.5, 1.0, 5.0, 10, 50, 100, 500 and 1000 µg/ml 1st main study: 0, 5, 10, 50, 100 and 200 µg/ml 2nd main study: 0, 5, 10, 50, 100, 150 and 200 µg/ml	Negative	Negative	Difethialone did not induce mutagenic effects in CHO cells at the HGPRT locus either in the presence or absence of metabolic activation. High concentrations (≥ 50 µg/ml) were found to be cytotoxic.	(Weill, 1988b)

4.9.1.2 In vivo data

Table 18. *In vivo* genotoxicity

Type of test Method/ Guideline	Species Strain Sex no/group	Frequency of application	Sampling times	Dose levels	Results	Remarks	Reference
Bone marrow mutagenicity EPA 84-2b	Mouse ICR 15 male 15 female in the dose group. 5 male 5 female for micronucleus bioassay	3 days consecutively	24 hours after the last dose	20 mg/kg bw	No induced increase in micronucleated polychromatic erythrocytes in comparison with the vehicle control. Positive control significantly increased the number of micronucleated cells in both sexes confirming method sensitivity.	No induced significant increase in micronuclei in bone marrow polychromatic erythrocytes. Negative mouse micronucleus test.	(Murli, 1992b and 1992c)

Results for *in vitro* bacterial gene mutation, *in vitro* cytogenicity in mammalian cells and *in vitro* mammalian cell gene mutation tests (Weill, 1988a, Murli, 1992a, Weill 1988b) were negative. The mouse micronucleus test (Murli 1992b and 1992c) was also negative.

In toxicokinetic studies faecal elimination of unchanged Difethialone accounted for approximately 90% of administered dose. No identifiable metabolites of concern were found in the remaining fraction (see 4.1. Toxicokinetics).

4.9.2 Human information

No data

4.9.3 Other relevant information

No data

4.9.4 Summary and discussion of mutagenicity

Results for *in vitro* bacterial gene mutation, *in vitro* cytogenicity in mammalian cells and *in vitro* mammalian cell gene mutation tests as well as *in vivo* mouse micronucleus test were all negative.

4.9.5 Comparison with criteria

Results for three *in vitro* and one *in vivo* genotoxicity studies were all negative. Hence, criteria for classification is not met.

4.9.6 Conclusions on classification and labelling

Difethialone does not fulfil the EU criteria for classification as a mutagenic substance.

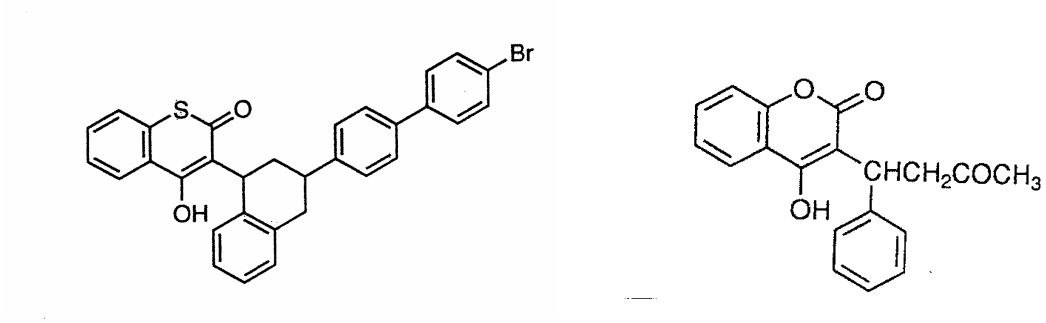
TC-C&L conclusion on mutagenicity

TC C&L concluded in November 2006 (ECBI/20/07, Rev 1) that classification for mutagenicity was not required.

4.10 Carcinogenicity

Carcinogenicity and long-term toxicity studies are not available. Difethialone is structurally and functionally almost similar to the molecule warfarin at the functional coumarin (left-hand) end of the molecule.

Figure 2. Comparison between the structure of difethialone and warfarin



difethialone warfarin

The molecules both have significant structural similarity to the forms of vitamin K shown in Figure 1. It can be seen that this structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K. The major differences in the active substances lie in the 'tail', which has varying degrees of lipophilicity. There is long term experience in humans with warfarin, widely used in anti-clotting therapy in humans.Its therapeutic use is described in more detail in 4.7. Repeated dose toxicity. There is no indication of any higher incidence of cancer in humans following long term warfarin therapy (IPCS, 1995 Environmental Health Criteria 175).

Evidence is presented to show that it would not be possible to perform a meaningful long-term study in any species because of the accumulative nature and high toxicity of the active substance. Furthermore, so-called antidotal treatment would not actually expose the target organ (the liver) to the active substance, studies in marginally less-susceptible non-rodent species would mean that the study would have to be impossibly large to have a statistical chance of detecting any increase in tumours, the 90 day study in rats showed no indications of either hyperplasia or hypertrophy (which could lead to non-genotoxic carcinogenesis) at near-

term lethal levels of administration and the mutagenicity studies were negative (Competent Authority Report on difethialone, Document III-A, Section 6.5-01/ 6.7-01).

4.10.1 Non-human information

Carcinogenicity and long-term toxicity studies are not available.

4.10.2 Human information

See introduction

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

4.10.5 Comparison with criteria

Based on the available data, no classification for carcinogenicity seems to be warranted for difethialone.

4.10.6 Conclusions on classification and labelling

Based on the available data, no classification for carcinogenicity for difethialone seems to be warranted.

