Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Bromadiolone (ISO); 3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one

EC number: 249-205-9
CAS Number: 28772-56-7

CLH-O-0000005446-71-01/F

Adopted

14 March 2014

14 March 2014

CLH-O-0000005446-71-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: Bromadiolone (ISO); 3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one

EC number: 249-205-9
CAS number: 28772-56-7

The proposal was submitted by **Sweden** and received by the RAC on **12 February 2013.** All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 March 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 April 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Veda Marije Varnai**

Co-rapporteur appointed by the RAC: **Jose Luis Tadeo**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **14 March 2014** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **Bromadiolone** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry										
Dossier submitters proposal	607-716-00-8	bromadiolone (ISO); 3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one	249-205-9	28772-56-7	Repr. 1A Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H300 H310 H330 H372 H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 H410		STOT RE 1; H372 (blood): C ≥ 0,02 % STOT RE 2; H373 (blood): 0,01 % ≤ C < 0,1 % M=1 M=1
RAC opinion					Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H300 H310 H330 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 (blood) H410		Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372 (blood): C ≥ 0,005 % STOT RE 2; H373 (blood): 0,0005 % ≤ C < 0,005 % M=1 M=1

Resulting Annex VI entry if agreed by COM					Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H300 H310 H330 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 (blood) H410		Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372 (blood): C ≥ 0,005 % STOT RE 2; H373 (blood): 0,0005 % ≤ C < 0,005 % M=1 M=1

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

Bromadiolone belongs to a group of compounds known as the anticoagulant rodenticides, i.e. those with an anti-vitamin K (AVK) mode of action (MoA) which are used mainly as active substances in biocidal products for pest control of rats, mice and other rodents. Some of the substances had an existing harmonised classification. However, at the time of writing, only Warfarin is currently classified for toxicity to reproduction in category 1A.

Eight AVK rodenticides were previously discussed by the Technical Committee on Classification and Labelling of Dangerous Substances (TC C&L) of the European Chemicals Bureau (ECB) (2006 – 2008). However, the work was transferred to ECHA and to that end Member State Competent Authorities (MSCAs) were requested to prepare CLH proposals.

CLH proposals for eight AVK rodenticides, Coumatetralyl (Denmark), Difenacoum (Finland), Warfarin (Ireland), Brodifacoum (Italy), Flocoumafen (The Netherlands), Difethialone (Norway) Chlorophacinone (Spain) and Bromodialone (Sweden), were submitted by eight different Dossier Submitters (DS). The dossiers were handled as a group but the Committee for Risk Assessment (RAC) proceeded to evaluate the proposals on a substance by substance basis comparing the human data available for Warfarin (and other AVKs) and relying on a weight-of–evidence approach as required by Regulation 1272/2008 (CLP).

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

Bromadiolone is not highly flammable, does not contain any chemical groups known to possess oxidizing properties, does not undergo self-ignition below its melting point (~210°C), and is thermally stable up to at least 150°C in air and nitrogen-atmosphere. Therefore, it is not classified for physico-chemical properties.

Comments received during public consultation

This endpoint was not commented on.

Assessment and comparison with the classification criteria

Since Bromadiolone does not have explosive, oxidising or self-ignition properties, RAC supported the non-classification for physico-chemical properties, as proposed by the DS.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Three acute oral toxicity studies (two in rats, one in dogs), two acute dermal toxicity studies (one in rats, one in rabbits), and one acute inhalation toxicity study (in rats) are available.

Acute oral toxicity
Two acute oral toxicity studies in rats (in accordance with US EPA Guideline 81-1, equivalent to OECD 401) showed that Bromadiolone was very toxic with an oral LD_{50} of >0.56 mg/kg:

- in the first study in rats LD_{50} values were estimated to be between 0.56 and 0.84 mg/kg bw;

- in the second study in rats LD_{50} values were estimated to be 1.43 mg/kg bw for males, 1.25 mg/kg bw for females and 1.31 mg/kg bw for combined sexes.

Most deaths occurred between post-exposure days 3 and 10 in the first study, and day 6 and 14 in the second study. The necropsies confirmed the occurrence of internal haemorrhage.

Acute oral toxicity studies in dogs (in accordance with US EPA Guideline 86-1) showed no apparent difference between males and females, with acute LD_{50} estimated to be 8.1 mg/kg for combined sexes. Deaths occurred between post-exposure days 6 and 12, due to internal haemorrhage.

An acute dermal toxicity study in rabbit (in accordance with US EPA Guideline 81-2, equivalent to OECD 402) showed LD_{50} values of 1.3 mg/kg bw for males, 2.38 mg/kg bw for females and 1.71 mg/kg bw for combined sexes. Deaths occurred between post-exposure days 6 and 14, due to internal haemorrhage.

Acute dermal toxicity
An acute dermal toxicity study in rats (in accordance with OECD 402) showed LD_{50} values of 20.62 mg/kg bw for males, 32.08 mg/kg bw for females and 23.31 mg/kg bw for combined sexes. Deaths occurred between post-exposure days 5 and 14, due to internal haemorrhage.

Acute inhalation toxicity
An acute inhalation toxicity study in rats (in accordance with US EPA Guideline 81-3, equivalent to OECD 403) was conducted with Bromadiolone as an undiluted powder (4h exposure period, nose only). Study results showed LC_{50} values of 0.46 µg/L for males, >0.33 and <0.46 µg/L for females and 0.43 µg/L for combined sexes. Deaths occurred between post-exposure days 4 and 9, due to internal haemorrhage.

It was concluded by the DS that Bromadiolone is acutely toxic by the oral, dermal and inhalation routes, causing death as a result of internal haemorrhages and that it should be classified in the acute toxicity hazard category 1 (oral, dermal and inhalational route) with the hazard statements H300, H310 and H330 (respectively).

Comments received during public consultation

Two Member States supported the proposed classification.

Assessment and comparison with the classification criteria

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

- Acute Tox. Cat. 1 - H300: Fatal if swallowed (acute oral LD_{50} between 0.56 and 1.43 mg/kg in rats are lower than ATE ≤ 5 mg/kg)
- Acute Tox. Cat 1 - H310: Fatal in contact with skin (acute dermal LD_{50} of 1.71 mg/kg bw for combined sexes in rabbits and of 23.31 mg/kg bw for combined sexes in rats are both lower than ATE ≤ 50 mg/kg)
- Acute Tox. Cat 1 - H330: Fatal if inhaled (LC_{50} value of 0.00043 mg/L for combined sexes in rats is lower than ATE ≤ 0.05 mg/L for dusts and mists).

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

Summary of the Dossier submitter's proposal

There was no proposal for specific target organ toxicity – single exposure because no data was available.

Comments received during public consultation

This endpoint was not commented on.

Assessment and comparison with the classification criteria

In the opinion of RAC, the blood coagulation system is affected after single exposure since it was the main cause of mortality in acute studies. However, classification for STOT-SE for Bromadiolone is not warranted since it is covered by the classification as Acute Tox. 1.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

The results of two skin irritation studies conducted in rabbits were considered by the DS. Study results showed average scores of zero for both erythema and oedema in all animals at 24, 48 and 72 h after application.

