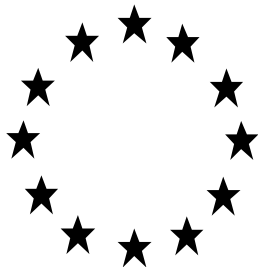
Competent Authority Report

According to Directive 98/8/EC



Bromadiolone

CAS 28772-56-7

Active substance in Biocidal Products, Product Type 14 (Rodenticides)

The Bromadiolone Task Force

DOCUMENT III-A

Section 6: Toxicological and Metabolic Studies

Rapporteur Member State: Sweden

Final CAR April 2011

KEMI

Kemikalieinspektionen

Swedish Chemicals Agency

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Section A.6.1 Acute toxicity

Section A6.1.1 **Annex Point IIA VI 6.1.1**	**Acute Toxicity** Acute Oral LD_{50} in rat	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (1996) Report: Acute Oral Toxicity Study of Test Substance Technical Bromadiolone in Rats, XXXXX. Report 96/299-001P	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 401	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot / Batch number	2239	
3.1.2 Specification	As given in section 2	
3.1.3 Description	Yellow Powder	
3.1.4 Purity	96.00% Technical Bromadiolone	X1
3.1.5 Stability	Stable under test conditions	
3.2 Test Animals		
3.2.1 Species	Rats	
3.2.2 Strain	CRL:(WI) BR (Wistar) Rats	
3.2.3 Source	CHARLES RIVER (EUROPE) LABORATORIES INC. LAB-TECH KFT. István u.11. Budapest, Hungary	
3.2.4 Sex	Male and Female	
3.2.5 Age/weight at study initiation	Young healthy adults: Male 187g – 215g Female 147g – 176g	
3.2.6 Number of animals per group	5 animals/sex/group	
3.2.7 Control animals	No	

Section A.6.1 Acute toxicity		
Section A6.1.1 **Annex Point IIA VI 6.1.1**	**Acute Toxicity** Acute Oral LD_{50} in rat	
3.3 Administration/ Exposure	Oral	
3.2.2 Postexposure period	14 days	
	Oral	
3.2.3 Type	Single dose by gavage	
3.2.4 Concentration		
3.2.5 Vehicle	1% Methylcellulose solution	
3.2.6 Concentration in vehicle	0.003, 0.006, 0.009, 0.012, 0.015 % w/v	
3.2.7 Total volume applied	Single dose of either 0.3, 0.6, 0.9, 1.2, or 1.5 mg/kg	X2
3.2.8 Controls	None	
3.4 Examinations	Clinical observations, mortality, body weight, necropsy	
3.5 Method of determination of LD50	Probit analysis according to Finney Method	
3.6 Further remarks	None	
	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	1.2 mg/kg dose group – All animals were symptom-free to the 10th day after the treatment. One male and two female animals showed slight squatting position, piloerection and slight then moderate paleness one or tow days thereafter. Besides in females dyspnoe were observed on the day before the death. The male and one female animals died on the 13th day of the observation period, one female died on the 14th day. 1.5 mg/kg dose group – all animals showed squatting position, paleness and piloerection. Dyspnoea occurred in three males and one female. Activity decreased was found in two male and in all females.	
4.2 Pathology	Haematomas and haemorrhages, blood-filled thoracic cavity, blood discharge on the nasal region.	
4.3 Other	No consistent effects on bodyweight and bodyweight gain	X3
4.4 LD_{50}	Males 1.43 mg/kg; Females 1.25 mg/kg; Males + females 1.31 mg/kg	
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A.6.1 Acute toxicity		
Section A6.1.1 **Annex Point IIA VI 6.1.1**	**Acute Toxicity** Acute Oral LD_{50} in rat	
5.1 Materials and methods	The study was conducted according to OECD 401 study in rats. Test substance was applied in form of 0.003, 0.006, 0.009, 0.012, 0.015 w/v% suspension in 1% Methylcellulose solution. The single oral dosage was carried in doses of 0.3, 0.6, 0.9, 1.2, 1.5 mg/kg in male and female animals by gavage. Clinical observations were made five times on the day of the test substance administration during the first five hours, then daily. Body weight was measured weekly. Gross necropsy was performed in all animals immediately after the death or terminally. Statistical analysis was performed for the following date: Body weight, and the calculation of LD50 was Probit analysis, according to Finney's method. The statistical analysis was done with SPSS PC+ software package. The heterogeneity of variance between groups was checked by Bartlet's homogeneity of variance test.	X4
5.2 Results and discussion	Slight –severe paleness, dyspnoea, squatting position, decreased activity, piloerection appeared in both sexes mainly in the highest dose (1.5 mg/kg) dose group, but those appeared in some animals of the 1.2 mg/kg group, too. Sporadically bleedings, nosebleed, swellings in different areas, cyanotic skin, abnormal gait, incoordination and tremor were observed in the highest dose group. The onset of signs usually was on the 4^{th}-7^{th} days after treatment, but in some animals of the 1.2 mg/kg dose group in the 10^{th}-11^{th} days. Death of male animals occurred between 6-13^{th} days after the treatment. Female animals died between 7-14^{th} days. Some animals did not show severe signs of illness, but they died. The survival animals did not show any symptoms on the last day of the observation period. The body weight gain and the man body weight decreased both in the male and female animals of the 1.5 mg/kg dose group at the end of the first week. During the necropsy of animals survived, in some cases yellowish-brown areas where observed in the abdominal fatty tissue which can be in connection with an previous haemorrhage. In the died animals the haematomas, haemorrhages, bloody discharge on the nasal region and the blood-filled thoracic cavity are alterations which can be in connection with the effect of the bromadiolone. Some animals died without showing severe clinical symptoms, and their thoracic cavity was filled with blood. These findings may refer to a presumable vessel wall damaging effect of the test substance. Due to a vessel wall damage a bleeding started in the thoracic cavity and this might have caused the sudden death of these animals.	X5
5.3 Conclusion	Bromadiolone caused haemorrhages, haematomas all the body in CRL:(WI) BR rats after single oral application. The cause of death might be due to oligaemic shock.	X6
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	

Section A.6.1 Acute toxicity		
Section A6.1.1 **Annex Point IIA VI 6.1.1**	**Acute Toxicity** Acute Oral LD_{50} in rat	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2005	
Materials and Methods	Applicants version accepted with amendments: X1 There is no justification for the lower purity (96%) in this study, compared to the 98% purity level in section 2. However, this minor decrease in purity should not affect the results of the study. X2 A constant dosage volume of 1ml/100g bw was applied. X4 All deaths occurring during the observation period were recorded.	
Results and discussion	X3 Inconsistent with the observed dose- dependent decrease in mean bw and mean bw gain in both sexes. X5 Applicants version replaced by: The animals of the first three dose groups (0.3, 0.6 and 0.9 mg/kg) were symptom-free. Among the animals exposed to 1.2 mg/kg, three animals showed paleness, slight squatting position and piloerection,. Two of these animals (both female) also showed signs of dyspnea. The onset of symptoms occurred on the 10^{th} day and on the 13^{th}-14^{th} day these three animals died. Seven animals in the 1.2 mg/kg dose group were symptom-free. In the 1.5 mg/kg dose group, the onset of symptoms occurred on the 4^{th}-7^{th} day and deaths occurred day 6- 10. All animals in this dose group showed squatting position, paleness and piloerection. Dyspnea was seen in eight animals and decreased activity was seen in seven animals. Sporadic observations of external bleeding, lachrymation, nosebleeds, swellings, cyanotic skin, abnormal gait, incoordination and tremor were also made in this dose group. Symptoms among animals in the 1.5 mg/kg dose group were in most cases observed until the death of the animal. The two surviving animals of the 1.5 mg/kg dose group were symptom-free on the last day of observation. 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 of surviving animals included pale kidneys, hydrometras and internal haemorrhaging. In animals that died during the experimental period, necropsy findings included haematomas, haemorrhages, bloody discharge on the nasal region and blood-filled thoracic cavities. Some animals died without showing severe clinical symptoms. Mortality rates can be seen in Table A6_1-1.	
Conclusion	X6 Applicants version replaced by: Acute oral LD_{50} Males: 1.43 mg/kg, Females: 1.25 mg/kg, Males and Females: 1.31 mg/kg. In this study Bromadiolone has an acute oral combined sexes LD_{50} of 1.31 mg/kg with the 95% confidence limits at 1.17 – 1.49 mg/kg. According to directive 67/548/EC, Bromadiolone thereby requires a classification as Very Toxic (T+).	
Reliability	1	
Acceptability	Acceptable	

Section A.6.1 Acute toxicity		
Section A6.1.1 **Annex Point IIA VI 6.1.1**	**Acute Toxicity** Acute Oral LD_{50} in rat	
Remarks	Table A6_1.1 Exposure to 0.3 mg/kg caused symptoms in both females and males. In the 1.2 mg/kg dose group one male with pale kidneys and two females with hydrometra were reported. No animals were given 0 mg/kg.	

Table A6_1-1. Table for Acute Toxicity

Dose [unit]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0	-	-	
0.3 mg/kg	0/10	-	Male only- 1 case of pale kidneys and 1 hyrometra
0.6 mg/kg	0/10	-	Females only -1 point-like haemorrhages in lung and 3 hydrometra
0.9 mg/kg	0/10	-	Females only – 3 hydrometra
1.2 mg/kg	3/10	Days 13 – 14	Areas of haemorrhages – Nasal and thoracic cavity filled with blood.
1.5 mg/kg	8/10	Days 6 – 10	Haematoma and haemorrhages - Nasal and thoracic cavity filled with blood.
LD_{50} value	Males 1.43 mg/kg; Females 1.25 mg/kg; Males + females 1.31 mg/kg with 95% confidence limits 1.17 – 1.49 mg/kg		

Section A6.1.2 **Annex Point IIA VI 6.1.2**	**Acute Toxicity** Acute Dermal LD_{50} in rat	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX, (1996) Report: Acute Dermal Toxicity Study of Test Substance Technical Bromadiolone in Rats, XXXXX. Report 96/299-002P	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 402	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.2.2 Lot / Batch number	2239	
3.2.3 Specification	As given in section 2	
3.2.4 Description	Yellow powder	
3.2.5 Purity	96.00% technical bromadiolone	X1
3.2.6 Stability	Stable under test conditions	
3.3 Test Animals		
3.2.2 Species	Rats	
3.2.3 Strain	CRL:(WI) BR (Wistar) Rats	
3.2.4 Source	CHARLES RIVER (EUROPE) LABORATORIES INC. LAB-TECH KFT. István u.11. Budapest, Hungary	
3.2.5 Sex	Male and female	
3.2.6 Age/weight at study initiation	Young healthy adults: Male 219g – 274g Female 176g – 208g	
3.2.7 Number of animals per group	5 animals/sex/group	
3.2.8 Control animals	No	

Section A6.1.2 **Annex Point IIA VI 6.1.2**	**Acute Toxicity** Acute Dermal LD_{50} in rat	
Administration/ Exposure	Dermal	
3.2.9 Postexposure period	18 days	X2
	Dermal	
3.2.10 Area covered	10 % of body surface	
3.2.11 Occlusion	Semi-occlusive	
3.2.12 Vehicle	1% Methylcellulose solution	
3.2.13 Concentration in vehicle	0.5, 1.0, 1.5, 2.0, or 2.5 % w/v	
3.2.14 Total volume applied	Single dose of either 5.0, 10.0, 15.0, 20.0, or 25.0 mg/kg	X3
3.2.15 Duration of exposure	24 hours	
3.2.16 Removal of test substance	Washed with body temperature water	
3.2.17 Controls	None	
3.4 Examinations	Clinical observations, mortality, body weight, necropsy	X4
3.5 Method of determination of LD_{50}	Probit analysis according to Finney Method	
3.6 Further remarks	None	
	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	Activity decrease, squatting position, piloerection, dyspnoea, paleness, wounds, swollen areas, cyanotic areas, bleeding, paralysed limbs, lateral lying position, abnormal gait, restlessness	
4.2 Pathology	Haematomas and haemorrhages in various places, blood-filled thoracic cavity, stomach, small intestine and urinary bladder	
4.3 Other	Body weight decrease in males only	X5
4.4 LD_{50}	Males 20.62 mg/kg; Females 32.08 mg/kg; Males + females 23.31 mg/kg	
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.1.2 **Annex Point IIA VI 6.1.2**	**Acute Toxicity** Acute Dermal LD_{50} in rat	
5.1 Materials and methods	The study was conducted according to OECD 402 study in rats. Test substance was applied inform of 0.5, 1.0, 1.5, 2.0 and 2.5% w/v% suspension in 1% methylcellulose solution. The single dermal treatment was carried out in doses of 5, 10, 15, 20, and 25 mg/kg in male and female animals with 24 hour exposition. Clinical observation was made twice on the day of the test substance administration in the first and fifth hours then daily for 18 days. The observation period was extended due to the expectable death of some animals which showed symptoms on the 13th day. Body weight was measured immediately after the death or terminally.	X6
5.2 Results and discussion	In the lower tow dose groups, the males animals were symptom free and no death occurred in these groups. In the male dose groups, 15, 20 and 25 mg/kg most frequent clinical signs due to dyspnoea, paleness, wounds, swollen areas, and cyanotic parts of the body and bleedings on different regions of the body. The symptoms appeared in male animals between the 5th and 13th days. One male died in the 15mg/kg dose group on the 10th day. In the 20 mg/kg dose group 2 males died on the 6th day and one on 7th day. In the 25 mg./kg group 2 males died on the 7th day and on the 13th day of the observation period. In female animals, symptoms were similar to males, but in the some cases these appeared in the lowest group. Besides general symptoms, wounds and bleedings in some cases, paralysed limbs, lateral lying position, abnormal gait and restlessness was found in female animals. The symptoms were observed between 5th and 17th days. In males the body weight gain decreased in the 15 mg/kg dose group in the first week, but no effect on body weight was seen in the other dose groups. In females, there were no effects on body weight gain. Acute dermal LD_{50} Males + females 23.31 mg/kg; typical anticoagulant	X7
5.3 Conclusion	The single dermal 24 hour exposition with bromadiolone caused haemorrhages, haematomas all over the body in rats. The death of animals might be due to the oligaemic shock.	X8
3.2.2 Reliability	1	
3.2.3 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2005	

Section A6.1.2 **Annex Point IIA VI 6.1.2**	**Acute Toxicity** Acute Dermal LD_{50} in rat	
Materials and Methods	Applicants version adopted with amendments: X1 No justification for the lower purity (96%) in this study compared to the 98% purity level in section 2. However, this slight decrease in purity should not have a major impact on the results of this study. X2 The observation period has been extended to 18 days, causing a deviation from OECD 402, in which a 14 day observation period is recommended. However, this had no significant impact on the results. X3 Dosage volume 0.1 ml/100g, that is approx. 0.2-0.25 ml/rat. X4 Clinical observations were performed 1h and 5hrs after the first exposure and thereafter daily. Body weights were recorded on day 0, day 7, day 14 and day 18. Gross necropsy was performed in all animals immediately after the death or terminally. All deaths occurring during the observation period were recorded. X6 Body weights were recorded on day 0, day 7, day 14 and day 18. Gross necropsy was performed in all animals immediately after the death or terminally. All deaths occurring during the observation period were recorded. The animals were housed individually and received food and water *ad libitum.*	
Results and discussion	X5 No dose related effects on mean body weight or mean body weight gain were reported. X7 Applicants version replaced by: In the two lowest dose groups (5.0 mg/kg and 10 mg/kg) six animals/group (in both cases all males and one female) were symptom-free. In the 15 mg/kg dose group one male was symptom free and in the 20 mg/kg group two males were symptom free. In the 25 mg/kg group, two males and one female were symptom free. The most frequent symptoms observed were paleness, decreased activity, squatting position, exophthalmus and piloerection. There were also cases of dyspnea, nosebleeding, swellings, wounds and paralysis. In most cases the onset of symptoms occurred on the 5^{th} - 7^{th} day, but in some cases symptoms appeared on the 10^{th}-14^{th} day. Deaths occurred between the 5^{th}- 14^{th} day. For mortality rates see Table A6_1-1. No dose- related effects on mean body weight or mean body weight gain were reported. Common necropsy findings were haematomas, haemorrhages, blood-filled nasal and thoracic cavity, hydrometras and pale kidneys.	
Conclusion	X8 Applicants version replaced by: Acute dermal LD_{50} males: 20. 62 mg/kg bw, females: 32.08 mg/kg bw combined sexes LD_{50} :23.31 mg/kg bw. Based on the results of this study, it has been established that the acute dermal combined sexes LD_{50} value for bromadiolone in rat is 23.31 mg/kg bw with the 95% confidence limits at 15.46 – 241.79 mg/kg.. Since the upper 95% confidence limit is located at 241.79 mg/kg, the statistical safety of the dermal LD_{50} value, obtained following analysis based on the results from this study, is low. Based on the acute dermal LD_{50} :23.31 mg/kg bw, according to directive 67/548/EC, bromadiolone requires a classification as Very Toxic (T+).	
Reliability	1	
Acceptability	Acceptable	

Section A6.1.2 **Annex Point IIA VI 6.1.2**	**Acute Toxicity** Acute Dermal LD_{50} in rat	
Remarks	LD_{50} in Table A6_1.1 are values for acute oral toxicity, but should be dermal. No animals were given 0 mg/kg. Necropsy findings in the 10 mg/kg group were not seen in females only, lung haemorrhages were also seen in two males of this dose group. In the 15 mg/kg dose group no blood filled nasal or thoracic cavity has been registered in the submitted necropsy report. Instead haemorrhages in the testes and bloody nasal discharge were reported in this dose group. In the 25 mg/kg group necropsy findings also included a blood filled urinary bladder. Also, most lung haemorrhages in all dose groups were classed as pinprick, not point-like as specified in the table.	

Table A6_1-1. Table for Acute Toxicity (modify if necessary)

Dose [unit]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0	-	-	
5 mg/kg	1/10	Day 9	Haemotona. 3 with point-like haemorrhages in lung. 2 with hydrometra. 1 with pale kidneys
10 mg/kg	2/10	Day 7	Females only -4 with point-like haemorrhages in lung and 1 with hydrometra. 1 with pale kidneys.
15 mg/kg	3/10	Days 5 – 10	Haematoma and haemorrhages - Nasal and thoracic cavity filled with blood. 3 with point-like haemorrhages in lung and 1 with pale kidneys
20 mg/kg	4/10	Days 6 – 13	Haematoma and haemorrhages - Nasal and thoracic cavity filled with blood. 3 with hydrometra
25 mg/kg	6/10	Days 7 – 14	Haematoma and haemorrhages - Nasal and thoracic cavity filled with blood. 2 with point-like haemorrhages in lung. 1 with hydrometra. 1 with pale kidneys
LD_{50} value	Males 1.43 mg/kg; Females 1.25 mg/kg; Males + females 1.31 mg/kg with 95% confidence limits 1.17 – 1.49 mg/kg		

Section A6.1.3 **Annex Point IIA 6.1.3**	**Acute Toxicity - Inhalation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Compound is of very low vapour pressure and stable at NTP. It decomposes without boiling >200ºC. It is not applied by vaporisation, spraying or dusting as a fine powder. It is used in the form of a large bait block up to 250g, which is non-friable in nature. The potential for inhalation is therefore negligible. This is borne out by exposure assessment figures.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2008	
Evaluation of applicant's justification	X1: The exposure assessment figures provided by the applicant may be subject to change during the evaluation.	
Conclusion	Justification is acceptable	
Remarks		

Section A6.1.4(1) **Annex Point IIA V1 6.1.4**	**Acute Dermal Irritation** Primary dermal in rabbits	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (1999) Primary Dermal Irritation Study in Rabbits, XXXXX. Report 4743-98	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-5, and OPPTS No. 870.2500	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	980803	
3.1.2 Specification	As given in section 2	
3.1.3 Description	Yellow powder	
3.1.4 Purity	96% technical bromadiolone (code number LX125-01)	X1
3.1.5 Stability	Stable	
3.2 Test Animals		
3.2.1 Species	Albino rabbit	
3.2.2 Strain	New Zealand White	
3.2.3 Source	Ray Nichols Rabbitry; Lumberton, Texas	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	12-13 weeks	
3.2.6 Number of animals per group	3 male and 3 female	
3.2.7 Control animals	No	
Administration/ Exposure	Dermal	
3.2.8 Application		

3.2.9 Preparation of test substance	0.5g of test substance moistened with 2.0 ml of deionised water.	
3.2.10 Test site and Preparation of Test Site	An area at least 8 cm x 8 cm was clipped free of hair on the dorsal area of the trunk.	
3.2.11 Occlusion	Semi-occlusive	
3.2.12 Vehicle	Deionised Water	
3.2.13 Concentration in vehicle	0.5g in 2 ml of deionised water	
3.2.14 Total volume applied	Single dose of 0.5g	
3.2.15 Removal of test substance	Room temperature tap water and a clean cloth to remove residue	
3.2.16 Duration of exposure	4 hours	
3.3.17 Postexposure period	72 hours	
3.3.18 Controls	None	
3.4 Examinations		
3.4.1 Clinical signs	Yes	
3.4.2 Dermal examination	Yes	
3.4.3 scoring system	Draize Technique	
3.4.4 Examination time points	60 min, 24h, 48h, 72h	
3.4.5 Other examinations	Observation for erythema, oedema and any other skin defects or irritation.	
3.5 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Average score		
4.1.1Erythema	Average score for all animals at 24, 48, 72 h = 0.0	X2
4.1.2 Oedema	Average score for all animals at 24, 48, 72 h = 0.0	
4.3 Reversibility	No effects to reverse.	
4.4 Other examinations	No other signs of irritation or other dermal effect	
4.5 Overall result	Test Substance is rated non-irritating and is assigned to Toxicity Category IV	X3
	5 APPLICANT'S SUMMARY AND CONCLUSION	

5.1 Materials and methods	Primary dermal irritancy study in rabbits was conducted according to OPPTS 870.2500. Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x8 cm. A single intact exposure site was selected as the test site while the contralateral intact site serves as a control site. On day 0, 0.5g of test substance moistened with 2ml of deionised water was applied to each test site and covered with a surgical gauze patch measuring 2.5 x 2.5 cm and four single layers thick. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing which was secured on both edges with strips of tape-to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible. The test sites were observed for erythema, and edema formation, and any other dermal defects or irritation at 1, 24, 48, and 72 hours after unwrap.	X4
5.2 Results and discussion	Erythema and edema were not observed at any time throughout the study. No other signs of irritation were observed during the study. Primary dermal irritancy score of 0 – non-irritant.	X5
5.3 Conclusion	The primary irritation index of 0 out of a possible 8 was obtained from 1, 24, 48 and 72 hour observations and was used to give 96% Technical bromadiolone (LX125-01) as non-irritating.	X6
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	X7

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 4th 2005	
Materials and Methods	Applicants version accepted with amendments: X1 There is no justification for the lower purity (96%) in this study compared to the 98% purity level in section 2. This minor decrease in purity level should however not have any major impact on the outcome of the study. X2 According to EU Method B.4, the test site should be examined immediately after the (protective) patch has been removed. This immediate examination was not performed in this study, which is unfortunate taking into account animal well-fare issues. X4 In EU Method B.4, it is strongly recommended that the *in vivo* test be performed initially using one animal. In this study all six animals were treated simultaneously, which is unfortunate. Also, the study report in document IV does not include any information on observations of systemic adverse effects or effects on body weight. Therefore it is presumed that such observations were not performed, thus causing the study to deviate from the from EC Method B.4. X7 A number of deviations from EU Method B.4 have been identified, see X2 and X4.	
Results and discussion	X3 Categorization of toxicity into category I- IV, is not included in the classification and labelling procedures presented in directive 67/548/EU. X5 The use of primary irritancy scores are not included in the classification and labelling procedures presented in directive 67/548/EU.	
Conclusion	X6 Applicants version replaced with: Based on the results of this study and the criteria for classification and labelling presented in directive 67/548/EU, bromadiolone is classified as non-irritant.	
Reliability	1	
Acceptability	Acceptable	
Remarks		

Table A6.1.4-1. Table for skin irritation study			
score (average animals investigated)	time	Erythema	Oedema
average score Draize scores (0 to maximum 4)	60 min	0	0
	24 h	0	0
	48 h	0	0
	72 h	0	0
other times	*State time*	-	-
average score	24h, 48h, 72h	0	0
reversibility: *		-	-
average time for reversibility		-	-

* c : completely reversible
n c : not completely reversible
n : not reversible

Section 6.1.4(2) **Annex Point IIA6.1.4**	**Acute Eye Irritation** Acute eye irritation in rabbits.	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (1999) Primary Eye Irritation Study in Rabbits, XXXXX. Report 4742-98	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-4, and OPPTS No. 870.2400	X1
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Bromadiolone	
3.1.1 Lot/Batch number	980803	
3.1.2 Specification	Min. 96.0%	

Section 6.1.4(2) **Annex Point IIA6.1.4**	**Acute Eye Irritation** Acute eye irritation in rabbits.	
3.1.3 Description	Yellow powder	
3.1.4 Purity	98%	
3.1.5 Stability	Stable	
3.2 Test Animals		
3.2.1 Species	Albino rabbit	
3.2.2 Strain	New Zealand White	
3.2.3 Source	Ray Nichols Rabbitry; Lumberton, Texas	
3.2.4 Sex	Male and female	X2
3.2.5 Age/weight at study initiation	12 weeks	
3.2.6 Number of animals per group	3 male and 3 female	X3
3.2.7 Control animals	No	
3.3 Administration/ Exposure		
3.3.1 Preparation of test substance	Applied undiluted	
3.3.2 Amount of active substance instilled	0.1 ml by volume (0.032 mg)	
3.3.4 Exposure period	24 hours	
3.3.5 Postexposure period	4 days	
3.4 Examinations		
3.4.1 Ophthalmoscopic examination	Yes	
3.4.2 Scoring system	Draize Technique.	
3.4.3 Examination time points	60 min, 24h, 48h, 72h	
3.4.4 Other investigations	All treated eyes washed at 24 hrs	
3.5 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	No clinical signs outside of those associated with the eye, were observed.	
4.2 Average score		
4.2.1 Cornea	0.44 over 24, 48 and 72hrs .	
4.2.2 Iris	0 over 24, 48 and 72hrs	
4.2 3 Conjunctiva		

Section 6.1.4(2) **Annex Point IIA6.1.4**	**Acute Eye Irritation** Acute eye irritation in rabbits.	
4.2.4 Redness	0.44 over 24, 48 and 72hrs	
4.2.5 Chemosis	0.16 over 24, 48 and 72hrs	
4.3 Reversibility	Yes Cornea opacity – disappeared by 24 hrs. Conjunctiva, redness - disappeared by 4 days after treatment. Conjunctiva, chemosis - disappeared by 72 hrs.	
4.4 Other	Conjunctiva, discharge disappeared by 4 days	
4.5 Overall result	Not an irritant	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Primary eye irritancy study in rabbits to OPPTS 870.2400 protocol	X4
5.2 Results and discussion	The maximum average irritation score of 12.3, obtained at 1 hour after treatment, was used to rate bromadiolone as mildly irritating. Toxicity categories are determined by the presence and duration of corneal involvement, iridic irritation and 'positive' conjunctival positive. Any corneal involvement of iridic irritation with a score of 1 or is considered positive. Any conjunctival irritation with a score of 2 or more is considered positive. Since all positive effects had cleared prior to Day 4, the test substance is assigned to toxicity category III.	X5
5.3 Conclusion	Bromadiolone was rated mildly irritating. No irritation was observed in any eyes by Day 4.	X6
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section 6.1.4(2) **Annex Point IIA6.1.4**	**Acute Eye Irritation** Acute eye irritation in rabbits.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April 2006	
Materials and Methods	X1 EC Test Method B.5 should be used, not EPA Guidelines. X2 Initial body weights females 2.500- 2.825 kg males 2.550- 2.575 kg X3 A sequential use pattern was not performed, which is unfortunate. X4 Applicants version replaced with: Primary eye irritation of Bromadiolone was studied in three albino rabbits/sex. An acclimatisation period of five days passed before initiating the study. The rabbits were housed individually under a 12 hr light/dark cycle and fed a conventional laboratory animal diet. 0.1 ml Bromadiolone (0.032 mg) was placed in the conjunctival sac of the right eye of each animal. The left eye functioned as a control. Both eyes were washed with deionized water after the examination after 24 hrs exposure. Grades of ocular reaction were recorded at 1, 24, 48 and 72 hrs and on day 4 after treatment.	
Results and discussion	X5 Applicants version replaced by: Cornea opacity was reported in 5/6 animals but disappeared by 48 hrs. No effects on the iris were reported. In the conjunctiva, redness was reported in all six animals but disappeared by 4 days after treatment. Conjunctival chemosis was seen in all animals but disappeared by 72 hrs. No clinical signs other than those associated with the eye, were observed. Bromadiolone is thus mildly irritant and the effects are reversible.	
Conclusion	X6 Applicants version replaced by: Although the results show that bromadiolone is mildly irritating to the eye, the results do not fulfil the criteria for R36 classification set in directive 67/548/EEC, and thus Bromadiolone is not considered to be an ocular irritant.	
Reliability	1	
Acceptability	Acceptable	
Remarks	Table A6.4.1-1 includes some incorrect data and is therefore replaced with a revised version found below.	

Table A6.4.1-1. Results of eye irritation study

	Cornea (opacity)	Iris	Conjunctiva	
			redness	chemosis
Score (average of 6 animals investigated)	0-4	0-2	0-3	0-4
60 min	0.17	0	2	1.33
24 h	0.16	0	0.83	0.33
48 h	0.16	0	0.33	0.16
72 h	0	0	0.16	0
Average 24h, 48h, 72h	0.11	0	0.44	0.16
Affected area	4	-	-	
-#Maximum average score (Draize method - including area affected, max 110)	3.4	0	8.98	
Reversibility*	c	-	c	c
average time for reversion	24 hrs	-	48 hrs	24 hrs
* *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				
# Max average score is based on Draize method. For Cornea, figure is derived using average value of opacity after 1 hr x area affected(1hr) x 5= 0.17 x 4 x 5=3.4.				
For Iris it is zero since all values are 0.				
For the conjunctiva, figure is derived from [redness average (1hr) + chemosis average(1hr) + discharge average (1hr)]x2 =(2 + 1.33 + 1.16)x2 = 8.98				
The total score for the eye is the sum of all these scores with a maximum possible score being 110.				
So, maximum average score = 3.4+8.98 = 12.38				
This is slightly different to the 12.3 reported in the study, but still falls within the same rating range.				
Using a slightly modified rating system from Kay and Calandra((1962) Interpretation of eye irritation tests, J.Soc Cosmetic Chemists 13 : 281-289) as indicated in report, this would indicate the substance is mildly irritating.				