TC-C&L conclusion on carcinogenicity

TC C&L concluded in November 2006 (ECBI/20/07, Rev 1) that classification for carcinogenicity was not required.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

Difethialone was shown to have anticoagulant effects in the rat at doses of 4 µg/kg bw/day in a 90-day oral repeated dose toxicity study (Mally, 1986). No lethality was observed following doses of 2 and 4 µg/kg bw/day. All males fed 8 µg/kg bw/day were moribund at week 13. Doses of 16 µg/kg bw/day and above resulted in the death of all animals. The deaths occurred between weeks 1 and 2 at 128 µg/kg bw/day, weeks 4 and 5 at 32 µg/kg bw/day and weeks 6 and 8 at 16 µg/kg bw/day.
In the oral repeated toxicity study on beagle dogs (Harling *et al*. 1986), no toxicologically significant effects were observed at dose levels of 5 or 10 µg/kg bw/day. The high dose of 20 µg/kg bw/day elicited some reactions after 13 weeks which were consistent with the anticoagulant mode of action, with non-lethal haemorrhagic events (pale gums, reduced haemoglobin levels).
No repeated dose inhalation or dermal toxicity studies were available.
Classification as STOT RE 1; H372 is warranted based on the 90-day oral repeat dose toxicity data and on an extrapolation from the acute toxicity data for the dermal and

inhalation routes of exposure.

Specific concentration limits

The oral LOAEL established in a 90 day repeated dose study is as low as 4 µg/kg bw/day in rats (based on haemorrhagic changes seen at necropsy) with a steep dose response curve. All males fed 8 µg/kg bw/day were moribund at week 13, and doses of 16 µg/kg bw/day and above resulted in the death of all animals. Using this information, the SCL was calculated as follows (ECHA, 2009: Guidance on the Application of the CLP Criteria, section 3.9.2.6.):

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

SCL for STOT RE

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

No comments were received addressing this endpoint.

Assessment and comparison with the classification criteria

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

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

One or two generation studies are not available for difethialone. Testing anticoagulant substances in multi-generation studies is associated with practical difficulties related to the fact that several events in the reproductive cycle are associated with incidental or inevitable haemorrhage.

The short-term studies (up to 90-days duration) in rats and dogs have shown no adverse effects on the reproductive organs (macroscopic condition, organ weight analysis and histology).

However, doses were low and the function of the reproductive organs was not examined. Thus, based on short term studies, it cannot be concluded that there are no effects on fertilty.

Warfarin, another well known coumarin derivate with the same chemically active group as difethialone is not classified as toxic to fertility.

4.11.1.2 Human information

Difethialone is structurally and functionally almost similar to the molecule warfarin at the functional coumarin (left-hand) end of the molecule. Warfarin is not classified as toxic to fertility. While the traditional use of Warfarin has been associated with heart and blood disorders in the elderly, there is a significant cohort of patients, both male and female, of reproductive age with conditions such as mitral valve replacement or deep vein thrombosis (DVT). There have been no indications of any adverse effects on human fertility (i.e. mating performance) of either sex undergoing treatment with anticoagulants (IPCS, 1995. Environmental Health Criteria, No. 175).

However difethialone may be more potent *(*second-generation anticoagulants are stronger vitamine-K antagonists than warfarin; the dissociation of enzyme /inhibitor complexes is expected to be extremely low (IPCS, 1995. Environmethal Health Criteria, No. 175) and have effects on fertility that are not foreseen by cross reading warfarin data.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 19. Summary table of relevant developmental toxicity studies

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Dose	Critical effects dams fetuses	NOAEL maternal toxicity	NOAEL teratogenicity embryo toxicity	Reference

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Dose**	**Critical effects dams fetuses**	**NOAEL maternal toxicity**	**NOAEL teratogenicity embryo toxicity**	**Reference**
Oral	Preliminary EPA 83-3	Rat ICO OFA Sprague-Dawley Female 5 per group	12 doses - Day 6 to 17	0, 10, 20, 30, 50 or 70 µg/ kg bw per day	Death occurred in the high dose group and overt clinical signs of toxicity evident at 50 µg/kg bw per day among dams. No signs of embryo toxicity or macroscopic evidence of teratogenicity.	30 µg/kg bw per day	>70 µg/kg bw per day(	(Briffaux, 1985a)
Oral	EPA 83-3	Rat ICO OFA Sprague-Dawley Female 25 per group	12 doses - Day 6 to 17	0, 12.5, 25.0 or 50.0 µg /kg bw per day	No mortalities or clinical signs of reaction following dosing with control or three doses at 12.5, 25 or 50 µg/kg bw per day among dams. No adverse effect on foetus. Number of live foetuses, average litter weights and sex ratio showed no treatment–related changes. Examination of entire foetus and of organs and skeleton revealed no treatment–related changes in the incidence of teratogenic indicators.	≥50 µg/kg bw per day	≥50 µg/kg bw per day	(Briffaux, 1986a)
Oral	Preliminary EPA 83-3	Rabbit Hybrid Albino 5 pregnant animals per group	12 doses - Day 6 to 17	0, 0.5, 2.0, 4.0, 6.0 or 8.0 µg/ kg bw per day	Deaths occurred in the two highest dose groups among the dams and abortions were also noted in these two groups but there were no signs of maternal toxicity at doses of 4 µg/kg bw/day or lower. No signs of embryo toxicity or macroscopic evidence of teratology were seen at any dose level.	4 µg/kg bw per day	>8 µg/kg bw per day	(Briffaux, 1985b)
Oral	EPA 83-3	Rabbit Hybrid Albino	14 doses - Day 6 to	0, 1.25, 2.50 or 5.00 µg	No maternal or foetal toxicity and no teratogenic effects on	≥5 µg/kg bw per	≥5 µg/kg bw per	(Briffaux, 1986b)