The first study is of low reliability since abraded sites on the skin were used, the reporting of methods and results was inadequate and the assessment times do not cover full range of EC guideline criteria. However, no indications of irritation were found. Studies on the product supported the absence of skin irritation according to the DS but the concentration of Bromadialone in the products was not given.

The second study had some minor deviations from the OECD TG 404 (e.g. minor decrease in purity level, the test site was not examined immediately after protective patch was removed, all six animals were treated simultaneously). In that study, approximately 0.5 g of Bromadiolone was moistened with 2.0 ml of deionised water and applied to an area of at least 8 cm x 8 cm on the dorsal area of the trunk, No indication of irritation was observed.

The DS considered that these deviations should not affect the outcome of the 2nd study, and concluded that Bromadiolone does not cause irritation when it is in contact with rabbit epidermis. Bromadiolone does not fulfil the CLP criteria for classification as a skin irritant.

Comments received during public consultation

This endpoint was not commented on.

Assessment and comparison with the classification criteria

The results of skin irritation studies in rabbits showed zero average score for erythema and oedema in all animals at all time points (24, 48 and 72 h).

Also, in a skin sensitisation Buehler test in guinea pigs no visible changes were found after induction and challenge applications of Bromadiolone at a concentration of 5%. In the pilot test, 0.5 ml of test formulation with 0.001, 0.1, 0.1, 1, 5, 10, 25 or 50% Bromadiolone produced no reaction on guinea pigs' skin.

Although in the acute dermal toxicity study in rabbits skin reactions were observed (erythema was found in one male and one female and oedema in one male), they appeared at the highest dose applied (4.0 mg/kg bw, 24-hour exposure) at which four males and all five females died.

Since no skin reactions were observed in dermal irritation studies in rabbits and in a skin sensitisation study in Guinea pigs and erythema and oedema in acute dermal toxicity study in rabbits were found only at the dose with 90% mortality (at dermal exposure duration of 24 hours), RAC supports the conclusion of the DS that Bromadiolone should not be classified for skin irritation.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Two eye irritation studies in rabbits were presented by the DS in the CLH report. The first study was not considered suitable for the evaluation of eye irritation properties of Bromadiolone, primarily due to poor reporting. Study results do not indicate substantial eye irritating properties, which is supported by studies on the product according to the DS but the concentration of Bromadialone in the products was not given..

The second study was conducted in accordance with US EPA Guideline 81-4, equivalent to OECD TG 405. Study results showed that Bromadiolone was mildly irritating to the rabbit eye, but that the irritation reaction was reversible by post-exposure day 4. Therefore, the DS concluded that Bromadiolone does not fulfil the CLP criteria for classification as an eye irritant.

Comments received during public consultation

This endpoint was not commented.

Assessment and comparison with the classification criteria

RAC evaluated the results obtained in the second study. The results summarised in the table below have been obtained from the CAR IIIA document

	Cornea	**Iris**	**Conjunctiva**		
			Redness	Chemosis	Discharge
60 min	0.17	0	2	1.33	1.17
24 h	0.17	0	0.83	0.33	0.33
48 h	0.17	0	0.33	0.17	0.17
72 h	0	0	0.17	0	0
Average (24, 48 and 72h)	**0.11**	**0**	**0.44**	**0.17**	**0.17**
Reversibility	complete (in 72 h)	-	complete (in 4 days)	complete (in 72 h)	complete (in 72 h)

The average scores following grading at 24, 48 and 72 hours after instillation of the test material were lower than 1 for corneal opacity and iritis, lower than 2 for conjunctival redness and chemosis, and were fully reversible within 4 days of the study. Therefore, RAC agreed with the DS that Bromadiolone does not warrant classification for eye corrosion/irritation, because the observed effects did not meet the classification criteria under CLP.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The skin sensitising potential of Bromadiolone was tested in three Buehler tests in guinea pigs conducted in accordance to US EPA Guideline 81-6, equivalent to OECD TG 406. The study results are as follows:

- in the first study Bromadiolone did not elicit reactions typical of skin sensitisation, but the study is of low reliability due to OECD test guideline deviations and inadequate positive control response
- in the second study no results could be obtained due to stained skin by test substance
- in the third study which is of high reliability, Bromadiolone did not cause skin sensitisation. Topical inductions and challenge were performed by Bromadiolone in concentration of 5%, since at concentrations ≥10% mortality was observed in the pilot test. No skin

sensitisation reactions were noted in treated or control animals, while positive reactions were seen in 15/20 positive control animals treated with potassium dichromate.

Overall, the DS concluded that Bromadiolone does not cause skin sensitisation and does not fulfil the CLP criteria for classification as a skin sensitiser.

Comments received during public consultation

This endpoint was not commented.

Assessment and comparison with the classification criteria

RAC evaluated the results obtained in the third Buehler test. In that study zero scores at 24 and 48h after challenge were recorded in all treated and vehicle control animals (according to Magnusson and Kligman grading scale), while 75% of positive control animals showed positive results (with the mean score of 1.85 and 1.80 at 24 and 48 hours after challenge, respectively).

RAC therefore supports DS proposal that Bromadiolone should not be classified as skin sensitiser.

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

Summary of the Dossier submitter's proposal

Five oral repeated-dose studies conducted on various species (pig, ferret, dog, rat and rabbit) were presented in the CLH dossier.

Studies in pigs and ferrets were non-guideline studies, but demonstrated that pigs were less sensitive than ferrets. In ferrets dosed at 5 ppm, five consecutive doses induced haemorrhages (within 4-5 days) and death (on day 6). The LC_{50} value in ferrets was calculated to be 7.6 ppm (approximately 0.4 mg/kg bw/day). Pigs 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 oral toxicity study in Beagle dogs (3 animals/sex/group) was conducted in accordance to US EPA Guideline 82-1, equivalent to OECD TG 409. Three dogs per sex per group were dosed with bromadilone at 0, 8, 20 or 50 µg/kg bw/day in capsules. The following results were obtained:

- At 50 µg/kg bw/day all animals died within 21 to 32 days with the exception of one female who survived despite marked increases in coagulation and prothrombin times, reduced erythrocyte counts and haemoglobin levels, and a marked increase in leukocyte numbers;

- At 20 µg/kg bw/day severe haemorrhages were observed, four (out of six) animals were sacrificed on humane grounds on days 64 to 84 of the study; prothrombin time was 5-fold increased in males and 25-fold increased in females by the end of the study; in males significantly lower erythrocyte counts were observed at week 4;

- At 8 µg/kg bw/day no treatment related clinical signs were observed; body weights, weight gains and food consumption were similar to controls; prothrombin and coagulation times were not significantly different from controls and no treatment-related macroscopic or histopathological changes were found at necropsy.