Revised version of Table A6.4.1-1			**Results of eye irritation study**		
	Cornea (opacity)	Iris	Conjunctiva		
			redness	chemosis	Discharge
Score (average of 6 animals investigated)	0-4	0-2	0-3	0-4	
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 24h, 48h, 72h	0.11	0	0.44	0.17	0.17
Affected area	4	-			
-#Maximum average score (Draize method - including area affected, max 110)	3.4	0		8.98	
Reversibility*	c	-	c	c	c
Time for complete reversion	72hrs	-	4days	72hrs	72hrs

* *c : completely reversible*
n c : not completely reversible
n : not reversible

 # Max average score is based on Draize method. For Cornea, figure is derived using average value of opacity after 1 hr x area affected(1hr) x 5= 0.17 x 4 x 5=3.4.

For Iris it is zero since all values are 0.

For the conjunctiva, figure is derived from [redness average (1hr) + chemosis average(1hr) + discharge average (1hr)]x2

=(2 + 1.33 + 1.16)x2 = 8.98

The total score for the eye is the sum of all these scores with a maximum possible score being 110.

So, maximum average score = 3.4+8.98 = 12.38

This is slightly different to the 12.3 reported in the study, but still falls within the same rating range.

Using a slightly modified rating system from Kay and Calandra((1962) Interpretation of eye irritation tests, J.Soc Cosmetic Chemists 13 : 281-289) as indicated in report, this would indicate the substance is mildly irritating.

Section A6.1.5 (1) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Skin sensitisation in guinea pigs:Buehler Test	
	1 REFERENCE	**Official use only**
1.1Reference	XXXXX (1999) Dermal Sensitization Study in Guinea Pigs, XXXXX report 4770-98	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-6, and OPPTS No. 870.2600	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	Lot No 2/98	
3.1.2 Specification	As given in section 2	
3.1.3 Description	Deep Purple Liquid	
3.1.4 Purity	Bromadiolone 2,5	
3.1.5 Stability	Stable	
3.1.6 Preparation of test substance for application	For induction: Topical application: used undiluted For challenge: Topical application: used undiluted	
3.1.7 Pretest performed on irritant effects	Yes	
3.2 Test Animals		
3.2.1 Species	Guinea pigs	
3.2.2 Strain	Hartley Albino	
3.2.3 Source	Charles River Laboratories, Wilmington MA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Age not stated Male 352 – 392g Female 357 – 393g	
3.2.6 Number of animals per group	Naïve Control group 10 (5 male, 5 female) Treatment Group 10 (5 male, 5 female)	

Section A6.1.5 (1) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Skin sensitisation in guinea pigs:Buehler Test	
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	State study type: Non - Adjuvant	
3.3.1 Induction schedule	Day 1 - topical application Day 8 – topical application Day 15 – topical application *see table in appendix*	
3.3.2 Way of Induction	Topical	
	Semi-occlusive	
3.3.3 Concentrations used for induction	Topical: Bromadiolone 2.5% undiluted	
3.3.4 Concentration Freunds Complete Adjuvant (FCA)		
3.3.5 Challenge schedule	Day 29	
3.3.6 Concentrations used for challenge	bromadiolone 2.5 % undiluted	
3.3.7 Rechallenge	No	
3.3.8 Scoring schedule	24h, 48h after challenge	
3.3.9 Removal of the test substance	No removal of test substance	
3.3.10 Positive control substance	Separate test with 2-mercapto-benzothiazole	
3.4 Examinations		
3.4.1 Pilot study	Yes	
3.5 Further remarks	No mortalities	
	4 RESULTS AND DISCUSSION	
4.1 Results of pilot studies	Induction and challenge at 0.4 ml 2-mercapto-benzothiazole of 10% w/v solution in acetone Mean score of 0.5 for the test group after challenge compared to 0.2 for naïve control group confirming that guinea pigs were sensitive to the positive control material	
4.2 Results of test		
4.2.1 24h after challenge	Not possible to score as treatment stained skin	
4.2.2 48h after challenge	Not possible to score as treatment stained skin	
4.2.3 Other findings		
4.3 Overall result	Not possible to score as treatment stained skin	
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.1.5 (1) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Skin sensitisation in guinea pigs:Buehler Test	
5.1 Materials and methods	EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-6, and OPPTS No. 870.2600	X1
5.2 Results and discussion	The test substance deeply stained the test sites in all animals during the entire study, so scoring for erythema was impossible. Therefore, no evidence for sensitisation or non- sensitisation could be obtained.	X2
5.3 Conclusion	The test substance deeply stained the test sites in all animals during the entire study, so scoring for erythema was impossible. Therefore, no evidence for sensitisation or non- sensitisation could be obtained. However, there is no evidence of skin sensitising effect in any other dermal administration study, or in any commercial or experimental use. Moreover, normal rodent control operations demand a high level of hygiene, and especially the wearing of gloves.	X3
5.3.1 Reliability	3	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April 2006	
Materials and Methods	X1 Skin sensitisation in guinea pigs: Buehler Test	
Results and discussion	X2 Applicants version replaced by: The test substance deeply stained the test sites in all animals during the entire study, so scoring for erythema was impossible.	
Conclusion	X3 Applicants version replaced by: The test substance deeply stained the test sites in all animals during the entire study, so scoring for erythema was impossible. Therefore, no dermal sensitisation or non- sensitisation results could be obtained.	
Reliability	4	
Acceptability	Not Acceptable	

Table A6.1.5-1 Detailed information including induction/challenge/scoring schedule for skin sensitisation test

state test applied, delete other (modify if necessary, i.e. day of treatment)

Inductions	**GPMT**		**Buehler test**	**Observations/Remarks** *give information on irritation effects*
	day of treatment	application	day of treatment	
Induction 1	-	Topical	1	Skin deeply stained, unable to assess.
Induction 2	-	Topical	8	Skin deeply stained, unable to assess.
Induction 3	-	Topical	15	Skin deeply stained, unable to assess.
challenge	-	Topical	29	Skin deeply stained, unable to assess.
scoring 1			30	Skin deeply stained, unable to assess.
scoring 2			31	Skin deeply stained, unable to assess.

Table A6.5.1-2 Result of skin sensitisation test (modify if necessary)

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control**
scored after 24h	**9 / 10**	**N/A**	**9/10**
scored after 48h	**1 / 10**	**N/A**	**8/10**

Section A6.1.5 (2) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Buehler Test in Guinea Pigs	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX, (2004) Skin sensitisation of test item bromadioone technical in guinea pigs by Buehler method, XXXXX, Study code: 04/916-104T	
1.2 Data protection	Yes	
1.2.1 Data owner	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.1 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes – OECD 406	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.3 Description	Not stated	
3.1.4 Purity	97.6%	X1
3.1.5 Stability	Not stated	X2
3.1.6 Preparation of test substance for application	*for induction & Challenge:* Bromadiolone was powdered in a mortar and homogenised with ethanol then mixed with methyl cellulose (1%). The final ethanol concentration of formulation was 4%. The test item was used for the dermal induction and challenge in concentration of 5%.	X3
3.1.7 Pretest performed on irritant effects	Yes	

Section A6.1.5 (2) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Buehler Test in Guinea Pigs	
Test Animals		
3.1.8 Species	Guinea pigs	
3.1.9 Strain	Dunkin Hartley	
3.1.10 Source	LAB-ALL Bt. Budapest, 1174 Hunyadi u. 7.	
3.1.11 Sex	female	
3.1.12 Age/weight at study initiation	Weight: 311-350g	
3.1.13 Number of animals per group	2 animals per concentration,	X4
3.1.14 Control animals	Yes	
3.2 Administration/ Exposure	Non-Adjuvant	
3.2.1 Induction schedule	day 0 – day –7 – day 14	
3.2.2 Way of Induction	Topical	
	Occlusive	
3.2.3 Concentrations used for induction	5% test substance / 0.5 ml	X5
3.2.4 Challenge schedule	day 28; see table in appendix	
3.2.5 Concentrations used for challenge	5% test substance / 0.5 ml	
3.2.6 Rechallenge	No	
3.2.7 Scoring schedule	24h, 48	
3.2.8 Removal of the test substance	Not stated	
3.2.9 Positive control substance	Potassium dichromated	
3.3 Examinations		
3.3.1 Pilot study	yes	
3.4 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Results of pilot studies	0.5ml of test formulation in concentrations of 0.001, 0.1, 0.1, 1, 5, 10, 25 and 50% produced no reaction on the skin of guinea pigs after the first treatment. At higher doses, 50, 25, and 10% of test item, the animals died, on either 2md, 3rd, and 4th weeks of the experiment.	
4.2 Results of test		
4.2.1 24h after challenge	After the challenge with bromadiolone in 5%, positive response was not observed on the animals of the test group. The mean scores was 0.00. No effects seen on control groups.	X6

Section A6.1.5 (2) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Buehler Test in Guinea Pigs	
4.2.2 48h after challenge	After the challenge with bromadiolone in 5%, positive response was not observed on the animals of the test group. The mean scores was 0.00. No effects seen on control groups.	X6
4.2.3 Other findings	Body weight: No significant difference was found between the groups.	
4.3 Overall result	Bromadiolone was classified as non-sensitiser.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Main study: Dermal induction exposure (Day 0) An area of approximately 5x5 cm on the scapular region of the animals were clipped free of hair and shaven close with care. The test animals were treated with 0.5ml of the determined concentration of the test item (5%). The control group was treated with 0.5ml vehicle. The exposed areas were covered for 6 hours with a porous gauze fastened with 'Leucoplast'. Main Study: Dermal Induction exposure (Day 7 and 14) The same application as on day 0 was carried out on the same test area of the same flank on day 7, and 14. After the dermal induction treatment the animals were left untreated for 14 days prior to challenge. Main study: Challenge exposure (Day 28) Two weeks after the latest dermal induction treatment, the animals were exposed to challenge dose, dermally. 24 hours before the challenge treatment the left and the right flank areas of each animal were prepared for application. The left shaved flank area of all animals was treated with 0.5ml of the test item at 5%. The right shaved flank area of all animals was treated with 0.5ml of the vehicle. It was exposed for 6 hours.	X7
5.2 Results and discussion	After the challenge with bromadiolone in 5%, positive response was not observed on the animals of the test group. The mean scores was 0.00 according to the 24th and 48th hour results. No effects seen on control groups at the 24th and 48th hour results. There were changes in the body weights.	X8
5.3Conclusion	Bromadiolone is not sensitising to the skin of Guinea-pigs under the conditions of the study.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section A6.1.5 (2) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Buehler Test in Guinea Pigs	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April 2006	
Materials and Methods	Applicants version accepted with amendments: X1 The purity level has been changed to 99.2 % in the final study report and deviates thereby from the 98% purity presented in section 2. However, this should not have a major impact on the outcome of the study. X2 According to section 2, bromadiolone is stable at room temperature. X3 According to EU test Method B.6 the use of water, surfactant, 80% ethanol /water solution and acetone as vehicle is recommended. However, since the phys-chem. Data in section 2 reveals that solution in these vehicles may not be possible for bromadiolone, therefore the use of the ethanol/methyl cellulose vehicle is justified. X4 In the pilot study each exposure group consisted of 2 animals. In the main study the test group consisted of 20 animals, the vehicle control of 10 animals and the positive control included 20 animals. X5 According to EU Method B.6 the concentration used for the challenge exposure should be the highest non-irritating dose. In the pilot study the highest exposure concentration at 50 % did not induce ant sensitizing effect. However, in the pilotstudy animals exposed to concentrations above 10% died, therefore the 5% exposure concentration is justified. Vehicle consisted of ethanol (4%) and methyl cellulose. X6 In the previously performed reliability study 20 animals were exposed to potassium dichromate and positive reactions were seen in 15 of 20 animals with the mean score of 1.85 and 1.80 at 24 and 48 hrs. X7 Pilot study: 0.5ml of test formulation in concentrations of 0.001, 0.1, 0.1, 1, 5, 10, 25 and 50% produced no reaction on the skin of guinea pigs (2 guinea pigs/dose level) after the first treatment. At the 50%, 25% and 10% concentrations both animals died, durin the 2^{nd} - 4^{th} week. Main study: Body weights were recorded at the start and end of the study. The test group consisted of 20 animals, the vehicle control of 10 animals and the positive control included 20 animals. At 24 hrs and 48 hrs after patch removal all animals were examined and dermal irritation was scored using the Draize scoring system.	
Results and discussion	Applicants version accepted with amendments: X8 In the previously performed reliability study 20 animals were exposed to potassium dichromate and positive reactions were seen in 15 of 20 animals with the mean score of 1.85 and 1.80 at 24 and 48 hrs. No dermal effects seen in the vehicle control group at the 24 and 48 hours after patch removal. There were no differences in mean body weight in the test group compared to controls at the start or end of the study. In this case the Buehler test is accepted instead of the preferred Guinea Pig Maximisation Test, taking into consideration that products including the active have been on the market for many years, yet there is no data indicating sensitisation reactions following human exposure to bromadiolone.	
Conclusion	Applicants version accepted	
Reliability	1	

Section A6.1.5 (2) **Annex Point IIA VI 6.1.5**	**Skin sensitisation** Buehler Test in Guinea Pigs	
Acceptability	Acceptable	
Remarks		

Table A.6.1.5-1 Detailed information including induction/challenge/scoring schedule for skin sensitisation test

state test applied, delete other (modify if necessary, i.e. day of treatment)

Inductions	**Buehler test**	**Observations/Remarks** *give information on irritation effects*
	day of treatment	
Induction 1	day 0	No visible changes
Induction 2	Day 7	No visible changes
Induction 3	Day 14	No visible changes
challenge	28	No visible changes
scoring 1	24	0
scoring 2	48	0

Table A.6.1.5-2 Result of skin sensitisation test (modify if necessary)

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control**
scored after 24h	**0 /10**	**0 /20**	**15 /20**
scored after 48h	**0 /10**	**0 /20**	**15 /20**

Section A6.2 Metabolism studies in mammals

Section A6.2 Annex Point IIA VI 6.2	Metabolism studies in mammals	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	A large amount of data exists in the public domain relating to the metabolism of anticoagulants especially with regard to warfarin as a result of its therapeutic use in humans. Since the anticoagulants are a closely related group of analogues with clear and similar physico-chemical and toxicological properties, it is considered valid to consider that data on one compound as being applicable to others. Comparison of pharmacokinetic properties of warfarin, brodifacoum and difenacoum has shown this extrapolation to be valid for those compounds (Breckenridge, Cholerton et al 1985). Consequently, it is considered that adequate data exist already and that further studies cannot be justified on animal welfare grounds. Flocoumafen was rapidly absorbed into the blood from a single oral dose. Elimination was very slow with less than 0.5% of the dose being detected in urine up to 7 days after dosing and 23-26% in the faeces. Approximately half of the dose was found in the liver that was eliminated with a half-life of 220 days. (Huckle K R et al (1989). Anticoagulant rodenticides including brodifacoum are rapidly absorbed via the gastro-intestinal tract. After a single dose of the analogue difenacoum the highest concentration was found in the liver (41.5% of administered dose) 24 hrs after dosing. Elimination from the liver was biphasic, with the half-life for the first phase being 8 days and for the second phases 118 days. A similar biphasic pattern was seen in the kidney. Thin layer chromatography of solvent extracts of livers from rats killed 1 and 14 days after dosing showed that the bulk of radioactivity consisted of metabolites (Parmar et al 1987). The major route of elimination of anticoagulants in rats and sheep after oral administration is via the faeces, primarily as metabolites rather than parent compound (WHO 1995) When administered orally to male Spague-Dawley rats at doses ranging from 0.1-0.33 mg/kg bw, brodifacoum exhibited a remarkably steep dose-response curve; 0.1 mg/kg bw failed to induce any effect on plasma prothrombin level within 24 hrs whereas 0.2 mg/kg bw reduced the prothrombin complex activity to 7% of normal values and 0.33 mg/kg bw reduced it to 4% of normal. Concentrations in the liver were rapidly established, remained relatively constant for at least 96 hrs and exceeded serum concentrations by 20-fold. The mean serum/liver concentration ratio was approximately 20. Disappearance from the liver is relatively slow with a half-life of 156 hrs or more (Bachman and Sullivan 1983) Brodifacoum was found in rats‘ faeces as unchanged parent compound. In	

Section A6.2 Metabolism studies in mammals

Section A6.2 Annex Point IIA VI 6.2	Metabolism studies in mammals

sheep, about 20 and 30% of an oral dose of 0.2 or 2.0 mg/kg bw respectively were eliminated in the faeces within 8 days. No residues of brodifacoum were detected in the abdominal fat of sheep 8 days after an oral administration of 0.2 or 2.0 mg/kg bw. Brodifacoum was detected in livers of sheep 128 hrs after oral administration of either 0.2 or 2.0 mg/kg bw in concentrations of 0.64 and 1.07 mg/kg dry weight respectively (Laas et al 1985).

In mongrel dogs, the elimination of brodifacoum follows a classical decay curve with a distributive half-life of 1.4 days and an elimination half-life of 8.7 days (Murphy et al 1985). An elimination half-life in serum has been determined at 6±4 days in dogs (Woody et al 1992).

Six weeks after intravenous administration of a single dose of 1 mg/kg bw to male New Zealand White rabbits, the prothrombin complex activity was still lower than 30% of normal. It was also shown in this study that in the rabbit the maximal antagonist dose of vitamin K_1 by warfarin was produced by a dose of 63 mg/kg whereas a similar result was obtained with only 1 mg/kg bw of brodifacoum (Park and Leck 1982)

In horses gavaged with brodifacoum-containing bait equivalent to 0.125 mg brodifacoum /kg bw , the peak plasma concentration occurred 2-3 hrs after dosing. Detectable levels of brodifacoum were still present in two horses 9 days after treatment. Pharmacokinetic evaluation indicated that brodifacoum has a half-life of 1.22 ±0.22 days, a body clearance of 1073.1± 53.21 mg/kg bw and closely approximates a one-compartment model in the elimination phase (Boermans et al 1991).

In humans, the half-life of brodifacoum in patients with haemorrhages was found to range from 16-36 days (Weitzel et al., Donovan et al.).

Mice eliminated most of the administered ^{14}C in faeces and urine within 4 days after dosing. Rats however were much slower and radiocarbon continued to appear 8 days after dosing. The acute oral LD_{50} is 2.3 mg/kg for rats and 141 mg/kg for mice; this difference may in part be related to the diphacinone elimination rates between the species. (Ching C. Yu, et al 1982)

The acute oral toxicity of brodifacoum to sheep was examined in a trial of 40 sheep. There was a low mortality in the high dose group attributed to the precipitation of insoluble brodifacoum in the sheep's alimentary canal. The lack of correlation of the level of brodifacoum in the liver suggests that the liver is not suitable tissue for quantifying exposure of an animal to brodifacoum. The liver may be a metabolic site that is saturable. (M.E.R. Godfrey, F.J.Laas and C.G. Rammell (1985)

In a repeated dose of flocoumafen on rats the high dose group showed clinical signs consistent with anticoagulant toxicity. Excretion of radioactivity via the urine was a very minor route at both dose levels. The major route is via faeces. Radioactivity in the tissues for both low and high dose groups was in the order of liver>>kidney>>skin>muscle>fat>blood. (K.R. Huckle et al 1988)

In a study that examines the pharmacokinetics of difenacoum in rats the *cis:trans* diastereomer ratio were calculated as 2.95 by fluorescence detection and 3.15 by ultraviolet absorption. This method provides a simple, rapid and sensitive technique for the analysis of diastereomers of difenacoum in blood and liver tissues (M. J. Kelly et al 1993).

Analysis by gas chromatography/mass spectrometry after chromic acid oxidisation of liver extracts does not differentiate between brodifacoum

Section A6.2 Metabolism studies in mammals

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and bromadiolone because they yield the same product, but conversely offers the advantage of screening with one protocol for the 2 most commonly used rodenticides. This technique also offers comparable sensitivity and much improved selectivity as contrasted with existing HPLC methods. (Allen C. Ray et al 1989)

From the toxicity study male mice are more susceptible to toxic effects of difenacoum than female mice and the fall in PCA is a reliable index of the pharmacological effects of coumarin anticoagulants. (M. J. Winn et al 1986).

The above data are consistent with other published and unpublished references, and are reliable enough to be used for risk assessment.

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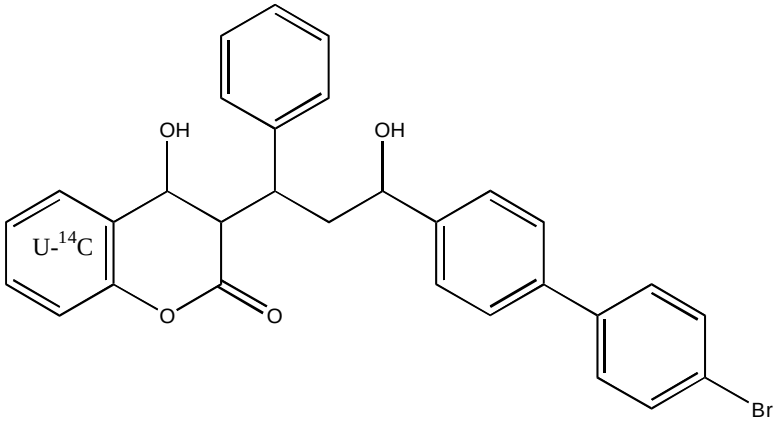
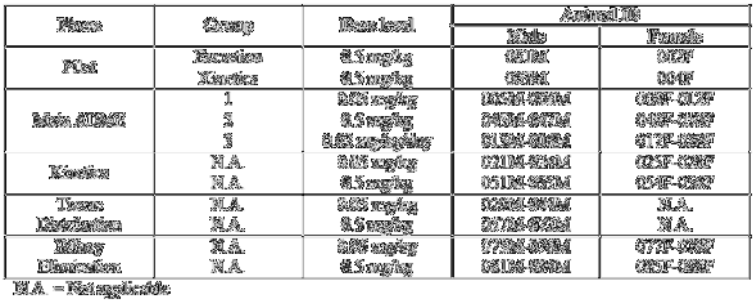
	K.R. Huckle, D.H. Hutson and P.A. Warburton (1988) Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. *Xenobiotica 1988 Vol 18, No. 2, 1465-1479.* M. J. Kelly, J. Chambers and A.D. MacNicoll (1993). Simple and rapid method for the determination of the diastereomers of difenacoum in blood and liver using high performance liquid chromatography with fluorescence detection. *Journal if Chromatography 620, 105-112.* Allen C. Ray, Michael J. Murphy, Michael D. DuVall and John C. Reagor (1989). Determination of brodifacoum and bromadiolone residues in rodent and canine liver. *Am J Vet Res, Vol 50, No. 4, April 1989, 546-550.* M. J. Winn, J. A. D. Clegg and B .K. Park. (1986). An investigation of sex-linked differences to the toxic and to the pharmacological actions of difenacoum: studies in mice and rats. *J. Pharm Pharmacl. 1987 , 39:219-222.*
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2006
Evaluation of applicant's justification	Read-across between 4- hydroxycoumarine anticoagulants can not be accepted for toxicokinetic endpoints. It has previously been established that structurally analogous rodenticides, such as Bromadiolone, Brodifacoum and Difenacoum all include the 4- hydroxycoumarine group and that the affinity of this group to the VKOR is the mechanism of action behind the anticoagulant properties of these compounds. Thus, it can be concluded that these compounds have a comparable structure-activity relationship. However, knowing that there is a structure-activity relationship, dose not mean that the pharmacokinetic properties of these compound are comparable, since the pharmacokinetic properties are not only affected by the structure of the active moiety of the molecule, but are influenced by the structure and composition of the entire molecule. Therefore, read-across can not be accepted when considering the metabolism end-point. Further supporting the non-acceptance of read-across, pharmacokinetic studies on structural analogues Brodifacoum and Difenacoum, show that these compounds deviate pharmacokinetically.(Breckenridge et al., 1985 Also, since several justifications for non-submission of data included in this dossier, state that the dermal absorption of bromadilone is low, to accept theses justifications, the studies on dermal absorption must be performed.
Conclusion	Justification not accepted, a metabolism study, fulfilling the requirements of the EU Test Methods documents, must be performed. This was requested by the applicant, and the study was received in june 2008. This study is evaluated under point A6.2 (1).
Remarks	The applicant had submitted a series of study summaries for other substances that was not evaluated since read-across could not be accepted. They have not been included in this version of Doc III.

Section A6.2 (1) Annex Point IIA6.2	METABOLISM Metabolism of[^{14}C]- Bromadiolone in the Rat	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2008) The Metabolism of [^{14}C]-Bromadiolone in the Rat, XXXXX, Study No. 184056, Report No. 29088	
1.2 Data protection	Yes	
1.2.1 Data owner	The Bromadiolone Task Force	
1.2.2 Companies with letter of acces	N/A	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes OECD Guideline 417	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2 Bromadiolone	
3.1.1 Lot/Batch number	[^{14}C]-Bromadiolone: Batch No. CFQ14848 Non-radiolabelled Bromadiolone: Lot No. L22678	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	[^{14}C]-Bromadiolone: white solid	
3.1.2.2 Purity	[^{14}C]-Bromadiolone: 98.3 % (subsequent analysis of this material showed the purity to be unsuitable for use and therefore the material was re-purified at Charles River prior to each dose formulation) Non-radiolabelled Bromadiolone: >99 %	
3.1.2.3 Stability	Not specified	
3.1.2.4 Radiolabelling	^{14}C as indicated below: 	X

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
3.1.3 Analysis	The test item and study samples were analysed by HPLC using the following equipment and conditions. **HPLC Method 1** Equipment: HPLC Model: Agilent 1100 Series HPLC Radiodetector: Packard Model 150TR Flow Scintillation Analyser Scintillant: Ultima-Flo™M Flow Cell Volume: 500 µL Fraction Collector: Gilson Model 202 Fraction Collector Pre-column: Phenomenex Security Guard, 3 x 4mm HPLC Column: Zorbax ODS (250 x 4.6 mm; 5 µm) Data Handling: Atlas 2002 (Thermo LabSystems) Product Version 6.18 Conditions: Column Temperature: 40°C UV Detection: 260 nm Flow Rate: 1.0 mL/min Mobile Phase A: 0.1 % formic acid in Milli-Q water Mobile Phase B: 0.1 % formic acid in Acetonitrile Gradient **HPLC Method 2** In addition, all liver samples were analysed using the following equipment and conditions. This was performed to confirm if the major peak identified in HPLC Method 1 could be tentatively identified as Bromadiolone. Equipment: HPLC Model: Agilent 1100 Series HPLC Radiodetector: Packard Model 150TR Flow Scintillation Analyser Scintillant: Ultima-Flo™M Flow Cell Volume: 500 µL Fraction Collector: Gilson Model 202 Fraction Collector Pre-column: Phenomenex Security Guard, 3 x 4mm HPLC Column: Zorbax ODS (250 x 4.6 mm; 5 µm) Data Handling: Atlas 2002 (Thermo LabSystems) Product Version 6.18 Conditions: Column Temperature: 25°C UV Detection: 260 nm Flow Rate: 1.0 mL/min Mobile Phase A: Methanol:Milli-Q Water:Acetic Acid (5:94.2:0.8, v/v/v) Mobile Phase B: Methanol:Milli-Q Water:Acetic Acid (94.2:5:0.8, v/v/v)	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
	Gradient	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Sprague Dawley	
3.2.3 Source	Charles River (UK) Limited	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Age: ca 7-8 weeks at dosing Body weight: 176-355 g at dosing	
3.2.6 Number of animals per group		
3.2.7 Control animals	No	
3.2.8 Husbandry	A standard laboratory diet of known formulation (SDS Rat and Mouse Maintenance Diet No. 1, Special Diets Services, 1 Stepfield, Witham, Essex) and domestic mains tap water were available *ad libitum*. Holding and study areas had automatic control of light cycles and temperature. Light hours were 0700-1900 h. Ranges of temperature and humidity measured during the study were 19-22 °C and 45-61 %, respectively.	
3.3 Administration/ Exposure		
3.3.1 Concentration of test substance	Pilot: 0.5 mg/kg Main ADME: 0.05 mg/kg (Group 1), 0.5 mg/kg (Group 2 and 0.02 mg/kg/day (Group 3) Kinetics: 0.05 and 0.5 mg/kg Tissue distribution: 0.05 and 0.5 mg/kg Biliary elimination: 0.05 and 0.5 mg/kg	
3.3.2 Specific activity of test substance	The specific activity of the supplied [^{14}C]-Bromadiolone was not confirmed. The value supplied by the Sponsor (8.47 MBq.mg^{-1}, 229 µCi.mg^{-1}) was accepted. The [^{14}C]-Bromadiolone was used neat with no radiodilution for each dose formulation.	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
3.3.3 Dose administration	The dose was given by gastric gavage at target dose volumes of 7.5 mL/kg (pilot phase), 0.5 mL/kg (low dose), 5.0 mL/kg (high dose and repeat non-radiolabel dose) and 0.2 mL/kg (radioactive repeat dose). The actual dose received by each animal was determined by reference to the weight of the dose formulation dispensed, the radioactive concentration of the dose formulation and the specific activity of the [^{14}C]-Bromadiolone. **Main ADME Experiment** The absorption, distribution, metabolism, and excretion of [^{14}C]-Bromadiolone was investigated. Three separate treatment groups were used: Group 1: Single oral low dose (4 males, 4 females) Group 2: Single oral high dose (3 males, 3 females) Group 3: 14 Days oral low dose with non-radiolabelled Bromadiolone followed by a single low dose with [^{14}C]-Bromadiolone (4 males, 4 females). **Pharmacokinetic Experiment** Four male and 4 female rats were given a single oral low dose of [^{14}C]-Bromadiolone. An additional 3 male and 3 female rats were given a single oral high dose of [^{14}C]-Bromadiolone. **Tissue Kinetics Experiment** Sixteen male rats were given a single oral low dose of [^{14}C]-Bromadiolone. An additional sixteen male rats were given a single oral high dose of [^{14}C]-Bromadiolone. **Biliary Elimination Experiment** 4 male and 4 female bile duct cannulated rats were given a single oral low dose of [^{14}C]-Bromadiolone. An additional 4 male and 4 female bile duct cannulated rats were given a single oral high dose of [^{14}C]-Bromadiolone.	
3.3.4 Exposure period	The test material was generally administered as a single oral dose. IN the main ADME experiment, bromadiolone was administered once daily for 14 days and then a single oral dose was administered on day 15. The post exposure period was up to 168 h.	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
3.3.5 Samples and sampling time	**Main ADME Experiment** Urine was collected into containers cooled by solid CO_2 at 6, 12 and 24 h post dose and then at 24 h intervals until the end of the study period. Faeces were collected into containers cooled by solid CO_2 at 12 and 24 h post dose and then at 24 h intervals until the end of the study period. Cages were rinsed with water at the time of faeces collection and the washings retained for analysis of total radioactivity. After 7 days the rats were humanely killed (CO_2 narcosis) and the total radioactivity in the following samples determined: Adrenals — Muscle Bone — Perirenal fat Brain — Plasma Gastrointestinal tract + contents — Residual carcass Heart — Spleen Kidneys — Testes/ovaries Liver — Thyroid Lung — Whole Blood **Pharmacokinetic Experiment** Blood samples (ca 0.2 mL) were collected into heparinised tubes by venepuncture of a tail vein, at the following times: 0.25, 0.5, 1, 2, 4, 6, 8, 24, 72, 120, 168 h post dose At 168 h post dose the rats were humanely killed (CO_2 narcosis) and a terminal blood sample obtained. The whole blood was centrifuged at 3000 rpm, 4 °C for 10 mins in order to separate plasma. The plasma was analysed for total radioactivity. **Tissue Kinetics Experiment** At each of 1, 4, 24 and 168 h post dose, four rats from each group were humanely killed (CO_2 narcosis) and the total radioactivity determined in the same tissues as detailed above. **Biliary Elimination Experiment** Bile was collected for the periods: Predose, 0-10, 10-20, 20-30 and 30,60 min and 1-2, 2-4, 4-8, 8-24 and 24-48 h post dose. Samples were collected into containers cooled by solid carbon dioxide (CO_2). Urine and faeces was collected for the periods 0-4, 4-24 and 24-48 h post dose. Cages were washed with water at each collection time and the wash retained. At the end of the collection period, the animals were killed and the carcass and gastrointestinal tract separately retained. Levels of total radioactivity were determined in each sample collected.	
	4 RESULTS AND DISCUSSION	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
4.1 Pilot phase	Following administration of a single oral administration of [^{14}C]-Bromadiolone to male and female rats at a target dose level of 0.5 mg/kg, the majority of the total radioactivity was excreted via the faeces with 60 % of the dose recovered in both the male and female animal over the 168h collection period. Urinary excretion accounted for 2.1 and 1.2 % of the dose recovered in the male and female animal, respectively, over the same period. Recovery of total radioactivity in expired air was negligible, indicating that the site of labelling was metabolically stable. Following this analysis, there was no requirement to carry out any further expired collections in the subsequent phases. Excretion of the recovered radioactivity was fairly rapid with the majority of the dose being excreted in the first 48 h post dose, 54.7 % in the male and 51.6 % in the female. The radioactivity remaining in the carcass at 168 h post dose accounted for 38.3 % of the dose in the male and 36.1 % in the female. Including cagewash and carcass, excretion was quantitative with recoveries of 100.6 % in the male and 97.5 % in the female. Following oral administration, the concentration of total radioactivity in plasma following a single oral administration of [^{14}C]-Bromadiolone to rats at a target dose level of 0.5 mg/kg was highest at 4 h post dose, with values of 64 and 41 ng equiv/mL in the male and female animal, respectively. The concentration of total radioactivity then decreased to approximately half of that seen at peak with values of 24 ng equiv/mL in the male and 23 ng equiv./mL in the female. By 168 h post dose, the concentration of total radioactivity in the plasma had decreased to 3 ng equiv/mL in the male and 4 ng equiv/mL in the female. The concentration of total radioactivity in whole blood following a single oral administration of [^{14}C]-Bromadiolone to rats at a target dose level of 0.5 mg/kg followed a similar pattern to that seen in plasma but at levels approximately half that seen in plasma. From these data, it was determined that the plasma only would be analysed in the subsequent phases of the study.	

