CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Bromadiolone

EC Number: **249-205-9**

CAS Number: **28772-56-7**

Index Number:

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Bromadiolone (ISO)***
EC number:	***249-205-9***
CAS number:	***28772-56-7***
Annex VI Index number:	**-**
Degree of purity:	***≥ 96.9% (w/w)***
Impurities:	***No impurities present at ≥1% and none of the impurities present at lower levels are considered relevant for the classification of the substance***

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	Not included	Not included
Current proposal for consideration by RAC	See Table 3	See Table 4
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)		

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				Data lacking
2.2.	Flammable gases				Data lacking
2.3.	Flammable aerosols				Data lacking
2.4.	Oxidising gases				Data lacking
2.5.	Gases under pressure				Data lacking
2.6.	Flammable liquids				Data lacking
2.7.	Flammable solids				Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids				Data lacking
2.10.	Pyrophoric solids				Data lacking
2.11.	Self-heating substances and mixtures				Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases				Data lacking
2.13.	Oxidising liquids				Data lacking
2.14.	Oxidising solids				Data lacking
2.15.	Organic peroxides				Data lacking
2.16.	Substance and mixtures corrosive to metals				Data lacking
3.1.	Acute toxicity - oral	Acute Tox. 1; H300	Specific concentration limits are not applicable for acute toxicity classification according to regulation EC 1272/2008. Rather, the relative potency of substances is implicitly taken into account in the additivity formula.		
	Acute toxicity - dermal	Acute Tox. 1; H310	Specific concentration limits are not applicable for acute toxicity classification according to regulation EC 1272/2008. Rather, the relative potency of substances is implicitly taken into account in the additivity formula.		
	Acute toxicity - inhalation	Acute Tox. 1; H330	Specific concentration limits are not applicable		

			for acute toxicity classification according to regulation EC 1272/2008. Rather, the relative potency of substances is implicitly taken into account in the additivity formula.		
3.2.	Skin corrosion / irritation				conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation				conclusive but not sufficient for classification
3.4.	Respiratory sensitisation				data lacking
3.4.	Skin sensitisation				conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				data lacking
3.6.	Carcinogenicity				data lacking
3.7.	Reproductive toxicity	Repr. 1A; H360D	Specific concentration limit is needed; to be added when discussions on method for deriving SCLs are finalized		
3.8.	Specific target organ toxicity –single exposure				data lacking
3.9.	Specific target organ toxicity – repeated exposure	STOT RE. 1; H372	C≥ 0.01% STOT RE 1; H372 0.001 ≤C < 0.01%, STOT RE 2; H373		
3.10.	Aspiration hazard				data lacking
4.1.	Hazardous to the aquatic environment	Acute Cat. 1 H400 Chronic Cat. 1 H410	M-factor 1		
5.1.	Hazardous to the ozone layer				conclusive but not sufficient for classification

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

Labelling: Signal word: Danger
Hazard statements: H360D, H330, H310, H300, H372, H400, H410

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness				Data lacking
Oxidising properties				Data lacking
Flammability				Conclusive but not sufficient for classification
Acute toxicity	T+: R26/27/28	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		
Acute toxicity – irreversible damage after single exposure				Data lacking
Repeated dose toxicity	T; R48/23/24/25	0.025%≤C<0.25% T; R48/23/24/25 0.0025%≤C<0.025% Xn; R48/20/21/22		
Irritation / Corrosion				conclusive but not sufficient for classification
Sensitisation				conclusive but not sufficient for classification
Carcinogenicity				data lacking
Mutagenicity – Genetic toxicity				conclusive but not sufficient for classification
Toxicity to reproduction – fertility				data lacking
Toxicity to reproduction – development	Repr. Cat. 1; R61	R61 Specific concentration limit is needed; to be added when discussions on method for deriving SCLs are finalized		
Toxicity to reproduction – breastfed babies. Effects on or via lactation				conclusive but not sufficient for classification
Environment	N, R50/53	C≤25%: R50/53 25%≤C≤2.5%: R51/53 2.5%≤C≤0.25%: R52/53		

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

Labelling: Indication of danger: T+, N
R-phrases: R: 61-26/27/28-48/23/24/25-50/53
S-phrases: 53-45-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Bromadiolone is not currently classified in Annex I of Council Directive 67/548/EEC or according to Annex VI of Regulation (EC) no 1907/2006 (REACH). There is, however, a proposal for classification which has been developed in the biocides review programme, see 2.4.2 below. The classification proposal of bromadiolone was agreed by TC C&L in November 2006.

2.2 Short summary of the scientific justification for the CLH proposal

Physico-chemical hazards

Bromadiolone is shown not to contain any chemical groups associated with explosive properties. Also, available data is sufficient to conclude that bromadiolone is not highly flammable, and since it does not contain any metals or metalloids it can be concluded that it is not expected to emit flammable gases in contact with water. Bromadiolone appears to have been safely handled in various testing and it appears possible to conclude that it should not be classified as a pyrophoric solid, and it is also shown not to contain any chemical groups known to possess oxidizing properties. Based on the structural properties, experience in use and available data on thermal stability and relative self-ignition temperature it seems possible to conclude that bromadiolone shall not be classified as "self-reactive substances and mixtures", "self-heating substances and mixtures" or "substances and mixtures corrosive to metals".

Human health hazards

Bromadiolone is acutely toxic at low doses. The oral LD50 is between 0.56 and 0.84 mg/kg bw which are below ≤ 25 mg/kg bw for required labelling with the symbol T+ and the risk phrases R 28 'Very toxic if swallowed'. The dermal LD50 is 1.71 mg/kg bw or 23.3 mg/kg bw which are both ≤ 50 mg/kg bw which requires labelling with the symbol T+ and the risk phrase R27 'Very toxic in contact with skin'. The LC50 value was estimated to be 0.43 µg/L i.e ≤ 0.25 mg/L which requires labelling with the symbol T+ and the risk phrase R26 'Very toxic by inhalation'. The oral LD50 is also less than the ATE ≤ 5 mg/kg bw for an acute toxicity hazard category 1. The dermal LD50 is 1.75 mg/kg bw (sexes combined) and 23.3 mg/kg (sexes combined) which are far below the ATE ≤ 50 mg/kg bw for an acute toxicity hazard category 1. The inhalational LC50 of 0.43 µg/l is far below the ATE ≤ 0.05 mg/l for an acute toxicity hazard category 1.

A death rate of 100% occurred after repeated dose treatment with bromadiolone at 50 µg/kg bw in both rats and dogs which is far below the classification limit of 5 mg/kg bw for R48/25: Toxic: danger of serious damage to health by prolonged exposure if swallowed. No repeated dose studies were available for the dermal or inhalational route with bromadiolone. However, based on the oral data and extrapolation from the acute data for dermal and inhalational data, classification also as R48/23/24 would be warranted. Serious effects were observed in the 28 day rat study and 90-day dog study at levels below the criterion of "oral, rat ≤ 10 mg/kg bw/day for 90-days" used for classification with STOT Rep. 1 H372 for the oral route. For classification for the dermal and inhalatory routes, oral data can be used. Further, there is a large margin between the oral dose levels indicating severe effects and the limit value for STOT RE 1. Also, the acute LD50 values for all three routes were already below the limits for classification as toxic after repeated exposure. Based on these findings, we propose to classify bromadiolone with STOT RE 1 without a specific route

and stating the blood as the main affected organ: H372: "Causes damage to the blood through prolonged or repeated exposure".

No human data exists for bromadiolone. Bromadiolone was teratogenic in rabbit, inducing CNS effects in offspring, similar to what has been observed in humans after Warfarin treatment. Due to the structural similarity to and the same mode of action as the known developmental toxicant warfarin, read across to warfarin is applied and Repr. Cat. 1; R61 (according to the DSD) and Repr. 1A, H360D (according to CLP) is therefore required.

Since bromadiolone is very toxic at low doses, specific concentration limits are needed for acute-, repeated-, and developmental toxicity.

Environmental hazards

According to the CLP criteria a substance should be assigned to hazard class Acute Category 1 if EC_{50} of the most sensitive organism is lower than 1 mg/L. RMS concludes, taking into account expert judgement, that this criterion is fulfilled for bromadiolone. Consequently, bromadiolone fulfils the criteria for hazard class Chronic Category 1, since it is not rapidly degradable and has potential to bioaccumulate.

2.3 Current harmonised classification and labelling

No decision on harmonised classification.

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

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

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

2.4.2 Current self-classification and labelling based on DSD criteria

The following classification proposal has been used in the biocides review programme (Directive 98/8/EC):

Classification	as in Directive 67/548/EEC
Class of danger	T+, N
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 the aquatic environment.
S phrases	S45: In case of accident or if you feel unwell, seek medical advice immediately. Show 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 sheet.

Specific concentration limits	C≥0.5% 0.25%≤C<0.5% 0.025%≤C<0.25% 0.0025%≤C<0.025%	T+;R61-26/27/28 - T; R48/23/24/25 T+; R26/27/28 – T; R48/23/24/25 T; R23/24/25 – T; R48/23/24/25 Xn; R20/21/22 – R48/20/21/22

Bromadiolone is thermally stable below 200°C, its melting point. It is not classified as highly flammable and does not undergo self ignition below its melting point. It is not considered to be explosive or to have oxidising properties. There is no record that it has reacted with any storage container during many years of industrial production. It is concluded therefore, that there are no hazards associated with its physico-chemical properties under normal conditions of use.

The safety phrases proposed are based on the classification and risk phrases. The human health classification is based on toxicological studies summarised in III-A section 6 which indicate that bromadiolone is very toxic by inhalation, when swallowed or in contact with skin in acute accidental or intentional exposure and harmful by repeat exposure. Based on the structural similarities to and the same mechanism as warfarin, read-across from this substance is proposed, which would lead to classification for developmental toxicity. Regarding human health effects a provisional classification with R61 was decided in November 2006 by the TC C&L, but without a final decision on the category to be used (Repr.Cat 1 or Repr.Cat 2). The proposed classification for bromadiolone for acute and repeated dose toxicity was agreed upon.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Not applicable for biocides.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

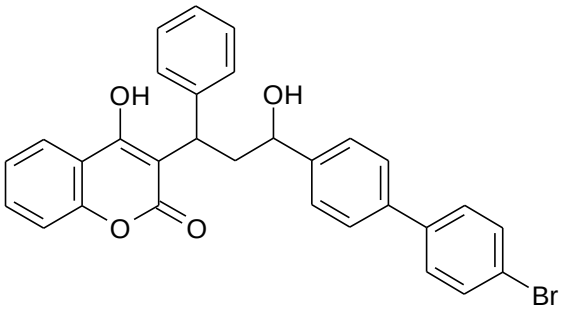
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	249-205-9
EC name:	3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2-benzopyrone
CAS number (EC inventory):	28772-56-7
CAS number:	28772-56-7
CAS name:	2H-1-Benzopyran-2-one, 3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-
IUPAC name:	3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one
CLP Annex VI Index number:	
Molecular formula:	$C_{30}H_{23}BrO_4$
Molecular weight range:	527.40 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
bromadiolone	Confidential (is found in IUCLID section 1.2)	Confidential (is found in IUCLID section 1.2)	

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No impurities present at ≥1% and none of the impurities present at lower levels are considered relevant for the classification of the substance (all impurities are listed as Confidential in IUCLID section 1.2)	-	-	-

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None	-	-	-	-

Current Annex VI entry:

1.2.1 Composition of test material

The test material used in the studies reported for the physico-chemical properties were of purified (99.2 - ~100%w/w) or technical (≥ 98%w/w) quality. No further information (e.g. content and identity of impurities and diastereomeric ratio) is available on the composition of the batches used in testing of the physico-chemical properties.

1.3 Physico-chemical properties

Table 9: 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	Solid (white powder)	Farrell, 2002-A3.3.1/01 (LiphaTech) Drake, 2005-A3.4/02 (Task Force)	
Melting/freezing point	172.0-202.1°C (98.8% purity) 198.3-199.8°C (~100% purity)	Mullee and O'Connor, 2006 - A3.1.1/02 (Task Force) Pesselman, 1990a-A3.1.1/02 (LiphaTech)	Broad melting range due to the mixture of diastereomers.
Boiling point	Decomposes, without boiling, above the melting point.	Jackson, 2002-A3.1.2 (LiphaTech) Mullee and O'Connor, 2006a - A3.1.1/02 (Task Force)	
Density	1.45 g/cm^3 at 20-21°C	Sarff and Locke, 2001-A3.1.3/01 (LiphaTech) Mullee and O'Connor, 2006a - A3.1.1/02 (Task Force)	
Vapour pressure	2.13 x 10^{-8} Pa at 25°C (extrapolated) 0.05 x 10^{-3} Pa at 45°C (direct measurement)	Pesselman, 1991a-A3.2/01 (LiphaTech) Fabrini, 1997-A3.2/01 (Task Force)	Extrapolated value derived from vapour pressure curve generated by measurements in the range 87-97°C
Surface tension	71.2-72.1 mN/m at 20-21°C and a concentration of 1.47-17.4 mg/l	Mullee and O'Connor, 2005 - A3.13/01 (Task Force) de Campos, 2007-A3.13/01 (LiphaTech)	

Water solubility	In buffered solutions at 20°C: pH 4-5: 0.10-0.11 mg/l pH 7: 18.4 mg/l pH 9: 0.18 g/l pH 10: 1.23 g/l In purified water: 12.5 mg/l at 25°C 2.48 mg/l at 20°C	Pesselman, 1992-A3.5/02 (LiphaTech) Hahn, 2002a-A3.5/01 (LiphaTech) Mullee and O'Connor, 2006a - A3.5/02 (Task Force)	
Partition coefficient n-octanol/water	pH 4-5: log P_{ow} = >5 (20-25°C) pH 6-7: log P_{ow} = 3.8-4.1 (20-25°C) pH 9-10: log P_{ow} = 2.5-3.2 (20-25°C) In purified water: log P_{ow} = 4.3 at 23°C (pH not stated)	Pesselman, 1991b-A3.9/02 (LiphaTech) Sarff, 2002b-A3.9/01 (LiphaTech) Mullee and O'Connor, 2006b - A3.9/02 (Task Force)	
Flash point	Not applicable as bromadiolone as manufactured is a solid with a melting point >40°C		Valid justification
Flammability	Not highly flammable Bromadiolone is also not flammable in contact with water nor has it pyrophoric properties	Tremain, 2003-A3.11/01 (LiphaTech)	
Explosive properties	Bromadiolone is not considered explosive	Tremain, 2003-A3.15/01 (LiphaTech)	Theoretical considerations based on the structure
Self-ignition temperature	No self-ignition below the melting point	Tremain, 2003-A3.11/01 (LiphaTech)	
Oxidising properties	Bromadiolone is not considered oxidizing	Tremain, 2003-A3.16/01 (LiphaTech)	Theoretical considerations based on the structure
Granulometry	No data available		
Stability in organic solvents and identity of relevant degradation products	No data available		Data not considered relevant as bromadiolone as manufactured does not contain organic solvents and as bromadiolone is not formulated in organic solvents.
Dissociation constant	pKa_1=4.5 (deprotonation of the hydroxyl-group in the coumarine moiety of the enolic form of bromadiolone)	ACD/PhysChem Suite	Predicted values as experimental testing is technically not feasible due to the low water solubility.

	pKa_2=9.06 (deprotonation of the carbon between the ketone and the lactone in the coumarine moiety of the keto form of bromadiolone)		
Viscosity	Not applicable as bromadiolone as manufactured is a solid with a melting point >40°C		Valid justification

2 MANUFACTURE AND USES

2.1 Manufacture

Information on the manufacture of bromadiolone is provided in the confidential annex of the biocides Competent Authority Reports.