In surviving animals there were no toxicologically significant effects in the ophthalmological examinations, ECGs and blood pressure measurements, or clinical chemistry and urinalysis (sampled at week 4 or week 13). There were no effects of treatment on relative organ weights. There were no indications of other toxic effects, and histopathology revealed no hypertrophy or hyperplasia of the liver. The results from two preliminary studies support the findings in the main study.

In the main study, LOAEL of 20 µg/kg bw/day (based on haemorrhagic events) and NOAEL of 8 µg/kg bw/day were determined.

A 28-day oral (gavage) toxicity study in Wistar rats was performed in accordance to OECD TG 407. The study was performed in two parts, Study 1 and Study 2, in 5 rats per sex, per dose: Study 1 (doses of 0, 0.1, 0.5, and 1 mg/kg bw/day) and Study 2 (doses of 0, 0.0025, 0.05 mg/kg bw/day).

All animals dosed at 0.05, 0.1, 0.5 and 1 mg/kg bw/day died prematurely. At necropsy haemorrhagic events , serious hepatitis (at all dose levels), and centrilobular hepatic necrosis (at 0.05 and 0.5 mg/kg/day in males) were observed.

At 0.0025 mg/kg bw/day (Study 2) there were no significant treatment-related effects on average body weights or daily mean food intake. In males there was a decrease in the mean corpuscular haemoglobin concentration (MCHC) and in the monocyte count whereas in females there was a decreased red blood cell distribution width. Prothrombin time was not reported to be significantly different in treated rats compared to controls. At necropsy, calcium deposits, uterus dilation and focal proliferation of mononuclear phagocyte system cells in the liver were slightly more common in treated rats than in controls, but were considered of no biological significance.

Based on Study 1 and Study 2, a LOAEL of 0.05 mg/kg bw/day (based on 100% mortality due to internal haemorrhages), and a NOAEL of 0.0025 mg/kg bw/day were determined.

A 90-day oral toxicity study in New Zealand white rabbits was performed in accordance to OECD 409. Bromadiolone was administered by gavage at dose levels of 0, 0.1, 0.5, and 1 µg/kg bw/day to 6 animals/sex/group. No treatment-related mortality was observed. At 0.5 µg/kg bw/day two females died due to acute bacterial invasion, not considered to be due to treatment. Bromadiolone infrequently produced diarrhoea in treated rabbits.
Paleness of the skin or mucous membranes was seen in females in all dose groups, and in males dosed at 0.5 and 1 µg/kg bw, with a clear dose-response relationship in both genders.

There were no treatment related effects on body weight gain, food consumption, ophthalmologic examination or blood marrow smears. No prominent pathological organ changes were observed at necropsy: pituitary weight was increased in all dose groups compared to controls (21-34%) without a clear dose-response, and pin-prick sized lung haemorrhages, lung abscesses and nutmeg-like pattern in the liver were slightly more common in rabbits treated at 1 µg/kg bw/day compared to controls. A significant prolongation in partial prothrombin time (PPT) was measured only in males and females dosed at 1 µg/kg bw/day.

Based on the above, a LOAEL of 1 µg/kg bw/day (based on prolonged PTT), and a NOAEL of 0.5 µg/kg bw/day were determined.

Overall, the significant prolongation in PTT observed at a level of 0.001 mg/kg bw/day in rabbits occurred below the criterion used for classification as STOT RE 1; H372 for the oral route. Based on this finding, the DS proposed to classify Bromadiolone as 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".

An SCL of 0.01% for STOT RE 1 (H372) was proposed by the DS using the following calculation: 0.001 mg/kg bw/day (effective dose) / 10 mg/kg bw/day (limit) * 100% = 0.01%. An SCL for STOT RE 2 (H373) between 0.001% and 0.01% was proposed using the same data and method of calculation. This calculation was performed according to the method described in the Guidance on the Application of the CLP Criteria.

Comments received during public consultation

Two Member States supported the proposed classification and specific concentration limits.

Assessment and comparison with the classification criteria

RAC supports dossier submitter's proposal for NOAEL and LOAEL values in repeated dose toxicity studies:

- LOAEL of 0.020 mg/kg bw/day based on severe haemorrhagic effects, and NOAEL of 0.008 mg/kg bw/day at which no toxicologically significant effects were found, including on (partial) prothrombin time, in 90-day oral toxicity in dogs;

- LOAEL of 0.05 mg/kg bw/day (based on 100% mortality due to haemorrhages), and NOAEL of 0.0025 mg/kg bw/day at which no substance-related toxicity was found, including the effects on (partial) prothrombin time, in a 28-day oral toxicity study in rats;

- LOAEL of 0.001 mg/kg bw/day (based on prolonged PTT), and NOAEL of 0.0005 mg/kg bw/day at which no substance-related toxicity was found, including the effects on (partial) prothrombin time, in a 90-day oral toxicity study in rabbits.

RAC supports the dossier submitter's proposal for STOT RE 1; H372 (Causes damage to the blood through prolonged or repeated exposure) classification, since:

- serious effects were observed in the 28-day rat study (mortality) and 90-day dog study (severe haemorrhagic effects) at levels more than one order of magnitude below the guidance values (C ≤ 10 mg/kg bw/day for 90-day oral study in rats and C ≤ 30 mg/kg bw/day for 28-day oral rat study if Haber's rule is applied) used for classification as STOT RE 1;
- oral data can be used for classification for the two other routes of exposure (dermal and inhalation): acute LD_{50} values in dermal and inhalation toxicity studies (in which deaths occurred due to internal haemorrhages) were below the limits for classification for STOT RE 1, and there is a large margin between the oral dose levels indicating severe effects and the limit value for STOT RE 1.

Regarding the DS proposal for setting SCLs based on the significant prolongation in PPT without other adverse effects, RAC is of the opinion that this parameter *per se* is not an effect severe enough to be considered as a basis for the effective dose in the SCL calculation.

Since adverse effects observed at LOAELs derived from repeated-dose toxicity studies were either too severe (the majority of exposed dogs in the 90-day oral toxicity study presented severe haemorrhages, and 100% mortality was observed in the 28-day oral toxicity study in rats) or too mild (prolonged PTT without other adverse effects in the 90-day oral toxicity study in rabbits), specific concentration limits for STOT-RE could be derived from the rabbit teratogenicity study (Biocidal product applicant Task Force). In this study, at the lowest dose applied of 2 µg/kg bw/day, no mortality was observed, but haemorrhages (bleeding around body orifices, kidney haemorrhage) occurred in 3 out of 18 treated rabbits (17%). This dose level was set as an effective dose (ED), and calculation of the SCLs was performed applying Haber's rule (90 days:22 days = 4.1):

ED = 0.002 mg/kg bw/day (haemorrhages in 3 out of 18 treated rabbits)

SCL for STOT RE 1 = ED/GV1(41) x 100% = 0.005%
SCL for STOT RE 2 = ED/GV1(410) x 100% = 0.0005%

Conclusion

RAC, therefore, proposes the following classification and specific concentration limits based on the LOAEL of less than 0.002 mg/kg bw/day in the rabbit teratogenicity study (applying Haber's rule):

STOT RE 1; H372 (Causes damage to the blood through prolonged or repeated exposure)
This classification should apply for all routes of exposure.