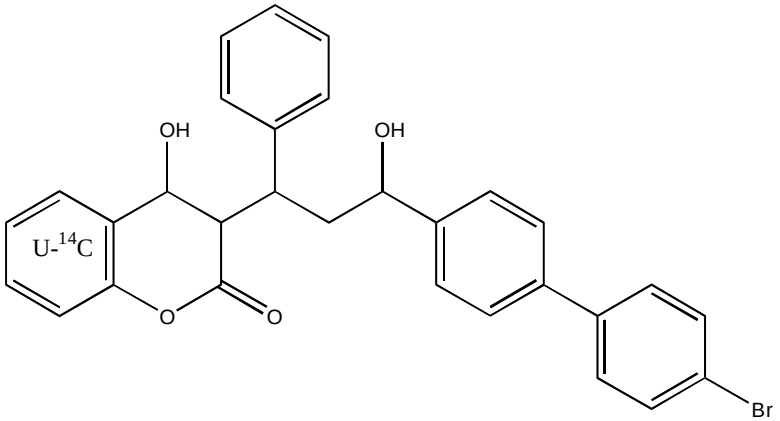
Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
4.2 Main ADME experiment	**Group 1 – Low Dose** Following a single oral administration of [^{14}C]-Bromadiolone to male and female rats at a target dose level of 0.05 mg/kg, the majority of the excreted total radioactivity was found in the faeces with a mean of 54.3±1.4 % of the administered dose recovered in males and 49.0±1.5 % in females. Urinary excretion accounted for a mean of 5.1±0.4 % of the radioactivity recovered in males and 3.4±0.1 % in females over the 168 h collection period. The liver accounted for a mean of 35.7±1.3 % of the administered radioactivity in males and 43.5±1.6 % in females at 168 h post dose. The majority of the excreted radioactivity was eliminated by 72 h post dose in males and 96 h post dose in females. Including cagewash, tissues, gastrointestinal tract and carcass, recovery was quantitative with means of 104.4±1.0 % in males and 105.5±2.3 % in females. **Group 2 – High Dose** Following a single oral administration of [^{14}C]-Bromadiolone to male and female rats at a target dose level of 0.5 mg/kg, the majority of the excreted total radioactivity was found in the faeces with a mean of 65.5±3.1 % of the administered dose recovered in males and 56.7±2.3 % in females. Urinary excretion accounted for a mean of 3.7±0.2 % of the radioactivity recovered in males and 2.5±0.2 % in females over the 168 h collection period. The liver accounted for a mean of 15.0±1.0 % of the administered radioactivity in males and 27.3±1.5 % in females at 168 h post dose. The majority of the excreted radioactivity was eliminated by 72 h post dose in males and females. Including cagewash, tissues, gastrointestinal tract and carcass, recovery was quantitative with means of 102.3±3.7 % in males and 104.6±1.8 % in females. **Group 3 – Repeat Dose** Following repeated (once daily for 14 days dosing of Bromadiolone) followed by a single oral administration of [^{14}C]-Bromadiolone on day 15 to both male and female rats at a target dose level of 0.02 mg/kg/day The majority of the excreted total radioactivity was found in the faeces with a mean of 55.9±3.0 % of the administered dose recovered in males and 51.8±3.4 % in females. Urinary excretion accounted for a mean of 3.3±0.6 % of the radioactivity recovered in males and 1.4±0.0 % in females over the 168 h collection period. The liver accounted for a mean of 26.4±3.5 % of the administered radioactivity in males and 37.1±0.7 % in females at 168 h post dose. The majority of the excreted radioactivity was eliminated by 72 h post dose in males and females. Including cagewash, tissues, gastrointestinal tract and carcass, recovery was quantitative with means of 100.4±2.7 % in males and 100.8±3.5 % in females. The highest mean concentration of total radioactivity in tissues at 168 h post dose 15 were found in the liver with a value of 121.7±14.9 ng equiv/g in males and 213.4±12.3 ng equiv/g in females. High concentrations of total radioactivity were also found in the kidneys, thyroids and adrenal glands.	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
4.3 Pharmacokinetic experiment	**Low Dose** Following administration of a single oral dose of [^{14}C]-Bromadiolone to male and female rats at a target dose level of 0.05 mg/kg, the highest mean concentration of total radioactivity in plasma was noted at 6 h in males and 8 h in females with values of 6±2 and 3±1 ng equiv/mL in males and females, respectively. The concentration of total radioactivity then declined slowly to mean values of 3±1 ng equiv/mL in males and 2±0 ng equiv/mL in females at 24 h post dose. By 72 h post dose, the mean concentration of total radioactivity had dropped below the limit of reliable measurement in both males and females. **High Dose** Following a single oral administration of [^{14}C]-Bromadiolone to both male and female rats at a target dose level of 0.5 mg/kg, the highest mean concentration of total radioactivity in plasma was noted at 4 h in males and 8 h in females with values of 164±5 and 118±22 ng equiv/mL, respectively. The concentration of total radioactivity then decreased to mean concentrations approximately half those seen at peak at 24 h post dose with values of 50±7 ng equiv/mL in males and 66±20 ng equiv/mL in females. The mean concentration of total radioactivity then declined slowly to a mean of 4±1 ng equiv/mL in males and 7±2 ng equiv/mL in females by 168 h post dose.	

Section A6.2 (1) Annex Point IIA6.2	METABOLISM Metabolism of[^{14}C]- Bromadiolone in the Rat	
4.4 Tissue kinetics experiment	**Low Dose** Following a single oral administration of [^{14}C]-Bromadiolone to male rats at a target dose level of 0.05mg/kg, the concentration of radioactivity in the gastrointestinal tract and contents at each timepoint reflected the passage of the orally administered dose. Concentrations of radioactivity in the gastrointestinal tract are therefore not discussed along with the general tissue concentrations. At 1 h post dose the highest mean concentration of total radioactivity was found in the liver, which contained a total mean concentration of 100.1±84.9 ng equiv/g. Measurable concentrations of total radioactivity were also noted in the adrenal glands, kidneys, thyroid and spleen (3-10 ng equiv/g). All remaining tissues contained concentrations below the mean plasma level (3±2 ng equiv/g). At 4 h post dose, tissue concentration generally increased with the highest mean concentration of total radioactivity found in the liver (503.1±88.5 ng equiv/g). High concentrations of total radioactivity were also noted in the kidneys, thyroid, adrenal glands, lungs and spleen (21-66 ng equiv/g). All remaining tissues contained total radioactivity below the mean plasma level (9±3 ng equiv/g). By 24 h post dose the highest mean concentration of total radioactivity was found in the liver, with a value of 449.6±50.1 ng equiv/g which had decreased from the previous timepoint. High concentrations of total radioactivity were also noted in the lungs, kidneys, adrenal glands and spleen (21-66 ng equiv/g). All remaining tissues contained total radioactivity similar to or below the mean plasma level (4±03 ng equiv/g). At 168 h post dose general tissue levels had decreased from previous values and were below the limit of reliable measurement in plasma. The highest mean concentration of total radioactivity was found in the liver, with a value of 309.7±20.4 ng equiv/g, followed by the kidneys, with a mean concentration of 50.4±4.8 ng equiv/g. High concentrations of total radioactivity were also noted in the thyroid, adrenals, lungs and spleen (14-32 ng equiv/g). The general carcass level (3.7±0.5 ng equiv/g) was also above the general limit of reliable measurement. **High Dose** Following a single oral administration of [^{14}C]-Bromadiolone to male rats at target dose levels of 0.5mg/kg At 1 h post dose the highest mean concentration of total radioactivity was found in the liver, with a value of 2251.7±513.4 ng equiv/g, followed by the kidneys, with a mean concentration of 265.4±90.9 ng equiv/g. All remaining tissues contained total radioactivity below the mean plasma level (166±93 ng equiv/g). At 4 h post dose, tissue concentrations increased with the highest mean concentration of total radioactivity found in the liver, with a value of 4056.3±367.3 ng equiv/g, followed by the kidneys, with a mean concentration of 488.6±64.5 ng equiv/g. High concentrations of total radioactivity were also noted in the adrenal glands and lungs (359.9±97.4 ng equiv/g and 255.0±23.0 ng equiv/g. All remaining tissues contained mean total radioactivity levels below the plasma level (253±33 ng equiv/g).	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
	By 24 h post dose, concentrations of total radioactivity in most tissues fell from the previous timepoint with the highest mean concentration found in the liver, with a value of 2383.7±105.4 ng equiv/g, followed by the adrenal glands, with a mean concentration of 376.0±42.5 ng equiv/g. High concentrations of total radioactivity were also noted in the kidneys, thyroid, lungs and spleen (225-372 ng equiv/g). All remaining tissues contained mean total radioactivity levels below the general carcass level (129.0±32.5 ng equiv/g) including the measured plasma concentration (62±6 ng equiv/g). At 168 h post dose, concentrations of total radioactivity continued to decline. The highest mean concentration of total radioactivity was found in the liver, with a value of 1429.7±194.6 ng equiv/g, followed by the adrenal glands, with a mean concentration of 265.8±51.6 µg equiv/g. High concentrations of total radioactivity were also noted in the kidneys, thyroid, lungs and spleen (162-369 ng equiv/g). All remaining tissues contained mean total radioactivity levels below the general carcass level (99.6.0±22.0 ng equiv/g) including the measured plasma concentration (9±2 ng equiv/g).	
4.5 Biliary elimination experiment	**Low Dose** Following a single oral administration of [^{14}C]-Bromadiolone at a target dose level 0.05 mg/kg to male and female bile duct cannulated rats, the major route of excretion was via the bile in both male and female rats, with total mean radioactive recoveries of 30.1±2.3 % and 21.7±1.5 %, respectively. Faecal excretion accounted for a mean of 19.6±4.0 % in males and 21.0±1.5 % in female. Urinary excretion accounted for a mean of 2.5±0.6 % in males and 2.4±0.2 % in females. Approximately half of the administered dose remained in the animal at 48 h post dose with 6.1±0.4 % in males and 6.9±0.9 % in female recovered in the gastrointestinal tract and 40.5±5.1 % in males and 50.9±3.0 % in female recovered in the carcass. Including cagewash, gastrointestinal tract and carcass, recovery was quantitative with mean values of 98.9±2.9 % in males and 103.0±2.8 % in females recovered over the 48 h collection period. **High Dose** Following a single oral administration of [^{14}C]-Bromadiolone at a target dose level 0.5 mg/kg to male and female bile duct cannulated rats, the major route of excretion was via the bile in both male and females, with a mean of 41.9±2.8 % and 32.9±7.9 % of the administered dose recovered over the 48 h period, respectively. Faecal excretion accounted for a mean of 19.4±2.9 % in males and 17.9±2.5 % in females. Urinary excretion accounted for a mean of 0.5±0.2 % in males and 0.6±0.6 % in females. A significant portion of the administered dose remained in the animal at 48 h post dose with 4.8±0.9 % in males and 4.7±1.6 % in female recovered in the gastrointestinal tract and 34.9±1.9 % in males and 37.7±7.0 % in female recovered in the carcass. Including cagewash, gastrointestinal tract and carcass, recovery was quantitative with mean values of 101.6±0.8 % in males and 94.9±15.3 % in females recovered over the 48 h collection period.	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
4.6 Chromatographic analysis	Chromatographic analysis demonstrated that a significant component of the radioactivity eliminated in the faeces was tentatively identified as unchanged Bromadiolone and this was also the major component retained in both the liver and kidney.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	This study was designed to investigate the metabolism of [^{14}C]-Bromadiolone in the rat following single and repeated oral administrations at target dose levels of 0.05, 0.5 and 0.02 (repeated) mg/kg, respectively. A pilot phase was set up in order to establish a methodology for the main study, such as the need for carbon dioxide traps and the requirement for whole blood and plasma analysis. It was deduced that carbon dioxide traps were not required for the remainder of the study. Plasma analysis was favoured over blood analysis. The study was divided into various groups. For the main ADME experiment there were 3 groups of animals. In group 1, 4 male and 4 female rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.05 mg/kg. Animals were housed singly in all-glass metabowls and urine and faeces samples were collected at intervals up to 168 h post dose. In group 2, a further 3 male and 3 female rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.5 mg/kg. Samples were collected as detailed in group 1. In group 3, four males and 4 females each received 14 days of a 0.02 mg/kg dose with non-radiolabelled Bromadiolone followed by a single dose of [^{14}C]-Bromadiolone on Day 15. Samples were collected as detailed in group 1. For the pharmacokinetic experiment, 4 male and 4 female rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.05 mg/kg. The animals were housed singly in polypropylene and stainless steel cages and blood samples obtained for plasma up to 168 h post dose. A further 3 male and 3 female rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.5 mg/kg. Samples were collected as detailed for the low dose. For the tissue kinetics experiment, 16 male rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.05 mg/kg. The animals were housed singly in polypropylene and stainless steel cages and 4 rats sacrificed for tissues at each of 1, 4, 24 and 168 h post dose. A further 16 male rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.5 mg/kg. Samples were collected as detailed for the low dose. For the biliary elimination experiment, 4 male and 4 female bile duct cannulated rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.05 mg/kg. Animals were housed singly in all-glass metabowls and urine, bile and faeces samples were collected at intervals up to 48 h post dose. A further 4 male and 4 female bile duct cannulated rats each received a single oral administration of [^{14}C]-Bromadiolone at a target dose level of 0.5 mg/kg. Samples were collected as detailed in the low dose group above.	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
5.2 Results and discussion	Following oral administration up to 50 % of the administered dose remained in the carcass at 168 h post dose with the majority of this radioactivity associated with the liver. 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. Following administration at both low and high dose levels and following repeated administration, the majority of the excreted radioactivity was found in the faeces. Investigations using bile duct cannulated rats showed that biliary elimination plays a major role in the excretion of absorbed radioactivity. Absorption of the radioactivity associated with [^{14}C]-Bromadiolone was fairly slow with peak plasma levels of total radioactivity not being seen until 4-8 h post dose. Peak tissue concentrations of radioactivity were observed at 4 and 24 h post dose. Chromatographic analysis demonstrated that a significant component of the radioactivity eliminated in the faeces was tentatively identified as unchanged Bromadiolone and this was also the major component retained in both the liver and kidney. Where the radioactivity had been absorbed and excreted, in the bile and urine, no Bromadiolone was noted in these samples. This indicates that the Bromadiolone found in the faeces was from an unabsorbed portion of the oral dose.	
5.3 Conclusion		
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Not Relevant	
Materials and Methods	Applicant's version accepted with the following comment: X The originally submitted study report did not contain information on where the radioactive label was inserted into the bromadiolone molecule. This information (see below) has been provided separately by the notifier and will be included in a revised study report.  	

Section A6.2 (1) **Annex Point IIA6.2**	**METABOLISM** Metabolism of[^{14}C]- Bromadiolone in the Rat	
Results and discussion	Applicants version accepted	
Conclusion	Applicants version accepted with the following addition: The RMS concludes based on the above presented study: Absorption is estimated as > 70% (71 to 77% for single dose animals) based on, urinary and biliary excretion and carcass amounts (GI excluded). Bromadiolone was extensively metabolised with little parent compound excreted in bile and urine. Around 20% of Bromadiolone was excreted unchanged in faeces. Urinary excretion of radioactivity ranged between approx. 1-5%, but no unchanged parent bromadiolone was found. The pattern of absorption and excretion were similar in single dosed and repeatedly dosed animals as well as for males and females, although there was an indication that radioactivity was more readily excreted from male rats and at higher dose levels. A significant amount was retained in the body of the animals at study end (27 to 61% in bile cannulated animals). The study is inconclusive for proposed metabolism scheme and structural identifications of major metabolites, since no such experiments were performed.	
Reliability	1	
Acceptability	Acceptable.	
Remarks	This study did not investigate the structure of metabolites and consequentially no pattern of metabolism could be proposed which is a requirement according to the TNsG on core data set for active ingredients. The information on formed metabolites *in vivo* would have been useful for further justifying the waiving of carcinogenicity studies, i.e to see if metabolites of concern were formed *in vivo*. However, the a.i is not genotoxic *in vitro* (but it should also be noted that no *in vivo* genotox studies were performed), and the representative use pattern of the product does not suggest exposure via food residues. Also, given the highly toxic nature of the substance, it is further unlikely that significantly high exposure to the substance would occur over time. Therefore, the data given in the above study is considered sufficient for human risk assessment, considered the proposed use and exposure of bromadiolone presented in this dossier.	

Section A6.2 (2) **Annex Point IIA6.2**	**METABOLISM** *Rat liver- Brodifacoum, difenacoum, bromadiolone and coumatetralyl*	
	1 REFERENCE	**Official use only**
1.1 Reference	G. Parmar, H. Bratt, R. Moore & P.L.Batten (1985). Evidence for common binding site *in vivo* for the retention of Anticoagulants in Rat Liver. Hum Toxicol, 6: 431-432.	
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Published paper based on a study carried out by Imperial Chemical Industries plc.	
1.2.2 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Difenacoum, brodifacoum, bromadiolone and coumatetralyl	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in published report.	
3.1.2.1 Description	Not stated	
3.1.2.2 Purity	Not stated	
3.1.2.3 Stability	A specific statement on stability is not provided within the paper.	
3.1.2.4 Radiolabelling	^{14}C-labelled compound	
3.2 Test Animals		
3.2.1 Species	Rat	
5.3.3 Strain	Alp:AP (Wistar derived)	
5.3.4 Source	Not stated in published report	
5.3.5 Sex	Male	
5.3.6 Age/weight at study initiation	Age not stated in published report. Weight range from 180g – 240g	
5.3.7 Number of animals per group	Up to 24 per group	
5.3.8 Control animals	no	
3.3 Administration/ Exposure	Oral	

Section A6.2 (2) **Annex Point IIA6.2**	**METABOLISM** *Rat liver- Brodifacoum, difenacoum, bromadiolone and coumatetralyl*	
3.3.1 Preparation of test site	Not applicable	
3.3.2 Concentration of test substance	Difenacoum = 2.70 µmol/kg Brodifacoum = 0.67 µmol/kg Bromadiolone = 1.76 µmol/kg Coumatetralyl = 20.55 µmol/kg	
3.3.3 Specific activity of test substance	Not relevant	
3.3.4 Volume applied	Not stated in published report	
3.3.5 Sampling time	Three animals were killed at days 1, 4, 8, 14, 28, 56, 84, 133 and 182 after dosing	
3.3.6 Samples	Hepatic concentration of radioactivity and prothrombin and kaolin cephalin times.	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	For all anticoagulants the elimination of radioactivity from the liver was biphasic. Initial rapid phase (T½ approx 2 days) lasting up to 8 days. Slower terminal phase with elimination half lives of: brodifacoum – 130 days; bromadiolone – 170 days and difenacoum – 120 days. Coumatetralyl was much lower at 55 days.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Test substance was administered orally and liver concentrations monitored for radioactivity.	
5.2 Results and discussion	For all anticoagulants the elimination of radioactivity from the liver was biphasic. Thin layer chromatographic analysis of solvent extracts of livers from rats killed 1 and 14 days post dosing showed that for brodifacoum, bromadiolone and coumatetralyl most of the radioactivity was present as the unchanged parent compound , whereas for difenacoum the bulk of the radioactivity consisted of metabolites	
5.3 Conclusion	Despite the differences in hepatic radioactivity concentration at day 1, the similarity in liver concentrations of the different anticoagulants during the slow, terminal phase suggests that they all interact at a common, saturable binding site.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	Applicants version accepted	
Results and discussion	Applicants version accepted	
Conclusion	Applicants version accepted	
Reliability	2	

Section A6.2 (2) **Annex Point IIA6.2**	**METABOLISM** *Rat liver- Brodifacoum, difenacoum, bromadiolone and coumatetralyl*	
Acceptability	Acceptable. However, this study is of limited use due to the lack of information concerning study procedures and substance purity and can only function as complementary information to other toxicokinetic studies.	
Remarks		

Section A6.2 (3) **Annex Point IIA6.2**	**METABOLISM** Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum	
	1 REFERENCE	**Official use only**
1.1 Reference	Allen C. Ray, Michael J. Murphy, Michael D. DuVall and John C. Reagor (1989). Determination of brodifacoum and bromadiolone residues in rodent and canine liver. Am J Vet Res, Vol 50, No. 4, April 1989, 546-550.	
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Public domain	
1.2.2		
1.2.3 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1Test material	Brodifacoum and bromadiolone	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in the published paper	
3.1.3 Description	Baits containing 0.005% a.i	
3.1.4 Purity	Not stated in the published paper	
3.1.5 Stability	A specific statement on stability is not provided within the paper.	
3.1.6 Radio labelling	No	
3.2 Test Animals		
3.2.1 Species	Rats and dogs	
3.2.2 Strain	Sprague-Dawley Rats – Mixed breed dogs	
3.2.3 Source	Not stated in the published paper	
3.2.4 Sex	Rats – 8 male and 4 female Dogs – male and female	
3.2.5 Age/weight at study initiation	Rats 400 to 450 g Dogs 11 to 20 kg	
3.2.6 Number of animals per group	Brodifacoum dosing: Single dose group – 3 rats Multiple dose group – 4 rats Single dose – 9 dogs Bromadiolone dosing:	

Section A6.2 (3) **Annex Point IIA6.2**	**METABOLISM** Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum	
	Single dose group – 3 rats Multiple dose group – 2 rats	
3.2.7 Control animals	No	
3.3 Administration/ Exposure	Oral	
3.3.1 Preparation of test site	None	
3.3.2 Concentration of test substance	Brodifacoum Rats: Single dose - 0.28 mg/kg bw Mulitple dose – (5 doses) total dose 7.5 to 11.25 mg/kg bw Dogs single dose – 1.1mg Bromadiolone Rats: Single dose – 1.25mg/kg bw Multiple dose (5 doses) total dose 6.75 to 10.6 mg/kg bw	
3.3.3 Specific activity of test substance	Rats: Commercial bait containing 50µg/g of either brodifacoum or bromadiolone Dogs: 1.1 mg of technical grade brodifacoum in polyethylene glycol 400/kg, PO, via gastric tube.	
3.3.4 Volume applied		
3.3.5 Sampling time	Rats every 2 to 6 hours for clinical signs up to 7 days. Dogs every 2 to 4 hours.	
3.3.6 Samples	Liver tissue	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	Identification of methylated products of chromic acid oxidisation of brodifacoum and bromadiolone was accomplished by gas chromatography/mass spectrometry. The primary oxidisation product 4-bromobenzoic acid was identified after trimethylanilinium hydroxide methylation by matching its retention time (4.8 mins) and mass spectrum with that of an authentic standard. Rats given low doses of either brodifacoum or bromadiolone had no outward signs of clinical illness. At necropsy, lesions were minimal, but 2 rats given brodifacoum had small amounts of blood in the thoracic cavity and 1 rat given bromadiolone had pulmonary congestion. In rats given the higher doses of both substances lesions were more pronounced and were confined to pulmonary congestion and abdominal haemorrhages. Bait intake decreased by the 4th day and signs of clinical toxicosis were not apparent until at least the 5th day. Dogs given brodifacoum also had lesions confined to pulmonary congestion and abdominal haemorrhages. Analysis of hepatic tissue from these dogs and rats indicated that residues from bromadiolone were usually less than those for brodifacoum and that he kidney may be as suitable a sample for analysis as the liver.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The aim of the study was to simulate natural poisoning. Rats were fed commercial bait with 50 µg/g of either brodifacoum or bromadiolone to	

Section A6.2 (3) **Annex Point IIA6.2**	**METABOLISM** Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum	
	create a single dose equivalent to 0.28 mg/kg body weight of active substance or for multiple dose (up to 30g of bait every 24 hours for 5 days) giving a total dosage of between 7.5 to 11.25 mg/kg bodyweight. Once the animals showed clinical signs of poisoning they were terminated and tissue samples taken. These samples were analysed for active substance residues in the liver or kidney. For dogs a single dose was administered equivalent to 1.1 mg of technical grade material. When clinical signs were seen the dogs were given treatment with vitamin K_1. Livers were examined on those that died during the experiment.	
5.2 Results and discussion	Rats given low doses of either brodifacoum or bromadiolone had no outward signs of clinical illness. At necropsy, lesions were minimal, but 2 rats given brodifacoum had small amounts of blood in the thoracic cavity and 1 rat given bromadiolone had pulmonary congestion. In rats given the higher doses of both substances lesions were more pronounced and were confined to pulmonary congestion and abdominal haemorrhages. Bait intake decreased by the 4th day and signs of clinical toxicosis were not apparent until at least the 5th day. Dogs given brodifacoum also had lesions confined to pulmonary congestion and abdominal haemorrhages. Analysis of hepatic tissue from these dogs and rats indicated that residues from bromadiolone were usually less than those for brodifacoum and that the kidney may be as suitable a sample for analysis as the liver.	
5.3 Conclusion	Analysis by gas chromatography/mass spectrometry after chromic acid oxidisation of liver extracts does not differentiate between brodifacoum and bromadiolone because they yield the same product, but conversely offers the advantage of screening with one protocol for the 2 most commonly used rodenticides. This technique also offers comparable sensitivity and much improved selectivity as contrasted with existing HPLC methods. Bromadiolone was less persistent in liver and may be eliminated more rapidly than brodifacoum. In dogs the selected dose was in the lower end of the reported spectrum. Because the monitoring and therapy were aggressive, unknown predisposing factors may have contributed to the demise of the 3 dogs during the study.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	Applicants version accepted	
Results and discussion	Applicants version accepted	
Conclusion	Applicants version accepted	
Reliability	2	

Section A6.2 (3) **Annex Point IIA6.2**	**METABOLISM** Rats (single and multiple dose) - brodifacoum and bromadiolone; and dogs single dose brodifacoum	
Acceptability	Acceptable However, this study is of limited use due to the lack of information concerning study procedures and substance purity and can only function as complementary information to other toxicokinetic studies.	
Remarks		

Section A6.2(4) **Annex Point IIA6.2**	**Percutaneous absorption (*in vitro* test)**	
	REFERENCE	**Official use only**
Reference	Toner F (2008) The In Vitro Percutaneous Absorption of Radiolabelled Bromadiolone in Two Test Preparations Through Human Skin, Charles River Laboratories, Study No. 780513, Report No. 28712.	
Data protection	Yes	
Data owner	The Bromadiolone Task Force	
Companies with letters of access	N/A	
Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	GUIDELINES AND QUALITY ASSURANCE	
Guideline study	Yes OECD Guideline 428	
GLP	Yes	
Deviations	No	
	MATERIALS AND METHODS	
Test material	As given in section 2 Bromadiolone	
Lot/Batch number	Non-radiolabelled bromadiolone: batch no. L22678	
Specification	As given in section 2	

Section A6.2(4) **Annex Point IIA6.2**	**Percutaneous absorption (*in vitro* test)**	
Description	Bromadiolone was tested by being incorporated into bait:saline (1:1, w/w) formulation (Test Preparation 1) and a representative wax block formulation (Test Preparation 2).	
Purity	Non-radiolabelled bromadiolone: >99 %	
Stability	Not specified	
Radiolabelling	[^{14}C]-Bromadiolone was synthesised and supplied by GE Healthcare and repurified by Charles River Laboratories, Tranent, Edinburgh, EH33 2NE, UK. The repurified test item was assigned the batch no. 212109-MCO584-32-1. The test item was stored at ca-80 °C in the dark.	
Test System	In vitro study	
Species	Human skin	
Strain	N/A	
Source	Seven samples of full-thickness human skin (4 breast and 3 abdomen) were obtained from patients (aged 28 to 60 years old) either attending the Plastic Surgery Unit at a local hospital or from a commercial Tissue Bank.	
Sex	Samples were obtained from female patients	
Age/weight at study initiation	N/A	
Number of samples per group	A total of 10 samples of human skin, obtained from 5 different donors, were dosed topically with [^{14}C]-Bromadiolone in the test preparation.	
Controls	No	
Preparation of Test Solutions	Formulation of Test Preparation 1 [^{14}C]-Bromadiolone in ethyl acetate solution was transferred in a single 320 µL aliquot into a round bottom vial containing several glass beads and a magnetic stirring bar. The solvent was removed by drying under a gentle stream of nitrogen gas. Blank grain bait formulation (1005.20 mg), that had previously been removed from the centre of the block and ground until it was a fine powder, was transferred to the vial and mixed by vortex mixing for ca 5 min. Physiological saline (1000.98 mg) was then added and mixed by vortex mixing for ca 5 min. The test preparation was then analysed by taking five weighed aliquots (12.8 µL) into scintillation vials. These samples were then mixed with scintillant (10 mL) and then analysed by liquid scintillation counting. The target concentration of Bromadiolone in the test preparation (0.0025%, w/w) was not achieved. Therefore, the test preparation was not accepted for dosing and was diluted with Blank Test Preparation 1 without Bromadiolone (415.75 mg). Formulation of Test Preparation 2 [^{14}C]-Bromadiolone in ethyl acetate solution was transferred in two 400 µL aliquots (total: 800 µL) into a vial containing a magnetic stirring bar. After the addition of each aliquot, the solvent was removed by drying under a gentle stream of nitrogen gas. Blank wax (1245.38 mg) that had previously been scraped from the outside of the blank bait block was transferred to the vial. The vial was heated to melt the wax and mixed thoroughly. Five weighed aliquots (12.8 µL) were then taken and placed into Combusticones® for analysis by combustion/ liquid scintillation counting.	