2.2 Identified uses

Bromadiolone is used as a rodenticide for pest control, mainly for the control of rats and mice, by both professional and amateur users. It belongs to Product type 14 (rodenticides) Main Group 03, according to the Biocidal Products Directive 98/8/EC. The content of bromadiolone in typical products is 0.005% w/w.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Theoretical considerations based on the structural properties (i.e. as described in EEC A.14 and CLP Appendix I, part 2, paragraph 2.1.4.3)	Bromadiolone contains no chemical groups associated with explosive properties		Tremain, 2003-A3.15/01 (LiphaTech)
EEC A.10 (flammable solids) The initial screening test was conducted which is identical to the one described in the recommended test method (UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, Part III, sub-section 33.2.1.4.3.1)	Bromadiolone did not ignite during the test period	The moisture content of the substance was 0.34%. Bromadiolone should not be classified as highly flammable according to DSD or a flammable solid according to CLP	Tremain, 2003-A3.11/01 (LiphaTech)
OECD 113 (DSC; both air and N_2-atmosphere)	Bromadiolone is stable up to at least 150°C in air and N_2-atmosphere	Data not sufficient for classification purposes under DSD or CLP. However, experience in use indicates that bromadiolone as manufactured is not a pyrophoric substance	Woolley and Mullee, 2003-A3.10/01 (LiphaTech) Mullee B.J., O'Connor D.M., 2006b-A3.10/02 (TaskForce)
EEC A.16 (relative self-ignition temperature of solids)	Bromadiolone does not have a self-ignition below its melting point (~210°C)	Data not sufficient for classification purposes under DSD or CLP	Tremain, 2003-A3.11/01 (LiphaTech)
Theoretical considerations based on the structural properties	Bromadiolone contains no chemical groups known to possess oxidizing properties		Tremain, 2003-A3.16/01 (LiphaTech)

3.1 Physico-chemical hazards

3.1.1 Summary and discussion of physico-chemical hazards

Technical bromadiolone has been tested for physico-chemical hazards according to the requirements of the DSD. Accordingly bromadiolone has been shown not be highly flammable, have a relative self-ignition temperature below its melting point and to be thermally stable up to at least 150°C in air and N_2-atmosphere based on experimental testing. Furthermore it has been judged not to be explosive or to possess oxidizing properties based on the structural properties of bromadiolone, which are acceptable grounds for data waiving according to the DSD.

3.1.2 Comparison with criteria

Explosivity

Bromadiolone is shown not to contain any chemical groups associated with explosive properties, which are sufficient data waivers both under DSD and CLP. Bromadiolone shall thus not be classified as an explosive under DSD or CLP.

Flammability

The available data is sufficient to conclude that bromadiolone is not highly flammable (F, R11) under DSD and that it is not a flammable solid under CLP (H228).

Moreover, based on the fact that the structure of bromadiolone does not contain any metals or metalloids it can be concluded that it should not be classified as a "substance and mixture which, in contact with water, emit flammable gases" (H260 or H261). Under DSD, data waiving possibilities for this parameter seems not to be explicitly stated. However, based on the structure and the fact that bromadiolone has been tested for water solubility it seems possible to conclude that it should also not be assigned the risk phrase "contact with water liberates extremely flammable gases" (R15) under DSD.

Moreover, based on the fact that bromadiolone appears to have been safely handled in various testing it appears possible to conclude that it should not be assigned the risk phrase "Spontaneously flammable in air" (R17) under DSD and that it should not be classified as a pyrophoric solid (H250).

Oxidizing properties

Bromadiolone is shown not to contain any chemical groups known to possess oxidizing properties, which is a sufficient data waiver under DSD. Furthermore, as bromadiolone only contains oxygen, fluorine or chlorine that is chemically bonded to carbon or hydrogen it shall also not be classified as an oxidizing solid under CLP.

Other physico-chemical hazards

All physico-chemical hazard classes under DSD have been addressed above.

There is not sufficient information available to conclude on the classification of technical bromadiolone under any other physico-chemical hazard classes in CLP. However, based on the structural properties, experience in use and available data on thermal stability and relative self-ignition temperature it seems possible to conclude that bromadiolone shall not be classified as "self-reactive substances and mixtures" (H240, H241 or H242), "self-heating substances and mixtures" (H251 or H252) or "substances and mixtures corrosive to metals" (H290).

3.1.3 Conclusions on classification and labelling

No classification is proposed for technical bromadiolone in relation to its physico-chemical properties based on the available data and information.

4 HUMAN HEALTH HAZARD ASSESSMENT

The summaries included in this proposal are partly copied from the Competent Authority Reports, Document IIA prepared in the context of the possible inclusion of bromadiolone in Annex I to Council Directive 98/8/EC (June 2010 and older versions, applicant Task Force, March 2008 and

older versions, applicant Lipha Tech, RMS Sweden). Summaries are only copied if relevant for the classification and labelling of the substance. References to individual studies (given as study numbers) should be seen as references to the respective CAR, Document IIA.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The active substance, bromadiolone, belongs to a group of substances, the anti-vitamin K rodenticides (AVKs). A summary of the mode of action for this group, taken from the WHO IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva, 1995 ISBN 92 4 157175 6) is presented below.

Anticoagulant rodenticides such as Bromadiolone function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver. Death is due to haemorrhage.

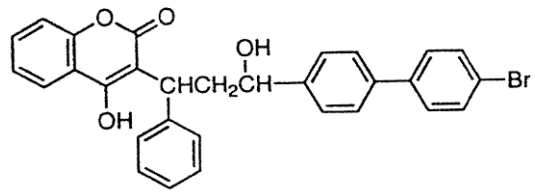
Blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin) to the insoluble fibrous protein fibrin, which binds platelets and blood cells to form a solid mass referred to as a blood clot, sealing the site of the haemorrhage and preventing further blood loss. Fibrinogen is present in the blood, but thrombin is not. Thrombin is formed at the site of injury from prothrombin (factor II) which is present in the blood. Conversion of prothrombin to thrombin occurs via the coagulation cascade, in which the blood clotting factors are employed. Without these blood factors clotting cannot take place, and the haemorrhage will not be controlled by clot formation. If the blood vessel is large and/or serves a vital organ, the haemorrhage will be fatal. The synthesis of a number of blood coagulation factors (factors II [prothrombin], VII [proconvertin] IX [Christmas factor], X [Stuart-Prower factor] and the coagulation inhibiting proteins C and S) is dependent upon vitamin K, which acts as a co-enzyme.

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.

The anticoagulant rodenticide active substances such as bromadiolone work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place.

Bromadiolone is a hydroxycoumarin (Fig 4.1.1-3) with significant structural similarity to the forms of vitamin K. This structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K.

Figure 4.1.1-3 The structure of bromadiolone



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.

A series of toxicokinetic studies were performed on bromadiolone to meet Guideline requirements.

In the most recent study on bromadiolone (A 6.2(1), applicant Task Force), bromadiolone was absorbed fairly slowly after oral administration with peak levels noted at 4-8h post dose in all rats administered 0.5 or 0.05 mg/kg bw. The absorption was between 71-77% of the administered dose, based on (carcass, bile- and urinary excretion). The major route of excretion was via the faeces accounting for ca 50-60% of the dose. Bile investigations showed that biliary elimination plays a major role in the excretion. Pilot studys showed that only neglible amounts of radioactivity were excreted in exhaled air, and was therefore not further investigated. The pattern of excretion and retention of radioactivity following both low and high dose administration and repeated low dose administration were similar although there was an indication that radioactivity was more readily excreted from male rats and at higher dose levels. Low dose animals excreted, in 168h, 54.3% (males) and 49.0% (females) in faeces and 5.1% (males) and 3.4% (females) in urine. High dose animals excreted, in 168h, 65.5% (males) and 56.7% (females) in faeces and 3.7% (males) and 2.5% (females) in urine. Repeated dose animals excreted, in 168h, 55.9% (males) and 51.8% (females) in faeces and 3.3 (males) and 1.4% (females) in urine. A large amount was retained in the animal at 7 days post dose, accounting for 33-48% of the dose, and was mainly retained in the liver. The amount ranged between 15 and 37% with the highest levels after repeated dosing and in females. No parent bromadiolone was observed in urine or bile. The amount bromadiolone in faeces was around 20% of the oral dose and could therefore be accounted for as unabsorbed. The chromatographic analysis of faeces, liver and kidney showed that the majority of the radioactivity present was chromotographically similar to bromadiolone. Several metabolite fractions were identified but not structurally determined. No metabolic pathway was therefore proposed in this study.

Groups of male rats were dosed orally with 14C-bromadiolone on a single occasion at a level of 5.0 mg/kg bw (A 6.2-01, applicant Lipha Tech). Three areas were investigated, mass balance, biliary excretion and protein binding. Samples of urine, faeces and bile (from cannulated rats) were collected up to sacrifice at 48 hours after dosing. Blood was collected at 1, 2 and 4 hours after dosing. Extracts were prepared from faeces and gastro-intestinal tract samples. At 1, 2 and 4 hours after dosing radioactivity was extensively (>98.8%) bound to plasma proteins. No change in the degree of binding was observed up to 4 hours. The only tissue sample examined was the gastro-intestinal tract; radioactivity in the G.I tract at 48 hours accounted for 18.0% of the administered dose. Distribution in other tissues or loss in expired carbon dioxide was not measured in the study, hence no exact oral absorption value could be set. Faecal excretion accounted for 53.3% of the radioactive dose after 48 hours while only 0.86% of dose was present in the urine in the first 48 hours following dosing. Radioactivity in the bile duct of cannulated rats accounted for 46.5% of the dose after 48 hours, with urine and faeces from these animals containing 19.4% of the dose.

Bromadiolone was rapidly absorbed by rats. Absorbed radioactivity was excreted relatively slowly and almost entirely via the bile and faeces. Urinary excretion represented a minor route of elimination. Analysis of faecal and gastro-intestinal tract extracts showed a single major metabolite, up to 10 minor components and polar radioactivity remaining at the origin of the TLC plate plus unchanged bromadiolone. The unchanged parent, bromadiolone, accounted for ca 22% of the dose in faeces and a further ca 6% of the dose in the G.I. tract. The single major metabolite accounted for ca 15% of the dose in the faeces and ca 4% of the dose in the G.I. tract. Polar radioactivity accounted for > 80% of the sample radioactivity in bile. Treatment of bile with β-glucuronidase reduced the polar fraction to 45% of the sample radioactivity, with unchanged bromadiolone and the single major metabolite amongst the components released. MS analysis suggested the single metabolite was a hydroxylated anologue of bromadiolone; hydroxylation was proposed on the benzylic carbon atom. This is consistent with other similar molecules in the AVK class. None of the metabolites of this class of compounds has been shown to be more, or as, toxic as the unchanged parent.

A second study (A 6.2-02, applicant Lipha Tech) investigated liver and plasma levels after low (0.8 mg/kg) and high (3 mg/kg) doses administered orally to groups of four female rats. Blood, liver and kidney samples were collected from animals sacrificed at 1, 3, 6, 9, 12, 24, 48, 72 and 97 hours after dosing. The bromadiolone plasma concentration recorded 1 h after dosing at 0.8 mg/kg was 0.12 µg/mL. Maximum levels were attained between 6 and 9 h after dosing. Plasma concentrations fell in a biexponential way with terminal half-lives of 25.7 h after dosing at 0.8 mg/kg and 57.5 h after dosing at 3 mg/kg. Plasma clearance was 0.1 and 0.12 L/h/kg and volume of distribution was 3.7 and 10.3 L/kg for the 0.8 and 3.0 mg/kg treatment levels, respectively. Hepatic bromadiolone concentrations increased rapidly after dosing, reaching maximum levels after 9 hours and remaining relatively constant during the first 24 hours. After 72 hours bromadiolone levels were 1.08 and 1.60 µg/g for the low and high dose treatments respectively. Liver to plasma ratios ranged between 15 and 35 for the 0.8 mg/kg dose and between 14 and 46 for the 3.0 mg/kg dose. Levels in the kidneys were 0.08 and 0.35 µg/g, for the 0.8 and 3.0 mg/kg doses; slightly higher than the plasma levels. The study concluded that bromadiolone is eliminated primarily via the liver. This is consistent with other findings and studies with similar molecules where biliary excretion via faeces after elimination from the liver is the major route of elimination.

In a third study (A 6.2-04, applicant Lipha Tech) groups of three male rats were sequentially sacrificed at 1, 3, 7, 14, 28, 50, 100, 150 and 200 days after oral dosing with bromadiolone at 0.2 mg/kg bw. At each point the liver was analysed for bromadiolone. Bromadiolone concentrations reached a maximum mean level of 0.98 µg/g one day after dosing. Initial elimination half life (in first 28 days) was calculated to be 17 days. This slowed in subsequent period and the half-life calculated over 50 to 200 days was 318 days.