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Dose	Critical effects dams fetuses	NOAEL maternal toxicity	NOAEL teratogenicity embryo toxicity	Reference
		Female 18 per group	19	/kg bw per day	the foetus were observed.	day	day	
Oral	EPA 83-3	Rabbit New Zealand White Female 16 per group	13 doses - Day 7 to 19	0, 2.5, 5.0, 10.0 or 20.0 µg /kg bw per day	Maternal toxicity (fatal haemorrhage) was evident at 10 µg/kg bw/day and above. No embryofoetal toxicity and no developmental toxicity indicative of teratogenicity at any dose level.	5 µg/kg bw per day	>10 µg/kg bw per day	(Tyl et al. 1994)

Difethialone and other coumarin derivates including warfarin have the same chemically active group and the same mode of action, leading to vitamin K (vitamin K hydroquinone) deficiency (see figure 1). It is clear from several studies that vitamin K is essential for clotting proteins in the liver, however, extrahepatic tissues/organs i.e. cartilage, bone and the nervous system also contain vitamin K dependent proteins. One would anticipate that similar health effects occur following exposure to various coumarin derivates. However, the potency of the different substances may differ.

Warfarin is a well documented human teratogen. Therapeutic use of warfarin in the first trimester is associated with a characteristic embryopathy called the "fetal warfarin syndrome". The available data indicate a risk for classical warfarin embryopathy due to warfarin treatment in the sensitive period (first trimester) of 4% relative to the number of pregnancies. The syndrome is characterised by skeleton anomalies. The most common consistent feature is a hypoplastic nose with a depressed or narrowed nasal bridge causing respiratory distress. Other common bony abnormalities are observed in the axial and appendicular skeleton where the most prominent being radiological stippling, particularly of the vertebral column and most dramatically in the lumbosacral area. Kryphoscoliosis, abnormal skull development, and brachydactyly (unusually short fingers and toes) have been irregularly observed. Other, nonskeletal abnormalities reported in association with the syndrome include opthalmological malformations, developmental delay, low birth weight (premature birth), mental retardation, nail hypoplasia, hypotonia, ear anomalies, hypertelorism, excessive haemorrhage and death. Similar effects have also been observed following treatment with other coumarins; acenocoumarol, phenindione and phenprocoumon (Macina and Schardein, 2007, Ville et al., 1993).

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 a Specialist Expert

group 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."

Based on the opinion of the Specialised Experts, the Technical Committee for Classification and Labelling (TC C&L) agreed with R61: however, Repr. Cat. 1 or Cat. 2 was still under discussion (for difethialone no human data exist and animal data were negative).

As industry did not agree with a simple read-across from the classification of warfarin, further rat studies on developmental toxicity (OECD 414 guideline study) of warfarin and on placental transfer of warfarin and flocoumafen have been conducted by the industry as a follow-up to the TC C&L discussions.

The study on placental transfer of warfarin and flocoumafen was evaluated by the Netherlands and included in the CLH report on flocoumafen. The study demonstrates that flocoumafen (which has a structure and molecular weight similar to difethialone), like warfarin, is able to pass the placenta (for further information on the study: See CLH report on flocoumafen, ref. Johnson, 2009). It is not possible however to quantitatively extrapolate data on foetal exposure between the AVK rodenticides.

Results from the OECD 414 guideline study with warfarin is summarised below.

Table 20. Summary of the OECD 414 guideline study with warfarin sodium:

Reference/ notifier	:	Kubaszky (2009)	Exposure	:	day 6-15 (TP 1) day 6-19 (TP 2)
Type of study	:	Teratogenicity	Doses	:	0, 0.125, 0.150, 0.200, 0.250 mg/kg bw per day
Year of execution	:	2007	Vehicle	:	aqueous CMC
Test substance	:	Warfarin sodium	GLP statement	:	Yes
Route	:	Oral by gavage	Guideline	:	OECD 414
Species	:	Rat Crl:(Wi) BR-Wistar	Acceptability	:	Yes
Group size	:	25 dams per group, except	$NOAEL_{mat}$	:	0.125 mg/kg bw per day

	high dose group 12 dams/group	$NOAEL_{dev}$	: < 0.125 mg/kg bw per day

The study was performed according to OECD 414 guideline. There were two treatment regimens. In the first group (TP1) the sperm positive females were exposed to warfarin from day 6-15 post coitum (i.e. during the period of organogenese according to the old guideline) and the second group from day 6-19 post coitum (treatment according to the new guideline (2001)). One deviation from the guideline was that a high dose group (0.250 mg/kg bw/day) was added after the other groups had started without an extra control group. This dose group was included to demonstrate clear maternal toxicity.

Warfarin caused adverse effect in dams at dose levels of 0.150 mg/kg bw/day and higher, in a dose related manner. These effects included piloerection, paleness, reduced activity, internal haemorrhage and open vaginal orifice. Treatment was associated with increased incidence of maternal death and bleeding from the vagina, with necropsy findings of blood in the uterus.

The incidences of external and visceral foetal haemorrhages were higher in all treated groups compared to the controls (although not in a dose-related manner), and this effect is most probably caused by the anticoagulant effect of warfarin.

Cataracts (malformation) were found in one animal of the 0.200 mg/kg TP1 dose group, and in all dose groups (except in the control group and at 0.250 mg/kg) of the TP2 dose group. No other clear indications of malformations were observed. From the litter data that were included in the evaluation of the study, there were no clear indications of skeletal abnormalities related to the treatment with warfarin. However, one litter of the 0.150 mg/kg TP1 treatment group (which was excluded from statistical analysis) had 4 of 7 foetuses with short nose and wide frontal bone at external examination. Skeletal examination was performed on two of these foetuses and the malformations were confirmed.