Section A6.2(4) **Annex Point IIA6.2**	**Percutaneous absorption (*in vitro* test)**	
	[^{14}C]-Bromadiolone in wax (ca 40 mg) was added to a cast (0.41 cm^2). The cast was placed onto a heated stirring plate and ca 40 mg of the blank bait that had previously been removed from the centre of the block and finely ground was added to the cast. The cast was left on the heated stirring plate for ca 30 min to allow the test item to partition between the wax and the grain. The cast was removed from the heated stirring plate, allowed to cool and the disc was removed from the cast. A total of sixteen discs were prepared in this manner.	
Administration/ Exposure		
Preparation of skin samples	Human skin samples were removed from storage and allowed to thaw at ambient temperature. The thickness of the uncut skin membranes was measured using a micrometer. Split-thickness membranes were prepared by pinning the full-thickness skin, stratum corneum uppermost, onto a raised cork board and cutting at a setting equivalent to 200-400 µm depth using a Zimmer® electric dermatome. The membranes were then laid out onto aluminium foil and the thickness of the membranes measured using a micrometer. The split-thickness membranes were stored at ca -20°C. An automated flow-through diffusion cell apparatus was used. The flow-through diffusion cells were placed in a steel manifold heated via a circulating water bath to maintain the skin surface temperature at ca 32 °C. The cells were connected to multi-channel peristaltic pumps from their afferent ports with the receptor fluid effluent dropping via fine bore tubing into scintillation vials on a fraction collector. Sections of split-thickness skin membrane, ca 1.5 x 1.5 cm, were cut out, positioned on the receptor chamber of the diffusion cell, which contained a magnetic flea and the donor chamber was tightened into place with screws. The prepared cells were then placed in the heated manifold and connected to the peristaltic pump. The Variomag magnetic stirrer was switched on to mix the contents of the receptor chamber. An equilibration period of ca 15 min was allowed while receptor fluid was pumped through the receptor chambers at ca 1.5 mL/h. The effluent was then collected for ca 30 min and retained as blank samples for use in the tritiated water barrier integrity assessment.	
Concentration of test substance	**Test preparation** | **1** | **2** Concentration of Bromadiolone by radioactivity (% w/w) | 0.0024 | 0.004	
Specific activity of test substance	The specific activity and radiochemical purity of the test item were stated to be 124 mCi/mmol (234 µCi/mg) and 98.0 %, respectively.	
Application	Test Preparation 1 [14C]-Bromadiolone in Test Preparation 1 was applied over the stratum corneum surface of the exposed skin using an M50 Gilson Microman positive displacement pipette set to deliver ca 12.8 mg (ca 20 mg/cm2). The donor chambers of the cells were not occluded and were left open to the atmosphere. To accurately quantify the radioactivity applied to the skin samples, 7 weighed aliquots (ca 12.8 mg) of the test preparation were collected into scintillation vials at the time of dosing. These representative mock dose samples were mixed with methanol (1 mL) and scintillant (10 mL) and analysed by liquid scintillation counting.	

Section A6.2(4) Annex Point IIA6.2	Percutaneous absorption (*in vitro* test)	
	Test Preparation 2 For Test Preparation 2, physiological saline was applied over the stratum corneum surface of the exposed skin using an M25 Gilson Microman positive displacement pipette set to deliver 6.4 µL. This was carried out in order to moisten the skin to ensure good contact between the disc and the skin. Test Preparation 2 (rodenticide disc containing [14C]-Bromadiolone) was placed onto the stratum corneum surface of the exposed skin. To accurately quantify the radioactivity applied to the skin samples, 6 discs of the test preparation were collected into Combusticones® for analysis by combustion/liquid scintillation counting.	
Volume applied	Test preparation 1: 20 mg/cm2 Test preparation 2: 6.4 µL	
Size of test site	The surface area of exposed skin within the cells apparatus was 0.64 cm2.	
Exposure period	The skin samples were exposed to the test material for 8 h and then monitored for a further 16 h (i.e. 24 h in total)	
Sampling time	Receptor fluid was collected in hourly fractions from 0 to 8 h post dose and then in 2 hourly fractions from 8 to 24 h post dose. All receptor fluid samples were mixed with scintillant (10 mL) and analysed by liquid scintillation counting.	
Samples	Terminal Exposure – 8 h Post Dose The exposed skin surface was washed and dried (skin wash). For Test Preparation 2, the wax disc was removed immediately prior to washing and the discs were retained in Combustocones® for subsequent combustion/ liquid scintillation analysis. An aliquot (50 µL) of concentrated commercial soap (Simple Antibacterial Handwash) was applied to the exposed skin and cleansed with tissue paper in a gentle rubbing motion. The skin was then rinsed with ten 0.5 mL aliquots of commercial soap solution diluted with water (2%, v/v). The soapy water was aspirated three times with a pipette and collected into a pre-weighed vial. The skin was dried with a tissue swab. This washing process was then repeated and the skin dried with an additional tissue swab. The tissue swabs were pooled and placed in a vial, mixed with ethanol (10 mL), aliquots taken and mixed with scintillant (10 mL) for analysis by liquid scintillation counting. The skin wash samples had ethanol added (10 mL). The mass of skin wash was determined and duplicate weighed aliquots (1 mL) taken, mixed with scintillant (10 mL) and analysed by liquid scintillation counting. The pipette tip was cut up and placed into a scintillation vial and methanol (1 mL) and scintillant (10 mL) was added and the samples analysed by liquid scintillation counting. Terminal Post Exposure Monitoring – 24 h Post Dose At 24 h post dose (i.e. after a 16 h monitoring period), each diffusion cell was disconnected from the receptor fluid pump lines. The underside of the skin was washed (receptor rinse) with receptor fluid (ca 1-2 mL), which was mixed with scintillant (10 mL) and then analysed by liquid scintillation counting. The receptor rinse represented the absorbed test item, which was in the receptor chamber but had not been collected in the 22-24 h receptor fluid fraction. The diffusion cell was dismantled and the skin removed from the cell. The skin was dried with additional tissue paper swabs. These swabs	

Section A6.2(4) Annex Point IIA6.2	Percutaneous absorption (*in vitro* test)	
	were analysed as described for the 8 h tissue swab samples. The donor and receptor chambers were transferred into pre-weighed pots (cell wash) containing a known weight (ca 40 mL) of water. The pots containing the cells were left to extract the test item for ca 30 min. During this time, the samples were sonicated for ca 10 min. The donor and receptor chambers were removed from the cell wash pots and duplicate weighed aliquots (1 mL) taken, mixed with scintillant (ca 10 mL) and analysed by liquid scintillation counting. The stratum corneum was removed with 20 successive tape strips. Each tape was placed into a separate vial, methanol (1 mL) and scintillation fluid (10 mL) were added and the sample analysed by liquid scintillation counting. The skin under the cell flange (unexposed skin) was cut away from the exposed skin with scissors. The samples were placed into individual vials and Solvable® (1 mL) added to each sample for solubilisation. To aid solubilisation, a number of unexposed skin samples were heated to 60 °C for ca 1 h. All samples were mixed with stannous chloride (50 µL) and scintillant (10 mL) and analysed by liquid scintillation counting.	
	RESULTS AND DISCUSSION	

Section A6.2(4) Annex Point IIA6.2	Percutaneous absorption (*in vitro* test)	
Recovery of labelled compound	**[^{14}C]-Bromadiolone in Test Preparation 1** All samples had a mass balance within 100 ± 10 % and the mean ± SD for absorbed dose and dermal delivery were 0.03 ± 0.01 % and 0.36 ± 0.30 %, respectively. The mean mass balance was 98.16% (SD, 2.78%) of the applied dose. **[^{14}C]-Bromadiolone in Test Preparation 2** All samples had a mass balance between 95 % and 118 % and the mean ± SD for absorbed dose and dermal delivery were 0.01 ± 0.01 % and 0.04 ± 0.04 %, respectively. The mean mass balance was 107.31 % (SD, 7.76 %) of the applied dose.	
Percutaneous absorption	**[^{14}C]-Bromadiolone in Test Preparation 1** At 8 h post dose, 96.79 % of the applied dose was washed off (52.49 %, 44.29 % and 0.01 % recovered in the skin wash, 8 h tissue swab and pipette tip, respectively). At 24 h post dose, a further 0.64 % of the applied dose was removed (cell wash and 24 h tissue swabs contained 0.63 % and 0.01 % of the applied dose, respectively). The material recovered in the cell wash was almost certainly material that had been dislodged from the skin at 8 h post dose during the washing procedure. Therefore, the total dislodgeable dose was 97.44 % of the applied dose. The mean total unabsorbed dose was 97.80 % of the applied dose. This consisted of the dislodgeable dose, unexposed skin (0.03 %) and the radioactivity associated with the stratum corneum (0.33 %). The first five tape strips contained 0.19 % of the applied dose. These initial tape strips may be considered to be on the stratum corneum surface and not associated with the stratum corneum. There was a steady decrease in the recovery of radioactivity associated with the stratum corneum. Tapes 6-10, 11-15 and 16-20 contained a further 0.07 %, 0.04 % and 0.03 %, respectively. The absorbed dose (0.03 %) was the sum of the receptor fluid (0.03 %) and the receptor rinse (<0.01 %). Dermal delivery (0.36 %) was the sum of the absorbed dose and the exposed skin (0.33 %). For [14C]-Bromadiolone at 24 h post dose the mass balance, total dislodgeable dose, unabsorbed dose, dermal delivery and absorbed dose were 487.28, 483.65, 485.47, 1.81, 0.17 ng equiv./cm2, respectively. Steady state absorption was not observed over the 24 h assessment period. Therefore, a lag time could not be calculated. **[^{14}C]-Bromadiolone in Test Preparation 2** At 8 h post dose, 107.20 % of the applied dose was washed off (105.89 %, 0.39 %, 0.92 % and 0.00 % recovered in the discs, skin wash, 8 h tissue swab and pipette tip, respectively). At 24 h post dose, a further 0.05 % of the applied dose was removed (cell wash and 24 h tissue swabs contained 0.05 % and <0.01 % of the applied dose, respectively). The material recovered in the cell wash was almost certainly material that had been dislodged from the skin at 8 h post dose during the washing procedure. Therefore, the total dislodgeable dose was 107.25 % of the applied dose. The mean total unabsorbed dose was 107.27 % of the applied dose. This consisted of the dislodgeable dose, unexposed skin (<0.01 %) and the radioactivity associated with the stratum corneum (0.02 %). The first five tape strips contained 0.01 % of the applied dose. These initial tape strips may be considered to be on the stratum corneum surface and not associated with the stratum corneum. There was a decrease in the recovery of radioactivity associated with the stratum corneum. Tapes 6-20 contained <0.01 %. The absorbed dose (0.01 %) was the sum of the receptor fluid (0.01 %) and the receptor	

Section A6.2(4) Annex Point IIA6.2	Percutaneous absorption (*in vitro* test)	
	rinse (<0.01 %). Dermal delivery (0.04 %) was the sum of the absorbed dose and the exposed skin (0.04 %). The mass balance, total dislodgeable dose, unabsorbed dose, dermal delivery and absorbed dose were 7066, 7062, 7063, 2.90, and 0.51 ng equiv./cm2, respectively. Steady state absorption was not observed over the 24 h assessment period. Therefore, a lag time could not be calculated.	
	APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.2(4) Annex Point IIA6.2	Percutaneous absorption (*in vitro* test)	
Materials and methods	Bromadiolone is commercially available formulated in a wax block rodenticide bait formulation. As part of the safety evaluation of Bromadiolone, a study was required to assess the rate and extent of absorption of Bromadiolone following topical application of [14C]-Bromadiolone incorporated into bait:saline (1:1, w/w) formulation (Test Preparation 1) and a representative wax block formulation (Test Preparation 2). The method utilised was OECD Guideline 428. Split-thickness human skin membranes were mounted into flow-through diffusion cells. Receptor fluid, tissue culture medium containing polyoxyethylene 20-oleyl ether (ca 6 %, w/v), sodium azide (ca 0.01 %, w/v), glucose (ca 1 %, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL), was pumped underneath the skin at a flow rate of ca 1.5 mL/h. The skin surface temperature was maintained at ca 32 °C throughout the experiment. A tritiated water barrier integrity test was performed and any human skin sample exhibiting a kp value greater than 3.5 x 10-3 cm/h was excluded from subsequent absorption measurements. Test Preparation 1 containing [14C]-Bromadiolone was applied at an application volume of 20 mg/cm2 in a saline paste. The addition of the saline to the grain was justified as this allowed the test preparation to be more easily applied over the skin surface. This also allowed better contact between the skin surface and the test preparation and was intended to mimic the operator sweating when handling the grain or wax. This was assumed to be representative of the worst case scenario for operator exposure. Test Preparation 2 was applied under infinite dose conditions in the form of a disc to human split-thickness skin membranes mounted into flow-through diffusion cells *in vitro*. Absorption was assessed by collecting receptor fluid in hourly fractions from 0 to 8 h post dose and then in 2-hourly fractions from 8 to 24 h post dose. At 8 h post dose, exposure was terminated by removal of the disc (Test Preparation 2 only), washing the skin surface with a concentrated commercial soap followed by rinsing with a dilute soap solution and drying the skin surface with tissue paper (tissue swabs). At 24 h post dose (i.e. after a 16 h monitoring period), the underside of the skin was rinsed with receptor fluid (receptor rinse). The skin was then removed from the flow-through diffusion cells, dried and the stratum corneum was removed with 20 successive tape strips. The remaining skin was divided into exposed and unexposed skin and solubilised with Solvable® tissue solubiliser. The discs were analysed by combustion/ liquid scintillation counting. All other samples were analysed by liquid scintillation counting.	
Results and discussion	In conclusion, following topical application of [14C]-Bromadiolone in Test Preparation 1 (0.0025 %, w/w) applied to human skin in vitro, the absorbed dose of [14C]-Bromadiolone was 0.03 % (0.17 ng equiv./cm2). The dermal delivery of [14C]-Bromadiolone was 0.36 % (1.81 ng equiv./cm2). The majority of the dose was removed by washing the skin; the total dislodgeable dose was 97.44 % of the applied dose. The mass balance was complete for [14C]-Bromadiolone from the test preparation (98.16 %). Following topical application of [14C]-Bromadiolone in Test Preparation 2 (0.005 %, w/w) applied to human skin in vitro, the absorbed dose of [14C]-Bromadiolone was 0.01 % (0.51 ng equiv./cm2). The dermal delivery of [14C]-Bromadiolone was	

Section A6.2(4) Annex Point IIA6.2	Percutaneous absorption (*in vitro* test)	
	0.04 % (2.90 ng equiv./cm2). The majority of the dose was removed by washing the skin; the total dislodgeable dose was 107.25 % of the applied dose. The mass balance was complete for [14C]-Bromadiolone from the test preparation (107.31 %).	
Conclusion		
Reliability	1	
Deficiencies	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2009	
Materials and Methods	Applicants version is acceptable	
Results and discussion	Applicant's version is adopted	
Conclusion	Bromadiolone was tested by being incorporated into bait:saline (1:1, w/w) formulation (Test Preparation 1) and a wax block formulation (Test Preparation 2).The dermal absorption for test preparation 1 (0.0025 %, w/w) was approximately 0.36% based on the sum of the absorbed dose and the exposed skin (incl. tapestrip 1-20). The dermal absorption for test preparation 2 (0.005 %, w/w) was approximately 0.04% based on the sum of the absorbed dose and the exposed skin (incl. tapestrip 1-20).	
Reliability	1	
Acceptability	Not acceptable for risk assessment. Even if the quality of the study is acceptable it is not performed on the active substance itself. It is not possible to predict exactly how the other ingredients in the products may influence the absorption. Anyhow, when using the biocidal products the exposure will be to the products and not to the active substance. Therefore, no new study will be necessary. If calculation of exposure to the active substance is needed a default value of 10% can be used based on the MW (>500) and the log P_{ow} (>4).	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2002) 28-day Preliminary study of 90-day repeated dose oral toxicity study of test item Bromadiolone Technical in rats. XXXXX. Report number 01/617-100P	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 407	
2.2 GLP	Yes	
2.3 Deviations	The purity of the test item tested is 99.4%. This will not affect the integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	White crystal	
3.1.2.2 Purity	99.4%	
3.1.2.3 Stability	Stable under test conditions	
3.2 Test Animals		
3.2.1 Species	Rats	
3.2.2 Strain	CRL:(WI) BR (Wistar) Rats	
3.2.3 Source	CHARLES RIVER (EUROPE) LABORATORIES INC. LAB-TECH KFT. István u.11. Budapest, Hungary	
3.2.4 Sex	Male and Female	
3.2.5 Age/weight at study initiation	Study 1 Male 180 - 199g; Female 152 - 172g. Study 2	X1

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
	Male 179 - 193g; Female 153 - 174g.	
3.2.6 Number of animals per group	Study 1 & Study 2 5 animals/sex/group	X2
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	Oral	
3.3.1 Duration of treatment	28 days	
3.3.2 Frequency of exposure	Daily	
3.3.3 Postexposure period	None	
Oral		
3.3.4 Type	gavage	
3.3.4.1 Concentration	Study 1 gavage 1 ml/100g bw	X3
3.3.4.2 Vehicle	Study 1 1% methylcellulose Study 2 Distilled water Ethanol 96% (w/v)	X4
3.3.4.3 Concentration in vehicle	Study 1: 0.1, 0.5 and 1.0 mg/kg/day Study 2: 0.0025 and 0.05 mg/kg/day	X5
3.3.4.4 Total volume applied	Study 1: 2.8, 14.0 and 28.0 mg/kg Study 2: 0.07 and 1.4 mg/kg	
3.3.4.5 Controls	Study 1: vehicle Study 2: physiologic saline containing same amount of ethanol as the high dose group	
3.4 Examinations		
3.4.1 Observations	Yes	
3.4.1.1 Clinical signs	Yes – before treatment and then once daily	
3.4.1.2 Mortality	Yes – once daily	X6
3.4.2 Body weight	Yes – weekly and terminal.	
3.4.3 Food consumption	Yes - weekly	
3.4.4 Water consumption	No	
3.4.5 Ophthalmoscopic examination	No	
3.4.6 Haematology	Yes – surviving animals termination of Study 2 Parameters: Haematocrit, haemoglobin concentration and erythrocytes, erythrocyte count and volume, total and differential leukocyte count,	

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
	platelet count and volume, prothrombin time.	
3.4.7 Clinical Chemistry	Yes – surviving animals termination of Study 2 Parameters: sodium, potassium, calcium, chloride, glucose, total cholesterol, urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, phosphorus, gamma glutamyl transpeptidase.	
3.4.8 Urinalysis	No	
3.5 Sacrifice and pathology		
3.5.1 Organ Weights	Yes organs: liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain and heart.	X7
3.2.2 Gross and histopathology	Yes all dose groups, Gross lesions, sternum, skin and mammary gland (in females), salivary glands (submandilar), femur & bone marrow, spinal cord (cervical, lumbal, thoracic level), pituitary, thymus, trachea, lungs with mainstream bronchi), heart, thyroid, parathyroid, oesophagus, stomach, caecum, duodenum, ileum, jejunum, colon, rectum, urinary bladder, liver, pancreas, spleen, kidneys, adrenals, prostate, testes with epididymides, ovaries, uterus with vagina, brain (coronal sections at three levels), eyes with optic nerve, Harderian glands and lachrymal gland, seminal vesicle, muscle (quadriceps), ischiadic nerve, aorta, submandibular and mesentherial lymph nodes.	
3.2.3 Other examinations	None	
3.2.4 Statistics	Statistical analysis was done with SPSS PC+ software package for the following data: Body weight, food consumption. Haematological. And biochemical data, organ weight data. Frequency of toxic response data by sex and dose and the daily mean food consumption were calculated, The heterogeneity of variance between the groups was checked by Bartlett's homogeneity of variance test.	
3.6 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Observations	j	
4.1.2 Clinical signs	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 and eyes, sanguineous urine, cyanotic skin and haematoma, general haemorrhagic diathesis.	X8
4.1.3 Mortality	Study 1: All animals in all dose groups by day 21 Study 2: All animals in high dose group	X9

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
4.2 Body weight gain	Study 1: A depression of body weight gain found in the measurable groups at the termination of the study Study 2: the average body weights were similar to control value in the surviving animals at the dose level of 0.0025 mg/kg. In male rats the body weight gain was slightly less than in the control group on the last week, but was not considered relevant.	X10
4.3 Food consumption and compound intake	The daily mean food intake was similar in the surviving animals (study 2 - 0.0025 mg/kg/day) and the control animals, whereas it showed a dose dependant decrease in dead animals.	X11
4.4 Ophthalmoscopic examination	None	
4.2 Blood analysis		
4.5.1 Haematology	In dose group of 0.0025mg/kg, decreased Mean Corpuscular haemoglobin concentration and monocyte count were found in male animals whereas the Red blood cell distribution width decreased in females in comparison with the controls. The haematological parameters showed slight differences from the control values. All those were within the physiological range.	
4.2.2 Clinical chemistry	In dose group of 0.0025mg/kg, slightly increased calcium and chloride concentration were observed in male animals. In females, no difference from the control occurred. The alterations of biochemical parameters were considered as species-specific variation because of the low degree of deviation from the control value.	
4.2.3 Urinalysis	Not investigated	
4.3 Sacrifice and pathology		
4.6.1 Organ weights	No significant differences from the control were found in the organ weight of male and female animals at the dose level 0.0025 mg/kg/day in study 2.	
4.3.2 Gross and histopathology	Dead animals: General haemorrhagic diathesis – 0.05, 0.1, 0.5 and 1.0 mg/kg/day Serious hepatitis – 0.05, 0.01, 0.5 and 1.0 mg/kg/day Centrilobular necrosis – 0.05 and 0.5 mg/kg/day (male only) Surviving animals: control and 0.0025 mg/kg/day Alveolar emphysema and focal haemorrhages in the lungs Liver focal proliferation of the mononuclear phagocyte system Kidney calcium deposits Uterus dilation	
4.7 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
5.1 Materials and methods	A study was conducted according to OECD 407. A control and dose group of 0.1, 0.5, 1.0 mg/kg were used in this study. Treatment was carried out by gavage daily. The concentrations of test item suspensions were adjusted to maintain the constant treatment volume (1ml/100g bw). The application volume was adjusted weekly according to the body weight changes. Control animals were treated with vehicle substance in the same manner. Clinical observations were made once daily. Body weights and food consumption were measured weekly or at the death of the animals. A gross necropsy were conducted in dead animals after death and in the control animals at termination (day 21) of the full study. Study 2 A control and dose groups of 0.0025mg/kg and 0.05 mg/kg were used. The test article was dissolved in ethanol (strain solution, 1mg/ml ethanol) and diluted to the appropriate concentration (0.0005mg/ml and 0.01 mg/ml) with distilled water. The constant treatment volume was 0.5ml/100 g bw. Control animals were treated with physiologic saline containing the same amount of ethanol as the solution of high dose group. Blood sampling fro haematological, clinical chemistry evaluation and a gross necropsy were conducted at the end of the treatment period in surviving animals. Dead animals were necropsied after death. Full histopathology was performed on the preserved organs and tissues of the control and high dose groups at the end of the treatment period. In addition, the liver, kidneys and organs showing macroscopic changes were examined microscopically in the low dose group.	X12 X13
5.2 Results and discussion	Bromadiolone caused the following findings after repeated oral application at dose levels of 0.05, 0.1, 0.5 and 1 mg/kg in rats: death, clinical symptoms (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, cyanotic skin and haematoma), general haemorrhagic diathesis and serious hepatitis. Centrilobular necrosis occurred in the liver in some male animals in dose groups of 0.5 mg/kg and 0.05mg/kg. A depression of body weight and food intake was found in the measurable groups.	X14
5.3 Conclusion		
5.3.1 LO(A)EL	Male and female: 0.05 mg/kg/day	
5.3.2 NO(A)EL	Male and female: 0.0025 mg/kg/day	
5.3.3 Other		
5.3.4 Reliability	1	
5.3.5 Deviations	No haematological, biochemical and organ weight evaluations were made in study 1. The popliteal lymph nodes were not removed at necropsy. This did not effect the integrity of the study.	

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	Applicants version accepted with amendments: X1 Young, adult animals were used. Animals were fed R/M-Z+H extruded complete diet and water *ad libitum.* X2 In study 1 the control group included 10 animals, 5 of which were intended for observation during the reverse phase. The highest dose group included 15 animals of which 5 only were observed for mortality and 5 were intended for observations during the reverse phase. In study 2 the control and both exposure groups consisted of 5 females and 5 males. Acclimatization time study 1: 12 days, study 2: 7 days. X3 In study 2, 0.5 ml/100g bw was administered by gavage. X4 In study 2, a stock solution containing Bromadiolone in 96% (w/v) ethanol was prepared and diluted daily with distilled water. The control animals were treated with physiological saline containing the same amount of ethanol as the solution given to the animals in the high dose group (1ml/100 ml). According to EC Method B.7, the control animals should be handled in an identical manner to the test group subjects and should receive the vehicle in the highest volume used. In this study the control group received a ethanol and saline solution, while the test groups were given bromadiolone in a ethanol- distilled water solution. Although this is unfortunate, it should not have any major effects on the outcome of the study. X5 Deviation from EC Method B.7 in which it is specified that generally, at least three test groups and one control should be used. However, study 1 included three higher exposure levels, therefore in this case two exposure groups are acceptable. According to EC Method B. 7, two to four fold dose level intervals are frequently optimal and an addition of a fourth test group is often preferable to using very large intervals e.g. more than a factor of ten, between the dosages. In study 2 the dose interval is 20-fold, thus a third exposure group would have been useful, but is not crucial. X6 Deviation from EC Method B.7, in which it is specified that check for morbidity and mortality should be performed twice a day. Also, according to EC Method B.7, in the fourth exposure week sensory reactivity to stimuli, assessment of grip strength and motor activity should be conducted. This was not performed in this study. X7 Organ weights were only recorded for animals surviving study 2. X12 Study 1- Each exposure group and control group consisted of five male and five female young adult Wistar rats. The animals were housed five animals/cage. X13 Treatment was carried out by gavage daily. Clinical observations were made once daily. Body weights and food consumption were measured weekly and immediately after the death of the animal.	

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
Results and discussion	X8 These symptoms were common among animals of all three exposure groups in study 1 and in the high (50 µg/kg) dose group in study 2. No clinical observations were reported in animals of the low dose group (2.5µg/kg) in study 2. The onset of symtoms in study 1 occurred day 0- 17 and in study 2 on day 16- 24. X9 No animals died in the low dose group (2.5µg/kg) in study 2. In the high (50 µg/kg) dose group in study 2, all animals died between day 17- 27. X10 In male rats of the low (2.5µg/kg) dose group in study 2, the body weight gain was significantly less than in the control group during the last week, but there was no difference in the summarized mean body weight gain during the exposure period in the low (2.5µg/kg) dose group compared to controls, indicating that this minor deviation is not of biological significance. X11 The daily mean food consumption was similar in the low dose group (2.5µg/kg) in study 2 compared to the controls. In the high (50µg/kg) dose group food consumption was lower compared to control values on day 21 (females only) and day 27 (males only, all females had died by day 27). The decrease in food consumption was probably caused by the exposure to Bromadiolone. X14 Applicants version replaced by: 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.	
Conclusion	LO(A)EL: 0.05 mg/kg/day for both sexes, based on the 100% mortality level in this dose group. NO(A)EL: 0.0025 mg/kg/day for both sexes	
Reliability	1	
Acceptability	Acceptable	

Section A6.3 Short-term repeated dose toxicity		
Section A6.3.1 **Annex Point IIA VI 6.3**	**Repeated dose toxicity** 28-day repeated dose oral toxicity in rats	
Remarks	The mg/kg dose levels should be specified in Table A6_3-1. Also, in study 2 the high dose group consisted of 5 animals, not 10 as specified in Table A6_3-1. Actual results should be presented.	