A non-guideline study in three cows was completed (A 6.2-03, applicant Lipha Tech). Bromadiolone was administered by intra-ruminal injection at dose levels of 1.0 or 3.0 mg/kg bw on a single occasion. Blood and milk samples were taken at intervals up to 13 days after dosing and analysed for bromadiolone. Maximum plasma levels for bromadiolone reached 0.46 and 1.86 µg/mL after 24 hours for low and high dose groups respectively. The levels declined thereafter to < 0.1 µg/mL after 92 and 192 hours, respectively. Clotting times were affected 24 hours after treatment, increasing to 2 – 2.5 times the T0 values between 120 and 216 hours after dosing. Concentrations of bromadiolone in milk were less than the limit of detection (0.05 ppm) at all sampling intervals at both treatment levels.

4.1.2 Human information

No data.

4.1.3 Summary and discussion on toxicokinetics

Bromadiolone was absorbed fairly slowly after oral administration with peak levels noted at 4-8h post dose in all rats administered 0.5 or 0.05 mg/kg bw (A.6.2(1), applicant Task Force). It is extensively bound to plasma proteins (>98.8 %, A 6.2-01, applicant Lipha Tech). Bromadiolone has a short plasma half-life (2.3 days, A 6.2-02, applicant Lipha Tech), but a longer liver half-life (approximately 318 days, A 6.2-04, applicant Lipha Tech). The absorption was > 70% of the administered dose, based on carcass, bile- and urinary excretion, A.6.2(1), applicant Task Force. Bromadiolone is widely distributed (based on measurable levels in blood and organs). The major route of excretion was via the faeces accounting for ca 50-60% of the dose. Bile investigations showed that biliary elimination plays a major role in the excretion. Urinary excretion represents a minor route of elimination and there is no excretion via expired air. No parent bromadiolone was excreted in bile or urine. The main retention site was the liver.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
EPA 81-1, Oral, Rat Sprague-Dawley 10 males and 10 females/group. Single dose at 0, 0.17, 0.25, 0.38, 0.56, 0.84 and 1.26 mg/kg bw. Post exposure period, 21 days.	The LD_{50} was estimated to be between 0.56 and 0.84 mg/kg bw.	Not on pure active substance Data sufficient for classification purposes under DSD or CLP (key study)	A 6.1.1/01 (Lipha Tech)
OECD 401, Oral, Rat Wistar 5 males and 5 females /group. Single dose by gavage at 0.3, 0.6, 0.9, 1.2 and 1.5 mg/kg bw, 14 day post exposure period	The LD_{50} was estimated to be for males: 1.43 mg/kg bw, for females: 1.25 mg/kg bw and combined: 1.31 mg/kg bw	Clinical signs and necropsy findings consistent with internal haemorrhaging. Purity of the a.s. 96% Data sufficient for classification purposes under DSD or CLP (key study)	A6.1.1(Task Force)
EPA 86-1, Oral, Dog, Beagle 4 males and 4 females/group Single dose at 2, 5, 12.5 or 19.8 mg/kg bw. Post exposure period 31 days.	Acute median lethal dose estimated to be 8.1 mg/kg for combined sexes. No apparent difference between males and females.	Data sufficient for classification purposes under DSD or CLP (key study)	A 6.1.1/02 (Lipha Tech)
EPA 81-2, Dermal Rabbit New Zealand White 5 males and 5 females/group Single dose of 0.5, 1, 2 and 4 mg/kg bw applied in corn oil. 0.2-0.4 mg/cm2.	LD_{50} (95% confidence limits) Combined sex: 1.71 (1.18 to 2.5) mg/kg bw Males: 1.3 (0.7 to 2.4) mg/kg bw	Data sufficient for classification purposes under DSD or CLP (key study)	A 6.1.2/01 (Lipha Tech)

Exposure to as large an area as possible for 24 hrs. Post exposure period 21 days.	Females: 2.38 (1.58 to 3.59) mg/kg bw		
OECD 402, Dermal Rat Wistar 5 males and 5 females /group 10% of body surface exposed to 5.0, 10.0, 15.0, 20.0 or 25.0 mg/kg bw. Post exposure period 18 days	The LD_{50} was estimated to be for males: 20.62 mg/kg bw, for females: 32.08 mg/kg bw and combined: 23.31 mg/kg bw	Clinical signs and necropsy findings consistent with internal haemorrhaging. Purity of the a.s. 96% Data sufficient for classification purposes under DSD or CLP (key study)	A6.1.2 (Task Force)
EPA 81-3, Inhalation Rat Sprague-Dawley 5-8 males and 5-8 females/group One group of 5 males and females via whole body exposure. Other groups via nose–only methods, with variable numbers per group. Whole body: 89.2 mg/m³. Nose only: 0.20, 0.33, 0.46, 1.63, 3.35 and 23.3 µg/L. Exposure period 4 hours. Observation period 21 days.	Males: 0.46 µg/L (95% fiducial limits 0.40 to 0.52 µg/L) Females: > 0.33 and < 0.46 µg/L Combined sexes: 0.43 µg/L (95% fiducial limits 0.40 to 0.47 µg/L).	Not on pure active substance Data sufficient for classification purposes under DSD or CLP (key study)	A 6.1.3/01 (Lipha Tech)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Bromadiolone was very toxic to rats with an oral LD50 of >0.56 (A 6.1.1-01, applicant Lipha Tech). Most deaths occurred between day 3 and 10. Clinical signs were limited to rats dosed 0.84 mg/kg bw or higher. The symptoms were observed 1-2 days prior to death and included signs of internal haemorrhage (pale mucous membranes and physical weakness). The necropsies confirmed the occurrence of internal haemorrhage. Similar results were observed in data for applicant Task Force where bromadiolone also was highly toxic to the rat, with a combined sexes LD50 value of 1.31 mg/kg following oral exposure. Clinical signs of toxicity were reported day 4 and onward and included paleness, squatting, dyspnea and external haemorrhaging. Deaths occurred in the 1.3 mg/kg and 1.5 mg/kg dose group, between postexposure day 6- 14. A dose-dependent decrease in mean bw and mean bw gain and covering all dose groups, was observed in both sexes day 7 and 14. Necropsy findings included internal haemorrhaging. Some animals died without showing severe clinical symptoms.

Bromadiolone is slightly less toxic to dogs with a LD50 value of 8.1 mg/kg bw (A6.1.1-02, applicant Lipha Tech). Also in this study clinical observations were typical of a haemorrhagic syndrome and haemorrhagic events were confirmed by necropsy findings. Deaths occurred between 6 and 12 days after dosing.

A dog study was performed where one objective was to assess the acute oral toxicity and determine an antidotal regimen after single lethal dose (A 6.10-03, applicant Lipha Tech). This study was of low reliability but gives some information and is consistent with other studies. For the dogs treated

only with bromadiolone death occurred at 15 and 20 mg/kg bw and similar ante mortem signs of a haemorrhagic syndrome were observed. In the acute trials, using doses of at least five times the dose required to induce intoxication, an antidotal therapy with intravenous administration of vitamin K1 followed by repeated oral administration of the antidote was shown to be effective.

4.2.1.2 Acute toxicity: inhalation

A study on inhalation toxicity was performed in rats with bromadiolone as undiluted powder, 4h exposure (A6.1.3-01, applicant Lipha Tech). The LC50 value was estimated to be 0.43 µg/L (combined sexes) with deaths occurring between day 4 and 9.

4.2.1.3 Acute toxicity: dermal

The dermal toxicity study in rabbits resulted in a LD50 value of 1.71 mg/kg bw for combined sexes (A 6.1.2-01, applicant Lipha Tech). Deaths occurred from day 6 to day 14 in dose groups at 1.0 mg/kg bw and higher. Like in the oral studies clinical signs and necropsy were consistent with internal haemorrhaging. Similar results were also observed in data for applicant Task Force, where bromadiolone also was highly toxic to the rat following dermal exposure, with a combined sexes LD50 value of 23.3 mg/kg. The clinical signs and necropsy findings were consistent with internal haemorrhaging. In most cases the onset of symptoms occurred on the 5th - 7th post exposure day, but in some cases symptoms appeared on the 10th-14th day. Deaths occurred in this study between the 5th- 14th post exposure day and in all dose groups.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

No data.

4.2.3 Summary and discussion of acute toxicity

Bromadiolone is acutely very toxic by the oral, dermal and inhalation routes. Death is a result of internal haemorrhage, which is the declared mode of action of the active substance. Bromadiolone requires classification and labelling according to DSD and CLP criteria.

4.2.4 Comparison with criteria

The oral LD50 is between 0.56 and 0.84 mg/kg bw which are below ≤ 25 mg/kg bw for required labelling with the symbol T+ and the risk phrases R 28 ‘Very toxic if swallowed’. The dermal LD50 is 1.71 mg/kg bw or 23.3 mg/kg bw which are both ≤ 50 mg/kg bw which requires labelling with the symbol T+ and the risk phrase R27 ‘Very toxic in contact with skin’. The LC50 value was estimated to be 0.43 µg/L i.e ≤ 0.25 mg/L which requires labelling with the symbol T+ and the risk phrase R26 ‘Very toxic by inhalation’.

The oral LD50 is also less than the ATE ≤ 5 mg/kg bw for an acute toxicity hazard category 1. The dermal LD50 is 1.75 mg/kg bw (sexes combined) and 23.3 mg/kg (sexes combined) which are far below the ATE ≤ 50 mg/kg bw for an acute toxicity hazard category 1. The inhalational LC50 of 0.43 µg/l is far below the ATE ≤ 0.05 mg/l for an acute toxicity hazard category 1.

4.2.5 Conclusions on classification and labelling

Bromadiolone requires labelling with the symbol T+ and the risk phrases R 28 ‘Very toxic if swallowed’, R27 ‘Very toxic in contact with skin’ and R26 ‘Very toxic by inhalation’, according to 67/548/EEC criteria.

TC-CL conclusion, November 2006 the following classification was agreed: T+: R26/27/28 and specific concentration limits for acute toxicity was agreed in may 2007:
0.25% ≤ C< 0.5%: T+; R26/27/28-48/23/24/25
0.025% ≤ C< 0.25%: T; R23/24/25-48/23/24/25
0.0025% ≤ C< 0.025%: Xn; R20/21/22-48/20/21/22

According to the CLP criteria bromadiolone should be classified in the acute toxicity hazard category 1 (oral, dermal and inhalational route), hazard statement 300, 310, 330 and labelled with pictogram GHS09, signal word danger and hazard statement 300, 310, 330.

Specific concentration limits are not applicable for acute toxicity classification according to regulation EC 1272/2008. Rather, the relative potency of substances is implicitly taken into account in the additivity formula.

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

No data.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Not applicable.

4.3.2 Comparison with criteria

Not applicable.

4.3.3 Conclusions on classification and labelling

No proposal on specific target organ toxicity – single exposure because no data was available.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit, 6/group FHSLA, CFR 21, 191.11. In line with EC method B.4, 0.5 g test material	Average score 24, 48, 72 h for erythema: 0.00 Oedema: 0.00	No irritation observed. Bromadiolone is not classified as a skin irritant. Reversibility was not assessed.	A 6.1.4-01 (LiphaTech)
Rabbit, NZ White, 3 female and 3 male. EPA 81-5, OPPTS 870.500	Average score 24, 48, 72 h for erythema: 0.00 Oedema: 0.00	None Data sufficient for classification purposes under DSD or CLP (key study)	A6.1.4/01 (Task Force)

4.4.1.1 Non-human information

The skin irritation study is of low reliability (applicant Lipha Tech). Abraded sites on the skin are used, the reporting of methods and results is inadequate and the assessment times do not cover full range of EC guideline criteria. However, the study gives no indications on irritation, which is supported by the studies on the product.

The rabbit skin irritation study (applicant Task Force) had some minor deviations from EU Method B.4. However, these deviations should not affect the outcome of the study. The results indicate that Bromadiolone does not cause irritation when in contact with rabbit epidermis.

4.4.1.2 Human information

No data.

4.4.1.3 Summary and discussion of skin irritation

The results indicate that Bromadiolone does not cause irritation when in contact with rabbit epidermis.

4.4.1.4 Comparison with criteria

Average score was for 24, 48, 72 h for erythema: 0.00 Oedema: 0.00 i.e. bromadiolone does not fulfil the criteria to be classified as a skin irritant according to the 67/548/EEC criteria or the regulation EC 1272/2008 criteria.

4.4.1.5 Conclusions on classification and labelling

Bromadiolone does not fulfil the CLP or DSD criteria to be classified as a dermal irritant.

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit, 9 animals in total, Comparable to EC Method B.5	Average score 24, 48, 72h: Cornea: 0.0 (rinsed, unrinsed) Iris: 0.28 (unrinsed), 0.0 (rinsed) Redness: 0.72 (unrinsed), 0.0 (rinsed) Chemosis: 0.72 (unrinsed), 0.0 (rinsed) Iridial and conjunctival reactions had largely resolved by Day 4 and all reactions had resolved by Day 7.	Study of low reliability due to poor reporting.	A 6.1.4/02(Lipha Tech)
Rabbit, NZ White, 3 male and 3 female EPA 81-4, OPPTS 870.2400	Average score 24, 48, 72h: Cornea: 0.11, Reaction reported in 5/6 animals, reversed by 48hrs. Iris: 0.0 Conjunctiva Redness: 0.44, All animals affected, reversed by 4th post exposure day. Conjunctiva Chemosis: 0.17, All animals affected, reversed by 72hrs.	All signs of irritation were reversed by post exposure day 4. Data sufficient for classification purposes under DSD or CLP (key study)	A6.1.4-02Task Force

4.4.2.1 Non-human information

The eye irritation test is also of low reliability (applicant Lipha Tech). In this case the major deficiency is poor reporting. However, the study gives some information and the result does not indicate any substantial eye irritating properties. This is supported by studies on the product.

The eye irritation study by the applicant Task Force shows that bromadiolone is mildly irritating to the rabbit eye, but that the irritation reaction is reversible by post-exposure day 4.

4.4.2.2 Human information

No data.

4.4.2.3 Summary and discussion of eye irritation

The results of the *in vivo* rabbit studies show that bromadiolone is at most only mildly irritating to the eye.

4.4.2.4 Comparison with criteria

The average score at 24, 48, 72h was at most 0.11(cornea) reaction reported in 5/6 animals, reversed by 48hrs. 0.44(conjunctiva redness), all animals were affected, reversed by 4th post exposure day. 0.17 (conjunctiva chemosis), all animals were affected, reversed by 72hrs. Bromadiolone does therefore not fulfil the criteria to be classified as an eye irritant according to the 67/548/EEC criteria or the regulation EC 1272/2008 criteria.