SCLs
STOT RE 1; H372: C ≥ 0.005%.
STOT RE 2; H373: 0.0005% ≤ C < 0.005%.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In *in vitro* genotoxicity studies - Ames test, mammalian chromosome aberration test, and mammalian cell gene mutation test (CHO-HGPRT) – Bromadiolone did not induce mutagenic effects with or without metabolic activation.
In an *in vivo* bone marrow chromosome aberration test in ICR mice, Bromadiolone did not induce significant increases in micronucleated bone marrow polychromatic erythrocytes.

The DS concluded that no classification for mutagenicity is required.

Comments received during public consultation

This endpoint was not commented.

Assessment and comparison with the classification criteria

RAC therefore supports DS proposal that Bromadiolone should not be classified for mutagenicity under CLP.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No data are available for carcinogenicity. Bromadiolone is considered to be non-genotoxic and no classification is proposed

Comments received during public consultation

This endpoint was not commented.

Assessment and comparison with the classification criteria

Bromadiolone was not genotoxic/mutagenic in *in vitro* and *in vivo* studies. There were no indications of hypertrophy or hyperplasia in 90-day oral toxicity studies in dogs and rabbits.

RAC, therefore, supports DS proposal that Bromadiolone should not be classified for carcinogenicity under CLP.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Four reproductive toxicity studies were considered in CLH report:
- Two-generation oral (gavage) study in Wistar rats in accordance to OECD TG 416
- Oral teratogenicity toxicity in New Zealand white rabbit in accordance to OECD TG 414
- Oral teratogenicity toxicity in Sprague-Dawley rat in accordance to US EPA 83-3
- Oral teratogenicity toxicity in New Zealand white rabbit in accordance to US EPA 83-3.

Two-generation oral (gavage) study in Wistar rats in accordance to OECD TG 416:

According to the DS, the two-generation reproduction toxicity study in rats is of low reliability and suitable for classification since no clinical signs were observed at any dose level and no dose-related effects were reported (according to OECD test guideline "the highest dose level should be chosen with aim to induce toxicity but not death"). The dossier submitter therefore concluded that reproductive effects of Bromadiolone cannot be excluded based on the problematic dose selection.

Oral teratogenicity study in rabbits conducted in accordance to OECD TG 414 (2004):
This study is considered suitable for classificationand showed indications of developmental toxicity.

Maternal toxicity
Clinical signs and pathological signs on necropsy related to haemorrhages were observed in rabbits already exposed to the lowest dose applied, i.e. 2 µg/kg bw/day. A mortality rate of 27% was present at the next dose level, i.e. 4 µg/kg bw/day, and increased to 46% at the highest dose level (8 µg/kg bw/day). A maternal NOAEL < 2 µg/kg bw/day was determined.

Developmental effects
Significantly increased incidences of post-implantation loss and total intrauterine mortality were observed at 4 µg/kg bw/day compared to controls. At 8 µg/kg bw/day significantly increased incidence of one small placental lobe was observed. The DS also presented (in the table below) the type of malformation in relation to maternal toxicity observed in three foetuses from different litters, at doses of 4 and 8 µg/kg bw/day:

Dose (µg/kg bw/day)	**N of affected foetuses/N of foetuses in litter**	**Observed malformations**	**Maternal necropsy findings**	**Placenta findings**	**Maternal bw gain** (day 29)
4	1/8	absence of mesencephalon and proencephalon; rudimentary cerebellum; facial skull bones and skull cap missing, hypoplastic mandibule, arch I-III cervical vertebrae fused, on the skull base rudimentary vertebrae-like bones	haemorrhages in uterine horns, pin sized haemorrhages in lungs	NR	-1.1%
4	1/1	absence of vitreous body, absence of retinal folds (both sides)	NR	no data	-9.3%
8	1/6	internal hydrocephaly	haemorrhages in uterine horns, pin sized haemorrhages in lungs	tumour-like formation in one lobe	+2.5%

NR = none remarkable

There was no information in the study report relating to haemorrhages in the surviving foetuses. No comparison with historical control data was presented.

Although limited in number, the presence of severe malformations of which some has been reported as congenital effects of Warfarin (e.g. CNS), indicates that Bromadiolone could pose a risk for unborn child at maternally toxic concentrations. A teratogenicity/Embryotoxicity NOAEL of 2 µg/kg bw/day was determined.

Teratogenicity studies performed in rats and rabbits according to US-EPA Guidelines:
Both of these studies were negative (detailed data not shown):

- in a rat teratogenicity study 48% maternal mortality at the high dose was observed, and there were no mortalities and other treatment-related changes in lower dose groups. No embryotoxicity or macroscopic signs of teratogenicity were noted. Maternal NOAEL of 35 µg/kg bw/day and teratogenicity/embryotoxicity NOAEL of 70 µg/kg bw/day (highest dose tested) were determined.
- in a rabbit teratogenicity study maternal toxicity was noted at 8 µg/kg bw per day (haemorrhages), and no developmental toxicity was observed. Maternal NOAEL of 4 µg/kg bw/day and teratogenicity/embryotoxicity NOAEL of 8 µg/kg bw/day (highest dose tested) were determined.

The DS pointed out that the two studies above were performed in 1981 with shorter dosing periods, which could have contributed to different outcomes relative to the rabbit teratogenicity study from 2004.

It was concluded by the DS that Bromadiolone was teratogenic in rabbit, inducing CNS effects in offspring similar to those observed in humans after Warfarin treatment. Taking into account? the structural similarity and the same MoA as the known developmental toxicant Warfarin, classification as Repr. Cat. 1; R61 (according to the DSD) and Repr. 1A, H360D (according to CLP) was proposed.

It was noted that a specific concentration limit is needed for Bromadiolone for reproductive toxicity.

Comments received during public consultation

Seven industry organisations opposed classification for reproductive toxicity, mainly with the justification that read-across to Warfarin is not scientifically based since a new OECD 414 study (Kubaszky 2009) showed that the OECD TG 414 protocol is able to reveal reproductive effects of AVKs.

Six Member States supported proposed Repr. 1A (H360D) classification, due to the same MoA of Warfarin (known human teratogen) and other AVKs, similarity in physicochemical properties and the mammalian toxicity profile between Warfarin and second generation AVKs, and because the concern remained that studies performed according to OECD TG 414 cannot adequately show developmental effects of AVKs.