Table A6.3.1-1 Results of repeated dose toxicity study

Parameter STUDY 1	Control		low dose		medium dose		high dose		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	5	5	5	5	5	5	10	10		
Mortality	0	0	5	5	5	5	10	10	+	+
clinical signs*	0	0	↑	↑	↑	↑	↑	↑	+	+
body weight	0	0	↓	↓	↓	↓	-	-	-	-
food consumption	0	0	↓	↓	↓	↓	↓	↓	-	-
General Haemorrhagic diathesis										
microscopic pathology*	0	0	↑	↑	↑	↑	↑	↑	+	+
Organ y										

Parameter STUDY 2	Control		low dose		medium dose		high dose		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	5	5	5	5	-	-	10	10		
Mortality	0	0	0	0	-	-	10	10	+	+
clinical signs*	0	0	0	0	-	-	↑	↑	+	+
body weight	0	0	0	0	-	-	↓	↓	-	-
food consumption	0	0	0	0	-	-	↓	↓	-	-
General Haemorrhagic diathesis										
microscopic pathology*	0	0	0	0	-	-	↑	↑	+	+
Organ y										

Section A6.3.2 **Annex Point IIA VI.6.3**	**Short-term repeated dose toxicity (dermal) 28 days**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Study considered to be unnecessary in view of the well-known nature of the compound. Its mode of action is well understood and documented, and is common among all mammalian species. The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Dermal absorption is not considered to be a major route of entry, and the compound is poorly absorbed through the skin Under these circumstances, a dermal 28-day study is considered to be of no value, and an unnecessary waste of experimental animals.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	A 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. Based on the use pattern of the product, the most probable form of exposure to humans is via the dermal route. However, dermal exposure to bromadiolone is expected to be low since the use of gloves when handling the baits is expected, not only due to the fact that the operator is handling a highly toxic substance, but also since rodent borne diseases may also be present.	
Conclusion	Justification accepted.	
Remarks		

Section A6.3.3 **Annex Point IIA VI.6.3**	**Short-term repeated dose toxicity (inhalation) 28 days**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Study considered to be unnecessary in view of the well-known nature of the compound. Its mode of action is well understood and documented, and is common among all mammalian species. The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Inhalation is not considered to be a significant potential route of entry due to low v.p and solid bait nature of product. Under these circumstances, a inhalation 28-day study is considered to be of no value, and an unnecessary waste of experimental animals.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	A short term repeat dose oral study has been performed for bromadiolone in rats. However, route-to-route extrapolation is not feasible since no acute inhalation study has been performed. Still, exposure via inhalation is expected to be minor both during production and during the use of bait blocks. Also, bromadiolone has a low vapour pressure.	
Conclusion	Justification accepted.	
Remarks		

Section A6.4 Subchronic toxicity		
Section A6.4.1 **Annex Point IIA VI.6.4**	**Subchronic oral – Rat**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Bromadiolone is a well-known compound, which has been used extensively for many years. It belongs to a close group of analogues, which have closely similar properties. They are well understood and the mode of action is also well understood. The mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested, including humans in therapeutic use (warfarin) and in poisoning incidents in humans and animals. There are no other significant toxic effects The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Data are available on closely analogous compounds (difenacoum and brodifacoum) which have similar physico-chemical and toxicological properties and on which subchronic data are available. The results for the subchronic oral study in rats for Difenacoum is summarised below: Oral administration of brodifacoum 0.01, 0.02, 0.04 mg/kg/day in male and female rats and 0.08 mg/kg/day in female rats had no effect on, clinical, haematological or pathological parameters measured. Oral administration of brodifacoum at 0.08 mg/kg/day in male rats resulted in a slight increased incidence of haemorrhage in two animals and slight increase in clotting times indices.Report: BRODIFACOUM 90-day Feeding Study in the Rat. K Morris – September 1995. MRS Inc. report MLS/10020) A rat multigeneration feeding reproduction study is also submitted which shows that the compound can only be tolerated at low doses. Bromadiolone technical caused no adverse effect at dose levels of 1 µg/kg/day, 2,5 µg/kg/day and 5 µg/kg/day in CRL(WI) BR rats in this two generation reproduction toxicity study. There were no gross pathologic, organ weight and histopathologic alterations. Mortality was low and non dose related. Under these circumstances, a second species 90-day study is considered to be of no value, and an unnecessary waste of experimental animals.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	

Section A6.4 Subchronic toxicity		
Section A6.4.1 **Annex Point IIA VI.6.4**	**Subchronic oral – Rat**	
Evaluation of applicant's justification	A subchronic oral study on bromadiolone has been performed using the rabbit as test species. Justification to not perform the study on a rodent species can be accepted, since the acute oral study in the target species, the rat, shows that bromadiolone is highly toxic (T+) to the rat, thereby rendering long-term studies technically difficult to perform. Also, the mode of action by which bromadiolone causes toxicity is well established.	
Conclusion	Justification acceptable	
Remarks		

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2004) Draft report: 90-Day repeated dose oral toxicity study of Bromadiolone Technical in Rabbit, XXXXX. Study code: 03/735-101N	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, OECD 409	
2.2 GLP	Yes	
2.3 Deviations	The purity of the test item tested is 99.4%. This will not affect the integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	As given in section 2	
3.1.2.2 Purity	99.4%	
3.1.2.3 Stability	Stable	
3.2 Test Animals		
3.2.1 Species	Rabbit	
3.2.2 Strain	New Zealand white	
3.2.3 Source	Ferenc Sandor, Breeder H-2173 Kartal, Voros Hadsereg ut 131, Hungary	
3.2.4 Sex	Males and females	
3.2.5 Age/weight at study initiation	Age: 11 weeks at arrival Body weight: 2894-3467g male 2953-3528g female	
3.2.6 Number of animals per group	12 animals per group	
3.2.7 Control animals	Yes	

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
3.3 Administration/ Exposure	Oral	
3.3.1 Duration of treatment	90 days	
3.3.2 Frequency of exposure	7 days per week	
3.3.3 <u>Oral</u>		
3.3.3.1 Type	gavage	
3.3.3.2 Concentration	gavage 0.1, 0.5, 1µg/kg bw	
3.3.3.3 Vehicle	Distilled water	
3.3.3.4 Concentration in vehicle	2, 1 and 0.2 µg/ml	
3.3.3.5 Total volume applied	0.5 ml/1000 g bw	
3.3.3.6 Controls	vehicle	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes, one day bore the first treatment and then after dosing, once a day.	
3.4.1.2 Mortality	Yes, daily	X1
3.4.2 Body weight	Yes, on day of the receipt, one day before starting treatment, weekly and terminally	
3.4.3 Food consumption	Yes, daily	
3.4.4 Water consumption	No	
3.4.5 Ophthalmoscopic examination	Yes, before start of the treatment, then at the termination of the study	
3.4.6 Haematology	Yes. number of animals: all animals time points: before, midway and end of study Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, clotting time, partial prothrombin time, thromboplastin time, Mean Corpuscular volume, mean corpuscular haemoglobin, differential white blood cell count	
3.4.7 Clinical Chemistry	Yes, number of animals: all animals time points: before, midway and end of study Parameters: Glucose, urea conc, creatinine conc, total protein conc, cholesterol conc, Triglycerides, aspartate aminotransferase activity, alanine aminotranferase activity, alkaline phosphatase activitiy, sodium conc, potassium conc, calcium conc, chloride conc	
3.4.8 Urinalysis	yes	

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
	number of animals: all animals time points: before, midway and end of study Parameters: appearance, volume, osmolality, specific gravity, pH, protein, glucose, blood, leukocytes, nitrite, UBG, bilirubin, ketone, sediment	
3.5 Sacrifice and pathology		
3.5.1 Organ Weights	yes organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart, thyroids, parathyroids, uterus, pituitary	
3.5.2 Gross and histopathology	yes 4 animals/sex of the control and high dose groups. organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes peripheral nerve, bone marrow, skin, eyes, caecum, cerebellum, cerebrum, epididymides, gross lesion, lachrymal glands, muscle, oesophagus, ovaries, rectum, sciatic nerve, sternum and vagina	
3.5.3 Other examinations		
3.5.4 Statistics	Statistical analysis was performed by SPSSPC+ software package for the following data: - body weight data - food consumption data - organ weight data - haematological, biochemical, urine data The heterogeneity of variance between groups were checked by Bartlett's test.	
3.6 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Observations		
4.1.1 Clinical signs	Male 0.1 µg/kg dose group – diarrhoea was observed in one animal on four occasions. 0.5 µg/kg dose group – diarrhoea occurred in two animals on 4-5 occasions/animal. This appeared from 80 to 90 days. The paleness skin mucous skin mucous membrane in one animal was seen on the last three days of the treatment period. 1.0 µg/kg dose group – in two animals on 2-4 occasions/animal diarrhoea and in half of animals thin faeces occurred between the 81 and 88 days of the study. The paleness skin mucous membrane in five animals were recorded from day 78 to days 88-90. In one animal was found salivation on 85 and 86 days of study period. Female:	

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
	0.1 0.1 µg/kg dose group – diarrhoea occurred in three animals. This appeared in tow animals 37-42 days of study period. The paleness skin mucous membrane in one animal were recorded from day 84 to 88 days. 0.5 0.5 µg/kg dose group – diarrhoea occurred in two animals. This clinical sign appeared in one animal between the 28 and 29 days. The paleness skin mucous membrane in one animal were recorded from day 81 to 91 days. Decreased activity appeared in two animals on 3 occasions/animal 1.0 µg/kg dose group – diarrhoea occurred in two animals which appeared in one animal between 82 and 84 days. In all animals paleness skin mucous membrane was observable from 79 -86 days to the end of the study. In three animals thin faeces appeared on the last period of the study 3-12 occasions/animal.	
4.1.2 Mortality	During the study two female rabbits died, at 0.5 µg/kg on 15th and 87th of day of treatment.	
4.2 Body weight gain	Male In 0.5 and 1.0 µg/kg dose groups the 13-week mean body weight gain of the rabbits were below the control value with 19.8% and 14.3% respectively. Female The 13-week mean body weight gain was above the control value in 0.1 µg/kg dose. In 0.5 µg/kg dose group was similar to the control value. In case of high 1 µg/kg dose the 13-week mean bw gain of the rabbits were below the control value with 13.5%.	X2 X3
4.3 Food consumption and compound intake	1 µg/kg – food consumption was below the control value with significance on week 12. In male dose groups, the food consumption was below the control value with significance in the dose group of 1.0 µg/kg on week 12 as well as in the dose group of 0.5 µg/kg on weeks 4 and 12. Overall there was no affect on food consumption at any dose group.	X4
4.4 Ophtalmoscopic examination	No pathological alterations were observed during the study.	X5
4.5 Blood analysis		
4.5.1 Haematology	These revealed minor statistically significant changes in some parameters in males in all treated groups. In the base level and midway period. In the terminal period, minor changes in the these parameters occurred in females along with increased white blood cell count in all treated groups not exceeding the physiological range and there were no pathological findings in the differential white blood cell count. In terminal phase, the increase in prothrombin time values in males and females in the 1µg/kg dose groups may represent a possible effect of the treatment.	X6
4.5.2 Clinical chemistry	In males only a slight increase in calcium ion and decrease in ALP activity in the midway period in the 1µg/kg dose without any biological significance. In females there were slight fluctuation in ALT activities, potassium and creatinine concentrations in the 1µg/kg dose groups while glucose concentrations decreased in all treated groups of terminal	

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
	phase. There were no treatment related effects.	
4.5.3 Urinalysis	There were no treatment related urine analysis changes.	X7
4.6 Sacrifice and pathology		
4.6.1 Organ weights	No effects on organ weights in any dose group	
4.6.2 Gross and histopathology	No effects on organ weights in any dose group	X8
4.7 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study was conducted according to OECD 409. Doses were chosen on the basis of the results of the preliminary study in both sexes. Six animals of both sexes/group were used in the study. Treatment was carried out by oral gavage. Bromadiolone was dissolved in ethanol (strain solution, 1 mg/ml ethanol) and diluted to the appropriate concentration (2, 1 and 0.2 g/ml). The constant treatment volume was 0.5ml/1000g body weight. Control animals were treated with distilled water containing the same amount of ethanol as the solution of high dose group. Clinical observations were made once daily. Body weight, food consumption were measured weekly. The haematological and clinical investigations and urine analysis were preformed prior to the treatment, at midway, at the end of the treatment. The ophtalmoscopic examinations were performed prior to the treatment, at the end of the treatment period.	X9
5.2 Results and discussion	Two female animals died at 0.5 µg/kg two female animals died on 15th and 87th days of treatment period. The gross and histopathological examination revealed as cause of death acute bacterial invasion. There were no clinical signs in the control groups. Diarrhea was observed in all dose groups with low frequency. Salivation and decreased activity were found in male dose group 1µg/kg and female dose group 0.5 µg/kg respectively in one and two animals. The thin faeces appeared in male dose group 1µg/kg and female dose groups 0.1 and 1µg/kg. This sign was recorded in half of animals in the highest dose groups in both sexes. The paleness skin mucous membrane was found in all dose groups, except the male dose group 0.1µg/kg. This sign proved to be biologically significant alteration of highest dose group in both sexes. Haematological investigations showed increase in prothrombin time values in males and females in the 1µg/kg dose groups can be related to the effect of the test item. There were no treatment related organ weight changes when treated with bromadiolone.	X10
5.3 Conclusion		X11
5.3.1 LO(A)EL	1.0 µg/kg	
5.3.2 NO(A)EL	0.5 µg/kg	
5.3.3 Other		

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	

Section A6.4.1 **Annex Point IIA VI 6.4**	**Sub chronic oral toxicity** *90-day oral repeat dose toxicity in rabbit*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	Applicants version is accepted with amendments: X1 Deviation from EC Method B.28, in which it is specified that a check for mortality and morbidity should be performed at least twice daily. X9 In this study young, healthy rabbits were allocated to three dose groups (0.1, 0.5, 1µg/kg bw) and a control group. Gross necropsy findings and organ weights were recorded in all animals. Histopathological examination was performed in four animals in the highest (1.0 µg/kg) dose group and in four controls.	
Results and discussion	X2 There was no significant difference in mean body weight gain in males w. 0-13 in the 1.0 µg/kg dose group compared to controls. The only significant decrease in body weight gain was seen in males of the intermediate (0.5 µg/kg.) dose group compared to controls during w. 0- 13. This deviation from control values was thus not dose related. X3 According to the table in appendix 1.2, there was no significant difference in mean body weight gain in females w. 0- 13 in the 1.0, µg/kg dose group compared to controls. X4 A significantly lower food consumption during week 12 was only seen in males. X5 Results not summarized in document IV. X6 No specific statistically significant effect on haematological results was seen in all three male dose groups compared to controls. X7 Nitrite. and sediment results were not been reported for all samples. X8 Pin-prick sized lung haemorrhages, lung abscesses and nutmeg-like pattern in the liver were slightly more common in both females and males compared to controls. X10 Applicants version replaced with: 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 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	

Section A6.4.1 Annex Point IIA VI 6.4	Sub chronic oral toxicity *90-day oral repeat dose toxicity in rabbit*	
	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 urinanalysis 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 was 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.	
Conclusion	X11 Applicants version replaced with: LOEL: 0.1 µg/kg, for both sexes based on the occurance of diarrhoea in this dose group. However, since the diarrhoea did not lead to any systemic effects (such as dehydration or leukocytosis), other than hypoglycaemia in females, the effect is not classified as adverse. LOAEL: 1 µg/kg , for both sexes based on the prolonged prothrombine time seen in this dose group. NOEL: < 0.1 µg/kg for both sexes based on the occurance of diarrhoea in all three dose groups. NOAEL : 0.5 µg/kg for both sexes, based on the absence of adverse effects in this dose group.	
Reliability	1	
Acceptability	Acceptable	
Remarks	Table A6 4 1-1 is misleading since several arrows represent non-significant deviations from control values. No males died in the study, + should be replaced by -.	

Table A6.4.1-1 Results of repeated dose toxicity study

Parameter	**Control**		**low dose**		**medium dose**		**high dose**		**dose-response +/-**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
number of animals examined	6	6	6	6	6	6	6	6		
Mortality	0	0	0	0	0	2	0	0	+	+
clinical signs*									+	+
Diarrhoea	0	0	1	3	2	2	2	2		
Paleness skin mucous membrane	0	0	0	1	1	2	5	6		
Thin Faeces	0	0	0	1	0	0	3	3		
Salivation	0	0	0	0	0	2	1	0		
body weight			0%	↑	-6%	↑	-4%	↓	-	-
food consumption			0	↑	↑	↓	↑	↓	+	-
General Haemorrhagic diathesis										
microscopic pathology*			↑	↑	↑	↑	↑	↑	+	+
Lungs										
Emphysema	66%	50%	50%	83%	83%	50%	16%	16%		
Pinprick-sized haemorrhages	16%	33%					50%	33%		
Point-like haemorrhages				16%		16%		16%		
Reddish mottled	16%	16%	33%		16%		33%	33%		
Purulent inflammation								16%		
Yellowish-white compact formation 1 cm dia.			16%							
Yellowish-grey compact formation 0.5 cm dia.							16%			
Brain			-2%	7%	1%	9%	-4%	8%		
Liver			12%	18%	10%	5%	8%	12%		
Nutmeg-like pattern							16%	16%		
Enlarged, dark red								16%		
Spleen			-18%	41%	-34%	15%	-26%	30%		
Kidneys			8%	8%	9%	-2%	4%	-2%		
Pale (2 side)			16%	16%						
Pale (1 side)		16%						16%		
Thymus			41%	-3%	36%	7%	6%	-3%		
Heart			3%	30%	16%	10%	7%	17%		
Pituitary			4%	21%	23%	34%	-4%	25%		
Uterus			-	10%	-	5%	-	12%		
Small intestines										
Catarrhal inflammation			16%							
Sporadic reddish formations (dia. 1-2 mm) on the serosa							16%			

Ovaries Cyst (1 side) Sporadic military sized reddish-black formations (2 side)		33%	-	37% 16%	-	-28% 16%	-	-6%		
Testes			-12%	-	-2%	-	-18%	-		
Epididymides			4%	-	6%	-	8%	-		
Adrenals			15%	-6%	10%	-16%	-1%	-8%		
Thyroids			-27%	32%	-33%	52%	-4%	11%		

Section A6.4.2 **Annex Point IIA VI.6.4**	**Subchronic dermal**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Bromadiolone is a well-known compound, which has been used extensively for many years. It belongs to a close group of analogues, which have closely similar properties. They are well understood and mode of action is well understood. Mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested, including humans in therapeutic use (warfarin) and in poisoning incidents in humans and animals. There are no other significant toxic effects Its mode of action is well understood and documented, and is common among all mammalian species. The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. As discussed earlier dermal absorption is not considered to be a major route of entry, due to the fact that the compound is poorly absorbed through the skin. Under these circumstances, a dermal 90-day study is considered to be of no value, and an unnecessary waste of experimental animals.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	A subchronic oral study has been performed for bromadiolone using the rabbit as test species. Route-to route extrapolation can be performed since both acute oral and acute dermal data are available for bromadiolone.	
Conclusion	Justification accepted	
Remarks		

Section A6.4.3 **Annex Point IIA VI.6.4**	**Subchronic inhalation toxicity test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Bromadiolone is a well-known compound, which has been used extensively for many years. It belongs to a close group of analogues, which have closely similar properties. They are well understood and mode of action is well understood. Mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested, including humans in therapeutic use (warfarin) and in poisoning incidents in humans and animals. There are no other significant toxic effects The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. As discussed earlier, inhalation is not considered to be a significant potential route of entry due to low vapour pressure and solid bait nature of product. Hence, inhalation exposure to rodenticides is minimal and effects of inhalation will be no different to oral administration. Under these circumstances, an inhalation 90-day study is considered to be of no value, and an unnecessary waste of experimental animals.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	The vapour pressure for bromadiolone is low and inhalation exposure is not considered to be significant during production or when handling bait blocks.	
Conclusion	Justification accepted.	
Remarks		

Section A6.5 Chronic toxicity		
Section A6.5 **Annex Point IIA VI 6.5**	**Chronic toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The compound belongs to a well-known and closely analogous group of anticoagulants with very similar properties. All studies on vertebrates show the same effects, primarily loss of blood coagulation, and these are shown clearly in acute studies. There is little species differentiation in effects or dose response, and there are no positive findings in genotox studies. To avoid acute effects, doses in repeat dose studies must be kept very low, and the potential for exposure to rodenticides is limited by the nature of their use. A second species 90-day feeding study is therefore considered unjustified. Bromadiolone is a well-known compound, which has been used extensively for many years. It belongs to a close group of analogues, which have closely similar properties. They are well understood and mode of action is well understood. Mode of action is by inhibition of blood clotting and is seen in all other mammalian species tested, including humans in therapeutic use (warfarin) and in poisoning incidents in humans and animals. There are no other significant toxic effects Its mode of action is well understood and documented, and is common among all mammalian species. The technical active ingredient has a high level of purity and there are no other substances of concern included as impurities or additives. Data on a rat 2-generation reproduction study has been submitted which also addresses this point. In the two generation reproduction study, the rats were orally dosed at three dose levels: 1, 2.5 and 5µg/kg of Bromodiolone Technical. Only one female animal died on treatment day 81, and the cause of death was catharral pneumonia and oedema in the lungs. The body weight and food consumption of both sexes through out the whole study period was unaffected at the examined dose levels. Gross pathology revealed no alterations due to the effect of the test article. The prothrombin values were similar in the control and the treated dose groups. No organ weights alterations related to the test material was found in the parental and F1 generation. There were no pathological, organ weight and histopatholigic alterations related to the bromodiolone for the parents or the pups. By studying the effects seen in the two-generation reproduction study, a chronic study will be of no value because no ill-effects were seen.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	

Section A6.5 Chronic toxicity		
Section A6.5 **Annex Point IIA VI 6.5**	**Chronic toxicity**	
Evaluation of applicant's justification	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, namely the multi-generation study found under point 6.8.2. 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 are thus not justified.	
Conclusion	Justification accepted	

Section A6.6 Genotoxicity studies		
Section A6.6.1 **Annex Point IIA VI 6.6.1**	**Genotoxicity in vitro** Reverse mutation assay using *Salmonella typhimurium*	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2001) Draft report: Bromadiolone Technical: Testing of Bromadiolone Technical with Bacterial Reverse Mutation Assay, XXXXX. report 01/617-007M	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 471	
2.2 GLP	Yes	
2.3 Deviations	The purity of the test item tested is 99.4%. This will not affect the integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	White crystalline powder	
3.1.2.2 Purity	99.4% (bromadiolone)	X1
3.1.2.3 Stability	Stable under test conditions	
3.2 Study Type	Bacterial reverse mutation test	
3.2.1 Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100, TA 102	
3.2.2 Deficiencies / Proficiencies	Not applicable	
3.2.3 Metabolic activation system	S9 mix	

Section A6.6 Genotoxicity studies		
Section A6.6.1 **Annex Point IIA VI 6.6.1**	**Genotoxicity in vitro** Reverse mutation assay using *Salmonella typhimurium*	
3.2.4 Positive control	Non-activation Sodium azide – 2 µg/plate for TA100 and TA1535 9-Aminoacridine (9AA) – 50 µg/plate for TA1537 4-nitro-o-phenylene-diamine - 4 µg/plate for TA98 Methyl-methane-sulphonate - 2 µl/plate for E. coli WP2uvrA Activation 2-aminoanthracene - 2 µg/plate for all Salmonella strains 2-aminoanthracene - 50 µg/plate for E.coli strain	
3.3 Administration / Exposure; Application of test substance		
3.3.1 Concentrations	39.06, 78.13, 156.25, 312.50, 625, 1250, 2500 and 5000 µg/plate	
3.3.2 Way of application	Dissolved in dimethyl sulphoxide and mixed with basal medium	
3.3.3 Pre-incubation time	12-14 hours at 37ºC in Gyrotory water bath shaker.	
3.3.4 Other modifications	None	
3.4 Examinations	Inhibition and mutagenicity	
3.4.1 Number of cells evaluated	Not stated	
	4 RESULTS AND DISCUSSION	
4.1 Genotoxicity		
4.1.1 without metabolic activation	No significant increase	
4.1.2 with metabolic activation	No significant increase	
4.2 Cytotoxicity	Test item had a strong inhibition effect on the *Salmonella typhimurium* TA1537 test strain at 5000 – 625 µg/plate concentrations and also observed a weak inhibition effect on *Salmonella typhimurium* TA98 and TA100 at 5000 - 1250 µg/plate, while the background lawn of his/trp minus cells reduced at the above concentration levels.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.6 Genotoxicity studies		
Section A6.6.1 **Annex Point IIA VI 6.6.1**	**Genotoxicity in vitro** Reverse mutation assay using *Salmonella typhimurium*	
5.1 Materials and methods	The study was conducted according to OECD 471. Bromadioline was used to study for mutagenic activity by Reverse Mutation Assay Method. The test was carried out twice with a set of histidine-requiring auxotroph strains of Salmonella typhimurium TA98, TA10,, TA1537, TA1535 and the tryptophan-requiring auxotroph strain of E.coli in presence and absence of rat liver fraction activated by Phenobarbitone and β-naphtoflavone, using appropriate positive and negative controls. The test item was dissolved in DMSO solvent in 100mg/ml concentration, as a basal solution for the main study. Concentrations of the test item in the main study were 5000, 1250, 625, 312.50, 156.25, 78.13 and 39.06 µg/plate. Bacteria were exposed to the test item both in the presence and absence of an appropriate metabolic activation system. The bacterial strains were cultured in nutrient broth. The selective medium was a minimal medium with 2% glucose. An appropriate number of parallel tubes of molten top agar was 3 per control or concentration level and they were prepared and kept at 45°C. An equivalent number of minimal plates was also prepared. For activation studies instead of phosphate buffer, 0.5ml of the S9mix had to be added to each overlay tube. The plates were then incubated at 37°C for 48 hours. The colony number on the control, positive control and the test plat4es were determined, the mean values and standard deviations were calculated.	
5.2 Results and discussion	Summarize relevant results; discuss dose-response relationship. Test item had a strong inhibition effect on the *Salmonella typhimurium* TA1537 test strain at 5000 – 625 µg/plate concentrations and also observed a weak inhibition effect on *Salmonella typhimurium* TA98 and TA100 at 5000 - 1250 µg/plate, while the background lawn of his/trp minus cells reduced at the above concentration levels. The revertant colony numbers in the test plates were practically the same or lower, compared to the untreated control plates.	X2
5.3 Conclusion	The test material bromadiolone technical is considered to be non-mutagenic.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April 2006	
Materials and Methods	Applicants version accepted with amendments: X1 Purity is slightly higher than the 98% purity level presented in section 2. However, this should not affect the outcome of the study.	

Section A6.6 Genotoxicity studies		
Section A6.6.1 **Annex Point IIA VI 6.6.1**	**Genotoxicity in vitro** Reverse mutation assay using *Salmonella typhimurium*	
Results and discussion	Complementary information found in DOC IV, was sufficient to accept the study. Applicants version replaced with: X2 The number of revertant colonies in the test plates were comparable to the untreated control plates and the solvent control plates for all of the tested bacterial strains. The test substance had a inhibitiory effect on the *Salmonella typhimurium* TA1537 test strain at 5000 – 625 µg/plate concentrations and also showed weak inhibitory effects on *Salmonella typhimurium* TA98 and TA100 at 5000 - 1250 µg/plate. Due to the inhibitory effect of Bromadione on Salm. Typh. TA 1537, revertant colonies could only be registered in plates with less than 312.5 µg/plate Bromadilone. However, no increase of revertant colonies were seen at the three low concentrations or at high koncentrations in the TA98, TA100, or TA1535 strains, rendering mutagenicity in the TA 1537 at higher concentrations, to be highly unlikely.	
Conclusion	Applicants version replaced with: The test substance bromadiolone is considered to be non-mutagenic in the studied test system.	
Reliability	1	
Acceptability	Acceptable	
Remarks		

Table A6.6.1-1 Table for Gene Mutation Assay

***Salmonella typhimurium* TA98**

Concentration µg/plate	Number of mutant cells		Comments *give information on cytotoxicity or other*
	— S9	+ S9	
5000.00	9.3 ± 3.51	11.0 ± 2.00	Weak toxic effect
2500.00	8.7 ± 2.52	8.3 ± 3.06	Weak toxic effect
1250.0	7.3 ± 1.53	9.0 ± 1.00	Weak toxic effect
625.00	11.7 ± 3.79	14.3 ± 4.16	
312.50	17.7 ± 4.93	12.3 ± 1.15	
156.25	18.0 ± 6.08	20.2 ± 3.46	
78.13	20.3 ± 2.08	24.3 ± 2.08	
39.06	21.0 ± 2.65	25.3 ± 3.51	
Untreated	22.3 ± 3.79	29.7 ± 3.21	
Untreated +DMSO	20.3 ± 2.89	27.7 ± 8.08	
NPD (4µg)	468.0 ± 39.13		
2-AA (2µg)		1042.7 ± 46.65	

***Salmonella typhimurium* TA100**

Section A6.6 Genotoxicity studies

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI 6.6.1

Reverse mutation assay using *Salmonella typhimurium*

Concentration µg/plate	Number of mutant cells		Comments *give information on cytotoxicity or other*
	— S9	+ S9	
5000.00	35.7 ± 5.69	68.7 ± 17.04	Weak toxic effect
2500.00	49.3 ± 13.32	53.0 ± 6.08	Weak toxic effect
1250.0	41.7 ± 3.51	51.0 ± 7.51	Weak toxic effect
625.00	39.7 ± 0.58	64.0 ± 4.00	
312.50	67.0 ± 2.65	75.3 ± 6.03	
156.25	82.0 ± 4.00	84.0 ± 13.89	
78.13	107.7 ± 11.59	102.0 ± 2.00	
39.06	116.3 ± 5.03	105.0 ± 3.46	
Untreated	111.3 ± 9.07	120.0 ± 6.24	
Untreated + D.Water	116.0 ± 13.53	107.3 ± 7.09	
Untreated +DMSO	116.0 ± 2.00	101.3 ± 2.08	
SAZ (2µg)	425.0 ± 11.36		
2-AA (2µg)		1370.0 ± 77.66	

***Salmonella typhimurium* TA1535**

Concentration µg/plate	Number of mutant cells		
	— S9	+ S9	
5000.00	8.3 ± 2.08	6.0 ± 1.73	
2500.00	5.7 ± 1.15	8.7 ± 1.53	
1250.0	4.3 ± 0.58	5.7 ± 0.58	
625.00	6.0 ± 1.00	7.0 ± 1.00	
312.50	6.7 ± 1.53	8.3 ± 0.58	
156.25	8.0 ± 1.73	7.3 ± 1.53	
78.13	8.0 ± 1.00	6.0 ± 1.00	
39.06	6.7 ± 1.15	6.0 ± 1.00	
Untreated	7.3 ± 1.53	9.3 ± 1.53	
Untreated + D.Water	116.0 ± 2.08	7.3 ± 1.53	
Untreated +DMSO	7.7 ± 0.58	8.0 ± 1.00	
SAZ (2µg)	468.0 ± 16.09		
2-AA (2µg)		89.7 ± 4.51	

Section A6.6 Genotoxicity studies

Section A6.6.1 Annex Point IIA VI 6.6.1	Genotoxicity in vitro Reverse mutation assay using *Salmonella typhimurium*	

***Salmonella typhimurium* TA1537**

Concentration µg/plate	Number of mutant cells		Comments *give information on cytotoxicity or other*
	— S9	+ S9	
5000.00	0.0 ± 0.00	0.0 ± 0.00	Strong inhibition effect
2500.00	0.0 ± 0.00	0.0 ± 0.00	Strong inhibition effect
1250.0	0.0 ± 0.00	0.0 ± 0.00	Strong inhibition effect
625.00	0.0 ± 0.00	3.3 ± 2.08	Strong inhibition effect
312.50	0.0 ± 0.00	4.0 ± 1.00	
156.25	4.3 ± 1.53	4.7 ± 1.53	
78.13	4.7 ± 0.58	6.3 ± 0.58	
39.06	6.7 ± 0.58	7.0 ± 1.00	
Untreated	7.0 ± 1.00	8.0 ± 1.73	
Untreated +DMSO	5.7 ± 1.15	6.3 ± 1.53	
9-AA (50µg)	328.0 ± 27.50		
2-AA (2µg)		97.3 ± 12.10	