The findings from this study show that warfarin induces cataracts and haemorrhages. Hence, based on this study warfarin should be considered as teratogenic in rats. The incidence of possible other teratogenic effects which are specific in humans, like skull malformations, are not convincingly seen in this rat study.

Although the study is conducted according to the OECD guideline 414, if has some limitation that should be addressed:

- A high-dose group was added several weeks after the start of the study in order to demonstrate maternal toxicity. No extra control group was added together with this group.
- The intervals between the doses are very small (4 dose levels within a factor of 2), and thus dose-related effects are difficult to detect.

With regard to the doubt expressed by the Specialised Experts whether the standard OECD 414 test can detect coumarin-specific developmental effects, this new study shows that some of the developmental effects induced in humans by warfarin are also detectable in rats, but others are not.

Difethialone has not caused any observed teratogenic effects in the animal studies summarised in this report. However, difethialone and warfarin have a similar chemical structure resembling

vitamin K (see figure 1 and 2). Both substances inhibit the vitamin K (epoxide) reductase complex which mainly results in effects on coagulation and bone formation. The same mechanism is also considered relevant for the developmental effects of warfarin in humans and in the OECD 414 developmental study with rats. Based on this it is likely that also difethialone would induce similar developmental effects as warfarin if the foetuses were exposed at relevant concentrations.

Based on warfarin data, human foetuses seem to be much more vulnerable to vitamin K deficiency than rodent foetuses (Howe and Webster, 1994). This may be related to the fact that human foetuses have very low blood vitamin-K concentrations, with a mid-gestation mean value of 30 pg/ml and a maternal level of 395 pg/ml, compared with plasma levels of 8,600 pg/ml in 20 day rat foetuses and maternal rat levels of 22,000 pg/ml (Howe and Webster,1990, ref. Mandelbrot, L. et al. 1988, Guillaumont, M.J. et al. 1988). In humans this 13 times difference in vitamin K level between mother and foetus may explain why teratogenic effects are observed in foetuses at dose levels that are not toxic to the mother. In contrast, the difference in vitamin K levels is only 2.5 between mother and foetus in the rat. Hence, the dose causing adverse effects in the foetus are most likely closer to the maternal lethal dose in rats than in humans. It should be noted that in the OECD 414 guideline study with warfarin the interval between the dose levels is very small: 4 dose levels are chosen within a factor 2. It can not be excluded that similar developmental effects would have been observed in an OECD 414 guideline study with difethialone if the dosing of the compound had been closer to the dose toxic to the mother. It should be noted that in the OECD 414 guideline study with difethialone in rat no maternal toxicity was observed at the highest dose administered, and this may explain the negative findings. Also difethialone is far more potent than warfarin, which indicates an even steeper dose response curve. Our concern is that the animal studies performed with difethialone have not been conducted within the relevant dose range and that the smaller difference in vitamin K levels between the dam and the foetus in rats makes it difficult to detect teratogenic effects using the conventional developmental toxicity tests in rodents (as OECD Test guideline 414), at least for the more potent of the anticoagulant rodenticides.

The elimination of difethialone is shown to be via faeces as unchanged parent material whereas warfarin is metabolised extensively in liver (predominantly by hydroxylation) to compounds of either non- or clearly decreased anticoagulant activity. The excretion is mainly urinary (Competent authority report on warfarin under directive 98/8/EC, 2009).

Effects on or via lactation

The amount of difethialone transferred to milk is unknown. No human data are available. Neither two-generation study in rats nor other studies with follow-up of off-spring (F1) after maternal exposure to difethialone has been performed. Although difethialone has a high lipophilicity which may indicate that it has a potential to be transferred to milk, no conclusion can be drawn from the available information, and no classification is proposed.

4.11.2.2 Human information

No specific human data on difethialone and reprotoxicity.
See information on warfarin (animal and human data commented in the same section) in section 4.11.2.1

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Fertility
One and two generation studies are not available for difethialone. No effects on the reproductive organs were reported in short-term studies. Warfarin, another well known coumarin derivate with the same chemically active group as difethialone is not classified as toxic to fertility. Based on the available data, no classification for effects on fertility for difethialone seems to be warranted.

Development
Difethialone did not cause any observed teratogenic effects in the experimental animal studies. In human foetuses the vitamin K level in blood is 13 times lower than in the mother, while for the rat this difference is only 2.5. Hence, the anti-vitamin K dose causing adverse effects in rat foetuses are most likely closer to the maternal lethal dose than in humans. Whereas maternal toxicity (fatal haemorrhage) was observed in rabbit studies with difethialone, no maternal toxicity was observed in the OECD 414 study in rats (maternal toxicity was only observed in the preliminary study).

It should be noted that in the positive OECD 414 guideline study with warfarin the intervals between the dose levels are very small: 4 dose levels are chosen within a factor 2. Difethialone is far more potent than warfarin, which indicates an even steeper dose response curve. It can not be excluded that teratogenic effects would have been observed for difethialone if the dosing of the compound had been closer to the dose toxic to the mother.

Due to the difficulties in the design of an optimal study protocol for the detection of potentially teratogenic effects following exposure to difethialone, no clear conclusion can be drawn from these studies.