4.4.2.5 Conclusions on classification and labelling

Bromadiolone does not fulfil the CLP or DSD criteria to be classified as an eye irritant.

4.4.3 Respiratory tract irritation

No data.

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

Not applicable.

4.4.3.4 Comparison with criteria

Not applicable.

4.4.3.5 Conclusions on classification and labelling

No proposal on respiratory tract irritation because no data was available.

4.5 Corrosivity

The skin irritation/corrosion studies are mentioned in section 4.4.1

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
See section 4.4.1 above.			

4.5.1 Non-human information

The skin irritation/corrosion studies are mentioned in section 4.4.1.

4.5.2 Human information

No data.

4.5.3 Summary and discussion of corrosivity

No visible skin damage or irreversible skin damage was observed in the irritation/corrosion studies mentioned in section 4.4.1.

4.5.4 Comparison with criteria

No visible skin damage or irreversible skin damage was observed in the irritation/corrosion studies mentioned in section 4.4.1. Bromadiolone therefore does not fulfil the criteria to be classified as corrosive according to the 67/548/EEC criteria or the regulation EC 1272/2008 criteria.

4.5.5 Conclusions on classification and labelling

Bromadiolone does not fulfil the CLP or DSD criteria to be classified as corrosive.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea Pig, EPA 81-6 Buehler test	Number of animals sensitized/total number of animals: Vehicle controls – 6 males in three groups of 2 (test material challenge, vehicle challenge and unchallenged negative control). Test group-10 males. Positive control group- 6 males.	Bromadiolone did not elicit reactions typical of skin sensitisation. Study of low reliability (deviating from guideline and inadequate positive control response)	A 6.1.5/01 (Lipha Tech)
Guinea pig, EPA 81-6, Buehler test OPPTS 870.2600	No results could be obtained due to stained skin.	Study of low reliability due to staining of test site by test substance.	A6.1.5/1 (Task Force)
Guinea pig, OECD 406	Number of animals sensitised/total number of animals: Vehicle controls- 10 females, test group- 20 females, positive control group-20 females	Bromadiolone did not cause skin sensitisation. Study of high reliability Data sufficient for classification purposes under DSD or CLP (key study)	A6.1.5/2 (Task Force)

4.6.1.1 Non-human information

Despite the major deficiencies the study (applicant Lipha Tech) gives an indication that bromadiolone is not a skin sensitiser. This is supported by another study of high reliability, where five percent bromadiolone was applied as solution (in 4% ethanol and 1% methyl cellulose) for induction and challenge (applicant Task Force). Eight dose levels were used in a preliminary dose finding study ranging between 0.001% and 50%. No skin reactions were observed in the preliminary test. The preliminary study was extended and the animals were treated on the 2nd, 3rd and 4th weeks after the first treatment. After the 2nd treatment mortality was observed amongst animals of the higher doses >10%. Therefore 5% was used for induction/challenge. Positive reactions were seen in 15/20 animals in a reliability study, treated at induction/challenge with

potassium dichromate. No reactions were seen in control animals treated with vehicle when challenged with potassium dichromate. The skin sensitisation studies indicate that bromadiolone does not cause skin sensitisation.

4.6.1.2 Human information

No data.

4.6.1.3 Summary and discussion of skin sensitisation

Bromadiolone did not cause significant reactions in the skin sensitisation tests performed on guinea pigs.

4.6.1.4 Comparison with criteria

Positive reactions in the animals did not exceed 15% of the animals (no skin reaction was observed in the treated animals). Bromadiolone does therefore not fulfil the criteria to be classified as sensitising according to the 67/548/EEC criteria or the regulation EC 1272/2008 criteria.

4.6.1.5 Conclusions on classification and labelling

Bromadiolone does not fulfil the CLP or DSD criteria to be classified as sensitising.

4.6.2 Respiratory sensitisation

No data.

Table 16: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data			

4.6.2.1 Non-human information

No data.

4.6.2.2 Human information

No data.

4.6.2.3 Summary and discussion of respiratory sensitisation

Not applicable.

4.6.2.4 Comparison with criteria

Not applicable.

4.6.2.5 Conclusions on classification and labelling

No proposal on respiratory sensitation because no data was available.

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral 45 days, Pig, Belgium Landrace or Large White, Sex not reported. 3 animals/group and 3 in total (1/group) in one trial 0.5 mg bromadiolone per pig continuously for 45 days. Comparative study in which other groups were dosed with warfarin (5 mg/pig) or brodifacoum (1 mg/pig). In third phase of the study, three pigs were given 6.25, 12.5 or 25 mg bromadiolone/pig/day for 5 days followed by 15 day rest period followed by further 5 days of dosing	At 0.5 mg bromadiolone /pig/day there were no mortalities during 45 days administration. In the third phase, the first period of five daily doses of bromadiolone resulted in anorexia, difficulty rising and walking and slight increases in prothrombin times for the two animals dosed at 12.5 or 25 mg/pig/day (circa 0.5 or 1 mg/kg/day). Signs resolved during treatment free period. There was no evidence of haemorrhage or death for bromadiolone treated pigs. LO(A)EL>10 mg/kg bw/day. (single animal) NO(A)EL 10 mg/kg bw/day. (single animal)	The study has too many deficiencies to calculate a reliable NOAEL. Non-guideline study with poor reporting.	A 6.3.1/01 (Lipha Tech)
Oral 35 days (only 5 days exposure) Ferret 5 males and 5 females /group 2.5, 5, 10, 20 or 40 ppm in diet (80g/day diet allocation). Dosed for five consecutive days	Haemorrhages developed from day 4 and death occurred in all groups generally between day 6 and 11. Preceding signs were typical of haemorrhagic syndrome. Prothrombin times increased rapidly during dose administration but returned to normal levels within 9 days of cessation of dosing. LO(A)EL 2.5 ppm (lowest dose tested) NO(A)EL < 2.5 ppm	LC50 value 7.6 ppm corresponding to approximately 0.4 mg/kg bw/day. Study of low reliability (inadequate reporting of test substance and short exposure time).	A 6.3.1/02 (Lipha Tech)
EPA 82-1B, Oral 90 days Dog Beagle, 3 males and 3 females/group 0, 8, 20 or 50 µg/kg bw/day.	No toxicologically significant effects at dose level of 8 µg/kg bw per day. Higher doses elicited reactions typical of haemorrhagic syndrome including elevated clotting times, leading to death by haemorrhage. At 20 µg/kg bw/day 4/6 dogs died between day 64 and 85 of the study. LO(A)EL 20 µg/kg bw/day based on haemorrhagic events. NO(A)EL 8 µg/kg bw/day	Data sufficient for classification purposes under DSD or CLP (key study)	A 6.4.1/01 (Lipha Tech)
OECD 407, Oral (gavage) 28 days Rat, Wistar, 5 males, 5 females per group . Study 1: 0, 100, 500, 1000 µg/kg bw/day Study 2: 0. 2.5, 50 µg/kg bw/day	Study 1 All dose groups: Clinical symptoms, body weight depression, 100% mortality Study 2 2.5 µg/kg bw: no clinical symptoms	Data sufficient for classification purposes under DSD or CLP (key study)	A6.3.1(Task Force)

	50 µg/kg bw: General signs of toxicity, bleeding leading to 100% mortality LO(A)EL 50 µg/kg bw/day NO(A)EL 2.5 µg/kg bw/day		
OECD 409, Oral (gavage) 90 daysRabbit, New Zealand white 6 males, 6 females per group0, 0.1, 0.5, 1 µg/kg bw daily	Prolonged prothrombine time seen in the 1 µg/kg bw group.LO(A)EL 1.0 µg/kg bw per day based on prolonged PTT. NO(A)EL 0.5 µg/kg bw per day	Data sufficient for classification purposes under DSD or CLP (key study)	A6.4.1 (Task Force)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Two non-guideline studies in the pig and ferret (A 6.3.1-01 and A 6.3.1-02, applicant Lipha Tech) demonstrated that after ingestion of five consecutive doses, without administration of antidote, marked haemorrhagic events occurred within 4-5 days and death occurred by day 6 for ferrets dosed at 5 ppm. The LC50 value was calculated to be 7.6 ppm corresponding to approximately 0.4 mg/kg bw/day. Pigs were less sensitive and survived two dose periods of 5 days separated by a fifteen day rest period at dose levels of up to 1 mg/kg bw/day.

A 90-day study in dogs was conducted (A 6.4.1-01, applicant Lipha Tech). At the highest dose tested (50 µg/kg bw/day) all dogs developed a haemorrhagic syndrome and died between day 21 and 32 of the study. Lethal effects were seen in doses as low as 20 µg/kg bw/day. 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 rapidly leading to death. There were no indications of other secondary toxicities: histopathology of the dog revealed no hypertrophy or hyperplasia of the target organ, the liver.

A 28-day repeated dose oral toxicity study (A6.3.1., applicant Task Force) showed that bromadiolone caused general signs of intoxication such as decreased activity, vocalisation, piloerection and also caused paleness, dyspnea and bleeding from nose and eyes, in rats. Common symtoms observed following exposure to a repeated oral dose of 0.05, 0.1, 0.5 and 1 mg/kg in rats were decreased activity, vocalisation, tremor, squatting position, abnormal gait, decreased righting reflex, decreased grip and limb tone, decreased body tone, lachrymation, paleness, piloerection, dyspnoea, bleeding, (nose, eyes), sanuineous urine and cyanotic skin. Also, a depression of body weight and food intake was found in these groups compared to controls. The mortality rate in these groups was 100%. In the low dose group (2.5µg/kg) of study 2, no clinical symtoms were observed and no effects on food consumption were reported. The only effect on body weight gain was a significant lower bw gain in males during the last week of exposure. However, the summarized bw gain during the exposure period was not different from controls.

Haematological findings included a decreased Mean Corpuscular haemoglobin concentration and monocyte count in male animals of the low dose group (2.5µg/kg) compared to controls, and a Red blood cell distribution width decrease in females of the low (2.5µg/kg) dose group compared to controls. Also, in the low (2.5µg/kg) dose group a significantly increased calcium and chloride concentration was observed in males compared to controls. These minor deviations from control values were not of biological significance, since all values were within the physiological range.

Common necropsy findings in the 0.05, 0.1, 0.5 and 1.0 mg/kg/day dose groups were general haemorrhagic diathesis and serous hepatitis. Centrilobular hepatic necrosis and alveolar emphysema were also reported in these dose groups. In the low (2.5µg/kg) dose group in study 2, calcium deposits, uterus dilation and focal proliferation of MPS-cells in the liver were slightly more common than in the control group, but were not of biological significance. Study one showed that at doses at and above 100 µg/kg bw/day, clinical signs and decreased body weight were observed. The mortality rate were also 100%. In study two, a dose of 50 µg/kg bw/day led to 100% mortality with deaths occurring between day 17 and 27. No clinical signs were observed at the lower dose level.

A 90-day repeated dose toxicity study in rabbits was performed (A6.4.1, applicant Task Force). Two females in the 0.5 µg/kg dose group died during the treatment period. Histopathological examination revealed pleuritis, hepatitis and pneumonia in both animals and an acute bacterial invasion was, in both cases, seen as a probable cause of death. Diarrhoea was observed in four animals in each dose group but was not seen in the control animals. Also, in the highest dose group (1 µg/kg) six animals had thin faeces. Pale skin or mucous membranes were seen in females in all dose groups and among males in the intermediate (0.5 µg/kg) and high dose group (1µg/kg.) Haematological investigations showed a significant increase in prothrombin time (PTT) in males and females in the 1µg/kg dose groups compared to controls. This may indicate an effect on homeostasis caused by the exposure to Bromadiolone. Other minor haematological effects were seen in midway blood samples from animals in the highest dose group compared to controls but none were seen in both sexes and no effects persisted until terminal sampling. In terminal blood samples, females of the highest dose group showed significantly decreased MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) values compared to controls. However, no significant differences in MCHC (mean corpuscular haemoglobine concentration), RBC (red blood cell concentration), Htc (haematocrite) or HGB (haemoglobine concentration) were reported, indicating that the decreased MCV and MCH values were not of biological significance. Females in all three dose groups showed significantly higher WBC (white blood cell concentration) values in terminal blood samples compared to controls. However, the mean control value was low, compared to normal values for rats (5- 15 x 109 /L) and start and midway sample values, indicating that this deviation was not of biological significance.The only effect on clinical chemistry seen in terminal blood samples was a significant decrease in glucose values in females of all dose groups compared to controls, which may be due to the malabsorption caused by diarrhoea seen in the majority of the exposed animals between day 85- 90 of the exposure period. A significantly increased urine volume was seen terminally in females of the 1µg/kg dose group compared to controls but since the control value was low, this difference is not of biological significance. No other dose related effects on urine analysis were seen. There were no treatment related effects on body weight gain, food consumption, ophthalmologic examination or blood marrow smears. The only effects on organ weights among males were a significantly lower testes weight in the 1.0 µg/kg dose group compared to controls. In females, the pituitary weight was increased in all dose groups compared to controls. This effect was not seen in males. Pin-prick sized lung haemorrhages, lung abscesses and nutmeg-like pattern in the liver were slightly more common in females and males of the 1 µg/kg dose group compared to controls. However, no organ weight deviations or necropsy findings were dose related. Pale skin or mucous membranes were seen in females in all dose groups and among males in the intermediate (0.5 µg/kg bw) and high dose group (1 µg/kg). Haematological investigations showed a significant increase in prothrombin time in males and females in the 1 µg/kg dose groups compared to controls. These findings are consistent with the mode of action of bromadiolone as an anticoagulant. The NOAEL was set to 0.5 µg/kg bw based on the prolonged PTT at 1 µg/kg bw.