Additional key elements

In the open literature, a study on developmental effects of sub-lethal doses on Bromadiolone and Chlorophacinone was found (Rady et al., 2013). The study was not conducted in accordance with OECD guidelines, i.e. the active substance used is of unknown purity, the number of dams at each time point is not stated and the number of pups is unknown. Bromadiolone was orally applied to 10 pregnant dams per group on the 3rd gestational day at the dose level of 1/4 (0.2805 mg/kg bw) and 1/10 (0.1122 mg/kg bw) of its acute oral LD_{50} (purity of the test substance not stated). An unspecified number of dams (probably 3-4 per time point) was sacrificed at 9th and 18th day of gestation, and the others were allowed to deliver spontaneously. In dams, dose-dependent increase in liver enzymes, bilirubin, creatinine, urea and cholesterol, and histopathological changes in liver (degenerative changes at lower dose and centrilobular necrosis at higher dose) and kidneys (oedema, inflammation, swelling of the lining glomerular endothelia, proliferation) were observed, indicating maternal toxicity, although presumably not to the level of mortality. Developmental effects included generalised oedema and haemorrhages in foetuses and dose-dependent prolongation of gestational period, decrease in the number of foetuses and newborn rats, decrease in foetal body weight, prolongation of time to eye opening and fur coating, and shorter survival time after birth. The majority of these effects were statistically significant (Fisher's Least Significant Difference (LSD) test).

Overall, the study can only be used as supportive evidence indicating that the offspring may be more severely affected than the dams after a single administration of Bromadiolone during gestation at maternally sub-lethal doses.

Assessment and comparison with the classification criteria

RAC agrees with the dossier submitter that the two-generation oral (gavage) study in rats is not suitable for assessment of reproductive toxicity (both fertility and developmental toxicity) of Bromadiolone. Namely, no treatment-related effect was observed in parents and offspring at any dose levels (1, 2.5 and 5 µg/kg/day), and prothrombin time was similar in the control and 5 µg/kg bw/day group (the highest dose tested).

Effects on fertility

Although the aforementioned two-generation reproduction toxicity study in rats cannot provide relevant data for fertility assessment, a 90-day study in rabbits with Bromadiolone showed no adverse effects on the gonads. In addition, Warfarin did not show any effect on fertility after many years of human use or in a two generation reproduction study in rats with Vitamin-K supplementation.

RAC, therefore, supports the proposal of the DS not to classify Bromadiolone for fertility according to CLP.

Developmental toxicity

RAC agrees with the DS that the significantly increased incidence of post-implantational loss and total intrauterine mortality observed at 4 µg/kg bw/day, could indicate that Bromadiolone poses a risk for the unborn child at a level inducing maternal toxicity.

Although poorly reported, the study of Rady et al. (American-Eurasian Journal of Toxicological Sciences 2013;5:7-14; please see the study summary in the BD) in rats could present supportive evidence that *in utero* Bromadiolone exposure could lead to foetotoxic effects at the dose levels that do not induce mortality in mothers.

There is a debate as to whether the standard OECD TG 414 study design is able to reveal developmental toxicity effects of anticoagulant rodenticides, especially after a new study appeared (Kubaszky, 2009), carried out according to the updated OECD 414 Guideline. In Kubaszky (2009), an increased incidence of subcutaneous and internal foetal haemorrhages, foetal ocular effects and some indications of reduced ossification of skull bones were observed. Haemorrhages and skeletal malformations were also described in Mirkova and Antov (1983), although without clear reporting of a relationship with maternal toxicity.

Foetal haemorrhages were not observed in teratogenicity studies with Bromadiolone. Haemorrhages in uterine horns in rabbits were reported at doses that induced maternal mortality, but there was no information in the study report relating to haemorrhages in the surviving foetuses, either in control or exposed animals.

The study conducted by Kubaszky (2009) commissioned by the CEFIC Rodenticide Data Development Group (RDDG), was specifically dedicated to assess potential anticoagulant effects on foetuses In order to address the capability of OECD TG 414 protocol to detect adverse effects on embryos and foetuses due to maternal exposure to Warfarin. The study reported dose-related increases in foetal haemorrhages compared to 2% incidence of haemorrhages in foetuses from the control group. In teratogenicity studies with other AVKs no foetal haemorrhage in control animals was reported.

Increased incidence of skeletal malformations was not found in teratogenicity studies with Bromadiolone. Skeletal malformations (nasal hypoplasia, stippled epiphyses, growth retardation) are the most often reported malformations in neonates of mothers undergoing coumarin therapy during gestation. However, in animal studies with Warfarin that generally followed the OECD TG 414 test design, a clear effect on foetal bone and cartilage was not routinely observed. In the Kubazsky study (2009), skeletal malformations were also not a prominent teratogenic feature - only one litter at mid-dose (0.150 mg/kg bw/day) had foetuses showing facial skeletal malformations (malformed skulls with wide nasal and/or frontal bone/cartilage), unossified nasal bone, malformed vertebra and malformed sternum.

Foetal ocular effects were found in Warfarin exposed foetuses in the Kubaszky study (2009). Yellowish discolouration in the lens was observed, shown to be a central cataract – an extremely rare malformation in rats. An ocular effect of this type was not observed in teratogenicity studies with Bromadiolone, nor in other studies with Warfarin.

Overall, based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (Repr. Cat 1A), the reproductive toxicity of Bromadiolone has been analysed in detail. It is acknowledged that the animal developmental toxicity studies on Warfarin were weakly positive and that the animal developmental toxicity studies on Bromadiolone were negative. However, in comparison with Warfarin, Bromadiolone and other 2nd generation AVKs have higher acute and repeated dose toxicity, steeper dose-response curves, and much longer half-lives in the exposed organisms, making the evaluation of developmental effects of all 2nd generation AVK rodenticides difficult. Thus, relatively low doses in repeated exposure during gestation lead to maternal toxicity and lethality which hinders the detection of developmental toxicity at higher doses.

As there were no data on the outcome of maternal exposure to Bromadiolone in humans, classification as Repr. 1A is not considered to be applicable for Bromadiolone.

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

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

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

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

Setting specific concentration limits (SCLs):
Regarding a SCL for Bromadiolone, it is acknowledged that the specific data on developmental toxicity of Bromadiolone is too scarce to help in setting an SLC.

Classification as Repr. 1B for developmental toxicity for Bromadiolone is supported by the RAC. However, only for Warfarin is there sufficient data to set a SCL for developmental toxicity. Thus,

based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg/day) may cause developmental toxicity and could be regarded as an ED_{10} level. This human ED_{10} value would, if using the guidance for setting SCLs based on animal data, belong to the high potency group (<4 mg/kg/day). The guidance states that for an ED10 <4 mg/kg/day, the SCL is 0.03%, and for ED_{10} below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from an ED_{10} value obtained from animal studies (0.125 mg/kg/day; Kubaszky et al 2009), it would qualify Warfarin for the high potency group and result in a SCL of 0.003%. Thus, the RAC concluded on a SCL on 0.003% for the developmental toxicity of Warfarin (RAC opinion on Warfarin).

As the other AVK rodenticides were equally or more toxic than Warfarin, it is not considered appropriate to apply the generic concentration limit for these substances (0.3%), but rather to base the SCLs on the SCL proposed for Warfarin. Thus, the RAC is of the opinion that the SCL for Warfarin can be used as a surrogate SCL for the other AVK rodenticides, resulting in a SCL of 0.003% for all AVK rodenticides, including Bromadiolone.