Warfarin, a well documented human teratogen, is classified as a reproductive toxicant (Repr. Cat 1; R61 - Repr. 1A H360D). Since difethialone has the same chemically active group and the same well-known mode of action by which warfarin causes teratogenicity in humans and in experimental animals (through vitamin K hydroquinone deficiency), classification of

difethialone for developmental toxicity with Repr. Cat. 1; R61 (Directive 67/548/EEC) and Repr. 1A H360D (Regulation EC 1272/2008) similar to warfarin, should be considered.

Effects on or via lactation
The amount of difethialone transferred to milk is unknown. Although difethialone has a high lipophilicity which may indicate that it has a potential to be transferred to milk, no conclusion can be drawn from the available information and no classification is proposed.

4.11.5 Comparison with criteria

Fertility
Based on the available data, no classification for effects on fertility for difethialone seems to be warranted.

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

Warfarin, a well documented human teratogen, is classified as a reproductive toxicant (Repr. Cat 1; R61 - Repr. 1A H360D). Since difethialone has the same chemically active group and the same well-known mode of action by which warfarin causes teratogenicity in humans and in experimental animals, classification of difethialone for developmental toxicity similar to warfarin, should be considered.

Effects on or via lactation
No conclusion can be drawn from the available information, and no classification is proposed.

Specific concentration limits
Potential developmental effects of difethialone would be expected at very low doses, and the possibility of setting specific concentration limits for reprotoxicity 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 for reprotoxicity is based on read across from other substances, and no direct estimate of the reproductive toxicity potency is possible.

4.11.6 Conclusions on classification and labelling

Difethialone did not cause any observed teratogenic effects in 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 difethialone, no clear conclusion can be drawn from these studies.

Since difethialone has the same chemically active group and the same well-known mode of action by which warfarin causes teratogenicity in humans and in experimental animals (through vitamin K hydroquinone deficiency), classification of difethialone for developmental toxicity with Repr. Cat. 1; R61 (Directive 67/548/EEC) and Repr. 1A H360D (Regulation EC 1272/2008) similar to warfarin, should be considered.

Potential developmental effects of difethialone would be expected at very low doses, and the possibility of setting specific concentration limits for reprotoxicity should therefore be explored.

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

SE and TC-C&L conclusion on reprotoxicity

The Specialised Experts for Reproductive Toxicity unanimously recommended that the anticoagulant rodenticides should collectively be regarded as human **teratogens** and be classified as Repr. Cat. 1; R61 (read across from warfarin) (September 2006, ECBI/121/06, ECBI/51/07).

At the TC C&L Meeting in November 2006, a provisional classification with R61 was agreed, but without a final decision on the category to be used (Repr. Cat 1 or Repr. Cat 2). Member States were invited to react in writing during the follow-up period with arguments for the relevance to read across from warfarin.

In May 2007 the provisional classification for reprotoxicity was not confirmed. The TC C&L decided to await results from further studies on anticoagulant rodenticides (i.e. new rat data on developmental toxicity of warfarin (OECD 414 study) and placental transfer of warfarin and flocoumafen) before finalising the discussion on reprotoxicity. The intention was to ask the Specialised Experts on their opinion on the new data, followed by a final written procedure in the TC C&L. However, the studies were not made available in the follow up period, and no final decision on classification for reprotoxicity could be made.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

- *Effects on fertility*

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

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

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

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

Effects on Developmental toxicity

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

Warfarin, a well-documented human teratogen, is classified as a reproductive toxicant (Repr. 1A; H360D). Since Difethialone has the same chemically active group and the same well-known mode of action by which Warfarin causes teratogenicity in humans and in experimental animals, classification of Difethialone for developmental toxicity similar to that for Warfarin should be considered.

Effects on or via lactation

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

Specific concentration limits

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

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

Comments received during public consultation

Four MS agreed with the proposed classification for Difethialone as Repr. 1A; H360D. Three of these MS suggested that read-across from the human and animal data for warfarin should be considered. One of the MS specifically noted that it agreed that Difethialone should not be classified for fertility. A further MS suggested that the SCL for toxicity to reproduction should be harmonised with those for Warfarin.
Comments from the industry did not support the CLH proposal for classification for developmental toxicity. They provided two statements from an expert toxicologist to demonstrate that the basis for read-across for developmental toxicity from Warfarin to Difethialone is invalid.

Assessment and comparison with the classification criteria

Fertility /Lactation
In the opinion of RAC, classification of Difethialone is not warranted for adverse effects on sexual function and fertility or for effects on or via lactation due to lack of relevant data.
Developmental Toxicity
Based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (classified as Repr. 1A), the reproductive toxicity of Difethialone has been analysed in detail. It is acknowledged that the animal developmental toxicity studies on Warfarin are weakly positive and that the animal developmental toxicity studies on Difethialone are negative. However, in comparison with Warfarin, Difethialone and other 2nd generation AVKs have higher acute and repeated dose toxicity, steeper dose-response curves, and much longer half-lives in the exposed organisms, making the evaluation of developmental effects of all 2nd generation AVK rodenticides difficult. Thus, repeated exposure to relatively low doses during gestation lead to maternal toxicity and lethality which hinders the detection of developmental toxicity at higher doses.
As there were no data available on the outcome of maternal exposure to Difethialone in humans, classification as Repr. 1A was not considered to be applicable for Difethialone.

Based on the assumption that all AVK rodenticides, including Warfarin and other

anticoagulant coumarin-based pharmaceuticals (see below) share the same MoA, namely inhibition of vitamin K epoxide reductase, the assessment of Difethialone includes consideration of the total data base for the AVKs. A weight of evidence assessment resulted in the conclusion that Difethialone has the capacity to adversely affect the human *in utero* development. Therefore a classification as Repr. 1B is proposed with the reasoning given below.