E.coli WP2 uvrA

Concentration µg/plate	Number of mutant cells		Comments *give information on cytotoxicity or other*
	— S9	+ S9	
5000.00	22.0 ± 4.58	27.7 ± 0.58	
2500.00	21.7 ± 3.06	27.3 ± 6.35	
1250.0	21.0 ± 3.00	29.0 ± 3.00	
625.00	21.7 ± 4.51	28.0 ± 4.00	
312.50	22.0 ± 3.00	22.0 ± 2.65	
156.25	24.0 ± 4.36	25.0 ± 3.61	
78.13	23.3 ± 3.51	24.3 ± 1.53	
39.06	24.3 ± 5.51	25.7 ± 0.58	
Untreated	22.0 ± 1.00	21.0 ± 2.65	
Untreated + D.Water	28.7 ± 4.51	23.7 ± 2.52	
Untreated +DMSO	21.7 ± 3.06	27.0 ± 4.36	
MMS (4µl)	487.7 ± 9.07		
2-AA (50µg)		438.7 ± 24.68	

Section A6.6.2 **Annex Point IIA VI 6.6.2**	**Genotoxicity in vitro** Chromosome aberration test on Chinese Hamster Ovaries *in vitro*	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2001) Draft report: BROMADIOLONE: In Vitro Mammalian Chromosomal Aberration study of Test item Bromadiolone Technical. XXXXX. report 01/617-020C	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 473	
2.2 GLP	Yes	
2.3 Deviations	The purity of the test item tested is 99.4%. This will not affect the integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	White crystalline powder	
3.1.2.2 Purity	99.4% (Bromadiolone)	X1
3.1.2.3 Stability	Stable under test conditions	
3.2 Study Type	*In vitro* mammalian chromosome aberration test	
3.2.1 Organism/cell type	mammalian cell lines: Chinese hamster Ovary (CHO)	
3.2.2 Deficiencies / Proficiencies	Not Applicable	
3.2.3 Metabolic activation system	S9 mix	

Section A6.6.2 **Annex Point IIA VI 6.6.2**	**Genotoxicity in vitro** Chromosome aberration test on Chinese Hamster Ovaries *in vitro*	
3.2.4 Positive control	In the absence of S9 – ethylmethane sulphonate dissolved in DMSO at final concentration of 0.4 µl/ml. In the presence of S9 – N-Nitrosodimethylamine dissolved in DMSO at final concentration of 0.4 µl/ml.	
Administration / Exposure; Application of test substance		
3.2.5 Concentrations	1.0, 7.5 and 15.0 µg/ml	
3.2.6 Way of application	Dissolved in dimethyl sulphoxide incorporated into medium	
3.2.7 Pre-incubation time		
3.2.8 Other modifications		
3.3 Examinations	see tables in appendix for examinations and results	
3.3.1 Number of cells evaluated	200 per dish	
	4 RESULTS AND DISCUSSION	
4.1 Genotoxicity		
4.1.1 without metabolic activation	No No positive results seen	
4.1.2 with metabolic activation	No No positive results seen	

Section A6.6.2 **Annex Point IIA VI 6.6.2**	**Genotoxicity in vitro** Chromosome aberration test on Chinese Hamster Ovaries *in vitro*	
4.2 Cytotoxicity	Yes 100ug/ml with or without activation	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	OECD 473. The test item Bromadiolone was studied for clastogenic activity in Chinese hamster ovary cells both in the presence and in the absence of metabolic activation in two independent experiments. The test item was dissolved in Dimethylsulphoxide. Study 1 was performed at test item concentrations of 1.0, 7.5 and 15.0 µg/ml in the presence and in the absence of metabolic activation. the exposure period was 4 hours at 37°C. In study 2 the test item was applied a 1.0, 7.5 and 10 µg/ml concentrations in the presence and in absence of metabolic activation. In case of study 2 the exposure period was 1.5 normal cell cycle length.	X2
5.2 Results and discussion	Doses were selected on the basis of cytotoxicity investigations. There was no significant increase in the number of aberrations without gaps in the applied concentrations either in the absence or in the presence of metabolic activation in the study 1 and study 2. The positive controls, the Ethylmethanesulphonate and N-Nitrosodimethylamine revealed a clear clastogenic effect, thus validating the test. In untreated control the number of aberrations without gap was less than 5% proving the suitability of the used cell line.	
5.3 Conclusion	The test material bromadiolone is considered to be non-clastogenic in the metaphase chromosome aberration assay in Chinese Hamster Ovary cells.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section A6.6.2 **Annex Point IIA VI 6.6.2**	**Genotoxicity in vitro** Chromosome aberration test on Chinese Hamster Ovaries *in vitro*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	Complementary information found in DOC IV, was sufficient to accept the study. Applicants version accepted with amendments: X1 Purity is slightly higher than the 98% purity level presented in section 2. However, this should not affect the outcome of the study. X2 In study 2 the dose used were 1.0, 7.5 and 15 µg/ml. Duplicate cultures were used for all test concentrations and controls in study 1 and 2. In study 2 no positive control was used, which is unfortunate but does not invalidate the study. All positive control values were significantly increased compared to controls. No records of polyploidism or endoreduplication are included in the study report, this however does not invalidate the study.	
Results and discussion	Applicants version accepted	
Conclusion	Applicants version accepted	
Reliability	1	
Acceptability	Acceptable	
Remarks	Table A6 6 1-1 is replaced with a new version. In all table the highest dose should be 15 µg/ml, not 1.5 µg/ml.	

Bromodiolone Technical without S9				Treatment period: 4 hours				Vehicle: DMSO			
Non Activation Test Condition	Number of Metaphases	Number of Aberrant cells		Aberration Rate (Aber./100 Cells)	Number of Aberrations		Aberrations				
							Chromosome			Chromatid	
		Gap+	Gap-	Gap-	Gap+	Gap-	Gap	del	exchange	Gap	del	exchange
Control a	100	6	1	0.010	7	1	3	0	0	3	1	0
Control b	100		2	0.020	6	2	2	0	0	2	2	0
Vehicle control a	100	4	2	0.040	9	4	1	0	1	4	3	0
Pos. control [x] a	100	5	15	0.410	69	41	8	6	1	20	29	0
Pos. control [x] b	100	20	14	0.410	73	41	7	14	3	25	19	0
TEST ITEM												
1.0 µg/ml a	100	6	0	0.020	8	2	1	1	0	5	1	0
1.0 µg/ml b	100	5	2	0.020	7	2	2	0	0	3	2	0
7.5 µg/ml a	100	9	1	0.040	15	4	3	1	1	8	2	0
7.5 µg/ml b	100	4	2	0.020	6	2	1	0	2	3	0	0
1.5 µg/ml a	100	7	2	0.030	12	3	3	1	0	6	2	0
1.5 µg/ml b	100	9	2	0.020	13	2	4	0	1	7	1	0

Table A6.6.1-1Table for Cytogenetic in vitro-Test: Chromosomal Analysis (modify if necessary)

Bromadiolone Technical With S9				Treatment period: 4 hours			Vehicle: DMSO					
Non Activation Test Condition	Number of Metaphases	Number of Aberrant cells		Aberration Rate (Aber./100 Cells)	Number of Aberrations		Aberrations					
							Chromosome			Chromatid		
		Gap+		Gap-	Gap+	Gap-	Gap	del	exchange	Gap	del	exchange
Control a	100	4	2	0.020	6	2	1	0	1	3	1	0
Control b	100	4	1	0.010	5	1	1	0	0	3	1	0
Vehicle control a	100	3	1	0.020	5	2	1	0	1	2	1	0
Pos. control [x] a	100	18	15	0.270	49	27	6	5	2	16	16	4
Pos. control [x] b	100	19	12	0.330	61	33	8	5	3	20	24	1
TEST ITEM												
1.0 µg/ml a	100	7	1	0.010	9	1	4	0	0	4	1	0
1.0 µg/ml b	100	4	3	0.030	7	3	2	0	1	2	2	0
7.5 µg/ml a	100	7	2	0.020	9	2	2	0	0	5	2	0
7.5 µg/ml b	100	7	1	0.010	9	1	3	0	1	3	0	0
1.5 µg/ml a	100	5	3	0.030	8	3	2	0	1	3	2	0
1.5 µg/ml b	100	3	2	0.020	5	2	0	1	1	3	0	0

Bromadiolone Technical without S9				**Treatment Period: 18-20 hours**		**Vehicle: DMSO**					
Non Activation Test Condition	**Number of Metaphases**	**Number of Aberrant cells**		**Aberration Rate (Aber./100 Cells)**	**Number of Aberrations**	**Aberrations**					
						Chromosome			**Chromatid**		
		Gap+	Gap-	Gap-	Gap+	Gap-	Gap	del	exchange	Gap	del	exchange
Control a	100	5	2	0.020	7	2	2	0	0	3	2	0
Control b	100	8	0	0.000	8	0	2	0	0	6	0	0
Vehicle control a	100	6	2	0.020	9	2	1	0	0	6	2	0
TEST ITEM												
1.0 µg/ml a	100	7	2	0.020	9	2	1	0	2	6	0	0
1.0 µg/ml b	100	5	2	0.020	8	2	2	0	0	4	2	0
7.5 µg/ml a	100	5	4	0.040	9	4	2	0	1	3	3	0
7.5 µg/ml b	100	7	3	0.030	10	3	2	0	0	5	3	0
1.5 µg/ml a	100	13	3	0.030	17	3	6	2	1	0	0	0
1.5 µg/ml b	100	9	2	0.020	11	2	1	0	0	2	2	0

Bromadiolone Technical with S9				**Treatment Period:18-20 hours**				**Vehicle: DMSO**			
Non Activation Test Condition	**Number of Metaphases**	**Number of Aberrant cells**		**Aberration Rate (Aber./100 Cells)**	**Number of Aberrations**		**Aberrations**				
							Chromosome			**Chromatid**		
		Gap+	Gap-	Gap-	Gap+	Gap-	Gap	del	exchange	Gap	del	exchange
Control a	100	6	3	0.030	9	3	1	0	0	5	2	0
Control b	100	8	2	0.030	12	3	4	0	0	5	3	0
Vehicle control a	100	8	3	0.030	21	3	3	1	1	5	2	0
TEST ITEM												
1.0 µg/ml a	100	11	3	0.030	14	3	3	0	0	8	3	0
1.0 µg/ml b		11	5	0.050	17	5	3	2	1	9	3	0
7.5 µg/ml a	100	10	3	0.030	16	3	3	0	0	10	3	0
7.5 µg/ml b	100	7	2	0.030	10	3	2	0	0	5	3	0
1.5 µg/ml a	100	7	3	0.030	12	3	1	1	1	8	1	0
1.5 µg/ml b	100	11	4	0.040	16	4	2	1	1	10	2	1

Bromodiolone Technical without S9				Treatment period: 4 hours				Vehicle: DMSO			
Non Activation Test Condition	Number of Metaphases	Number of Aberrant cells		Aberration Rate (Aber./100 Cells)	Number of Aberrations		Aberrations				
							Chromosome			Chromatid		
		Gap+	Gap-	Gap-	Gap+	Gap-	Gap	del	exchange	Gap	del	exchange
Control a	100	6	1	0.010	7	1	3	0	0	3	1	0
Control b	100	4	2	0.020	6	2	2	0	0	2	2	0
Vehicle control a	100	5	2	0.040	9	4	1	0	1	4	3	0
Pos. control [x] a	100	20	15	0.410	69	41	8	6	1	20	29	0
Pos. control [x] b	100	22	14	0.410	73	41	7	14	3	25	19	0
TEST ITEM												
1.0 µg/ml a	100	6	0	0.020	8	2	1	1	0	5	1	0
1.0 µg/ml b	100	5	2	0.020	7	2	2	0	0	3	2	0
7.5 µg/ml a	100	9	1	0.040	15	4	3	1	1	8	2	0
7.5 µg/ml b	100	4	2	0.020	6	2	1	0	2	3	0	0
15 µg/ml a	100	7	2	0.030	12	3	3	1	0	6	2	0
15 µg/ml b	100	9	2	0.020	13	2	4	0	1	7	1	0

NEW VERSION Table A6.6.1-1 Table for Cytogenetic in vitro-Test: Chromosomal Analysis (modify if necessary)

Section A6.6.3 **Annex Point IIA VI 6.6.3**	**Genotoxicity in vitro** Mutagenic Evaluation in CHO/HPRT Assay	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2002) Draft report: BROMADIOLONE: Mutagenic Evaluation of Test Item Bromadiolone Technical in CHO/HPRT Assay. XXXXX. report 01/617-015C	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with Access to data	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 476	
2.2 GLP	Yes	
2.3 Deviations	The purity of the test item is 99.4%. This will not effect the integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	White crystalline powder	
3.1.2.2 Purity	99.4% (bromadiolone)	X1
3.1.2.3 Stability	Stable under test conditions	
3.2 Study Type	Mutagenic evaluation – CHO/HPRT Forward Mutation Assay	
3.2.1 Organism/cell type	Chinese Hamster Ovary (CHO)	
3.2.2 Deficiencies / Proficiencies	Not Applicable	
3.2.3 Metabolic activation system	S9 mix	
3.2.4 Positive control	In the absence of S9 – ethylmethane sulphonate dissolved in dimethyl sulphoxide at final concentration of 0.4 µl/ml. In the presence of S9 –7,12-Dimethylbenzantracene dissolved in dimethyl sulphoxide at final concentration of 20 µg/ml.	
Administration / Exposure; Application of test substance		
3.2.5 Concentrations	1.0, 10.0, 20.0 and 30 µg/ml	

Section A6.6.3 **Annex Point IIA VI 6.6.3**	**Genotoxicity in vitro** Mutagenic Evaluation in CHO/HPRT Assay	
3.2.6 Way of application	Dissolved in dimethyl sulphoxide.	
3.2.7 Pre-incubation time		
3.2.8 Other modifications		
3.3 Examinations		
3.3.1 Number of cells evaluated	200 / 60mm dish	
	4 RESULTS AND DISCUSSION	
4.1 Genotoxicity		
4.1.1 without metabolic activation	No	
4.1.2 with metabolic activation	No	
4.2 Cytotoxicity	Yes Preliminary dose selection: 80 µg/ml without; 40 µg/ml with. Definitive study: 30 µg/ml with.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study was conducted according to OECD 476 guidelines. 10^6 cells/dish were seeded for each treatment group. The exposure period was 5 hours at 37°C in both studies, the second study, duplicating the tests from the first study. After exposure, the cells were washed twice in Ham's F12 medium and incubated in the culture medium for 19 hours. Cells were then subcultured to assess cytotoxicity and to begin the phenotypic expression period.	
5.2 Results and discussion	The mutant frequency both with and without metabolic activation was not significantly higher than that of the vehicle control. 30 µg/ml was found to be cytotoxic in the definitive study, so the determination of mutant frequency excludes this test concentration. Both studies showed dose related decreases both in relative survival to treatment and relative population growth. The mutagenic response of the positive control items indicated the validity of the test.	X2
5.3 Conclusion	The test material bromadiolone is considered to be non-mutagenic in the CHO-HPRT Forward Mutation Assay, both with and without metabolic activation. There was one deviation from the study plan, in that both study groups had in addition, controls in the absence of metabolic activation.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section A6.6.3 **Annex Point IIA VI 6.6.3**	**Genotoxicity in vitro** Mutagenic Evaluation in CHO/HPRT Assay	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	Complementary information found in DOC IV, was sufficient to accept the study. Applicants version accepted with amendments: X1 Purity is slightly higher than the 98% purity level presented in section 2. However, this should not affect the outcome of the study.	
Results and discussion	Complementary information found in DOC IV, was sufficient to accept the study. Applicants version accepted with amendments: X2 In this study the 30 µg/ml plates with S9 were not evaluated due to high levels of cytotoxicity, thereby causing the study to deviate from EU Test Method B.17 in which it is stated that at least four concentrations should be used. However, this does not invalidate the study since no increase of mutant frequency was seen the other three dose groups or in the high dose group in plates without S9 activation.. Individual plate data .is missing in DOC IV. However, since no concerns were identified when evaluating the Mean and SD values in the summary tables, this data was not considered to be needed to accept the study. Also, it is not stated if the test colonies were checked for mycoplasma, since no positive results were found in the test cultures, mycoplasma infection is not probable.	
Conclusion	Applicants version accepted	
Reliability	1	
Acceptability	Acceptable	
Remarks		

Table A6.6.3-1. Table for Gene Mutation Assay

Concentration (µg/ml)		Mutant frequency in 10^6 clonable cells		Comments *give information on cytotoxicity or other*
		— S9	+ S9	
0	S1	5.146	4.461	
	S2	3.030	5.396	
Vehicle control	S1	4.117	6.691	
	S2	5.051	6.475	
Positive Controls	S1	709.302**	677.419*	Positive controls were -S9: ethylmethane sulphonate, -S9: 7,12-dimethylbenzanthracene.
	S2	800.000*	290.149*	
1.0	S1	5.780	4.461	
	S2	1.045	5.396	
10	S1	3.822	0.000	
	S2	2.405	6.250	
20	S1	6.961	11.92	
	S2	1.367	5.5561	
30	S1	5.2400.000	-	+S9 is cytotoxic
	S2			

Section A6.6.4 **Annex Point IIA VI 6.6.4**	**Genotoxicity in vivo mutagenicity (bone marrow assay for chromosomal damage or a micronucleus test)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	There have been no positive findings seen in *in vitro* studies, hence based on animal welfare grounds and on the fact that conducting an *in-vivo* mutagenicity study will not provide any further relevant data, it is deemed scientifically unjustified to conduct this study.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification accepted	
Conclusion	Applicants justification accepted	
Remarks		

Section A6.6.5 **Annex Point IIA VI 6.6.5**	**Genotoxicity in vivo mutagenicity or evidence of DNA damage in tissue other than bone marrow**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	There have been no positive findings seen in *in vitro* studies, hence based on animal welfare grounds and on the fact that conducting an *in-vivo* mutagenicity study will not provide any further relevant data, it is deemed scientifically unjustified to conduct this study.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification accepted	
Conclusion	Applicants justification accepted	
Remarks		

Section A6.6.6 **Annex Point IIA VI 6.6.6**	**Genotoxicity in vivo (germ cell effects)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	There have been no positive findings seen in *in vitro* studies, hence based on animal welfare grounds and on the fact that conducting an *in-vivo* (germ cell effects) study will not provide any further relevant data, it is deemed scientifically unjustified to conduct this study.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification accepted	
Conclusion	Applicants justification accepted	
Remarks		

Section A6.6.7 **Annex Point IIA VI 6.6.7**	**Genotoxicity in vivo (further test if metabolites of concern are formed in mammals)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	There have been no positive findings in *in vitro* genotox studies. No metabolites of concern are noted for bromadiolone in the literature or for any other analogue, hence based on animal welfare grounds and on the fact that conducting an *in vitro* genotox study will not provide any further relevant data, it is deemed scientifically unjustified to conduct this study.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification accepted	
Conclusion	Applicants justification accepted	
Remarks		

Section A6.7 Carcinogenicity		
Section A6.7 **Annex Point IIA VI6.7**	**Carcinogenicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X] Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The compound belongs to a well-known and closely analogous group of anticoagulants with very similar properties. All studies on vertebrates show the same effects, primarily loss of blood coagulation, and these are shown clearly in acute studies. There is little species differentiation in effects or dose response, and there are no positive findings in genotox studies. To avoid acute effects, doses in repeat dose studies must be kept very low and it is considered infeasible to keep alive animals receiving any appreciable dose for more than a few months. The potential for exposure to rodenticides is limited by the nature of their use, and there is no exposure as a result of residues of the substance, or as a result of long-term exposure to vapour. A carcinogenicity study is therefore considered unjustified.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Performing long-term exposure studies is technically difficult when study highly toxic substances such as bromadiolone, since dose levels, at which toxicity is identifiable but without rendering high levels of lethality, are hard to predict. Also, no genotoxic potential has been identified for bromadiolone in *in vitro* tests of genotoxicity.	
Conclusion	Justification accepted	
Remarks		

Section A 6.8 Reproductive toxicity		
Section A6.8.1 **Annex Point IIA6.8.1**	**Teratogenicity Study** Oral developmental toxicity to the rabbit	
	1 REFERENCE	**Official use only**
Reference	XXXXX (2004) Teratology study of the test item Bromadiolone technical in rabbits. XXXXX. Report number 03/735-105N	
Data protection	Yes	
1.1.1 Data owner	Bromadiolone Task Force	
1.1.2 Companies with letter of access	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.1.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
Guideline study	OECD 414	
GLP	Yes	
Deviations	The purity of the test item is 99.4%. This will not affect the integrity of the study.	
	3 MATERIALS AND METHODS	
Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	White crystal	
3.1.2.2 Purity	99.4%	X1
3.1.2.3 Stability	Stable	
Test Animals		
3.1.3 Species	Rabbits	
3.1.4 Strain	New Zealand White	
3.1.5 Source	Ferenc Sandor breeder, H-2173 Kartal, Vörös Hadsereg út 131, Hungary	
3.1.6 Sex	Female	
3.1.7 Age/weight at study initiation	At least 4 months, young, healthy and breeding mature. Weight: 3236 – 4979 g	
3.1.8 Number of animals per group	22 per dose group	
3.1.9 Control animals	Yes	
3.1.10 Mating period	Animals were artificially inseminated - designated as first day of pregnancy – day 0 in OECD guidelines.	

Administration/ Exposure	Oral	
3.1.11 Duration of exposure	Day 7-28 inclusive post mating for 2 and 4 µg/kg bw Day 7-20 inclusive for 8 µg/kg bw as animals started to die at day 18	X2
3.1.12 Postexposure period		X3
	Oral	
3.1.13 Type	Gavage	
3.1.14 Concentration	0.5 ml/kg	
3.1.15 Vehicle	Ethanol (99.9%)	
3.1.16 Concentration in vehicle	0, 2, 4 and 8 µg/kg/day	
3.1.17 Total volume applied	11 ml/kg	X4
3.1.18 Controls	1.6% ethanol	X5
Examinations		X6
3.1.19 Body weight	Yes Recorded on gestation days 1, 4, 7, 10, 13, 14, 16, 19, 21, 22, 25, 28 and 29 to an accuracy of 1g.	
3.1.20 Food consumption	Yes Measured between gestation days 1-4, 4-7, 7-10, 10-13, 13-14, 14-16, 16-19, 19-21, 21-22, 22-25, 25-28 and 28-29 by re-weighing the non-consumed diet to an accuracy of 1g.	
3.1.21 Clinical signs	Yes Mortality, Bleeding from orifices, mucous and general activity	
3.1.22 Examination of uterine content	Yes, Intrauterine deaths, Liver foetuses, Embryo/Foetal deaths or resorptions	
3.1.23 Examination of foetuses	Yes: Bodyweight, Crown-rump length	
3.1.23.1 General		
3.1.23.2 Skeletal	Yes Abnormalities of fetuses	
3.1.23.3 Soft tissue	Yes Abnormalities	
Further remarks		
	4 RESULTS AND DISCUSSION	
Maternal toxic Effects	Compound-related clinical signs were noted from the second week of treatment period in each treated group. The incidence and severity of these signs were dose-related. Bleeding from body orifices, pale mucous membranes and reduced activity were observed. In the 4µg/kg dose group animals died from the 23rd day of gestation and in the 8µg/kg dose group the animals started to die from the 18th day of gestation.	X7

Teratogenic / embryotoxic effects	Malformations seen in 2 animals at 4µg/kg/day of bromadiolone and in 1 animal at 8µg/kg/day of bromadiolone. One malformed fetus was found in the 4µg/kg dose group. In this case exencephalia, craniorachischisis, hypoplastic mandible and ectrodactyly on all paws were observed. In the placentas anemia and tumor-like formations were found incidentally. One lobe of the placentas was smaller in every dose group which was statistically significant in the 8µg/kg dose group. Three malformed fetuses were found in the visceral examination. One from the 4µg/kg dose group with absent mesencephalon, prosencephalon and rudimentary cerebellum. An other one from this dose group had missing vitreous body and retinal fold in both eyes, however this fetus was not evaluated because the low implantation of the doe. The third implantation found was an internal hydrocephaly from the 9µg/kg dose group. Bilobed gallbladder as variation occurred in three fetuses. In skeletal examination, fetuses with 9 or less coccygeal vertebrae were evaluated as malformed. Fetuses with shorter tail were found in two treated groups (2 and 8µg/kg) and in the control.	
Other effects	None noted	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
Materials and methods	The study was conducted according to EU Test Method B.. The teratology study o f the bromadiolone technical was performed on New Zealand white rats in their first pregnancy. The females were artificially inseminated and the day of insemination was considered as the first day of pregnancy. The does were treated orally by gavage with 2 and 4 µg/kg from the 7th up to and including the 28th day of pregnancy. The does of the 8µg/kg dose group were treated from the 7th day upto and including the 20th gestation days since the animals started to die in this dose group from the 18th day of pregnancy. The test item was dissolved in ethanol and diluted by distilled water, 1.6% ethanol treated animals served as controls.	X8
Results and discussion	No significant difference was found between the control and Bromadiolone technical treated groups in respect of the body weight and corrected body gain. On the second week of the treatment period decreased boy weight gain was found in all treated groups as compared to control. No significant difference was found between the control and the bromadiolone technical treated groups for food consumption. 2 µg/kg/day: three cases of toxic symptoms observed. Treatment related gross pathological alterations were not observed. 4 µg/kg/day: Toxic symptoms observed in seven cases. Six animals died from day 23 of gestation onwards. Treatment related gross pathological alterations were observed in twelve animals. One malformed fetus was found with exencephalia, craniorachischisis, hypoplastic mandible and ectrodactyly on all paws. Another fetus had missing vitreous body and retinal fold in both eyes. 8 µg/kg/day: Toxic symptoms observed in twelve cases. Ten animals died from day 18 of gestation onwards. One case of internal hydrocephaly was observed at visceral examination	X9
Conclusion		X10

5.1.1	LO(A)EL maternal toxic effects	2 µg/kg/day of bromadiolone	X11
5.1.2	NO(A)EL maternal toxic effects	Less than 2 µg/kg/day of bromadiolone	
5.1.3	LO(A)EL embryotoxic / teratogenic effects	Malformations seen in 2 animals at 4µg/kg/day of bromadiolone and in 1 animal at 8µg/kg/day of bromadiolone	X12
5.1.4	NO(A)EL embryotoxic / teratogenic effects	8 µg/kg/day of bromadiolone	X13
5.1.5	Reliability	1	
5.1.6	Deficiencies	No	
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		April 2006/October 2007	

Materials and Methods	X1 The bromadiolone purity is slightly higher in this study compared to the 98% purity level presented in section 2. However, this should not affect the outcome of the study. X2 All does were exposed daily during the exposure period. Control animals were exposed GD (gestation day) 7- 28. The discontinued exposure of the high dose group deviates from EU Method B. 31 in which it is stated that "normally, the test substance is administered to pregnant animals at least from implantation until one day prior to the scheduled day of kill, as close as possible to the normal day of delivery without risking loss of data resulting from early delivery". Assuming the exposure disruption of the high dose group was performed to diminish the loss of data due to maternal deaths, this deviation may be considered justified. However, this deviation must be taken into account when evaluating the results from this study. X3 Autopsies were performed for all does following euthanizing on GD 29 or directly following death, if death occurred prior to the finalisation of the study. X4 Total volume given to the 8 µg/kg/day dose group was 7 ml/kg, since the exposure was discontinued. X5 The test item was dissolved in 99.9% ethanol to form a stock solution of 1mg bromadiolone / ml. The stock solution was then diluted daily with distilled water to the final volume. The highest ethanol concentration in the administered bromadiolone solutions was 1.6 %, assuming the stock solution was diluted 1:62 giving a final solution of 0.016 mg/ml, thus explaining why this ethanol level was used for the control group. X6 Section 10.4 replaced with: Clinical observations were performed daily after oral administration. Body weights and food consumption were recorded at least every third day. The does were euthanized on GD 29 and autopsied. Pregnancy rates, nr. of implantations, embryotic and fetal deaths, preimplantational losses, nr. of corpora lutea, nr. of live fetuses, fetal weight, fetal sex distribution, fetal crown-rump length and placental weight were all recorded for does euthanized on GD 29. All fetuses of does euthanized on GD 29 were also examined for external, skelettal and visceral variations and malformations. The sculls from half of the fetuses in each litter were fixed in Sanomiya mixture for Wilson sectioning. Skeletons were examined following KOH-alizarin red S staining and all variations and malformations were recorded. Fetuses of does that died prior to GD 29 were not evaluated and in the 4 µg/kg dose group only 10 of the 14 litters evaluated for fertility parameters, were also evaluated for external and internal malformations. The reason for this is not stated on study report. X8 Applicants version replaced with: This teratology study was performed using individually housed, healthy, young primiparous New Zealand White rabbits. The rabbits were artificially inseminated and the day of insemination was considered as the first day of pregnancy. The three dose groups of 2 µg/kg, 4 µg/kg and 8µg/kg and the control group, all consisted of 22 rabbits/group. The bromadiolone exposed does were given 0.5 ml/kg bromadiolone in 1.6% ethanol diluted with distilled water ,orally by gavage starting GD (gestation day) 7 until GD 28. Since the first death occurred on GD 18 in the 8 ug/kg group, the exposure in this group was discontinued on GD 20. The control animals were given 1.6% ethanol diluted in distilled water. Clinical observations were performed daily after oral administration. Body weights and food consumption were recorded at least every third day. The does were euthanized on GD 29 and autopsied. Pregnancy rates, nr. of implantations, embryotic and fetal deaths, preimplantational losses, nr. of corpora lutea, nr. of live fetuses, fetal weight, fetal sex distribution, fetal crown-rump length and placental weight were all recorded for does euthanized on GD 29. All fetuses of does euthanized on GD 29 were also examined for external, skeletal and visceral

Results and discussion	X7 Section 11 is replaced with the summary under X9 X9 Applicants version replaced with: The maternal mortality rate in this study was 19/ 88 does. One animal in the control and 2 µg/kg group respectively died due to technical reasons, six animals in the 4 µg/kg group died, all due to toxicity, starting on GD 23 and onward. 11 animals died in the 8 µg/kg group, from GD 18 and onward. One doe in this dose group died due to technical reasons. The pregnancy rate was 18/22 in the low (2 µg/kg) and the medium (4 µg/kg) dose group and 19/ 22 in the control and high (8 µg/kg) dose group. Two does in the 4 µg/kg dose group aborted. Due to the high mortality rate, the fetuses of only 14 and 9 does in the 4 µg/kg and 8 µg/kg dose groups respectively, were evaluated. Clinical signs were observed, starting on the second week of exposure. In the 2 µg/kg/day dose group three cases of bleeding around the body orifices were reported. 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, haemorraging in the kidney and bloody discharge from body orifices (or in the thorax) in both dose groups. No significant effects were reported on gravid uterine weight, body weight, body weight gain or food consumption in any dose group compared to controls. 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. Misshaped thymus and rudimentary/absent intermediate lung lobe was seen in the control and all three exposure groups, at comparable frequencies. One case of rudimentary gall bladder was also seen among controls. In the 2 µg/kg/day dose group one animal with bilobed gallbladder was reported. In the 4 µg/kg/day dose group two malformed fetus were found. One with absent mesencephalon and proencephalone, rudimentary cerebellum, craniorachischisis, hypoplastic mandible and ectrodactyly on all paws. The other malformed fetus (from another litter) lacked vitreous body and retinal fold in both eyes. This fetus was not fully evaluated, due to a low nr. of implantations of the doe. In the 8 µg/kg/day dose group one fetus was reported with internal hydrocephaly and one fetus had a bilobed gallbladder.