Supplemental information - In depth analyses by RAC

RABBIT TERATOGENICITY STUDY

Oral teratogenicity toxicity in New Zealand white rabbits was performed in accordance to US EPA Guideline 83-3, equivalent to OECD TG 414.

Table 1. Maternal and developmental effects in New Zealand white rabbits

	Control	**Bromadiolone** (µg/kg bw/day)		
		2	**4**	**8[b]**
Maternal effects				
N of does	19	18	18	19
Clinical signs related to haemorrhage (N of does)	-	+ (3)	+	+
Mortality	0	0	27%	46%
Necropsy (N of does with haemorrhagic changes)	NR	H (3)	H	H
Fertility & Development				
Abortions	0	0	2	0
Pregnancy rate (%)	86	86	91	91
Corpora lutea	9.8	8.9	9.4	8.3
Preimplantation loss[a]	11%	14%	17%	8%
Mean foetal wt (g)	37.4	39.8	36.7	38.8
N of viable foetuses	165	138	98	68
N of dead foetuses	0	0	7%**	1%
Postimplantation loss[a]	1	0	10%**	1%
Total intrauterine mortality[a]	12	14	26%**	9%
N of examined foetuses	165	138	87	68
external malformations (%)	0	0	1	0
external anomalies (%)	4	1	8	3
skeletal malformations (%)	1	0	1	0
skeletal anomalies (%)	7	3	16	3
skeletal variations (%)	6	3	15[c]*	3
visceral malformations (%)	0	0	1	1
visceral anomalies (%)	1	1	1	3
visceral variations (%)	1	1	0	1

NR = not remarkable
H = haemorrhage

[a] Data compared to N of implantations
[b] Data for the highest dose group are of limited reliability due to shortened exposure period compared to other groups (day 7-20, instead of 7-28, since animals started to die at day 18) and due to high maternal mortality
*Significantly different from Control
**Significantly different from Control (Chi square test done by dossier submitter)
[c] According to CAR IIIA document skeletal variations at this dose level included displaced, fused or misshaped sternebrae and 13th rib anlage.

Without reported historical control data it is difficult to assess the relevance of increased incidence of skeletal variations. For example, displaced, fused or misshaped sternebrae were reported in 6 animals in the group dosed at 4 µg/kg bw/day, that would approximately correspond to 7% incidence. In historical control data for New Zealand White rabbits published in open literature (Horimoto et al. Historical control data on prenatal developmental toxicity studies in rabbits: Skeletal variations. A Study Group for Historical Control Data on Prenatal Developmental Toxicity Studies in Rabbits, 2012) the incidence for fused sternebrae only is in the range of 0-12.0% (for period 1994-2000), and for fused sternebrae (junction) in the range of 0-5.10% (for period 2001-2010). However, these data have been derived from laboratories in Japan and are not necessarily valid for NZW rabbit breed used in the study.

Observed severe skeletal, CNS and eye malformations, similar to malformations reported in human cases of Warfarin teratogenicity, were found in very low number of animals (three) and at doses with overt maternal toxicity (mortality). Lack of historical control data and reduced number of foetuses available for evaluation at these dose levels make the assessment of the teratogenic effect of Bromadiolone uncertain.

Reference:

Rady et al. (American-Eurasian Journal of Toxicological Sciences 2013;5:7-14)

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

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

Degradation

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

The DS considered Bromadiolone as hydrolytically stable because no significant degradation was observed at pH 7-9, after 30 days, a degradation of just 6.5% was observed at pH 5. Bromadiolone is also rapidly photodegradable with an experimental half-life of minutes. It was degraded rapidly in the atmosphere by reaction with ozone and OH radicals (DT_{50} = 2.0 and 2.1 hours, respectively), although the presence of this compound in air is not expected due to its low vapour pressure.

Bromadiolone is not readily biodegradable according to OECD TG 301B. 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 a second test, performed according to OECD guideline 301D the maximum degree of degradation was 31% which also fulfils the criteria for being not readily biodegradable. Inherent biodegradability was studied according to OECD 302D, the degradation was maximum 2% during the test, and

minimum 20% is required to pass the test. Bromadiolone is therefore considered as not inherently biodegradable.

Based on the available data, Bromodiolone was considered to be not-rapid/ready degradable.

Bioaccumulation

Two experimental log K_{ow} values were calculated with the shake-flask method and the results were 4.07 at pH 7, 20ºC and 3.8 at pH 7.1, 25ºC. Three experimental bioconcentration studies were included in the CLH. In the first study with bluegill sunfish, the maximum bioconcentration factor for Bromadiolone was 460 for whole fish. In non-edible tissues the maximum BCF was 1658 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 of these studies, the reliability was low due to major deficiencies in reporting and high mortality in the exposed group of fish. Also, a fish bioconcentration study with rainbow trout was performed, but it failed due to high mortalities. Because of these deficiencies, the experimental BCFs were not used to establish the potential of bioaccumulation of this substance and the BCFs were derived by using the equation $log\ BCF_{fish} = 0.85 * log\ K_{ow} - 0.70$. The resulting calculated BCF values were 339 (based on a log K_{ow} of 3.8) and 575 (based on a log K_{ow} of 4.07).

The DS concluded that Bromadiolone has potential to bioaccumulate. This conclusion is in agreement with the assessment made by the Technical Committee on New and Existing Substances (TCNES) subgroup on Identification of PBT and vPvB substances, where Bromadiolone is considered persistent and toxic, and although there was some uncertainty regarding the bioaccumulation criterion they finally concluded that Bromadiolone has potential to bioaccumulate.

Aquatic toxicity

Two acute toxicity studies for each trophic level were reported by the DS, i.e. in fish (*Oncorhynchus mykiss,* OECD TG 203)*;* invertebrates (*Daphnia magna,* OECD TG 202) and algae *(Desmodesmus subspicatus* and *Pseudokirchneriella subcapitata,* OECD TG 201). There are not available long-term tests in fish and invertebrates but the algae tests submitted in the CLH report can be considered acute (EC_{50}) and chronic (NOEC), although, only a NOEC value of 0.037 mg/L for *Desmodesmus subspicatus* appears in the CLH report as a chronic endpoint.