The reasons for this conclusion are:

- Difethialone shares the same MoA as expressed by other anticoagulant AVK rodenticides and coumarin-based pharmaceuticals (inhibition of vitamin K epoxide reductase, an enzyme involved with blood coagulation and foetal tissues development, including bone formation, CNS development and angiogenesis)
- Warfarin and 2 other coumarin pharmaceuticals (acenocoumarol, phenprocoumon) have been shown to cause developmental toxicity in humans.
- One of the 2nd generation AVK rodenticides (Brodifacoum) has been shown to cause foetal effects in humans, possibly after one or a few exposures.
- For AVK rodenticides with a long half-life in the body, even single exposures might suffice to trigger developmental effects. However, such studies are normally not conducted and effects of single dose exposure cannot be detected in standard OECD 414 test where instead the repeated exposures may lead to maternal mortality with steep dose-response.
- The standard animal studies do not pick up all developmental toxicity effects of the AVK rodenticides, most notably the face and CNS malformations that are characteristic for Warfarin and other AVK coumarin pharmaceuticals.
- The most sensitive window for face malformations in humans is the first trimester. Thus, even if some AVK rodenticides may have a lower degree of placental transfer than Warfarin, this will not affect the face malformation hazard.

Not all steps of the MoA in the target tissues liver and bone have been proven, thus introducing some uncertainty into the assessment. However, the RAC is of the opinion that the uncertainty is not sufficient to warrant a Repr. 2 classification.

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

Specific Concentration Limit

Classification as Repr. 1B for developmental toxicity for Difethialone is supported by the RAC. However, there is only sufficient data for Warfarin to set a SCL for developmental toxicity. Thus, based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg/day) may cause developmental toxicity and could be regarded as an ED_{10} level. This human ED_{10} value would, if using the guidance for setting SCLs based on animal data, belong to the high potency group (<4 mg/kg/day). The guidance states that for an ED_{10} <4 mg/kg/day, the SCL is 0.03%, and for ED_{10} below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from an ED_{10} value obtained from animal studies (0.125 mg/kg/day; Kubaszky et al 2009), it would qualify Warfarin for the high potency group and result in a SCL of 0.003%. Thus, the RAC concluded on a SCL on 0.003% for the developmental toxicity of Warfarin

As the other AVK rodenticides are equally or more toxic than warfarin, it is not considered appropriate to apply the generic concentration limit for these substances (0.3%), but rather to base the SCLs on the SCL proposed for Warfarin. Thus, the RAC is of the opinion

that the SCL for Warfarin can be used as a surrogate SCL for the other AVK rodenticides, resulting in a SCL of 0.003% for all 8 AVK rodenticides concurrently evaluated by RAC at this time, including Difethialone.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Difethialone was investigated, in various screening tests (Depin and Chavernac, 1986), for potential pharmacological activity other than its known anticoagulant properties. Specifically, the following endpoints were investigated: antianginal activity *in vivo* or *in vitro*; antihypertensive activity; sedative activity; anticonvulsant activity; antidepressant activity; antispasmodic activity in a variety of *in vitro* tests; analgesic, anti-inflammatory or gastric antiacid activity. The absence of sedative activity, anticonvulsant activity, antidepressant activity and the absence of any clinical signs in rodent and dog toxicity tests support the conclusion that difethialone shows no neurotoxic effects..

4.12.1.2 Immunotoxicity

No data

4.12.1.3 Specific investigations: other studies

Studies have been performed to investigate antidotal treatment of intoxicated rats or dogs.

Two non-guideline studies in dogs demonstrated the effect of antidotal vitamin K1 therapy (phytomenadione) following single lethal doses of difethialone (Lorgue and Mally, 1985 and Markewicz, 1991b).

In one of the studies (Markewicz, 1991b), beagle dogs were exposed to a single lethal dose of difethialone of 40 mg/kg bw. The exposure produced a significant increase in prothrombin times and clinical signs including evidence of acute haemorrhagic toxicity (increased prothrombin times) within one or two days of exposure. By day 3 when prothrombin times had increased three fold, the effects were considered maximal for allowing recovery, and the antidote was administered. After an initial anaphylactic/hypersensitivity response to intravenous infusion of vitamin K_1, there was a rapid reduction, to near pre-treatment levels, for prothrombin times. Administration of oral antidote at 10 mg/kg bw/day for the following week held prothrombin times fairly constant at nearly normal levels, but reduction of the antidote dose to 5 mg/kg bw/day resulted in some increased prothrombin times. The antidotal treatment continued to day 30 when prothrombin times had reverted to pre-treatment levels. It was concluded that vitamin K_1 was an effective antidote following overexposure to difethialone when administered in conjunction with monitoring of prothrombin time and signs of haemorrhage. This conclusion is supported by findings in the other study (Lorgue and Mally, 1985) in which beagle dogs were exposed to 20 or 100 mg/kg bw of difethialone. The

administration of vitamin K1 by IV injection followed by supportive oral administration for 7 or 8 days resulted in rapid and definitive recovery of the animals.

In another study in rats (25 ppm end use product given as a diet replacement for 1, 2 or 3 days) antidotal treatment was successful following 24 hour exposure but less successful with longer periods of exposure (the majority of rats died after 48 or 72 hours exposure to difethialone) (Markewicz , 1991a, see also 4.7.1.1).

4.12.1.4 Human information

No data

4.12.2 Summary and discussion

Difethialone showed no neurotoxic effects in various screening tests for potential pharmacological activity.