4.7.1.2 Repeated dose toxicity: inhalation

A repeat dose inhalation study is waived (applicant Lipha Tech). An acute inhalation study (A 6.1.3-01, applicant Lipha Tech) showed that bromadiolone is highly toxic by inhalation. The LC50 for male and female rats was 0.46 µg/L. Appropriate protection measures are required to ensure no exposure to the (powdered) technical material or to the products during the production process. There is evidence from a study with one product type (B 6.1.3, applicant Lipha Tech) that acute inhalation is high when a micronised form of the product is aerosolised to generate a toxic inhalable atmosphere (LC50 <0.523 mg/L air). However, the products are either extruded wax blocks or treated grains (the treated grain products have been shown to be dust free) and will not lead to exposure via inhalation. Repeated exposures will very likely result in death by induction of a haemorrhagic syndrome with associated acute clinical signs of reaction to treatment. 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 useful information to that obtained in various species in repeat dose and subchronic studies by the oral route. As the outcome of such a study can be predicted from the knowledge on mode of action and acute or short term exposure, performing a repeat administration study would contravene Directive 86/609/EC which militates against unnecessary testing using animals.

The repeat dose inhalation study is waived (applicant Task Force). No acute inhalation study has been performed and route-to-route extrapolation is not feasible. Bromadiolone has a low vapour pressure and exposure via inhalation is expected to be negligible both during production and during the use of bait blocks. Waiving of the repeat dose inhalation study has therefore been accepted.

4.7.1.3 Repeated dose toxicity: dermal

A repeat dose dermal toxicity study is waived (applicant Lipha Tech). The dermal acute study (A 6.1.2-01) showed high dermal toxicity with a LD50 value of 1.71 mg/kg bw for male and female rabbits. Primary irritation studies showed no dermal irritation at abraded or non-abraded sites after a 24 hour exposure period. Lethal effects were apparent following topical application during the sensitisation study preliminary investigations, although the material does not cause delayed contact hypersensitivity and it is unlikely that repeated application would elucidate any longer term allergenic effects that would take precedence over the lethal effects. The highly toxic nature of the material is such that repeated administration studies would certainly result in death at high doses. 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 useful information to that obtained in various species in repeat dose and subchronic studies by the oral route. As the outcome of such a study can be predicted from the knowledge on mode of action and acute or short term exposure, performing a repeat administration study would contravene Directive 86/609/EC which militates against unnecessary testing using animals.

The repeat dose dermal toxicity study is waived (applicant Task Force). The short term repeat dose oral study has been performed for bromadiolone in rats and route-to-route extrapolation based on data from the acute oral and dermal studies does not indicate that dermal exposure constitutes a greater risk than oral exposure. Even if the most probable form of exposure to humans is via the

dermal route, based on the use pattern of the product, dermal exposure is expected to be low as the use of gloves when handling the baits is expected, and the waiving has therefore been accepted.

4.7.1.4 Repeated dose toxicity: other routes

No data.

4.7.1.5 Human information

No data.

4.7.1.6 Other relevant information

No data.

4.7.1.7 Summary and discussion of repeated dose toxicity

Repeat-dose oral studies show that even at doses as low as 20 µg/kg/day in the dog, lethal effects begin to be seen after 64 to 85 days administration. The clinical signs are consistent with increased clotting time and haemorrhagic events. The effects can be reversed by treatment with vitamin K if given in time. Signs of toxicity following repeat dose exposure to bromadiolone were consistent with the anticoagulant properties of Bromadiolone. In the 90-day oral exposure study in rabbits, a significant increase in prothrombin time was seen in the 1 µg/kg dose group. The overall NOAEL for repeat dose effects is 0.5 µg/kg/day based on the absence of adverse effects in this dose group in the 90-day rabbit study.

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

Repeated oral dose treatment with bromadiolone in dogs, rats, ferrets and rabbits caused haemhorrage and death at low doses. The results were consistent with the anticoagulative effects of bromadiolone also seen in the acute oral, dermal and inhalation studies.

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

A death rate of 100% occurred after repeated dose treatment with bromadiolone at 50 µg/kg bw in both rats and dogs which is far below the classification limit of 5 mg/kg bw for R48/25: Toxic: danger of serious damage to health by prolonged exposure if swallowed. No repeated dose studies were available for the dermal or inhalational route with bromadiolone. However, based on the oral data and extrapolation from the acute data for dermal and inhalational data, classification also as R48/23/24 would be warranted.

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

Based on the oral repeated dose toxicity data plus extrapolation from the acute data for the dermal and inhalation route of exposure, bromadiolone should be classified with R48/23/24/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed.

Agreed by TC C&L, November 2006: T; R48/23/24/25

Specific concentration limits agreed by TC C&L, May 2007:
0.25% ≤ C< 0.5%: T+; R26/27/28-T; 48/23/24/25
0.025% ≤ C< 0.25%: T; R23/24/25-48/23/24/25
0.0025% ≤ C< 0.025%: Xn; R20/21/22-48/20/21/22

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 section 4.7.1.8 above.

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

Serious effects were observed in the 28 day rat study and 90-day dog study at levels below the criterion of "oral, rat ≤ 10 mg/kg bw/day for 90-days" used for classification with STOT RE 1 H372 for the oral route. For classification for the dermal and inhalatory routes, oral data can be used. Further, there is a large margin between the oral dose levels indicating severe effects and the limit value for STOT RE. 1. Also, the acute LD50 values for all three routes were already below the limits for classification as toxic after repeated exposure. Based on these findings, we propose to classify bromadiolone with STOT RE 1 without a specific route and stating the blood as the main affected organ: H372: "Causes damage to the blood through prolonged or repeated exposure".

Specific concentration limits

An SCL for STOT RE 1 is proposed of 0.01% based on the LOAEL at 0.001 mg/kg bw/day in the longest study in rabbits. Calculation: 0.001 mg/kg bw/day (effective dose) / 10 mg/kg bw/day (limit) * 100% = 0.01%. STOT RE 2 is proposed between 0.001% and 0.01% using the same data and method of calculation (limit: 100 resp. 10 mg/kg bw/day). This calculation is performed according to the method described in the Guidance on the Application of the CLP Criteria.

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

Bromadiolone should be classified as STOT RE 1; H372.

Proposed Specific concentration limits: STOT RE 1; H372 above 0.01% and STOT RE 2; H373 between 0.001 and 0.01%

4.9 Germ cell mutagenicity (Mutagenicity)

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
OECD 471, Bacterial Reverse Mutation Test, S. typhimurium: TA 98, TA 100, TA 102 TA 1535, TA 1537 0, 39.06, 78.13, 156.25, 312.50, 625, 1250, 2500 and 5000 µg/plate	Negative ±S9 mix.	Bromadiolone is considered to be non-mutagenic in the studied test system. Data sufficient for classification purposes under DSD or CLP (key study)	A6.6.1 (Task Force)
OECD 473, Mammalian Chromosome Aberration Test Mammalian cell lines CHO 0, 1.0, 7.5, 15.0 µg/ml	Negative ±S9 mix.	Bromadiolone is considered to be non-clastogenic in the metaphase chromosome aberration assay in Chinese Hamster Ovary cells. Data sufficient for classification purposes under DSD or CLP (key study)	A6.6.2 (Task Force)
OECD 476, Mammalian Cell Gene Mutation Test Chinese hamster ovary (CHO) 0, 1,0, 10.0, 20.0 and 30 µg/ml	Negative ±S9 mix.	Bromadiolone is considered to be non-mutagenic in the CHO-HPRT Forward Mutation Assay, both with and without metabolic activation. Bromadiolone was toxic at 80 µg/ ml without activation and 40 µg/ml with activation. Data sufficient for classification purposes under DSD or CLP (key study)	A6.6.3(Task Force)
EPA 84-2, Bacterial reverse mutation test S. typhimurium: TA 98, TA 100, TA 1535, TA 1537, TA 153810, 33.3, 100, 333, 1000 and 3330 µg/plate with S9 and 3.33, 10, 33.3, 100, 333 and 1000 µg/plate without S9.	Negative ±S9 mix.	Bromadiolone did not produce significant increases in the number of revertant colonies, either with or without metabolic activation. Evidence of cytotoxicity in some strains at doses of 1000 µg/plate or higher in the presence or absence of S9. Data sufficient for	A 6.6.1/01 (Lipha Tech)

		classification purposes under DSD or CLP (key study)	
EPA 84-2, Mammalian chromosome aberration test Whole blood human lymphocytes 30 hour without activation: 7.49, 9.99, 25, 50 and 74.9 µg/mL 30 hour with activation: 37.5, 50, 74.9 and 99.9 µg/mL	Negative ±S9 mix.	Bromadiolone did not induce chromosomal aberrations under conditions of metabolic activation or non-activation. Bromadiolone considered negative for mutagenicity. Cytotoxicity was seen in the assay without activation, at 74.9 µg/mL. With activation, doses up to 99.9 µg/mL provided sufficient cultures for analysis although at higher doses (in the range finding study) there was evidence of cytotoxicity. Data sufficient for classification purposes under DSD or CLP (key study)	A 6.6.2/01(Lipha Tech)
EPA 84-2Mammalian cell gene mutation test, Chinese hamster ovary (CHO) Cytotoxicity assay with and without activation: 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg/mL Main study without activation: 20, 40, 60, 80, 100, 125 and 150 µg/mL Main study with activation: 10, 20, 40, 60, 80 and 90 µg/mL	Negative ±S9 mix.	Bromadiolone did not induce mutagenic effects in CHO cells at the HGPRT locus either in the presence or absence of metabolic activation. Bromadiolone was toxic at 200 µg/mL without activation, and at 100 µg/mL with activation Data sufficient for classification purposes under DSD or CLP (key study).	A 6.6.3/01(Lipha Tech)
EPA 84-2, Micronucleus test (bone marrow), Mouse ICR, 5 of each sex per group. Additional 15 of each sex for completion of the micronucleus bioassay. Dosing of 400 mg/kg bw for 3 consecutive days, sampling 24 hours after the last dose	Bromadiolone did not induce an increase in micro-nucleated polychromatic erythrocytes in comparison with the vehicle control. The positive control did significantly increase the number of micro-nucleated cells in both sexes confirming method sensitivity.	Bromadiolone was considered negative in the mouse micronucleus test. Data sufficient for classification purposes under DSD or CLP (key study)	A 6.6.4/01 (Lipha Tech)

4.9.1 Non-human information

4.9.1.1 In vitro data

The results for genotoxicity in *in vitro* tests (applicant Task Force) were all negative. The doses tested in A.6.6.2 were selected on basis of a cytotoxicity test. The results of a CHO clonal cytotoxicity test showed that > 50 µg/ml resulted in less than 10% survival and 10 µg/ml resulted in 63% survival in presence of metabolic activation and 10 µg/ml resulted in 57% survival without metabolic activation. The doses used in A.6.6.3 were selected on basis of a cytotoxicity assay. CHO clonal toxicity data showed that 40 µg/l resulted in a relative survival of 33% in absence of S9 and 0% survival in presence of S9 mix. 20 µg/ml resulted in 62-66% ±S9 mix.

The results for in vitro bacterial gene mutation (A 6.6.1); in vitro cytogenicity in mammalian cells (A 6.6.2) and in vitro mammalian cell gene mutation (A 6.6.3) tests were all negative (applicant Lipha Tech).

4.9.1.2 In vivo data

The mouse micronucleus test (A 6.6.4, Lipha Tech) was also negative.

4.9.2 Human information

No data.

4.9.3 Other relevant information

No data.

4.9.4 Summary and discussion of mutagenicity

Bromadiolone was not genotoxic based on the above tests, hence no classification for mutagenicity is proposed.

4.9.5 Comparison with criteria

Bromadiolone was not genotoxic based on the above tests, hence no classification for mutagenicity according to the criteria in 67/548/EEC and regulation EC 1272/2008 is proposed.

4.9.6 Conclusions on classification and labelling

Bromadiolone was not genotoxic based on the above tests, hence no classification for mutagenicity according to DSD and CLP is proposed.

4.10 Carcinogenicity

No data.

Table 19: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
No data availale			

4.10.1 Non-human information

Bromadiolone is highly toxic (T+) to the target species, the rat, making it technically difficult to perform long-term exposure studies in which signs of toxicity are identified, but keeping the level of lethality low so as not to mask any toxic effects caused by test substance exposure. Also, one long-.term study has been performed for bromadiolone. This study was not accepted due to the fact that dose levels were so low that no toxic effects were seen. The performance of long-term studies on bromadiolone is thus not justified. Waiving of the chronic toxicity and carcinogenicity data was accepted when evaluated under the 98/8/EC directive. Also, no genotoxic potential has been identified for bromadiolone in *in vitro* tests of genotoxicity.

4.10.1.1 Carcinogenicity: oral

No data.

4.10.1.2 Carcinogenicity: inhalation

No data.

4.10.1.3 Carcinogenicity: dermal

No data.

4.10.2 Human information

No data.

4.10.3 Other relevant information

No data.

4.10.4 Summary and discussion of carcinogenicity

No data available. Read-across with warfarin and other structural related coumarin derivatives were not done for carcinogenicity. Bromadiolone is considered to be non-genotoxic. No classification is proposed for both legislation 67/548/EC and the new regulation EC 1272/2008.

4.10.5 Comparison with criteria

No data available. Read-across with warfarin and other structural related coumarin derivatives were not done for carcinogenicity. Bromadiolone is considered to be non-genotoxic. No classification is proposed for both legislation 67/548/EC and the new regulation EC 1272/2008.

4.10.6 Conclusions on classification and labelling

No data, no classification proposed according to DSD and CLP.