	In the control group skeletal malformations were reported in one fetus and comprised hemicentric, dumb-bell shaped vertebrae and vertebrae with small thoracic arches. Two control animals had 13th rib anlage and three animals hade fused sternabrae. In the 2 µg/kg/day dose group two animals with fused sternebrae were reported, three with 13th rib anlage and two animals had just 12 Cc. vertebrae. The 4 µg/kg/day dose group included one animal (the one with absent mesencephalon and proencephalone, rudimentary cerebellum, craniorachischisis, hypoplastic mandible and ectrodactylyl on all paws.) with facial skull malformations, hypoplastic mandible and fused cervical vertebrae. In this group displaced, fused or misshaped sternebrae were reported in six animals. Seven cases of 13th rib anlage were reported in this group, rendering the number of fetuses with skeletal variations significantly higher than control values. In the 8 µg/kg/day dose group one animal with 12 Cc. vertebrae, 13th rib anlage and misshaped sternebrae were seen respectively. According to EU Test Method B.31, a maternal mortality exceeding 10 % may invalidate the study. In this study 19/ 88 animals died, thus exceeding the 10% limit. However, due to the technical difficulties to identify the narrow dose interval in which rodenticide toxicity is detected but not lethal, the high mortality level is acceptable and does not constitute sufficient justification to invalidate the study. In conclusion, two fetuses with severe malformations and an increased incidence of skeletal variations were reported in the 4 µg/kg/day and one fetus with hydrocephalus was seen in the high dose group. When evaluating these results it is important to keep in mind that, since the exposure of the high dose group was discontinued, the total dose received by the 4 µg/kg/day was 88 µg/kg while the 8 µg/kg dose group received a total dose of 112 µg/kg, rendering the total dose difference between the high and middle dose group smaller than expected. Also, only the middle dose group, in which the most severe malformations were reported, was exposed during the last week of gestation.

Conclusion	X10 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. However, the possibility that the effects seen may have been due to non-specific influences such as generalised toxicity, cannot be excluded. The Commission Working Group of Specialised Experts on Reproductive Toxicity has unanimously recommended that all AVK rodenticides should collectively be regarded as human teratogens due to structural similarity to and the same mode of action as the known developmental toxicant warfarin (meeting in Ispra, September 19-20 2006). X11 The maternal LO(A)EL value of 2µg/kg/day is based on the recorded bleeding around body orifices in three animals in this dose group. X12 The LO(A)EL value of 4µg/kg/day for embryotoxic / teratogenic effects is based on the severe malformations (such as absent mesencephalon and proencephalone, rudimentary cerebellum and absent vitrous body) in 2 fetuses and the increased incidence of skeletal variations seen in this dose group. X13 Applicants version replaced with: NO(A)EL for embryotoxic / teratogenic effects of bromadiolone : 2µg/kg/day, based on the absence of dose-related teratogenic effects in this dose group.
Reliability	1
Acceptability	Acceptable
Remarks	The mean body weight gain values in table A6_8-1 are wrong, should be 368.3, 406.4, 297.3, 463.7g. In in table A6_8-3 the 13% incidence of skeletal variations in the 4 µg/kg dose group is incorrect- should be 15% and the basis of some values have not been explained properly, such as the 7% and 6% incidence of skeletal anomalies and variations in the control group.

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups) Maternal effects

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	study				
Number of dams examined		19	18	18	19	
Clinical findings during application of test substance						
Mortality of dams *state %*		0	0	27	46	
Abortions		0	0	2	0	
Body weight gain (mean) *day 0-end of test,*	-	-114.7g	-56.0g	-43.7g	3.8g	
Food consumption days 1-7 (mean)	-	200.1g	194.4g	198.4g	202.4g	
Food consumption days 1-14 (mean)	-	190.7g	185.9g	182.7g	188.0g	

Food consumption days 14-21 (mean)	-	180.1g	180.5g	184.5g	195.4g	
Food consumption days 21-29 (mean)	-	91.3g	103.5g	92.8g	107.3g	
Water consumption	-	-	-	-	-	
Pregnancies *pregnancy rate or %*		86	86	91	91	
Necropsy findings in dams dead before end of test						

Table A6_8-2. Table for teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	study				
Corpora lutea *Mean number/number of dams*		9.8/19	8.9/18	9.4/14	8.3/9	
Implantations *Mean number/number of dams*		8.8/19	7.7/18	7.8/14	7.7/9	
Resorptions *state total/number of dams*						
pre-implantation loss *state %*		9.8	13.4	18.9	8.4	
post-implantation loss *state %*		1.3	0	11.6	1.2	
total number of litters		19	19	20	20	
fetuses		165	138	106	69	
live fetuses		165	138	98	68	
dead fetuses		0	0	8	1	
fetus weight (mean) *[g]*		37.4	39.8	36.7	38.8	
placenta weight (mean) *[g]*		6.7	7.0	6.2	7.3	
crown-rump length (mean) *[mm]*		9.6	9.8	9.4	9.8	
Fetal sex ratio *[state ratio m/f]*		79/86	59/79	44/53	34/34	

Table A6_8-3. Table for Teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Modify if necessary and give historical data if available

Parameter	control data		low dose	Medium dose	high dose	dose-response + / -
	historical	study				
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 variants* *[%]*		6	3	13	3	
Visceral malformations* *[%]*		0	0	1	1	
Visceral anomalies* *[%]*		1	1	1	3	
Variants visceral* *[%]*		1	1	0	1	

Section A6.8.1 **Annex Point IIA VI.6.8.1**	**Teratogenicity Study - Rat**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Rat multigeneration reproduction study has been submitted, Bromadiolone technical caused no adverse effect at dose levels of 1 µg/kg/day, 2,5 µg/kg/day and 5 µg/kg/day in CRL(WI) BR rats in this two generation reproduction toxicity study. Reproductive performance of males and females were unaffected by the treatment with bromadiolone technical both in P and F1 generations. There was no effect on postnatal development of pups either in F1 or in F2 generations. Rabbit teratology study has also been submitted which showed that body weight, body weight gain and food consumption in all groups were not significantly different from the control group. 2 µg/kg/day: three cases of toxic symptoms observed. Treatment related gross pathological alterations were not observed. 4 µg/kg/day: Toxic symptoms observed in seven cases. Six animals died from day 23 of gestation onwards. Treatment related gross pathological alterations were observed in twelve animals. One malformed fetus was found with exencephalia, craniorachischisis, hypoplastic mandible and ectrodactyly on all paws. Another fetus had missing vitreous body and retinal fold in both eyes. 8 µg/kg/day: Toxic symptoms observed in twelve cases. Ten animals died from day 18 of gestation onwards. One case of internal hydrocephaly was observed at visceral examination Data from teratology studies in rats with the analogues brodifacoum and difenacoum are also available (Report: BRODIFACOUM: Development toxicity to the rabbit. K Morris – July 1995. MRS Inc. report MLS/10019 and DIFENACOUM: Development toxicity to the rat. K Morris – June 1995. MRS Inc. report MLS/10013) It is therefore considered that adequate data already exists to answer this point and that a further rat teratology study can not be justified and would be a waste of experimental animals	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006/October 2007	

Section A6.8.1 Annex Point IIA VI.6.8.1	Teratogenicity Study - Rat	
Evaluation of applicant's justification	The Commission Working Group of Specialised Experts on Reproductive Toxicity has unanimously recommended that all AVK rodenticides should collectively be regarded as human teratogens due to structural similarity to and the same mode of action as the known developmental toxicant warfarin (meeting in Ispra, September 19-20 2006). A new study on rats is therefore considered an unjustifiable waste of experimental animals.	
Conclusion	Justification accepted	
Remarks		

Section A6.8.2 **Annex Point IIA VI 6.8.2**	**Multigeneration Reproduction Toxicity Study** Two-generation reproduction Toxicity Study in Rats	
	1 REFERENCE	**Official use only**
1.1 Reference	XXXXX (2004) Two generation reproduction toxicity study of test item bromadiolone technical in rats. XXXXX. Report 03/735-202PR	
1.2 Data protection	Yes	
1.2.1 Data owner	Bromadiolone Task Force	
1.2.2 Companies with letter of access	PelGar International Ltd, Babolna Bioenvironmental Centre Ltd Activa s.r.l. Laboratories Agrochem S.L.	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD 416	
2.2 GLP	Yes	
2.3 Deviations	The purity of the test item is 99.4%. This will not affect the integrity of the study.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	02473	
3.1.2 Specification	As given in section 2	
3.1.2.1 Description	White crystal	
3.1.2.2 Purity	99.4%	
3.1.2.3 Stability	Stable	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	CRL: (WI) rats	
3.2.3 Source	Charles River (EUROPE) Laboratories Inc	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Male approximately 6 weeks – 227g mean weight Females approximately 8 weeks – 248g mean weight	X1
3.2.6 Number of animals per group	25 rats/sex/group	
3.2.7 Mating	See table below	
3.2.8 Duration of mating	2 weeks	
3.2.9 Deviations from standard protocol	None	
3.2.10 Control animals	Yes	

Section A6.8.2 **Annex Point IIA VI 6.8.2**	**Multigeneration Reproduction Toxicity Study** Two-generation reproduction Toxicity Study in Rats	
Administration/ Exposure	Oral	
3.2.11 Animal assignment to dosage groups	See table below	
3.2.12 Duration of exposure before mating	10 weeks	
3.2.13 Duration of exposure in general P, F1, F2 males, females	Daily dosing of the Parent (P) males began 10 weeks prior to the mating period, throughout 2 weeks mating period until termination. Dosing of the F1 male animals selected for mating began at weaning and it was continued (throughout mating period) until termination. Daily dosing of the parent (P) females began 10 weeks prior to the mating period, it was continued during the mating period, throughout pregnancy and up to the weaning of the F1 offspring (until termination on postpartal day 29) Dosing of the F1 female animals selected for mating began at weaning (postnatal day 29), and it was continued during the mating period, throughout pregnancy and up to the weaning of the F2 offspring (until termination on postpartal day 22). The animals were treated once daily, at similar times each day, in the morning. Animals were not treated on the day of termination.	
	Oral	
3.2.14 Type	Gavage	
3.2.15 Concentration	0.5 µg/ml for low dose and intermediate groups and 1.0 µg/ml for high dose groups	
3.2.16 Vehicle	Distilled water with ethanol – 0.1%	
3.2.17 Concentration in vehicle	1, 2.5 and 5 µg/kg/day	
3.2.18 Total volume applied	Females (P) 226 ml/kg (low dose) and 565ml/kg (medium and high dose) maximum (depending of mating period) Males (P) 168 ml/kg (low dose) and 420ml/kg (medium and high dose)	X2
3.2.19 Controls	Vehicle	
3.3 Examinations		
3.3.1 Clinical signs	Clinical signs were observed twice daily in treated and control animals during the study.	
3.3.2 Body weight	P – (Male and female) once per week prior to and during mating P – (Female) gestation days 1, 8, 15 and 21 and on postnatal days 1, 5, 8, 15, 22, 29 and then weekly. F1 – (Male) once per week prior to and during mating, on postnatal days 1, 8, 15, 22 and 29 and then weekly. F1 – (female)postnatal days 1, 5, 8, 15, 22, 29 and then once a week prior to and during mating, on gestation days 1, 8, 15 and 21 and on postpartal days 1, 5, 8, 15 and 22.	
3.3.3 Food/water consumption	Mean daily food consumption calculated weekly	
3.3.4 Oestrus cycle	Smears prepared daily during premating period and during mating period.	

Section A6.8.2 **Annex Point IIA VI 6.8.2**	**Multigeneration Reproduction Toxicity Study** Two-generation reproduction Toxicity Study in Rats	
3.3.5 Sperm parameters	Total number of cells Sperm with separated head and tail sperm motility sperm morphology	X3
3.3.6 Offspring	Mortality, bodyweight at postnatal days, 1, 5, 8, 15, 22 and 29, Offspring development: water maze performance, Selected offspring: sexual maturation	
3.3.7 Organ weights P and F1 Adults	brain liver spleen kidneys pituitary testes epididymides prostate seminal vesicles adrenals thyroids uterus ovary	
3.3.8 Histopathology P and F1 Adults	Liver Kidneys Uterus with cervix ovaries testis epididymis seminal vesicle prostate with coagulating gland	X4
3.3.9 Histopathology F1 not selected for mating, F2, Offspring	Liver Kidneys Spleen Thymus	
3.4 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Effects		
4.1.1 Parent males	Reproductive performance, organ weights, body weight and sex ratio of pups unaffected.	
4.1.2 Parent females	Reproductive performance, organ weights, body weight and sex ratio of pups unaffected.	
4.1.3 F1 males	No effect to postnatal development. Reproductive performance unaffected. Mortality was low and non dose related	
4.1.4 F1 females	No effect to postnatal development. Reproductive performance unaffected. Mortality was low and non dose related.	
4.1.5 F2 males	No gross pathologic , organ weight and histopathologic alterations. Mortality was low and non dose related.	

Section A6.8.2 **Annex Point IIA VI 6.8.2**	**Multigeneration Reproduction Toxicity Study** Two-generation reproduction Toxicity Study in Rats	
4.1.6 F2 females	No gross pathologic , organ weight and histopathologic alterations. Mortality was low and non dose related.	
4.2 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study was conducted according to OECD 416. Twenty five CRL☹WI)BR rats/sex/group were involved in the study in a control and at three dose levels: 1, 2.5 and 5 µg/kg treatment was carried out orally in a 2ml/1000g bw (1µg/kg) and in a 5ml/ 1000g bw (Control, 2.5µg/kg and 5µg/kg) once a day. All animals of the Parent generation and animals of the F1 generation selected for mating were treated at least fro 10 weeks prior to mating, throughout 2 weeks mating, gestation and lactation periods up to the autopsy. Treatment of F1 animals started after weaning, on postnatal day 29. Observations included mortality, clinical symptoms, body weight, food consumption, gross necropsy, organ weight measurement, sperm analysis and histopathological examinations. The dams were allowed to litter, and rear their youngs up to weaning on day 29 postpartum. Developmental tests were evaluated on litters (surface righting reflex, suckling, pinna detachment, eye opening, testicular descent, vaginal opening and water maze performance). On postnatal day 29, 1-2 males and 1-2 females per litter were selected for subsequent evaluation of reproductive performance following pairing in F1 generation and the observation of the F2 generation up to postnatal day 22. One randomly selected pup/sex/litter from both the F1 and F2 generations was subjected to gross pathology, histopathology and organ weight (brain, thymus, spleen, liver and kidneys) measurement.	X5
5.2 Results and discussion	Clinical symptoms related to the test item effect were found. One contol female animals died on treatment day 81 (gestational day 8). Decreased activity, piloerection, hunched back, paleness and dyspnoea were observed fro three days before the death. Gross pathology revealed edema and reddish mottled colour in the lungs and nut-meg like patterned liver. Histologically focal necrosis in the liver, catharral pneumonia and efema in the lungs were observed as the cause of death. The body weight, body weight gain and food consumption of both sexes of the P and F1 generations were unaffected at the examined dose levels during the premating period. There was no effect on body weight, body weight gain and food consumption of dams during gestation and lactation at the examined dose levels either in P and F1 generations. Bromadiolone did not influence on the estrus. There were no test item related alterations in the delivery data of dams when compared with the control value either in P or F1 generation. Gestation length was comparable in all groups. Reproductive performance of males and females were unaffected by bromadiolone. The Prothrombin time was similar in the control and 5µg/kg groups. Mortality of pups was low both in the F1 and F2 generation without any dose relevance. There was not effect on postnatal development of pups of either in F1 or F2 generations. No gross pathologic, organ weight and histopathological alterations related to the test item effect were found in pups either in F1 or F2 generations.	X6
5.3 Conclusion		X7
5.3.1 LO(A)EL		

Section A6.8.2 **Annex Point IIA VI 6.8.2**	**Multigeneration Reproduction Toxicity Study** Two-generation reproduction Toxicity Study in Rats	
5.3.1.1 Parent males	>5 µg/kg/day	
5.3.1.2 Parent females	>5 µg/kg/day	
5.3.1.3 F1 males	>5 µg/kg/day	
5.3.1.4 F1 females	>5 µg/kg/day	
5.3.1.5 F2 males	>5 µg/kg/day	
5.3.1.6 F2 females	>5 µg/kg/day	
5.3.2 NO(A)EL		
5.3.2.1 Parent males	5 µg/kg/day	
5.3.2.2 Parent females	5 µg/kg/day	
5.3.2.3 F1 males	5 µg/kg/day	
5.3.2.4 F1 females	5 µg/kg/day	
5.3.2.5 F2 males	5 µg/kg/day	
5.3.2.6 F2 females	5 µg/kg/day	
5.3.3 Reliability	1	
5.3.4 Deficiencies	No	

Section A6.8.2 **Annex Point IIA VI 6.8.2**	**Multigeneration Reproduction Toxicity Study** Two-generation reproduction Toxicity Study in Rats	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	Applicants version accepted with amendments: X1 The body weight range for each sex would be useful, but are not obligatory. Values in document IV show males ranging from 204- 243 g and females :213-269 g. The initial weight variations were thus within a 20% range limit. X2 Dosage volume 0.5 ml/100g/day in the intermediate (2.5 µg/kg), high dose group (5 µg/kg) and control group but 0.2 ml/100g/day in the lowest dose group (1 µg/kg). X3 It should be specified if both testicular spermatids and cauda epididymal sperm have been enumerated. X4 No histopathology was performed on vagina the of parent (P) or F1 (used for mating). X5 Dosing of P females occurred until PND 29 and F1 females were dosed until PND 22. Reproductive parameters (such as fertility index, nr. of pregnants, live birth index etc), estrus cycle parameters and haematological analysis were also recorded.	
Results and discussion	X6 Applicants version replaced with: No clinical symptoms related to the exposure to bromadiolone were observed in P, F1 or F2 animals. One control P animal died on treatment day 81 (gestation day 8). Gross pathology revealed oedema and reddish mottled color in the lungs and nut-meg like patterned liver. Histologically, focal necrosis in the liver, catharral pneumonia and oedema in the lungs, were observed and diagnosed as the cause of death. The mean body weight in males of the intermediate dose group (2.5µg/kg) was slightly lower than in the controls during day 43- 71 of the premating period. Also, food consumption was significantly lower in males in the 1 µg/kg and 2.5 µg/kg dose groups compared to controls on day 43 – 71 of the premating period. None of these findings were seen in the highest dose group, indicating that these effects are not dose- related to the bromadiolone exposure. In P animals in the highest dose group (5µg/kg) a significantly lower mean bw gain was seen during day 22- 29 of the lactation period. This decrease in bw gain was also seen in F1 parents in the high dose (5 µg/kg) and intermediate (2.5µg/kg) dose group on day 15- 22 of the lactation period. The decreased body weight gain in the P parents was not compensated by the increased food consumption, seen among P parents in the highest dose group compared to controls on lactation day 8- 15. In the P parents this may be explained by a greater milk production in the highest dose group compared to controls, due to the fact that the mean litter size in the highest dose group was slightly higher than in controls (mean nr. of total births in the 5 µg/kg dose group being 14.3 compared to 13.8 in controls). Litter standardisation was not performed in this study. None of the above described minor deviations of body weight gain compared to controls were dose related. F1 parents in all three dose groups had a significantly longer estrus period compared to controls. F1 parents in the low (1 µg/kg) and high (5 µg/kg) dose group had a significantly shorter diestrus period compared to controls. However, none of these deviations were dose related. No effects on estrus cycle were seen in the P generation. The number of dams that delivered offspring among F1 parents was significantly lower in the intermediate and high dose group compared to controls. However, since the pregnancy rate in the F1 generation was relatively low in exposure groups and controls (7- 13 pregnancies per dose group following mating of 25	

Section A6.8.2 Annex Point IIA VI 6.8.2	Multigeneration Reproduction Toxicity Study Two-generation reproduction Toxicity Study in Rats	
	pairs), even small deviations from the control values were statistically significant. This deviation is therefore not of biological significance. In the P generation the number of dams delivered was significantly lower only in the 2.5 µg/kg dose group compared to controls, and was therefore not dose related to the exposure to Bromadiolone. Sperm analysis in F1 parents showed significantly lower mean nr. of cells in the highest dose group (5µg/kg) compared to controls. This effect was not seen in the P parents. No other effects were seen on sperm morphology or motility, indicating that the decreased sperm count was not of biological significance. The PTT (Activated Partial Thromboplastin Time) in P males of the highest dose group (5µg/kg) was significantly longer compared to controls. No effects were seen on the PT (Prothrombine Time) of the P males in the highest dose group (5µg/kg). Since PT tests deficiencies of K-vitamin dependent coagulation factors, the normal PT values indicate that the K-vitamin dependent branch of homeostasis was not affected by the bromadiolone exposure. No effects on haematological analysis were seen in F1 parent males, further supporting the conclusion that the prolonged PTT was not of biological significance. F1 offspring in all three dose groups showed significantly fewer positive responses of pinna detachment pnd 3 compared to controls. No dose related effects on pinna detachment were seen in the F2 generation and no other dose related developmental effects were seen in F1 offspring, indicating that this finding is not of biological significance. In F2 offspring of the 1 µg/kg and 5 µg/kg dose group, a significantly lower incidence of positive response to surface righting on pnd 3 was seen compared to controls. The lower incidence of positive response was not dose related. Liver weight referring to bw and brain weight, was significantly lower in P parents and F1 offspring in the highest dose group compared to controls. This was not seen in F2 offspring, no other effects on organ weights were seen and the deviations were minor, indicating the absence of biological significance. Although, a number of minor deviations from control values have been identified and described above, none of these findings are considered to be caused by the exposure to bromadiolone. Thus, exposing rats to the levels of bromadiolone given in this study did not cause any measurable effects.	
Conclusion	X7 Applicants version replaced by: 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.	
Reliability	3	
Acceptability	Not acceptable for risk assessment, can be used as complementary information. However, it has been agreed in technical discussions that a 2-generation reproduction study can be waived based on technical difficulties and low exposure.	
Remarks	Table A6_8_2-2 is incomplete	

Table A6.8.2-1 Table for animal assignment for mating (modify as appropriate)

		Number of animals			
		Controls	**Low Dose**	**Medium Dose**	**High Dose**
Parents	**m**	25	25	25	25
	f	25	25	25	25
F_1	**m**	1 or 2 per litter	1 or 2 per litter	1 or 2 per litter	1 or 2 per litter
	F	1 or 2 per litter	1 or 2 per litter	1 or 2 per litter	1 or 2 per litter

Table A6.8.2-2. Table for reproductive toxicity study (modify if appropriate)

If effects are found in one generation, the figures for the other generation(s) should be given as well (as shown as an example for mortality). Give only information on endpoints with effects, delete other endpoints.

Parameter			control		low dose		medium dose		High dose			
		Genera-tion	m	f	m	f	m	f	m	f	m	f
Mortality	incidence	**P**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**		
		F_1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**		
		F_2	**3**	**7**	**7**	**6**	**2**	**5**	**2**	**5**		
Food consumption	% of control		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		
Body weight gain	% of control		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		
Clinical Observations *specify effects*	Incidence											
Organ weights	% of control		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		
Pathology												
Histopathologic examination *specify effects*	Incidence											
Reproductive Performance												
Mating index			**96**	**96**	**92**	**92**	**100**	**100**	**96**	**96**		
Fertility index			**92**	**92**	**78**	**78**	**88**	**88**	**96**	**96**		
Number of implantation sites	Mean											
Duration of pregnancy	Mean			**22**		**22**		**22**		**22**		
Birth index												
Live birth index				**97**		**100**		**100**		**100**		
Gestation index				**100**		**100**		**91**		**100**		
Litter size	Mean		**14**		**13**		**14**		**14**			
Litter weight	Mean		**95**		**84**		**93**		**97**			
Pup weight	Mean		**7.0**		**6.7**		**6.8**		**6.8**			
Sex ratio	Male/female		**146**	**150**	**107**	**118**	**141**	**130**	**167**	**162**		
Survival index												
Viability index			**97**		**99**		**98**		**98**			
Lactation index				**98**		**100**		**96**		**96**		
Sperm characterization												
Number	% of control		**100**						**106**			
Deformations	% of control		**100**						**80**			

Section A6.9 Neurotoxicity		
Section A6.9	**Neurotoxicity study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	There has been no evidence of neurotoxic effects in any studies conducted. Consideration of the chemical structure does not suggest that it would induce neurotoxic effects, such as an organophosphate. Hence, conducting 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.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification accepted	
Conclusion	Applicants justification accepted	
Remarks		

Section A6.10 Mechanistic study		
Section A6.10	**Mechanistic study – any studies necessary to clarify effects seen in toxicity studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The effects of administration of anticoagulants have been extensively investigated and summaries above and reported under metabolism data Please refer to section 6.2.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Anti-vitamin K rodenticides, such as bromadiolone, have a well known mechanism of action causing inhibition of the blood coagulation cascade. No indications of this mechanism being species specific have been identified.	
Conclusion	Justification accepted	
Remarks		

Section A6.11 Studies on other routes of administration (parental routes)		
Section A6.11	**Studies on other routes of administration (parental routes)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Compound is highly toxic by oral exposure. It is a large, lipophilic molecule which is poorly absorbed through the skin. It is of low water solubility and very low vapour pressure. The mode of action is common to all mammals and is well understood as a vitamin K antagonist, without secondary effects. It is only used as baits for the control of rodents. Manufacturing takes place in closed or controlled environments with full protective clothing and use as a rodenticide necessarily involves wearing gloves, overalls and other protective clothing because of the biological hazards involved and associated hygiene requirements. Data on other routes of administration are considered an unjustifiable waste of experimental animals since the compound is shown to be highly toxic by the oral route and other routes of administration are not relevant to the current and proposed uses of the compound.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Data on other routes of administration are considered an unjustifiable waste of experimental animals since the compound is shown to be highly toxic by the oral route and other routes of administration are not relevant to the current and proposed uses of the compound. Also, most rodenticide intoxication in humans occur following oral exposure to rodenticides, not via parental exposure.	
Conclusion	Justification accepted	
Remarks		

Section A6.12 Medical data in anonymous form		
Section A6.12.1 **Annex Point IIA VI.6.9.1**	**Medical surveillance data on manufacturing plant personnel <u>if available</u>**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	It is to take in account that the active involved is an anticoagulant: it gains this property when chemical is completed coupling intermediate with 4-hydroxycoumarin. It is to be clear that only at this point the chemical begin an anticoagulant. In addition the active is used only to prepare anticoagulant solutions at 2,5% or other percentage. All staff, composed by 7 operators, is followed from 1975 by a doctor specialised in "hygiene and preventive medicine" and "work medicine". At beginning in 1975, staff was controlled each 3 months with haematochimical and urine examen. After a period of ten years in 1985, since no kind of problems rise and all processes were well secured, medical surveillance was changed with: - six-monthly medical visit made by the competent doctor, - spyrometric annual control, - six-monthly haematochimical and urine examen. In 1995 another change was made: haematochimical and urine examen began annual. All surveillance plans is made by the upper doctor who inspect also the production facilities with some surprise visit during working. All upper results control are communicated to local authorities each year. No accidents occur from 1975 till today: this can demonstrate process safety and operator medical surveillance (This information is from Section A2.10). These records are covered by personnel privacy provisions.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2007	
Evaluation of applicant's justification	The justification provide some information even if it is scarce. However, this data should be provided <u>if available</u> and therefore no further information will be requested.	
Conclusion	Justification acceptable	

Section A6.12 Medical data in anonymous form		
Section A6.12.1 **Annex Point IIA VI.6.9.1**	**Medical surveillance data on manufacturing plant personnel <u>if available</u>**	
Remarks		

Section A6.12.2 (1) **Annex Point IIA VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents <u>if available</u>** *Human – acute bromadiolone intoxication*	
	1 REFERENCE	**Official use only**
1.1 Reference	Grobosch T, et al Journal of Analytical Toxicology, Vol.30, May 2006	
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Public domain	
1.2.2 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Bromadiolone	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in the published paper	
3.1.3 Description	*Not described*	
3.1.4 Purity	Not stated in the published paper	
3.1.5 Stability	A specific statement on stability is not provided within the paper.	
3.1.6 Radio labelling	No	
3.2 Test Animals		
3.2.1 Species	Human	
3.2.2 Strain	Not applicable	
3.2.3 Source	Accident victim	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	55 years	
3.2.6 Number of animals per group	1	
3.2.7 Control animals	No	
3.3 Administration/ Exposure	Oral	
3.3.1 Preparation of test site	Not applicable	
3.3.2 Concentration of test substance	Unknown	
3.3.3 Specific activity of	Not relevant	

Section A6.12.2 (1) **Annex Point IIA VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents <u>if available</u>** *Human – acute bromadiolone intoxication*	
test substance		
3.3.4 Volume applied	Not stated	
3.3.5 Sampling time	Specific sampling times not stated. However, the report does state that 7 serum samples were taken over the course of 500 hours (see figure 5 in lit. paper)	
3.3.6 Samples	Blood serum	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	Maximum concentration of bromadiolone was 440µg/l. The measurement of several samples allowed the determination of the half-life of bromadiolone in the patient, which was 140 h. Upon the consequent treatment with vitamin K_1 (and clotting factors Beriplex), the patient left the hospital after 22 days without any signs of serious bleeding during the time of hospitalisation.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Serum sample was analysed using an LC-MS method after liquid-liquid extraction (1-chlorobutane, buffer pH 4.2). In addition, systematic toxicological analysis was performed.. All the chemicals, reagents and solvents were of analytical grade. For serum sample preparation, a total of 0.5ml serum, 0.1ml potassium buffer (pH 4.2) and 0.4ml extraction reagent were mixed in a 1.5ml eppendorf cup for 2 minutes. The sample was centrifuged for 2 minutes at 15,000g and 0.2ml of the organic phase was evaporated to dryness under a stream of nitrogen at 30°C. The residue was redissolved in 120µl of methanol. For quantitation, serum was spiked at six concentrations of each analyte. All calibration samples were stored frozen at -18°C until analysis. Quantitation followed the internal standard method. Precision and accuracy for each determined compound was measured using in-house quality control samples. LC parameters: The HPLC was equipped with a binary pump, a system controller, a solvent degasser, an autosampler, an oven, and a UV-detector. An atlantis C18 (2.1 x 20 mm, 3µm, Waters) analytical column was used. The oven temperature was 40°C. The mobile phase consisted of methanol and a mixture of methanol/0.1% formaic acid (10:90, v/v) pumped at a flow rate of 0.6ml/min. The following gradient was used : 0-0.1min, 95%; 0.7-1.1 min, 50% B linear; 1.1-3.2 min, 6% B linear; 3.2-3.8 min, 6% B; and 3.8-4.2 min, 95% linear. Injection volume was 50µl. MS parameters: The MS (MS-2010-System) was obtained from Shimadzu. The ESI source was operated with a spray voltage of 4.5kV. Nitrogen was used at a flow-rate of 4.5 l.min. Block and curved dissolution line-temperature were set at 300°C. All other settings were default from the standard tuning. The MS apparatus was operated in the positive and negative-ion detection mode with a focus on the masses.	
5.2 Results and discussion	The report presents a novel screening method for the simultaneous	