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

Difethialone showed no neurotoxic effects in various screening tests for potential pharmacological activity. No classification warranted

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

5.1.1 Stability

No data available

5.1.2 Biodegradation

Not readily biodegradable:

Degradation in 10 days: < 6 %
Degradation in 28 days: < 6 %

Test method: ISO 14593 (equivalent to OECD 301B, CO_2 headspace test)

Reference: Daniel and Swarbrick, 2003a

Deficiencies: The nominal concentration of difethialone was 29 mg/L, which is above the water solubility of difethialone. However, assuming a maximum dissolved concentration of difethialone at its water solubility, it can be stated that no biodegradation occurred. The high nominal concentration of difethialone is not supposed to have influenced the outcome of the test.

The test was not conducted with a toxicity control. However, a respiration inhibition test with activated sludge was also conducted with difethialone, showing no effects up to 100 mg/L test substance concentration. It can therefore be concluded that difethialone would not have inhibited biodegradation in this biodegradation test.

Other degradation studies:

- Hydrolysis at pH 7 at 25 °C: DT50 = 175 days (11.2 % degradation after 30 days)

  Reference: Spare, 1986

- Photolysis at pH 7 at 12°C: DT50 = between 20 and 60 minutes

  Reference: Spare, 1987a and Lynn et al., 2003

- Anaerobic biodegradation in STP < 5 % degradation after 63 days

  Reference: Daniel and Swarbrick, 2003b

- Aerobic degradation in soil at 12°C: DT50 = between 417 and 976 days

  Reference: Spare, 1987b

5.1.3 Summary and discussion of degradation

Difethialone is neither aerobically nor anaerobically biodegradable in sewage treatment plants. It is not considered readily biodegradable. Difethialone is slowly degraded in soil under aerobic conditions with a mean half-life of 635 days at 12°C.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Difethialone is strongly and rapidly adsorbed to soil. The Freundlich sorption coefficient normalised for organic carbon content was more than 1 x 10^8 mL/g. This indicates difethialone as non-mobile in soil.

5.2.2 Volatilisation

Due to the low vapour pressure (< 1.33 x 10^{-5} Pa) and the low Henry's law constant (< 1.8 x 10-2 Pa m^3 mol^{-1}) difethialone is not expected to volatilise from soil and water to air.

5.2.3 Distribution modelling

Not available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

As the exposure of fish to difethialone from the use of rodenticide products is expected to be insignificant, a bioconcentration study was not considered necessary. According to the Technical Guidance Document on Risk Assessment, TGD, (European Commission, 2003) the BCF for fish can be predicted from the relationship between Pow and BCF in cases where measured BCF values are not available. For substances with a log Pow higher than 6, a parabolic equation is suggested (equation 75 in the TGD, part II):

$$BCF_{fish} = -0.20 * \log Pow^2 + 2.74 * \log Pow - 4.72$$

The resulting estimated BCF_{fish} for difethialone is 39974, based on the measured log Pow of 6.29.

5.3.1.2 Measured bioaccumulation data

No data available.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on the measured log Pow and the calculated BCF factor difethialone is assumed to bioaccumulate in aquatic species.

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

96 h LC_{50} = 0.051 mg/L (*Oncorhynchus mykiss)*

(NOEC at 96 h = 0.022 mg/L)

Reference: Suprenant and Nicholson, 1986b

Deficiencies: Static conditions, nominal concentrations, fall in dissolved oxygen concentration below 60 %.

The dissolved oxygen concentration was 57 to 86%. However, it was less than 60% only in two test vessels at 72 to 96 hours. The toxic effects of difethialone occur already after 24/48 hours and hence the decrease in oxygen content after 72 to 96 hours is not considered to have affected the outcome of the test.

Concentrations were not measured during the test. Due to the relatively low water solubility of difethialone (0.39 mg/L) and the high log Pow (6.29) test concentrations may have dropped during the study due to adsorption to glassware. Maybe an even lower LC50 would have been achieved in semi-static or flow-through tests. However, test concentrations in another fish test, resulting in a LC50 of < 0.15 mg/L, were measured analytically and showed recovery rates between 74 and 90 % after 24 hours.

5.4.1.2 Long-term toxicity to fish

No studies on chronic toxicity to fish are available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

24 h EC_{50} > 0.005 mg/L; 48 h EC_{50} = 0.0044 mg/L (*Daphnia Magna)*

(NOEC at 48 h = 0.0030 mg/L)

Reference: Suprenant and Nicholson, 1986a

Deficiencies: Static conditions, nominal concentrations

Due to the relatively low water solubility and the high log Pow of the substance test concentrations may have dropped during the duration of the study. Maybe an even lower EC_{50} would have been achieved in semi-static or flow-through tests However, analytical measurements in another test with fish (see above) show recovery rates between 74 and 90 % after 24 hours. The effect of difethialone on *Daphnia magna* in this test already occurs after 24 hours (45 % immobility after 24 hours, 70 % after 48

hours at 5 µg/L test concentration). A further possible decline in test substance concentration from 24 to 48 hours is not considered significant enough to have influenced the outcome of the test.

5.4.2.2 Long-term toxicity to aquatic invertebrates

No studies on chronic toxicity to aquatic invertebrates are available

5.4.3 Algae and aquatic plants

72 h ErC_{50} > 0.18 mg/L (72 h EbC_{50} = 65 mg/L)

72 h NOEC = 0.032 mg/L

Species: *Selenastrum capricornutum*

Reference: Swarbrick, 2003

Deficiencies: Nominal concentrations in the study; only the two highest test concentrations were measured analytically in an external stability control.