4.11 Toxicity for reproduction

Table 20: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
OECD 416, Rat Wistar Oral (gavage), 25 males and 25 females/dose group 10 weeks before mating, 2 weeks mating, pregnancy until termination on postpartal day 22 0, 1, 2.5 and 5 µg/day	No toxicity occurred at the highest dose level and a reliable NOAEL could therefore not be concluded on the basis of this study.	Data not sufficient for classification purposes under DSD or CLP	A6.8.2 (Task Force)
OECD 414, Oral (gavage) Rabbit New Zealand White 22 females per dose group Day 7-28 for 2 and 4 µg/kg bw Day 7-20 for 8 µg/kg bw 0, 2, 4, 8 µg/day	Maternal mortality was 22%. Clinical signs: bleeding around the body orifices. Two fetuses with severe malformations and increased incidence of skeletal variations were reported (4 µg/kg) and one with hydrocephalus in the high dose group. NO(A)EL (maternal) < 2 µg/kg/day NO(A)EL Teratogenicity/Embryotoxicity 2 µg/kg/day	Data not sufficient for classification purposes under DSD or CLP (key study)[1]	A6.8.1(Task Force)
EPA 83-3, Oral, Rat, Sprague-Dawley Female 25 per group10 doses, Day 6 to 15, 0, 17.5, 35 or 70 µg/kg bw per day	Death occurred in the high dose group (48%). Ante-mortem signs included vaginal haemorrhage, metrorrhagia, hypertonicity and pale eyes. There were no mortalities and no clinical signs of reaction to treatment in the other groups. No signs of embryo toxicity or macroscopic evidence of teratology NO(A)EL (maternal) 35 µg/kg bw per day µg/kg/day µg/kg/day NO(A)EL Teratogenicity/Embryotoxicity70 µg/kg bw per day (highest dose tested)	Data not sufficient for classification purposes under DSD or CLP (key study)[1]	A 6.8.1(Lipha Tech)
EPA 83-3, Oral, Rabbit New Zealand White, Female, 19-20 per group. 13 doses – Day 6 to 180, 2.0, 4.0 or 8.0 µg/kg bw per day	Maternal toxicity was evident at 8 µg/kg bw per day where metrorrhagia was found in 8/19 dams. No embryofoetal toxicity and no developmental toxicity indicative of teratogenicity.NO(A)EL (maternal) 4 µg/kg bw per day NO(A)EL Teratogenicity/Embryotoxicity 8 µg/kg bw per day (highest dose tested)	Data not sufficient for classification purposes under DSD or CLP (key study)[1]	A 6.8.1/02 (Lipha Tech)

[1]The study is performed according to the guideline, with a high reliability and considered a key study but are not sufficient for classification purposes for this type of chemicals due to reasons stated in setion 4.11.3

4.11.1 Effects on fertility

4.11.1.1 Non-human information

The two-generation reproduction toxicity study is of low reliability (applicant Task Force). According to EC Method B35 "the highest dose level should be chosen with aim to induce toxicity but not death". In this study, no clinical signs were seen in any dose group and no dose-related effects were reported. Therefore, reproductive effects following bromadiolone exposure can not be excluded based on the results from this study. However, the 90-day study in rabbits showed no adverse effects on the gonads. Also, since long term exposure studies are technically hard to perform for such highly toxic substances as bromadiolone, no new study was required. A multigeneration study is waived for both applicants. Due to the high toxicity and the anticoagulant effect this kind of study may not be possible to conduct with a reliable result. The progressive accumulation of the substance leads to an increased probability of death by haemorrhage. Several events in the reproductive cycle are associated with haemorrhage, which would increase the risk. The high toxicity also means that very low doses are needed to keep the animals alive, but if the doses are too low there will be no toxic response. The dose-response curve seems to be steep, which complicates the dose setting.

As mentioned before bromadiolone is structurally similar to warfarin and share the same mode of action. Warfarin is not classified as toxic to fertility. There is also long term experience in humans with warfarin with no association with adverse effects on fertility (i.e. mating performance) of either sex (IPCS Environmental Health Criteria 175, 1995).

Additionally, the 90 day study in dogs showed no adverse effects on the reproductive organs (macroscopic condition, organ weight and histology, applicant Lipha Tech).

4.11.1.2 Human information

No data.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

A teratogenicity study on bromadiolone was performed using the rabbit as test species (applicant Task Force). The mortality rate for the dams was 0, 0, 27 and 46% for the controls, 2 µg/kg, 4 µg/kg and 8 µg/kg groups respectively. The incidence and severity of clinical signs were dose related. Autopsy findings showed three animals with kidney haemorrhaging. In the 4 µg/kg/day and the 8 µg/kg/day dose groups common clinical observations were bleeding around body orifices (6/22 in the 4 µg/kg group and 10/22 in the 8 µg/kg dose group), reduced activity (1-2 does/dose group) and pale mucous membranes (5/22 in the 4 µg/kg group and 11/22 in the 8 µg/kg dose group). Autopsy findings revealed uterine haemorrhaging, reddish mottled lungs, haemorrhaging in the kidney and bloody discharge from body orifices (or in the thorax) in both dose groups. No dose related effects on fertility or fetal development were reported apart from a significantly increased incidence of post-implantational loss and total intrauterine mortality in the 4 µg/kg dose group compared to controls. Also, a significantly increased incidence of one small placental lobe was seen in the 8 µg/kg dose group compared to controls.

Table 4.11.2-1: Pregnancy outcome

	Control	**2 µg/kg**	**4 µg/kg**	**8 µg/kg (Only dosed to GD20)**
Number of does	19	18	14	*9*
Number of viable fetuses	165	138	98	*68*
Number of foetuses examined for malformations	165	138	87	*68*
Corpora lutea	187	161	132	*75*
Preimplantation loss[1]	11%	14%	17%	*8%*
Dead fetuses[1]	0	0	7%**	*1%*
Postimplantation loss[1]	1	0	10%**	*1%*
Total intrauterine mortality[1]	12	14	26%**	*9%*

[1]Data compared to no. of implantations, **=p<0.01 CH2

The maternal mortality rate was high, 19/88 does died. Clinical signs were observed starting on the second week of exposure. The symptoms were related to the anticoagulant property of bromadiolone and comprised of bleeding around body orifices, pale mucous membranes and reduced activity. Autopsy findings revealed uterine haemorrhaging, reddish mottled lungs. Haemorrhaging in the kidney and bleeding discharge from body orifices. The two fetuses with malformations had absent mesencephalon and proencephalone, rudimentary cerebrellum and absent vitrous body. The high dose group 8 µg/kg day were only dosed up to day 20 of gestation for the surviving animals due to mortalities from day 18. There are no information in the study report relating to any bleedings observed in the surviving foetuses. The observed malformations were not discussed, in the study report, in relation to historical control data.

Based on the severe fetal malformations reported in this study, following exposure to maternally toxic levels of bromadiolone, exposure to bromadiolone may constitute a possible risk to the unborn child.

Table 4.11.2-2: Malformations observed in relation to maternal toxicity observed

Doe	Dose group	No of foetuses malformed (total no of foetuses)	Observed malformation (s)	Maternal observations at autopsy	Placenta findings	Maternal body weight gain (day 29 compared to day 1 and corrected for uterine weight)
31905116	4 µg/kg	1/8	Absence of mesencephalon and proencephalon. Rudimentary cerebrellum. Facial scull bones and scull cap missing, hypoplastic mandibule, arch I-III cervical vertebrae fused, on the scull base rudimentary vertebrae –like bones	Haemhorrhages in uterine horns, pin sized haemhorraghes in lungs	No abnormalities reported	-1.1%
1105036	4 µg/kg	1/1	Absence of vitreous body, absence of retinal folds (both sides)	None reported	Data missing in report	-9.3%
64204506	8 µg/kg	1/6	Internal hydrocephaly	Haemhorrhages in uterine horns, pin sized haemhorraghes in lungs	Tumor-like formation in one lobe	+2.5%

Two teratogenicity studies in rat and rabbit were also performed by applicant Lipha Tech and both were negative. It should be noted though that these studies were performed in 1981 with shorter dosing period, which may contribute to the differences in the rabbit teratogenicity results.

4.11.2.2 Human information

No data.

4.11.3 Other relevant information

The structural similarity of bromadiolone and the same mode of action as the known developmental toxicant warfarin need attention. Warfarin causes a specific kind of embryopathy when administered to humans in the first trimester. The deformities consist of skeletal anomalies including severe nasal hypoplasia, stippled epiphyses and hypoplasia of the extremities. The mechanism behind these anomalies is considered to be the vitamin K-deficiency. Vitamin K is essential for clotting proteins in the liver. However, extrahepatic tissues/organs i.e. cartilage and bone also contain vitamin K dependent proteins. In humans the development of bone structure is early in the pregnancy, whereas for rats it is late or even postnatally. This means that teratogenicity studies in rats can be negative because they are not exposed at the most critical period with the current guideline methods. Another problem is the high toxicity, making it difficult to give high enough doses without maternal deaths. Studies have been conducted with warfarin together with high doses of vitamin K. This treatment leads to an extrahepatic vitamin K deficiency, while the vitamin K dependent processes in the liver of the dams would have been preserved. In these studies the teratogenic effects of warfarin was confirmed. Without vitamin K supplementation and adapted study protocol, results on the teratogenic effects of warfarin have been equivocal. Based on warfarin data, human fetuses also seem to be much more vulnerable to vitamin K deficiency than rodent fetuses.

In 2006 the Commission Working Group of Specialised Experts in the field of Reproductive Toxicity (ECBI/51/07) concluded: "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 the standard pre-natal developmental toxicity studies to detect warfarin teratogenicity the predictive value 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 human.

Considering all 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".

The CEFIC Rodenticide Data Development Group has recently performed a study on warfarin in rats, to establish if the current OECD 414 guideline can detect the teratogenic potential of warfarin. This study was not requested by the competent authority in Sweden. The study was performed on the initiative of the CEFIC Rodenticide Data Development Group and is summarised in the warfarin dossier.

4.11.4 Summary and discussion of reproductive toxicity

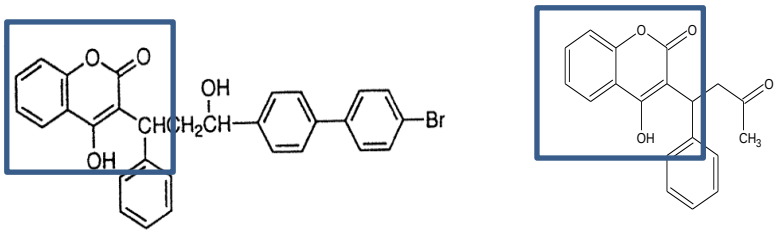
The bromadiolone study in rats (applicant Lipha Tech) had a treatment protocol corresponding to the TP1 protocol of the new warfarin study, where cataracts were only found in the top dose group with 1/99 findings i.e the sensitivity of the TP1 protocol to capture these effects seem rather low. No study similar to the TP2 protocol is available in rats in the bromadiolone dossiers and therefore it cannot be fully concluded whether bromadiolone can produce cataracts in the rat or not. Another factor complicating this issue is also that the dose span was rather narrow in the warfarin study and it is generally believed that the second generation AVK's are more toxic and thus have an even narrower window of effects. Therefore it is very difficult to assess any dose response of effects and also to distinguish if effects occur in presence or absence of maternal effects. Furthermore effects could be obscured by the high frequency of deaths in the mothers. No cataracts were reported in the rabbit studies for bromadiolone nor were bleedings in the fetuses reported.

The more recent teratogenicity study in rabbits shows some effects i.e. absent mesencephalon and proencephalone, rudimentary cerebrellum and absent vitrous body but the mortality in this study where high, leading to treatment of the high dose group for only 20 days and may therefore mask any clear trend of effects, further substantiating the difficulty of studying these highly toxic molecules in animal models. But these observed effects substantiate that there are significant concern that bromadiolone could cause warfarin like syndroms in humans and that read-across to warfarin should be made due to lack of human data and suitable animal studies. It also cannot be concluded that the warfarin study in rats show that there are a difference in effects in the OECD 414 studies of bromadiolone and warfarin. In addition the effects seen, though to a limited extent in the bromadiolone study, were on the CNS, which has also been described in the literature as congenital effects of warfarin.

The conclusion therefore is that bromadiolone is considered to be a possible developmental toxicant based on read across to human teratogenicity data for warfarin and requires the classification as Reprotoxic with the labelling R61, may cause harm to the unborn child and corresponding labeling according to CLP.

Read across rationale

Bromadiolone is a hydroxycoumarin with significant structural similarity to warfarin and forms of vitamin K. It is considered that this structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K. Human fetuses have much lower vitamin K levels than rat fetuses and mothers.



Bromadiolone Mw 527.40 Warfarin Mw 308.25

Fig. 4.11.4-1 Structural similarity of bromadiolone and warfarin, with the vitamin K similar part marked.

Anticoagulant rodenticides such as Bromadiolone function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver. Death is due to haemorrhage. The anticoagulant rodenticide active substances such as bromadiolone work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place (WHO Geneva, 1995 ISBN 92 4 157175 6).

The structural similarity of bromadiolone and the same mode of action as the known developmental toxicant warfarin need attention. Warfarin causes a specific kind of embryopathy when administered to humans in the first trimester (summarised below, detailed information can be found in the warfarin dossier). The deformities consist of skeletal anomalies including severe nasal hypoplasia, stippled epiphyses and hypoplasia of the extremities. The mechanism behind these anomalies is considered to be the vitamin K-deficiency. Humans are more sensitive

Two separate mechanisms have been proposed for the specific embryopathy of warfarin identified following first trimester exposure and the adverse CNS effects seen with second/third trimester exposure, according to the warfarin dossier.

Two vitamin K-dependent proteins have been characterised in the skeleton i.e. osteocalcin, (bone gla [γ-carboxyglutamic acid]), which is associated with hydroxyapatite crystals in the extracellular matrix, and matrix gla protein (MGP) that predominates in embryonic bone and cartilage extracellular matrix. It has been proposed that in the presence of Warfarin, γ-carboxylation of glutamate residues in osteocalcin is inhibited by preventing the reduction of vitamin K epoxide, resulting in poor calcium binding and the observed anomalies in bone formation. In normally developing cartilage MGP, which is synthesised in the growth plate cartilage, remains decarboxylated; this prevents the calcification of cartilage. In the presence of Warfarin, inappropriate calcification of cartilage occurs. Evidence has been provided to show that abnormal calcification of the nasal septum may be the underlying cause of this particular symptom of Warfarin embryopathy (Howe and Webster, 1992[1]). In this study rats were given daily s.c. doses of sodium warfarin (100 mg/kg) and vitamin K1 (10 mg/kg) for up to 12 weeks from birth. All rats survived without any signs of haemhorrage. The warfarin treated rats developed a marked maixillonasal hypoplasia with reduction in nasal bone length, large areas of calcification in the nasal septum, and abnormal calcium bridges in the epiphyseal cartilages of the vertebrae and long bones i.e. effects similar to the first trimester effects observed in warfarin syndrome in humans.