All the acute endpoints (EC_{50}) reported in the CLH dossier for the three trophic levels are higher than 1 mg/L: fish LC_{50} (96h) = 2.86 and > 8 mg/L; invertebrate EC_{50} (48h) = 2.0 and 5.79 mg/L and algae E_rC_{50} (72h) = > 1 and 1.14 mg/L. For fish the results were based on nominal concentrations because the analysis showed that the test concentrations were 95-102% of nominal. For daphnia one test was based on nominal concentration (99-107% of nominal) and the second test was based on mean measured concentrations. In the case of algae, which is the most sensitive species, the analysis of the concentrations of the test performed with *Desmodesmus subspicatus* had deficiencies and the results were based on nominal concentrations. However, the results of the test performed with *Pseudokirchneriella subcapitata* were based on geometric mean from the initial measurements (t = 0 h) and half of the LOQ of 0.3 mg/L (t = 72h). For both algae tests, the analysis results of the test substance at the end of the test were below the limit of quantification and therefore the degradation rate of the substance in the test is not known. The likely rapid photolytic degradation of the test substance in the study lead to the conclusion that the real EC_{50} is below 1 mg/L, and therefore, an EC_{50} < 1 mg/L was used to establish a classification under CLP of Aquatic Acute 1 and Aquatic Chronic 1, both with an M = 1. The chronic NOEC of 0.037 mg/L lead to the same classification, and will thus not influence the above environmental classification.

Comments received during public consultation

Three Member States supported the environmental classification proposed by the DS without any additional comments. One Member State suggested other E_rC_{50}s for the algae test in agreement

with the combined Assessment Report for Bromadiolone dated "30 May 2008, revised 16 December 2010",.

In their response to comments document (RCOM), the DS explained that the E_rC_{50s} for algae which appear in the CLH report are in agreement with the correct version of the combined Assessment Report for Bromadiolone dated "30 May 2008, revised 16 December 2010", so DS disagreed with this comment.

Assessment and comparison with the classification criteria

Degradation

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

RAC also agreed that Bromadiolone is not readily or inherently biodegradable under test conditions, with a level of degradation of 31% after 28 days in the best case.

Based on these data, RAC agreed with the DS that Bromadiolone should be considered **not rapidly degradable** according to CLP.

Bioaccumulation

There is an experimental log K_{ow} for Bromadiolone of 4.07, pH 7 and 20ºC which is above the cut-off values of log K_{ow}≥4 (CLP), therefore RAC agreed with the DS that Bromadiolone has **high potential for bioaccumulation**.

This decision, as it was explained by the DS, is in agreement with the assessment made by the TCNES subgroup on identification of PBT and vPvB substances.

Aquatic toxicity

Under CLP, the acute toxicity category should be based on the lowest EC_{50}, in this case, algae is the most sensitive species. Two algae tests were summarised in the CLH report. The first one was performed with *Desmodesmus subspicatus*, and toxicity values of E_rC_{50} > 1 mg/L, E_bC_{50} of 0.17 mg/L and a NOE_rC of 0.037 mg/L were reported, however, these values are based on nominal concentrations. Three concentrations were measured at the beginning and at the end of the test (96h) but at the end were below the lowest calibration point (assumed to be 0.004 mg/L), therefore, the endpoints based on nominal concentrations for this test are clearly underestimates. This test has been used as additional information, in order to support that the acute toxicity of algae is lower than 1. Many assumptions have been discussed but no final value was obtained (see "*In depth analyses by the RAC"* section).

In the second test performed with *Pseudokirchneriella subcapitata,* which has been used as key study, the toxicity results were E_rC_{50} 1.14 mg/L and E_bC_{50} of 0.66 mg/L based on geometric mean concentrations. In this case all concentrations were measured at the beginning and at the end of the test (72 h) and as well as the first test, the concentrations at the end were below limit of quantification. The geometric means were calculated from the measured concentrations at the beginning of the test and from half of the limit of quantification (LOQ: 0.3 mg/L) at all other measuring points (i.e. 48h and 72h) according to the Technical Guidance Note (TGN): "*Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern*" (CA-May08-Doc.6.5).

Bromadiolone is rapidly photodegradable with an experimental half-life between 3-36 min, so it is possible that the test concentrations used in the algae test may have decreased to below LOQ much earlier than after 72h and therefore the toxicity is underestimated. The light conditions in the *Pseudokirchneriella subcapitata* test may be estimated to 1/20 of that in the photolysis study, therefore it is reasonable to assume that extensive photolytic degradation of bromodialone took place during the study, and that the level of bromodialone has decreased to below LOQ much earlier than after 96h and 72h.

According to the OECD TG 201, for unstable substances, additional samplings for analysis at 24 hour intervals during the expose period are recommended in order to better define loss of the test substance, unfortunately these tests did not follow this recommendation. Nevertheless it is possible to assume that at 48h the measured concentrations are lower than the LOQ, because as it has been explained above, the photolytic half-life reported from the DS is expressed in minutes and the light conditions of the algae study may be estimated to 1/20 of that in the photolysis study. In fact in one photolysis study, a complete photolysis occurred after approximately two hours. According to this approach an E_rC_{50}=0.633 mg/L was obtained, clearly lower than 1 mg/L (see "*In depth analyses by the RAC"* section).

Furthermore, the E_bC_{50}s, which have been reported above, are lower than 1 mg/L, thus supporting the results of the discussed approach. Nevertheless, the classification shall be based on the E_rC_{50}s, which were reported to be slightly above 1 mg/L in the studies..

On the other hand, structurally similar substances, such a brodifacoum, have algae toxicity lower than 1 mg/L (ErC_{50} = 0.04 mg/L), which supports the view that the toxicity of Bromadiolone in algae is underestimated.

In conclusion and based on these explanations, regarding aquatic acute toxicity, RAC agreed with the DS and considered that it is highly likely that the actual test concentrations that cause 50% inhibition of algae growth were below 1 mg/L for both studies, therefore, the E_rC_{50} was assumed to be below 1 mg/L.

Regarding chronic toxicity, no adequate chronic data is available. Only chronic toxicity (NOEC) from the first test of algae (*Desmodesmus subspicatus*) appears in the CLH report. The chronic endpoints from this test as well as the acute endpoint were underestimated and the uncertainly of these values are high as it has been explained before. A surrogate approach from the most sensitive species as determined in the acute studies (algae, *Pseudokirchneriella subcapitata*) was carried out in order to conclude on aquatic chronic classification. According to the results of this surrogate approach, E_rC_{50} < 1 mg/L (0.633 mg/L) and taking into account that the substance is not rapidly degradable, a classification under CLP as Aquatic Chronic 1, H410 with an M-factor of 1 is justified.

In conclusion, RAC agreed with the DS's proposal to classify Bromadiolone according to the CLP criteria as Aquatic Acute 1 (H400) with an M-factor of 1 based on the acute toxicity in algae (EC_{50} < 1mg/L) and Aquatic Chronic 1 (H410) with an M-factor of 1 based on the surrogate approach for algae (*Pseudokirchneriella subcapitata*; E_rC_{50} < 1 mg/L (0.633mg/L)) and taking into account that the substance is not rapidly degradable.

In depth analyses by the RAC

Test 1: Growth inhibition test on algae (*Desmodesmus subspicatus*).