The measurements of the test concentrations were conducted in an external stability control, not in the test media with algae itself. This stability control had been stored deep-frozen for 16 month before analysing it. Concentrations of difethialone were below the limit of detection (0.13 mg/L) both at time 0 hours and 72 hours. Therefore these measurements cannot be regarded representative for this test.

Difethialone is photo-labile and has a strong adsorption tendency, and it can therefore be assumed that exposure concentrations were considerably lower than nominal concentrations. However, this has no influence on the environmental classification and labelling of difethialone as both fish and *Daphnia magna* have L/EC_{50} values below 1 mg/L.

Difethialone is not a highly coloured substance.

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Toxicity to terrestrial organisms

- Earthworms: LC_{50} > 252 mg/kg wwt

  Reference: Hughes and Paterson, 2003

- Birds: 30 days LD_{50} (Bobwhite quail) = 0.264 mg/kg bw (acute oral)

  Reference: Fletcher 1988a

- 30 days LC_{50} (Bobwhite quail) = 0.56 mg/kg food (short term dietary, 5 days feeding)

Reference: Fletcher, 1988b

No other studies with terrestrial organisms, e.g. higher plants or bees, are available.

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

The acute toxicity towards aquatic organisms is ≤ 1 mg/L with *Daphnia magna* being the most sensitive organism (48hours EC_{50} = 4.4 µg/L). Difethialone is not readily biodegradable and has a log Pow of 6.29 which is above the cut off in both the DSD (log Pow ≥ 3) and the CLP Regulation (log Pow ≥ 4).

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

An environmental classification with N; R50-53 'Very toxic to aquatic organisms, may cause long-term adverse effects in aquatic environments' is warranted. The acute toxicity towards aquatic organisms is ≤ 1 mg/L. *Daphnia magna* was the most sensitive organism with a 48hours EC_{50} of 4.4 µg/L. Difethialone is not readily biodegradable and has a log Pow of greater than 4.

Table 21. Proposed classification for TC C&L meeting in April 2006

Classification	Toxicity	Degradation	Bioconcentration	Escape Clause
N; R50-53	Fish acute LC_{50} 96 h = 51 µg/L Daphnia EC_{50} 48 h = 4.4 µg/L Algae ErC_{50} 72 h > 180 µg/L (Algae E_bC50 = 65 ug/L)	Not readily biodegradable based on data	log Pow = 6.29 BCF (calculated) >39000	Not relevant
Specific concentration limits:				
M-factor: 100 $C_n \geq 0.25\%$: N, R50-53 $0.025\% \leq C_n < 0.25\%$: N, R51-53 $0.0025\% \leq C_n < 0.025\%$: R52-53				

Table 22. Agreed classification for difethialone

TC-C&L conclusion for Environmental Effects

At a meeting in TC C&L in April 2006 (ECBI/92/06 Rev 1), the proposed environmental classification of N R50-53 was agreed. Specific concentration limits were set as proposed (M factor of 100), and S60-61 was assigned:

C ≥ 0.25%: N, R50-53 (S60-61)
0.025% ≤ C < 0.25%: N, R51-53 (S61)
0.0025%≤ C < 0.025%: R52-53 (S61)

Under the CLP regulation, considering the 2nd ATP criteria, this classification is accordingly "Aquatic Acute 1; H400, Aquatic Chronic 1; H410" with a M factor of 100.

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

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

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

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

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

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

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

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

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

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

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

The effect of Difethialone on *Daphnia magna*, which is the most sensitive species in the reported acute tests, occurs after 24 hours, and a further possible decline in test substance concentration from 24 to 48 hours is not considered significant enough to have influenced the outcome of the

test. As the *Daphnia* test has been used as the key study, the losses in the concentrations for fish and algae have no influence on the environmental classification.

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

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

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

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

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

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

different, the decline of the test concentration from 24h to 48h could be relevant (see "in depth analyses by the RAC" section).

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

In depth analyses by the RAC

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

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

Immobilisation data

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

Effect data

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

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

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

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

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

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

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

6 OTHER INFORMATION

Not available.

7 REFERENCES

Author(s)	**Year**	**Section No / Reference No** in CA report on difethialone[1]	**Title** **Source (where different from company)** **Company** **Report No.** **GLP (where relevant)** **(Un)Published**
Anon	2005	III-A 6.12.1/03	Worker Exposure Report Rodenticide Formulation at Liphatech S.A.S. Pont du Casse, France *This report contains confidential information.*
Belleville, M.J.	1991	III-A 6.10/01	Compared hepatic kinetics of brodifacoum and difethialone in the rat after oral administration of 0.06 mg/kg once weekly for 4 consecutive weeks. Lipha Research Center, Lyons, France, laboratory report no. L-AV/VN GLP/Unpublished
Belleville, M.J.	1986	III-A 6.2/01	Toxicokinetic and metabolism. 1. Study performed with the molecule marked ^{14}C. 2. Study performed with the cold molecule. Lipha Research Center, Lacassagne, France, laboratory report no.

Author(s)	**Year**	**Section No / Reference No** in CA report on difethialone[1]	**Title** **Source (where different from company)** **Company** **Report No.** **GLP (where relevant)** **(Un)Published**
			MET-RAD/LM2219-5-85-3 GLP/Unpublished
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The full CA report is available on CIRCA BC in a confidential version (interest group: Biocides TM) and the Assessment Reports in a public version:
https://circabc.europa.eu/faces/jsp/extension/wai/navigation/container.jsp

Study summaries on physic chemical properties, toxicology and ecotoxicology and fate are included in the IUCLID dossier on difethialone (attachments to section 13) together with the effects assessment of the substance (Doc IIA), the Assessment Report and an annex with confidential data.

8 ANNEXES