Inhibition of carboxylation of vitamin K-dependent clotting factors leading to intracranial haemorrhage is considered responsible for the CNS effects seen following exposure during the second and third trimesters (Pati and Holmbrecht, 1994[2]). No specific pattern of CNS abnormalities has been identified, and there is no correlation between time of exposure and CNS effects in humans (Hall et al., 1980). In a study where pregnant rats were treated with 100 mg/kg daily oral doses of sodium warfarin, supplemented with i.m vitamin K1(10 mg/kg), on day 9 to day 20 of gestation, haemhorrhage of fetal brain, face, eyes, ear and occasionaly limbs were observed. No haemhorraghes were observed in the mothers. The brain hemhorrhages were frequently intraventricular and caused various degrees of hydrocephaly. No bone defects were observed.The hemhorrhages was associated with treatment during second half of gestation. Localised hemhorrhage in the walls of cerebral hemispheres caused restricted areas of brain destruction, and

[1] Howe, A.M. and Webster, W.S. (1992), The Warfarin embryopathy: A rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology 46, 379-390.

[2] Pati S, and Helmbrecht G.D.(1994), Congenital schizencephaly associated with in utero warfarin exposure. Reprod Toxicol. Mar-Apr;8(2), 115-20.

haemhorraghes in the eye and ear were similarily associated with tissue distortion and destruction (Howe and Webster, 1990)[3]. Coumarins, during the first trimester, were associated with a high rate of spontaneous abortions, in addition to the incidences of specific embryopathy. Likewise, exposure during the first and second trimesters was also associated with a high rate of spontaneous abortion, stillbirths and related complications (developmental abnormality) (Hall et al., 1980[4]).

Teratogenicity studies of bromadiolone in animals are inconclusive due to the high toxicity observed. A study with bromadiolone and vitamin K supplementation to prevent mortality would have been useful but has not been performed. No human data on teratogenicity are available for bromadiolone. Therefore, based on the structural similarity of bromadiolone and warfarin, same mechanism of action and toxicity pattern, a read across to the human data on teratogenicity for warfarin is considered appropriate.

4.11.5 Comparison with criteria

No human data exist for bromadiolone. Bromadiolone was teratogenic in rabbit, inducing CNS effects in offspring, similar to what has been observed in humans after Warfarin treatment. Due to the structural similarity to and the same mode of action as the known developmental toxicant warfarin, read across to warfarin is applied and Repr. Cat. 1; R61 (according to the DSD) and Repr. 1A, H360D (according to CLP) is therefore required.

4.11.6 Conclusions on classification and labelling

Based on read across data from warfarin bromadiolone is considered to be a possible developmental toxicant. Classification as Reprotoxic Cat.1, with the labelling R61, may cause harm to the unborn child is required according to the DSD. Furthermore, Repr. 1A, H360D is required according to CLP.

Proposed specific concentration limits, taking into account acute, repeated dose toxicity and reproductive effects (DSD):

C≥0.5% T+;R61-26/27/28 - T; R48/23/24/25

0.25%≤C<0.5% T+; R26/27/28 – T; R48/23/24/25

0.025%≤C<0.25% T; R23/24/25 – T; R48/23/24/25

0.0025%≤C<0.025% Xn; R20/21/22 – R48/20/21/22

Proposed specific concentration limits, taking into account acute, repeated dose toxicity and reproductive effects (CLP): The discussion on how to set specific concentration limits for reproductive effects are currently under discussion and therefore no proposal is added at this point. But a specific concentration limit is needed for bromadiolone for reproductive toxicity.

[3] Howe, A.M. and Webster, W.S. (1990), Exposure of the pregnant rat to warfarin and vitamin K1: an animal model of intravetricular hemhorrhage in the fetus. Teratology 42, 413-420.

[4] Hall, J. G., Pauli, R. M., & Wilson, K. M. (1980). Maternal and fetal sequelae of anticoagulation during pregnancy. The American Journal of Medicine , 68, S. 122-140.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

There are no indications that bromadiolone may have neurotoxic properties. The toxicological studies do not indicate any neurotoxic effects. A neurotoxicity study would be scientifically unjustified and would not provide any new data. Based on this and animal welfare grounds it is deemed unnecessary to conduct a neurotoxicity study and applicant's justification was accepted.

4.12.1.2 Immunotoxicity

No data.

4.12.1.3 Specific investigations: other studies

The mechanism for bromadiolone as an anticoagulant is well known and no mechanistic studies were considered necessary.

4.12.1.4 Human information

Manufacturing plant personnel are subject to medical surveillance. No illnesses due to anticoagulants have been seen during the period 1987-1999, at two sites involved in rodenticide production (applicant Lipha Tech). Similarly, he applicant applicant Task Force states that no accidents or problems have occurred from 1975. .

Many poisoning incidents have been reported for second generation anticoagulants as a group to poisoning control centres etc., but only one of those cited in the dossier is clearly stated to be with bromadiolone. In this case two children were accidentally poisoned, with prolonged coagulation time as a result. Both children recovered after antidotal treatment with Vitamin K. 95 % of the reports for the group anticoagulants at an American poison control centre is stated to be accidental, and most of the remainder is classified as "intentional" and includes attempted suicides. A majority of the reports concerns children <6 years of age. During the time period 1996–1999 a total of 115 calls concerning bromadiolone were received by the Milan Poisons Center, 98 of which involved clinical cases among humans or animals. The most common route of exposure was through ingestion and in 55% of the cases children under the age of four years were exposed. The symptoms were reported in eleven human cases and included vomiting, gastric pyrosis and itching. Only one case was reported with haematological problems. Therapy included administration of activated charcoal, ipecac syrup, gastric lavage and vitamin K1 phytonadione. Other cases have been described in the literature where most symptoms included an increased tendency to bleed and effects thereof.

The closely-related active substance warfarin has been in use for over forty years as an anticoagulant drug in human medicine. It has been used in patients with clotting disorders, heart disease, atrial valve replacement, and more recently, deep vein thrombosis. Use is life-long for most patients with heart disease, clotting disorders or valve replacement.

There have been no reports of any increase in tumour incidence or of any adverse effects on human fertility. There have been no reports of neurotoxic or neurodegenerative disease, or neuro-muscular disease associated with the use of warfarin. Use during pregnancy is contraindicated.

4.12.2 Summary and discussion

Investigations of other effects do not indicate that any additional classification and labelling according to the DSA or CLP is warranted.

4.12.3 Comparison with criteria

Not applicable.

4.12.4 Conclusions on classification and labelling

See section 4.12.2.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 21a: Summary of available information on degradation

Method	Results	Remarks	Reference
Hydrolysis. US EPA 161-1	Stable to hydrolysis at pH 7 and 9	30 days test, 25°C Data sufficient for classification purposes under DSD or CLP (key study)	A 7.1.1.1.1 (LiphaTech)
Hydrolysis. OECD 111	Stable to hydrolysis at pH 7 and 9	120 days test, 50°C Data sufficient for classification purposes under DSD or CLP (key study)	A 7.1.1.1.1 (Task Force)
Photolysis. OECD draft and US EPA OPPTS 835.2210.	DT_{50} at 25°C 11.5 min. in sterile buffer solution and 14 min. in sterile pond water.	Not relevant for classification.	A 7.1.1.1.2-01 (LiphaTech)
Photolysis. US EPA OPPTS 835.2210.	DT_{50} 2.98 min. Biphasic degradation (fast degradation phase, summer) and 74.5 min. (slower degradation phase, summer)	Not relevant for classification.	A 7.1.1.1.2 (Task Force)
Ready biodegradability. CO_2 evolution measured as TOC. OECD 301B	No measurable degradation (0 % after 28 d), not readily biodegradable.	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.1.1.2.1 (LiphaTech)
Ready biodegradability. Closed bottle test. Oxygen content measured and degradation expressed as specific BOD in the percentage of ThOD. OECD 301D	Maximum degradation 31 %, not readily biodegradable	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.1.1.2.1 (Task Force)
Inherent biodegradability. CO_2 production measured as inorganic carbon. OECD 302D	Maximum degradation 2%, not inherently biodegradable	Not relevant for classification.	A 7.1.1.2.2 (Task Force)

5.1.1 Stability

The hydrolytic degradation of bromadiolone has been investigated under dark conditions at a temperature of 25°C in sterile aqueous buffer solution (1 mg/L) at pH values of 5, 7 and 9 (LiphaTech). The hydrolytic degradation of bromadiolone under sterile aqueous conditions after 30 days at a temperature of 25°C and a pH of 5 amounted to 6.3 %. Due to the poor correlation of the linear regression curve for degradation at this pH the estimated reaction rate constant is not considered reliable enough to calculate a DT_{50}. At pH values of 7 and 9 no significant degradation was observed. Correspondingly, bromadiolone is considered stable to hydrolysis. No significant

degradation products were observed. The study by Task Force was done at a temperature of 50°C, at the pH values of 7 and 9. They justified with a pre-test that testing of hydrolysis at pH 4 was not possible to perform accurately, due to the low solubility of bromadiolone at this pH. The results show that there was no hydrolysis of bromadiolone during the 120 days test.

The LiphaTech photolysis study shows that bromadiolone is degraded in aqueous solution when exposed to a representative artificial light source. The mean estimated photolysis DT_{50} at 25°C was 11.5 minutes in sterile buffer solution and 14 minutes in sterile pond water (estimated overall average 12.8 minutes). The values show good correlation to pseudo first order kinetics. A recalculation of the half-lives to minutes of natural summer sunlight at latitudes 40°N and 50°N resulted in DT_{50} values for the buffer solution of 29 and 28 minutes, respectively. For the sterile pond water the corresponding DT_{50} values were 35 to 36 minutes, respectively. In the study presented by Task Force a dilute (0.0039 mM) aqueous solution of bromadiolone was exposed to natural sunlight at 52° north. The first ten minutes 68 % of the bromadiolone was degraded which was followed by a slower degradation rate, and complete photolysis had occurred after approximately two hours.

LiphaTech also studied photolytic degradation. Photolysis of bromadiolone led to the formation of carbon dioxide (8.8 to 15.4 % of AR) and 15 other degradation products, separated by TLC. Six of these degradation products exceeded 10 % of AR, but none were identified. Maximum levels were between 10.9 and 33.3 % of AR. The applicant's argument for not identifying the major transformation products is that the majority of these metabolites were past their maximum levels after at the end of the study (after 15 days), indicating that they are transient in nature.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No information.

5.1.2.2 Screening tests

The ready biodegradability of bromadiolone has been investigated by LiphaTech in a laboratory study according to OECD guideline 301B (CO_2 evolution test). The test showed that bromadiolone is not readily biodegradable in the environment, according to the criteria of test. To be classified as biodegradable 60 % or more of the active substance should have been degraded after 28 days whereas in the test there was no measurable degradation of bromadiolone. In the corresponding test by Task Force the maximum degree of degradation was 31 %, which also fulfils the criteria for being not readily biodegradable. Inherent biodegradability was studied by Task Force according to OECD 302D whereby the test substance was incubated in a buffered, mineral salts medium which had been inoculated with a mixed population of micro-organisms. The degradation (inorganic carbon production) was maximum 2% during the test, and minimum 20% is required to pass the test. Bromadiolone is therefore considered as not inherently biodegradable.

5.1.3 Summary and discussion of degradation

No hydrolysis was found at pH 7 or 9, so hydrolysis of bromadiolone is not expected to be a significant process in the environment. Photolysis of bromadiolone in aqueous solution is rapid with a half-life of 12 hours or less. Photolytic degradation was studied by LiphaTech and led to the formation of carbon dioxide and significant levels of six unidentified degradation products which had either reached plateau levels or were declining at the end of the study (15 days). Bromadiolone

is not readily biodegradable under environmentally relevant conditions or during sewage treatment processes. It is also not inherently biodegradable. Therefore, the overall conclusion is that bromadiolone fulfills the criteria for being not biodegradable.

5.2 Environmental distribution

Table 21b: Summary of available information on distribution

Method	Results	Remarks	Reference
Adsorption/desorption in soil. US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 163-1/ OECD 106	K_{OC} was calculated to 1563 to 1709 mL/g	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.1.3 (LiphaTech)
Adsorption/desorption in soil. OECD 106	K_{OC} was calculated to 3530 to 41600 mL/g	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.1.3 (Task Force)

Bromadiolone is strongly adsorbed to soil and K_{OC} values range between 1563 and 41600 mL/g, including results from both applicants, which corresponds to 'slightly mobile' to "non-mobile" according to the SSLRC classification index. Laboratory soil column leaching and aged leaching studies performed by LiphaTech indicate that bromadiolone and any potential degradation products, even if released indirectly to soil in small quantities, are not likely to move through the soil profile and are unlikely to reach groundwater in significant quantities. The rapid photolysis rate in air ($t_{½}$ ca 2 hours), the low vapour pressure of bromadiolone and the low Henry's law constant together show that bromadiolone is not expected to volatilise to or persist in air in significant quantities. A strong tendency to adsorb to sediment combined with a high degree of photo-instability means that bromadiolone is unlikely to remain in the water column of surface waters. The information on studies on distribution is summarised in Table 21b.

5.2.1 Adsorption/Desorption

The sorption properties of bromadiolone have been investigated by LiphaTech in a laboratory adsorption/desorption study. The amount of bromadiolone adsorbed to soil was 66.0 to 81.2% during the adsorption phase. The Freundlich soil sorption coefficient normalised for organic carbon content (K_{OC}) was calculated to 1563 to 1709 mL/g (mean value 1632 mL/g). This indicates that bromadiolone is 'slightly mobile' according to the SSLRC classification index (K_{oc} 1000-4000 = slightly mobile and K_{oc} > 4000 = non-mobile). Also the Task Force has performed a sorption study, which was conducted with five different soils and the resulting K_{oc} values ranged between 3530 and 41600 with three out of five values being above 4000, which would lead to the conclusion that bromadiolone is practically non-mobile in soil.

5.2.2 Volatilisation

The vapour pressure of bromadiolone at ambient temperature has been determined by LiphaTech to be 2.13×10^{-8} Pa (OECD 104) and by. Furthermore, Henry's law constant for bromadiolone has been calculated to 8.99×10^{-7} Pa·m^3/mol (based on a water solubility of 12.5 mg/L). The corresponding data from Task Force is a vapour pressure of $1*10^{-7}$ Pa and Henry's law constant of 4.25×10^{-4} Pa*m^3/mol. References to these studies can be found in section 3 above. Based on these data bromadiolone is not considered volatile and is not expected to partition into air in significant

quantities. In addition, the photochemical oxidative degradation half-life of bromadiolone in air has been estimated using the Atmospheric Oxidation Program v1.90 (AOPWIN), which is based on the structural activity relationship (QSAR's) methods developed by Atkinson, R (1985 to 1996). The half-life for the hydroxyl reaction in air (based on a hydroxyl radical concentration of 1.5 x 10^6 OH radicals per cm^3) is estimated to 2.1 hours and the ozone reaction in air is estimated to 2.0 hours (LiphaTech reference A 7.3.1). A similar calculation has been performed by Task force with similar result (Task force reference A 7.3.1). In conclusion, bromadiolone is not expected to volatilise to or persist in air in significant quantities.