Table 1: Analytical results

Nominal concentration of Bromadiolone [mg/L]	**Measured concentration [mg/L]**		**Initial measured concentration as % of nominal**
	0 h	**96 h**	
Control	0.001	**	-
Solvent control	0.005	**	-
0.004	0.006	**	150
0.037	0.036	**	97.3
0.333	**	**	-

** Peak heights below lowest calibration point.

Considering that 0.004 mg/L is the lowest calibration point and equal to the reported LOQ and that all the concentrations are between 0.004 to 0.333 mg/L at the beginning of the study and are lower than the LOQ at the end of the study, the geometric mean could be calculated. This can be done by using the measured concentration at the beginning of the test and half of the limit of quantification (LOQ: 0.004 mg/L), i.e. 0.002 mg/l for all other time points (in this case 96 h) which were below the LOQ. This is according to the Technical guidance note (TGN) to calculate time weighted average (TWA): "*Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern*" (CA-May08-Doc.6.5: http://echa.europa.eu/documents/10162/16960215/bpd_guid_guidance_rapidly_degrading_substances_twa_2009_en.pdf).

The geometric mean concentrations would be (the reported values adopted from the Competent Auhtority Report (CAR) of bromadiolone, Document III-A, 2008):

Table 2: Analytical results (assumptions)

Nominal concentration of Bromadiolone [mg/L]	**Measured and assumed concentrations [mg/L]**		**Geometric mean concentrations [mg/L]**
	0 h (measured)	**96 h (assumed)**	
0.004	0.006	< LOQ	0.003
0.012	0.036	< LOQ	0.008
0.037	0.037	< LOQ	0.009
0.111	0.111	< LOQ	0.015
0.333	0.333	< LOQ	0.026
1.000	1.000	-*	<< 1.000*

*There is no information about the concentration at 96h, nevertheless, it is expected to be lower than 1 mg/L, considering that the reduction of the concentration in the second highest concentration is from 0.333 mg/L to a concentration below the LOQ. Therefore, the geometric mean is expected to be also lower than 1 mg/L.

Table 3: Inhibition of growth rate, days 0-3

Geometric mean concentrations [mg/L]	**72 h ln X_j**	**0 h ln X_i**	**ln X_j - ln X_i**	**mean specific growth rate μ_{i-j}**	**rate reduction[a]**	**% inhibition[a]**
Control	12.42	9.21	3.21	1.07	-	-
Solvent control	12.42	9.21	3.21	1.07	-	-
0.003	12.35	9.21	3.14	1.05	0.02	1.9
0.008	12.64	9.21	3.43	1.14	-0.07	-6.5
0.009	12.72	9.21	3.51	1.17	-0.10	-9.3
0.015	11.89	9.21	2.68	0.89	0.18	16.8
0.026	11.49	9.21	2.28	0.76	0.31	29.0
<< 1.000	11.15	9.21	1.94	0.65	0.42	39.3

[a] Relative to mean solvent control; negative values = growth stimulation.

Table 4: Inhibition of growth rate, days 0-4

Geometric mean concentrations [mg/L]	96 h ln X_j	0 h ln X_i	ln X_j - ln X_i	mean specific growth rate μ_{i-j}	rate reduction[a]	% inhibition[a]
Control	13.14	9.21	3.93	0.98	-	-
Solvent control	12.89	9.21	3.68	0.92	-	-
0.003	12.79	9.21	3.58	0.90	0.02	2.2
0.008	13.00	9.21	3.79	0.95	-0.03	-3.3
0.009	13.25	9.21	4.04	1.01	-0.09	-9.8
0.015	12.25	9.21	3.04	0.76	0.16	17.4
0.026	12.07	9.21	2.86	0.72	0.20	21.7
<< 1.000	11.74	9.21	2.53	0.64	0.28	30.4

[a] Relative to mean solvent control; negative values = growth stimulation.

In conclusion, as the geometric mean concentration for the last point tested which would be quite lower than 1 mg/L showed a % of inhibition higher than 30, it is expected that the E_rC_{50} would be still lower than 1 mg/L.

It is not possible to establish conclusive E_rC_{50} and NOE_rC values with this approach. It is just an approach which confirms the underestimation of the acute and chronic endpoints.

Test 2: Growth inhibition test on algae (*Pseudokirchneriella subcapitata*).

Table 1: Analytical results

Nominal concentration of Bromadiolone [mg/L]	Measured concentration [mg/L]		Geometric mean concentrations [mg/L]
	0 h	72 h	
Control	<LOQ	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ	<LOQ
12	12.5	<LOQ	1.37
6	6.79	<LOQ	1.01
3	3.41	<LOQ	0.72
1.5	1.73	<LOQ	0.51
0.75	0.82	<LOQ	0.35

The geometric mean concentration was calculated from the initial measurement and half of the LOQ at 72h, in accordance with the TGN: "*Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern*" (CA-May08-Doc.6.5).

However, Bromadiolone is rapidly photodegradable, and this degradation is much faster than what can be seen as a value below LOQ (0.3 mg/L) after 72h. The reported photolytic

half-life is expressed in minutes, and the light conditions of the algae study may be estimated to approximately 1/20 of that in the photolysis study. Therefore, it is possible that the test concentrations used in the algal test may have decreased to below LOQ much earlier than after 72h. According to the OECD TG 201, if the substance is unstable, additional samplings for analysis at 24 hour intervals during the exposure period are recommended to better define loss of the test substance. If a reduction below LOQ at 48 hour is assumed, according to the explanation above, the geometric mean concentrations would be in Table 2 as follow:

Table 2: Analytical results (assumptions)

Nominal concentration of Bromadiolone [mg/L]	**Measured concentration [mg/L]**			**Geometric mean concentrations [mg/L]**
	0 h	**48h**	**72 h**	
Control	<LOQ	<LOQ	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ	<LOQ	<LOQ
12	12.5	<LOQ	<LOQ	0.655
6	6.79	<LOQ	<LOQ	0.535
3	3.41	<LOQ	<LOQ	0.425
1.5	1.73	<LOQ	<LOQ	0.339
0.75	0.82	<LOQ	<LOQ	0.264

Table 3: Inhibition of growth rate, days 0-3

Geometric mean concentrations [mg/L]	**72 h ln X_j**	**0 h ln X_i**	**ln X_j - ln X_i**	**mean specific growth rate μ_{i-j}**	**% inhibition[a]**
0.655	10.523	9.038	1.486	0.495	68.539
0.535	11.911	9.038	2.873	0.958	39.167
0.425	13.060	9.038	4.023	1.341	14.820
0.339	13.395	9.038	4.358	1.453	7.728
0.264	13.705	9.038	4.667	1.556	1.169
Control	13.760	9.038	4.723	1.574	-
Solvent control	13.784	9.038	4.746	1.582	-

[a] Relative to mean solvent control; negative values = growth stimulation.

According to this new approach and assumptions, and using GraphPad Prism 6 to calculate the E_rC_{50}, the E_rC_{50} is 0.633 mg/L, which is clearly lower than 1 mg/L, as well as in the first test.

It is not possible to establish conclusive E_rC_{50} and NOE_rC values with this approach. It is just an approach confirming the underestimation of the acute and chronic endpoints.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

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