5.2.3 Distribution modelling

No information.

5.3 Aquatic Bioaccumulation

Table 22: Summary of available information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient. EEC A.8 (shake-flask method)	Log K_{ow}=4.07 at pH 7, 20°C log BCF_{fish} = 0.85·log K_{ow} – 0.70 BCF = 575	Data sufficient for classification purposes under DSD or CLP (key study)	A 3.9/01 (LiphaTech)
Partition coefficient. EEC A.8 and OECD 107 (shake-flask method)	Log K_{ow} =3.8 at pH 7.1, 25°C log BCF_{fish} = 0.85·log K_{ow} – 0.70 BCF = 339	Data sufficient for classification purposes under DSD or CLP (key study)	A 3.9 (Task Force)
Bioconcentration in fish. Bluegill sunfish (*Lepomis macrochirus*). General accordance with OECD 305E	BCF 460 for whole fish.	Not reliable due to high mortality (44%) of the test animals compared to 1% in the control.	A 7.4.3.3.1-01 (LiphaTech)
Bioconcentration in fish. Channel catfish (*Ictalurus punctatus*). No guideline compliance claimed.	BCF 74 for whole fish.	Not reliable due to high mortality (47%) of the test animals compared to 0% in the control and lack of analysis of the test substance.	A 7.4.3.3.1-02 (LiphaTech)
Bioconcentration in fish. Rainbow trout (*Oncorhynchus mykiss*). OECD 305	No results, early termination due to high mortalities.	Not reliable due to high mortality (>30%) of the test animals making early termination of the test necessary. Insufficient data was generated to produce uptake and depuration curves.	A 7.4.3.3.1 (Task Force)

Two studies have been conducted by LiphaTech of bioconcentration in the tissues of fish under artificial conditions in the laboratory. In a study with bluegill sunfish the maximum bioconcentration factor for bromadiolone was 460 for whole fish. In non-edible tissues the

maximum BCF was 1,658 and in edible tissues 161. In a second study with channel catfish, the bioconcentration factors in whole fish ranged from 24 (day 1) to 74 (day 14). In edible and non-edible tissues the maximum bioconcentration factors were 59 and 641, respectively. In both these studies the reliability was low due to major deficiencies in reporting and too high mortality in the exposed group of fish. Also, a fish bioconcentration study with rainbow trout was performed by Task Force, but it failed due to high mortalities of the fish. Taken together, the fish bioconcentration studies are of low reliability, but the references are included for information. Consequently, BCF was derived by calculation from log K_{ow}, using equation 74 in the TGD[5] (log BCF_{fish} = 0.85·log K_{ow} – 0.70) resulting in BCF values ranging between 339 (based on a log K_{ow} of 3.8 at pH 7.1, Task Force) and 575 (based on a log K_{ow} of 4.07 at pH 7, LiphaTech). The information on studies on aquatic bioaccumulation is summarised in Table 22.

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

See above (5.3).

5.3.1.2 Measured bioaccumulation data

See above (5.3).

5.3.2 Summary and discussion of aquatic bioaccumulation

Our conclusion is that bromadiolone has potential to bioaccumulate. This is also in line with a conclusion that was drawn as a result of a PBT assessment which was carried out for all existing anticoagulant biocidal active substances (i.e. which are included in the review programme under the directive 98/8/EC) bromadiolone is a potential PBT substance. According to this assessment, which was done by the TCNES Subgroup on Identification of PBT and vPvB Substances and finalised in 2008, bromadiolone is considered persistent and toxic, whilst there was some uncertainty regarding the bioaccumulation criterion and the conclusion therefore was that bromadiolone has potential to bioaccumulate. Hence the designation as potential PBT substance. A comparison with the criteria for bioaccumulating substances, i.e. a BCF > 100 (according to DSD) and BCF > 500 (according to CLP) indicates that bromadiolone fulfills these criteria.

5.4 Aquatic toxicity

Table 23: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference

[5] European Commission. 2003. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

Acute toxicity to fish. *Oncorhynchus mykiss.* Semi-static test. Limit test with only one test concentration. OECD 203	96 h LC_{50} > 8.0 mg/L (nominal) Analysis showed that test concentration was 96-102 % of nominal.	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.4.1.1-01 (LiphaTech)
Acute toxicity to fish. *Oncorhynchus mykiss.* Semi-static test. OECD 203	96 h LC_{50} = 2.86 mg/L (nominal) Analysis showed that test concentrations were 95-102 % of nominal.	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.4.1.1 (Task Force)
Acute toxicity to invertebrates. *Daphnia magna.* Flow-through test, dim light. US EPA 72-2 (comparable to OECD 202)	48 h LC_{50} = 2.0 mg/L (lethality, mean measured concentration)	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.4.1.2 (LiphaTech)
Acute toxicity to invertebrates. *Daphnia magna.* Static test, darkness. OECD 202	48 h EC_{50} = 5.79 mg/L (immobilization, nominal concentration) Analysis showed that test concentrations were 99-107 % of nominal.	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.4.1.2 (Task Force)
Growth inhibition of algae. *Scenedesmus subspicatus.* Static test. OECD 201	96 h E_bC_{50} = 0.17 mg/L (recalculated 72 h E_rC_{50} >1 mg/L) (nominal concentrations) Analysis had deficiencies, but for two test concentrations the values were 97-150 % of nominal. The analysis results of the test substance at the end of the test were below detection, so the degradation rate of the substance in the test is not known. The likely rapid photolytic degradation of the test substance in the test leads to the conclusion that the real EC_{50} is below 1 mg/L.	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.4.1.3 (LiphaTech)
Growth inhibition of algae. *Pseudokirchneriella subcapitata.* Static test. OECD 201	72 h E_rC_{50} 1.14 mg/L (geometric mean test concentration calculated from the initial measurements and half of the LOQ of 0.3 mg/L) The analysis results of the test substance at the end of the test were below detection, so the degradation rate of the substance in the test is not known. The likely rapid photolytic degradation of the test substance in the test leads to the conclusion that the real EC_{50} is below 1 mg/L.	Data sufficient for classification purposes under DSD or CLP (key study)	A 7.4.1.3 (Task Force)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Based on the results of acute toxicity studies, bromadiolone is toxic to fish (*Oncorhynchus mykiss*). In the LiphaTech study, rainbow trout (*Oncorhynchus mykiss*) was exposed to bromadiolone under semi-static conditions during 96 hours in darkness. The study was performed as a limit test with only one test concentration of 8.0 mg/L and a solvent control group. The result was that 96 h LC_{50} exceeded 8.0 mg/L, the single concentration applied and confirmed by analysis. Analysis of the test concentration was performed after 0, 24 and 96 hours and the measured concentration was found to be 96-102 % of the nominal concentration. No fish died at the limit concentration. In the Task Force study rainbow trout was exposed to bromadiolone during 96 h under semi-static conditions with a light regime of 12 hour dim light/12 hour darkness. 96 h LC_{50} was 2.86 mg/L (nominal concentration, test concentrations of bromadiolone were measured before and after each change of medium and the measured concentrations were all within the range 95-102 % of nominal).

5.4.1.2 Long-term toxicity to fish

Not available. Studies waived due to limited exposure to the aquatic environment.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of bromadiolone to invertebrates has been tested by LiphaTech in a 48-hour flow-through study with *Daphnia magna*. Analysis of the test concentration was performed after 0 and 48 hours and was found to be 74-92 % of the nominal concentration. The study was performed with a 16 hours photoperiod in dim light. In a corresponding study by Task Force the acute toxicity to *Daphnia magna* was determined in a static system. The study was conducted in the dark due to the photosensitivity of the test material. *Daphnia magna* was similar in sensitivity to fish, with a 48 h EC_{50} of 5.79 mg/L (Task Force) and a 48 h LC_{50} of 2.0 mg/L (LiphaTech). The LiphaTech endpoint was based on lethality rather than immobilisation and on mean measured concentrations of bromadiolone in the test media. It is possible that the value would be somewhat lower if the endpoint were based on immobility. The Task Force result is based on nominal concentrations, but the actual test concentrations in water were measured at 0 and 48 h and the recovery rate was 99-107 %.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Not available. Studies waived due to limited exposure to the aquatic environment.

5.4.3 Algae and aquatic plants

The effect of bromadiolone on algal growth has been tested by LiphaTech in a laboratory study using the green alga *Scenedesmus subspicatus* and 6 different concentrations of bromadiolone together with control and solvent control. Light conditions were 8000 lux continuous light. Concentrations of bromadiolone were reduced to below the limit of quantification under the conditions of the 96 h test. Also, the analysis of bromadiolone during the test had deficiencies and resulted in useful values for only two of the initial test concentrations, and the rest were below detection (including the highest initial concentration). The light intensity used represents about 80

% of the light intensity that was reported in the photolysis study and it is therefore reasonable to assume that extensive photolytic degradation of bromadiolone took place during the study, and that the level of bromadiolone has decreased to below LOQ much earlier than after 96 h.. However, given the conditions of the recommended guideline, it is difficult to avoid degradation of the test substance. Further, there is no feasible alternative to a static test regime, since it is not practicable to renew the test medium or arrange a flow through system with microalgae as test organisms. The 96 h E_bC_{50} for *Scenedesmus subspicatus* was 0.17 mg/L and the NOEC with respect to biomass yield was 0.037 mg/L. Levels of growth inhibition recalculated to specific growth rates were included by LiphaTech at a later stage and the resulting 72 h E_rC_{50} of >1 mg/L, with 39.3 % inhibition at 72 h at thishighest tested concentration, is presented for comparison. The NOEC value, however, remains unchanged. Due to the shortcomings of this study described above RMS considers that there is a large uncertainty regarding the reported effect values and that they lead to a significant underestimation of the toxicity.

In the Task Force study the toxicity of bromadiolone was determined over 72 h with *Pseudokirchneriella subcapitata* as test alga. The test was performed in a static system with continuous light at 5500 lux and DMSO was used to increase the solubility of bromadiolone. The resulting 72 h E_rC_{50} was 1.14 mg/L. Due to the rapid photolysis of the test substance, the test concentrations used to express the results were calculated by the Task Force according to the OECD Guidance document on aquatic toxicity testing of difficult substances and mixtures (the geometric mean test concentrations were calculated from the initial measurements and half of the LOQ of 0.3 mg/L). However, it is likely that the photolytic degradation is much faster than what can be seen as a value below LOQ after 72 h. The photolytic half-life reported from the Task Force photolysis study is expressed in minutes, and the light conditions of the Task Force algal study may be estimated to ca 1/20 of that in the photolysis study (full sunlight). Therefore, it is possible that the test concentrations used in the algal test may have decreased to below LOQ much earlier than after 72 h. Although this study is better performed than the LiphaTech study the RMS still considers that the resulting effect value (E_rC_{50}) may be a significant underestimation of toxicity.

Algae represented the most sensitive of the three aquatic trophic levels tested, in spite of the fact that the conditions necessary in algal growth inhibition tests are the ones most likely of all the aquatic acute toxicity tests to result in lowering of exposure concentrations, based on the photo-instability of bromadiolone in aqueous solution. Therefore, RMS considers that it is highly likely that the actual test concentrations that cause 50% inhibition of algal growth are below 1 mg/L, particularly in the LiphaTech study but also in the Task Force study.

Taken together, the RMS considers that it is highly likely that the real 72 h E_rC_{50} for bromadiolone is lower than 1 mg/l.

5.4.4 Other aquatic organisms (including sediment)

No information.

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

According to the CLP criteria a substance should be assigned to hazard class Acute Category 1 if EC_{50} of the most sensitive organism is lower than 1 mg/L. According to the conclusion above (5.4.3) which takes into account expert judgement (as denoted in the guidance document “Guidance on the application of the CLP criteria” section 4.1.3.3.1) this is considered fulfilled for bromadiolone. Consequently, bromadiolone also fulfils the criteria for hazard class Chronic Category 1, since it is not rapidly degradable and has potential to bioaccumulate.

Since the submission of this CLH report, ATP2 (second adaption to technical progress) to CLP has been published, and brings in new criteria for classification of long-term hazards for the aquatic environment (e.g. the use of chronic toxicity data). The only chronic toxicity data available for bromadiolone is the NOEC from the algal study, which is not a true chronic study, although it may according to the TGD be considered as chronic if chronic data from another taxonomic group is also available. In any case, the chronic NOEC of 0.037 mg/L would according to ATP 2 lead to the classification Chronic category 1, and will thus not affect the environmental classification.

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

The suggested classification for bromadiolone is Acute Category 1, M-factor 1 and Chronic Category 1, M-factor 1. This is in line with the classification of bromadiolone which was agreed by TC C&L in November 2006.

6 OTHER INFORMATION

7 ANNEXES

Annexed to the CLH report are robust study summaries of relevant studies in the form of excerpts of Document III level from biocides CA reports. The study summaries have been slightly edited in order to remove some sensitive information. The study summaries are grouped with respect to the two applicants and to subject (phys/chem, toxicology and ecotoxicology) and are available in a separate document to the CLH report. The following annexes are included:

Annex no.	Annex name	Contents
7.1	Study summaries of physico-chemical studies, LiphaTech	DOCUMENT III-A Section 3: Physical and Chemical Properties (LiphaTech)
7.2	Study summaries of physico-chemical studies, Task Force	DOCUMENT III-A Section 3: Physical and Chemical Properties (Task Force)
7.3	Study summaries of toxicology studies (human health), LiphaTech	DOCUMENT III-A Section 6: Toxicological and metabolic studies (LiphaTech)
7.4	Study summaries of toxicology studies (human health), Task Force	DOCUMENT III-A Section 6: Toxicological and metabolic studies (Task Force)
7.5	Study summaries of environmental studies, LiphaTech	DOCUMENT III-A Section 7: Ecotoxicological profile including environmental fate and behaviour (LiphaTech)
7.6	Study summaries of environmental studies, Task Force	DOCUMENT III-A Section 7: Ecotoxicological profile including environmental fate and behaviour (Task Force)