Section A6.12.2 (1) **Annex Point IIA VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents <u>if available</u>** *Human – acute bromadiolone intoxication*	
	identification and quantification of 10 vitamin K antagonists by LC-MS. The chosen conditions for the analysis allowed a fast elution of the substances within 5 minutes. The general conditions corresponded to the LC-MS standard configuration in the test laboratory; thus no extra time for set up was needed.	
5.3 Conclusion	Maximum concentration of bromadiolone was 440µg/l. The measurement of several samples allowed the determination of the half-life of bromadiolone in the patient, which was 140 h. Upon the consequent treatment with vitamin K, the patient left the hospital after 22 days without any signs of serious bleeding during the time of hospitalisation. The report presents a novel screening method for the simultaneous identification and quantification of 10 vitamin K antagonists by LC-MS. The chosen conditions for the analysis allowed a fast elution of the substances within 5 minutes. The general conditions corresponded to the LC-MS standard configuration in the test laboratory; thus no extra time for set up was needed.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Not applicable, since literature report.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2008	
Materials and Methods		
Results and discussion	The patient came to the hospital with a bleeding wound on the tongue. The coating of his tongue was green and his sputum was red. He had an increased international normalized ratio-value.	
Conclusion	This study gives some information on a clinical case, but mostly on an analytic method. More information is provided in the justification below (6.12.2 (2)). The article also give reference to some other clinical cases; E.Y.Chow, L.P. Haley, L.M. Vickars and M.J. Murphy. A case of bromadiolone (superwarfarin) ingestion. Can. Med. Assoc. J. 147:60-62 (1992). M.C. Greef, O. Mashile and L.G. MacDougall. Superwarfarin (bromadiolone) poisoning in two children resulting in prolonged anticoagulation. Lancet 28:1269 (1987).	
Reliability		
Acceptability	Acceptable together with the information in 6.12.2 (2)	
Remarks		

Section A6.12.2 (2) **Annex Point IIA VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents <u>if available</u>**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Please refer to report Bromadiolone / T13, Information about and toxicity of anticoagulant rat poisons: Case Histories from the Milan Poisons Centre 1996-1999. Summary for Milan Poisons Centre Anticoagulant poisoning alters the mechanisms, which regulate blood coagulation, interfering with hepatic biosynthesis of prothrombin and factors VII, IX and X, which are responsible for the coagulative chain. Bromadiolone is one of a group of second-generation anticoagulants, which are more dangerous to man and non-target species and should be used with extreme care. (Children of 10kg might develop clinical symptoms with an ingested dose of 1.5mg/kg, corresponding to 30g of commercial bait with an active ingredient of 0.005%, with effects lasting up to four weeks.) X1 The recommended therapy is the prevention of absorption and is dependant on the quantity ingested and the time since ingestion. If a few grains have been ingested powdered activated charcoal is sufficient. More significant quantities will require ipecac syrup as an emetic. The methods of emesis, gastric lavage and powdered activated charcoal for indications/contraindications are explained fully. The recommended antidote therapy for extended prothrombin time is Vitamin K1. X2 Anticoagulant intoxication accounts for 7% of poisoning by controlled substances. The total number of bromadiolone related calls during the period of the paper were 115 of which 85% were clinical cases involving humans and animals. 78% of calls came from hospitals of which 87% were from the ingestion route. The majority of incidents happen in the domestic environment (86%) of which 79% are accidental with children under the age of 4 years accounting for 55% of incidents with the most effected gender being male. The majority of cases in both humans and animals only exhibited gastroenteric symptoms such as nausea and vomiting, with only 1 animal and 1 human exhibiting reduced coagulation. The time span after ingestion with humans is such that gastroenteric decontamination is the most useful method and follow up data confirmed that this was the case. With humans Vitamin K is only administered if the prothrombin times are altered. During the period investigated there were no recorded deaths to humans. With animals the symptoms are usually seen before treatment commenced, hence vitamin K is used, but due to the time delay not always successful. X3	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	

Section A6.12.2 (2) **Annex Point IIA VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents <u>if available</u>**	
Evaluation of applicant's justification	Justification included adequate information to fulfill the requirements of this endpoint and is therefore accepted. X1 More should be deleted X2 Vitamin K1 phytonadione should be administered intramuscularly if ingestion is of medium entity and intraveniously diluted in physiological solution or 5% glucose, slowly (maximum 5% total dose per minute) so as to avoid complications such as rash, cyanosis, dizziness, hypotension and broncoconstriction. Once vitamin K1 supplementation is sufficient to restore prothrombine levels, fresh plasma or coagulations factors should be given to restore hemostasis. X3 Among the 98 clinical cases of bromadiolone intoxication, the symtoms seen were documented in 11 humans and one animal. Among the 11 human cases 10 showed signs of vomiting, gastric complications, pyrosis or itching. One human showed haematological problems and one dog dog showed bleeding of the mucous membranes.	
Remarks		

Section A6.12.3 **Annex Point IIA VI.6.9.3**	**Human Case Report** Health records from industry and other sources.	
	1 REFERENCE	**Official use only**
1.1 Reference	Davanzo F et al. (2001) Bromadiolone: Information about and toxicity of anticoagulant rat poisons: Case Histories from Milan Poisons Centre 1996-1999. l Servizo Di Anestrsia E Rianimazione Centro Antiveleni	
	2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
	3 MATERIALS AND METHODS	
3.1 Substance	Product is in a waxy block form and has a active ingredient purity of 2.5%.	
3.2 Persons exposed		
3.2.1 Sex	Both sexes were exposed but the gender most exposed was male.	
3.2.2 Age/weight	60% of cases were children aged 0 to 4 years old.	X1
3.2.3 Known Diseases	Not stated	
3.2.4 Number of persons	115 calls made to Milan Poisons Centre regarding clinical cases involving humans and animals with Bromadiolone. 98 clinical cases involving humans or animals.	
3.2.5 Other information		
3.3 Exposure	Oral/Inhalation/Dermal The contact routes were 87% by ingestion, 0% by inhalation, 13% by other routes.	
3.3.1 Reason of exposure	The circumstances of the intoxication were in most cases accidental (79%) but there were reports of voluntary intoxication (12%). Analysing the details of these circumstances, the most frequent cause was the incapacity to rationalise, i.e. by children in the age range 0-4 years.	
3.3.2 Frequency of exposure	Not stated	
3.3.3 Overall time period of exposure	Not stated	
3.3.4 Duration of single exposure	Not stated	
3.3.5 Exposure concentration/dose	measured, estimated, not available	X2
3.3.6 Other information		

Section A6.12.3 **Annex Point IIA VI.6.9.3**	**Human Case Report** Health records from industry and other sources.	
3.4 Examinations	The risk assessment at the time of the telephone consultation is based on the active ingredient, on the case history data of the human or animal involved, on the contact mode, on the circumstances and on the symptoms found.	
3.5 Treatment	In attempted suicides which showed altered coagulation, vitamin K therapy over several weeks was found beneficial. Therapy is based on the prevention of absorption. If a few grains of bait have been ingested it is sufficient to adminsister powdered activated charcoal. If more significant quantities have been ingested, it is useful to administer ipecac syrup as an emetic. Gastric Lavage Gastric lavage consists of the removal of gastric contents using a probe which is either swallowed by the patient or delivered to the stomach passing through the nasal channels. It is useful in the ingestion of significant quantities of product, especially with attempted suicide in adults.	X3
3.6 Remarks		
	4 RESULTS	
4.1 Clinical Signs	Coagulopathy develops after intoxication by bromadiolone. Haemorrhaging is the most common symptom of intoxication and may appear some time after exposure. Neurological effects Intracranial haemorrhage with cephalea Reduced state of awareness Convulsions Coma followed by death (Ornstein & al 1999) Gastroneteric symptomatlogy abdominal pains spontaneous vomitting gastroenteric bleeding with melanotic or haemorrhagic stools Genitoutinary symptomatology haematuria vaginal bleeding	X4
4.2 Results of examinations	Not stated	
4.3 Effectivity of medical treatment		
4.4 Outcome		
4.5 Other		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The paper presents the experience of the anticoagulant intoxication requiring consulting from the Milan Poisons Centre from the Milan Poison Centre from 1996 to 1998.	

Section A6.12.3 **Annex Point IIA VI.6.9.3**	**Human Case Report** Health records from industry and other sources.	
5.2 Results and discussion	80 calls regarding bromadiolone were received between 1996 to 1998. The gender most affected was male.	
5.3 Conclusion	Clinicla effects appear when there is a massive overdose with rapid and persistent diminution of the prothrombin activity associated or otherwise with the presence of haemorrhagic diathesis. Children in the age range of 0-4 years were the most likely to be intoxicated. Monitoring of INR or the prothrombin time must be effected at 24-48 hours after ingestion, in asymptomatic children. Treatment will involve the prevention of absorption but the method of prevention will depend on the amount ingested and how long after ingestion treatment is being received. Emesis is more efficient the earlier it is effected. Vitamin K1 phytonadione, is the antidote of choice which must be administered on to patients with extended PT or INR and must be administered via intramuscular injection if ingestion is of medium entity.	X5
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	Applicants version accepted with amendments: X1 60 % should be changed to 55% X2 Information not included in report X3 The recommended antidote therapy when extended prothrombin times are seen, is Vitamin K1. Vitamin K1 phytonadione should be administered intramuscularly if ingestion is of medium entity and intraveniously diluted in physiological solution or 5% glucose, slowly (maximum 5% total dose per minute) so as to avoid complications such as rash, cyanosis, dizziness, hypotension and broncoconstriction. Once vitamin K1 supplementation is sufficient to restore prothrombine levels, fresh plasma or coagulations factors should be given to restore hemostasis.	
Results and discussion	Section replaced with: X4 Among the 98 clinical cases of bromadiolone intoxication, the symtoms seen were documented in 11 humans and one animal. Among the 11 human cases 10 showed signs of vomiting, gastric complications, pyrosis or itching. One human showed haematological problems and one dog showed bleeding of the mucous membranes.	
Conclusion	Applicants version replaced with: X5 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 4 years were exposed. The symtoms were reported in 11 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. The information is acceptable.	

Section A6.12.3 **Annex Point IIA VI.6.9.3**	**Human Case Report** Health records from industry and other sources.	
Remarks	An additional case report is summarised in 6.12.2 (1).	

Section A6.12.4 **Annex Point IIA VI.6.9.4**	**Epidemiological studies on the general population, <u>if available</u>**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	No data available	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification accepted	
Conclusion	Applicants justification accepted	
Remarks		

Section A6.12.5 **Annex Point IIA VI.6.9.5**	**Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Please refer to report Bromadiolone / T13, Information about and toxicity of anticoagulant rat poisons: Case Histories from the Milan Poisons Centre 1996-1999. Summary for Milan Poisons Centre There are no specific studies available on these points. Symptoms of intoxication may appear some time after exposure. Thereafter they may develop rapidly. Clinical signs result from an increased bleeding tendency and include: 1. an increase in prothrombin time 2. bruising easily with occasional nose or gum bleeds 3. blood in the stools or urine 4. excessive bleeding from minor cuts and abrasions 5. pale mouth and cold gums 6. anorexia and general weakness More severe cases of poisoning include haemorrhage (usually internal), shock and coma.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	

Section A6.12.5 Annex Point IIA VI.6.9.5	Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available	
Evaluation of applicant's justification	X1 Applicants justification replaced with: Common clinical findings following anticoagulant intoxication, presented in the Milan Poisons Centre report (Davanzo F et al. (2001) Bromadiolone: Information about and toxicity of anticoagulant rat poisons: Case Histories from Milan Poisons Centre 1996-1999. l Servizo Di Anestrsia E Rianimazione Centro Antiveleni) **Clinical Chemistry:** Extension of INR, PT, and PTT (Chow et al., 1992) Repeat test every 6- 12 hours to monitor the effects on coagulation ability. Haematocrit and Haemoglobine may also be useful. **Clinical Signs:** Ecchymosis and purpura Epitaxis, gingivorragia Tachycardia and hypotension Respiratory insufficiency secondary to alveolar haemorrhage (Barnett et al.) Hemothorax (Kruse and Carlsson, 1992) Traecheal bleeding <u>Neurological effects</u> Intracranial haemorrhage with cephalea Reduced state of awareness Convulsions Coma followed by death (Orn Stein & al 1999) <u>Gastroneteric symptomatlogy</u> abdominal pains spontaneous vomiting (Smolinske et al., 1989) gastroenteric bleeding with melanotic or haemorrhagic stools (Kruse and Carlsson, 1992, Sheen et al., 1994) Haemoperitoneumfrom stillicidium (Morgan, Tomas Zewski, 1995) and intraperitonial haemorrhaging with hypovolemic chock and grave metabolic acidosis (Corke, 1997) <u>Genitoutinary symptomatology</u> haematuria vaginal bleeding Symtoms reported following bromadiolone intoxication in 10 humans- gastric pyrosis, dizziness, vomiting and /or itching. One case with haematological problems.	
Conclusion	Justification includes relevant data and is therefore accepted.	
Remarks		

Section A6.12.6 **Annex Point IIA VI.6.9.6**	**Sensitisation/allergenicity observations, if available**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	No evidence of allergenic effect seen in man or animals in commercial and experimental use by bromadiolone or analogues.	
	High toxicity of the product and hygiene issues surrounding rodent infestations mean that gloves must be worn at all times during rodent control operations. Skin contact is therefore minimal and no problems of allergenicity have been reported to date. No data are available by way of studies.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Justification accepted	
Conclusion	Justification accepted	
Remarks		

Section A6.12.7 **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Therapy is based on the prevention of absorption, if the time after the ingestion allows and on control of the anticoagulation parameters if required. Prevention of absorption: If a few grains of bait have been ingested it is sufficient to administer powdered activated charcoal, but if more significant quantities have been ingested, it is useful to administer ipecac syrup. **Emesis** (i.e. provoking vomiting with ipecac syrup) is more efficient the earlier it is effected. Ipecac syrup is administered at 7.5 %, not diluted, in the quantity indicated in the table below, followed after 5 minutes, by the administrations of at least 100ml of water. Ipecac syrup is recommended for children where ingestion of more than a few grains of anticoagulant is suspected and must be made within 1 hour after ingestion. Emesis should not be used (contraindications) if: The patient has an altered state of awareness which could reduce the cough reflex and increase the risk of vomit being inhaled. The patient has reduced blood pressure, due to risk of cerebral bleeding due to increased endocranial pressure induced by vomiting. The patients are already under anticoagulant therapy for some time, due to the potential risk of gastroenteric bleeding.	

Ipecac syrup doses	**Quantities**
Adults or children over 40-45kg	From 15 to 30 ml
Children from 1 to 12 years	15ml
Children between 6 and 12 months	5-10 ml
Administration of children below 6 months of age is not recommended	
The dose may be repeated after 30 minutes if vomiting is not induced	

If emesis fails, even after two doses of ipecac, it would be useful to think of gastrolavage, but considering the dose of toxic substance, not because the concentration of the emetic may cause toxicity.

Adverse effects of the ipecac include diarrhoea, repeated and protracted vomiting and slight disorientation and slowness.

Gastric Lavage:

This consists of the removal of gastric contents using a probe which is either swallowed by the patient or delivered to the stomach passing through the nasal channels. It is effected by delivering through the probe from 20 to 60 ml jets of water at a temperature of 37°C, ensuring that

Section A6.12.7 **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known**	
	there is equal extraction of liquid from the stomach to avaoid excessive absorption of liquids. The water must be at body temperature to avoid risks of hypothermia. Gastric lavage is only used as a last possibility of removing the toxic substance because the patient is often not compliant with this method. It can be useful when there is ingestion of significant quantities of product, especially with attempted suicide in adults. It must be carried out within 4 hours of ingestion, in order to be effective. **Powdered active charcoal (AC):** The administration of powdered AC is preferable, especially with anticoagulants which are well absorbed in powdered AC. It must be activated i.e. micronised to augment the absorption surface, and in powdered form because because only this physical state guarantees its efficacy. 1 g of substance is deactivated by 10g of AC. The usual dose is 1g AC per kg in children and 3g AC per kg in adults diluted in water and added over several administrations. Complications reported are: vomit, aspiration into the respiratory tract and corneal abrasions. AC should not be used if there is a reduced cough reflex, gastroenteric lesions, ingestion of solvents and the absence of intestinal peristalsis. Antidote therapy: Vitamin K1 phytonadione, is the antidote of choice which must be administered to patients with extended PT or INR, and must be administered via intramuscular injection if ingestion is a medium amount or administered intravenously diluted in physiological solution or glucose at 5%, slowly (do not exceed 5 % of the total dose per minute) if a significant dose has been taken. Adverse reactions arise if the intravenous infusion is done too quickly, with cutaneous rash, cyanosis, dizziness, hypotension and broncoconstriction. If the vitamin K1 is insufficient to restore prothrombin levels, the use of fresh plasma or coagulation factors is recommended to restore haemostasis. Reference: Davanzo F, Faraoni L, Pirina A, Sesana F, Pannacciulli E, Bromadiolone, Information about and toxicity of anticoagulant rat poisons: Case Histories from the Milan Poisons Centre 1996-1999.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2008	
Evaluation of applicant's justification	The antidote treatment is the most important since in most cases the poisoning is not discovered immediately. Oral vitamin K therapy is an option to follow up the acute treatment. It is important to monitor the clotting ability (prothrombin time) of the blood to continue the treatment long enough.	
Conclusion	Information is acceptable	
Remarks		

Section A6.12.7(2) **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known** *Human – Bromadiolone*	
	1 REFERENCE	**Official use only**
1.1 Reference	International Programme on Chemical Safety: WHO/FAO Datasheets on Pesticides. No. 88 Bromadiolone. (1996) *http://www.inchrm.org/documents.pds/pds/pest88_e.htm* (Accessed December 2005)	X1
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Public domain	
1.2.2		
1.2.3 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Bromadiolone	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in the published paper	
3.1.3 Description	*Not described*	
3.1.4 Purity	Not stated in the published paper	
3.1.5 Stability	A specific statement on stability is not provided within the paper.	
3.1.6 Radio labelling	No	
3.2 Test Animals		
3.2.1 Species	Human	
3.2.2 Strain	n/a	
3.2.3 Source	n/a	
3.2.4 Sex	n/a	
3.2.5 Age/weight at study initiation	n/a	
3.2.6 Number of animals per group	n/a	

Section A6.12.7(2) **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known** *Human – Bromadiolone*	
3.2.7 Control animals	n/a	
3.3 Administration/ Exposure	n/a	
3.3.1 Preparation of test site	n/a	
3.3.2 Concentration of test substance	n/a	
3.3.3 Specific activity of test substance	n/a	
3.3.4 Volume applied	n/a	
3.3.5 Sampling time	n/a	
3.3.6 Samples	B n/a	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	See below	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Bromadiolone is an anticoagulant rodenticide of high toxicity to most mammals. It may be absorbed from the gastrointestinal tract and from the skin. In patients with blood clotting impairment or liver diseases, or following exposure to large amounts of bromadiolone, blood clotting may be disturbed. Specific treatment of bromadiolone poisoning is discussed in the results and discussion section.	
5.2 Results and discussion	<u>Treatment</u> All suspected poisoned patients should receive medical attention immediately. If poisoning is recent (within 2-3 hours) gastric lavage has been recommended in the past. Repeated administration of activated charcoal is useful. Vitamin K_1 (phytomenadione) is the specific antidote of choice. Dosage is dependent on coagulation parameters, mainly prothrombin time. If the patient is bleeding severely, 25 mg of vitamin K_1 (phytomenadione) should be given by slow intravenous injection. Prothrombin time should be checked at 3- hourly intervals in severe casesa and 8-10 hours in less severe cases. If no improvement occurs, vitamin K_1 injection sh ould be repeated. In moderate to minor cases of poisoning, vitamin K_1 may be given in lower doses. Whole blood, fresh frozen plasma or factor concentrate should e used in cases of acute severe bleeding in order to rapidly restore the blood clotting factors. <u>Reported Cases</u> Accidental bromadiolone poisoning was reported in two children,	

Section A6.12.7(2) **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known** *Human – Bromadiolone*	
	resulting in prolonged anticoagulation. Descarboxyprothrombin levels were increased in both cases by 27% and 29.9% respectively (normal as a non-detectable level). Both poisoned children were treated with vitamin K_1. The first child rapidly recovered after treatment with high-dose intravenous factor IX-prothrombin complex and vitamin K1. The clotting profile became normal on the third day after admission. The second child gave a poor response to 10 mg intravenous vitamin K_1 and the dose was increased to 20 mg.	
5.3 Conclusion	Vitamin K_1 therapy can be effective in the treatment of bromadiolone poisoning.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	X1: the address to the website should be: http://www.inchem.org/documents/pds/pds/pest88_e.htm	
Results and discussion	The applicant's version is accepted	
Conclusion	The applicant's version is accepted	
Reliability	3	
Acceptability	Acceptable as supportive information only since the statements are not supported by references to original studies.	
Remarks		

Section A6.12.7(3) **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known** *Human – Bromadiolone*	
	1 REFERENCE	**Official use only**
1.1 Reference	International Programme on Chemical Safety: Health and Safety Guide NO. 94 Bromadiolone. (1995) WHO, Geneva. *www.inchem.org/documents /hsg/hsg/hsg094.htm* (Accessed December 2005)	
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Public domain	
1.2.2		
1.2.3 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Bromadiolone	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in the published paper	
3.1.3 Description	*Not described*	
3.1.4 Purity	Not stated in the published paper	
3.1.5 Stability	A specific statement on stability is not provided within the paper.	
3.1.6 Radio labelling	No	
3.2 Test Animals		
3.2.1 Species	Human	
3.2.2 Strain	n/a	
3.2.3 Source	n/a	
3.2.4 Sex	n/a	
3.2.5 Age/weight at study initiation	n/a	
3.2.6 Number of animals per group	n/a	
3.2.7 Control animals	n/a	
3.3 Administration/	n/a	

Section A6.12.7(3) **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known** *Human – Bromadiolone*	
Exposure		
3.3.1 Preparation of test site	n/a	
3.3.2 Concentration of test substance	n/a	
3.3.3 Specific activity of test substance	n/a	
3.3.4 Volume applied	n/a	
3.3.5 Sampling time	n/a	
3.3.6 Samples	B n/a	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	See below	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Bromadiolone is an anticoagulant rodenticide of high toxicity to most mammals. It may be absorbed from the gastrointestinal tract and from the skin. In patients with blood clotting impairment or liver diseases, or following exposure to large amounts of bromadiolone, blood clotting may be disturbed. Specific treatment of bromadiolone poisoning is discussed in the results and discussion section.	
5.2 Results and discussion	<u>Treatment</u> If poisoning has occurred recently (within a few hours), gastric lavage and the administration of charcoal in repeated dose is recommended. A venous blood sample should be taken to measure the haemoglobin level, prothrombin time, blood grouping, and cross matching. If a patient is bleeding severely, 25 mg of vitamin K_1 (phytomenadione) should be given by slow intravenous injection. The patient should be transfused with whole blood or plasma. Fresh, frozen plasma may be given. Prothrombin time should be checked at 3 hours intervals and injection of vitamin K_1 repeated if no improvement occurs. Administration of factor concentrate may be considered to avoid volume overload. In less severe cases of poisoning, vitamin K_1 may be given in lower doses together with fresh, frozen plasma for rapid restoration of blood clotting factors. Prothrombin time should be checked after 8-10 hours and vitamin K_1 administration repeated, if necessary. Once the prothrombin time has stabilized, treatment with oral vitamin K_1 (10 mg) should be considered four times daily. Oral treatment may be sufficient in minor cases. Patients should be kept in hospital until the prothrombin time has remained normal for three days. Patients should be discharged from hospital with the following	

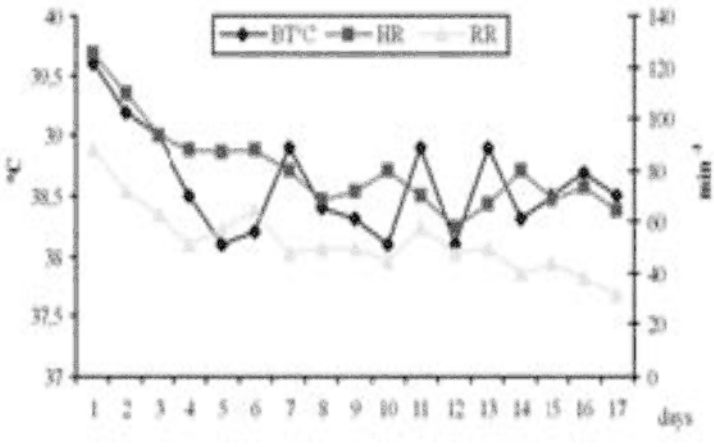
Section A6.12.7(3) **Annex Point IIA VI.6.9.7**	**Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known** *Human – Bromadiolone*	
	treatment: oral vitamin K (10 mg) twice daily for up to 60 days with close monitoring if the prothrombin time. It may be possible to reduce the length of treatment.	
5.3 Conclusion	Vitamin K_1 therapy can be effective in the treatment of bromadiolone poisoning.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Materials and Methods	The applicant's version is accepted	
Results and discussion	The applicant's version is accepted	
Conclusion	The applicant's version is accepted	
Reliability	3	
Acceptability	Acceptable only as supportive information since the statements are not supported by references to original studies.	
Remarks		

Section A6.12.8 **Annex Point IIA VI.6.9.8**	**Prognosis following poisoning**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Please refer to report Bromadiolone, Information about and toxicity of anticoagulant rat poisons: Case Histories from the Milan Poisons Centre 1996-1999. Summary for Milan Poisons Centre Bromadolone is an indirect anti-coagulant. Vitamin K1 is antidotal. In the case of suspected poisoning, determine prothrombin times not less than 18 hours after consumption. If elevated, administer vitamin K1 and continue until prothrombin times normalise. If the Vitamin K1 is insufficient to restore the prothrombin levels, the use of fresh plasma fresco or coagulation factors is recommended to restore hemostasis.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	X1 No deaths, following bromadiolone intoxication in humans, were recorded by the Milan Poisons Centre during 1996- 1999. There are also many references in the open literature to support the conclusion that the prognosis is good if diagnosis is made quickly and appropriate therapy is instituted.	
Conclusion	Information is acceptable	
Remarks		

Section A6.13 Toxic effects on livestock and pests

Section A6.13 (1) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pests** *Dogs - Bromadiolone*	
	1 REFERENCE	**Official use only**
1.1 Reference	*Rumen Binev et al, VETERINARSKI ARHIV 75 (3), 273-282, 2005*	
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Not applicable	
1.2.2		
1.2.3 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Bromadiolone	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in the published paper	
3.1.3 Description	Not stated in the published paper	
3.1.4 Purity	Not stated in paper	
3.1.5 Stability	A specific statement on stability is not provided within the paper.	
3.1.6 Radio labelling	No	
3.2 Test Animals		
3.2.1 Species	Dogs	
3.2.2 Strain	Caucasian mountain shepherd dog	
3.2.3 Source	Not stated in the published paper	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	18 months Weight approx 60kg	
3.2.6 Number of animals per group	1	
3.2.7 Control animals	No	
3.3 Administration/ Exposure	Not applicable; For some time the dog was occasionally observed eating dead and dying rodents.	
3.3.1 Preparation of test	Not applicable	

Section A6.13 Toxic effects on livestock and pets

Section A6.13 (1) Annex Point IIIA VI.2	Toxic effects on livestock and pets *Dogs - Bromadiolone*	
site		
3.3.2 Concentration of test substance	Unknown	
3.3.3 Specific activity of test substance	Anticoagulant	
3.3.4 Volume applied	Unknown	
3.3.5 Sampling time	At referral and at post-hospitalization days 4, 8, 12 and 16.	
3.3.6 Samples	Blood from v. cephalica antebrachii was sampled for determination of haemoglobin concentration (HGB), red blood cell (RBC) counts, white blood cell (WBC) counts and haematocrit (HCT) and differential WBC counts by the method of Pappenheim, erythrocyte sedimentation rate (ESR) by the method of Panchenko, Thrombin time (TT), activated partial thromboplastine time (APTT), prothrombin time (PT), protein induced by Vitamin K antagonism or absence (PIVKA) and activated clotting time (ACT) using diagnostic kits (BIOLABO, France), and a coagulometer (Amelung, Germany). The activities of transaminases ASAT and ALAT, uric acid, total bilirubin, urea, creatinine and blood sugar were assessed using an automated analyser (Reflotron Manual, Germany) using a Roche (Germany) diagnostic kit.	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	The physical examination revealed pale conjunctives, decreased locomotor activity and sensory perception, rapid exhaustion, bilateral symmetrical abdominal enlargement with drooping of the ventral wall and spinal lordosis. The patient was constantly sitting up in order to ease respiration. Body temperature was 39.6 ^{o}C, heart rate 115 min^{-1} and respiratory rate – 88 min^{-1}.  Fig. 1. Changes in body temperature (BT), heart rate (HR) and respiratory rate	

Section A6.13 Toxic effects on livestock and pets

Section A6.13 (1)
Annex Point IIIA VI.2

Toxic effects on livestock and pets
Dogs - Bromadiolone

(RR) of a dog following intoxication with the anticoagulant rodenticide bromadiolone.

During hospitalization (Fig. 1) an elevated BT was present during the first two days, but afterwards, until the end of the follow-up, it was within physiological range (37.5-39.0 °C).

A tachycardia was observed from the first (115 min^{-1}) until the 7_{th} (82 min^{-1}) day of hospitalization. From day 8 until the end of follow-up, RR was normalized (60-80 min^{-1}). The pulse was strong, full, hard and rhythmic. After percussion a decrease in absolute cardiac dullness was observed, while auscultation revealed stunt cardiac tones.

Table. 2. Clinical laboratory blood examinations data of a dog following intoxication with antico-agulant rodenticide bromadiolone.

Parameters	Days after the hospitalization				
	1	4	8	12	16
Haematological parameters					
HGB g/L	82	88	98	115	122
RBC T/L	5.19	5.24	5.54	7.39	8.56
HCT %	33.3	35.7	43.4	55.3	61.1
WBC G/L	19.1	15.2	16.4	13.8	12.1
Differential wbc counts %					
Eo	0	0	1	1	0
Mm	3	5	3	4	2
St	18	25	14	13	22
Sg	70	64	65	77	62
Lym	8	6	16	5	14
Mo	1	0	1	0	0
Biochemical parameters					
Blood sugar mmol/L	6.48	6.23	6.87	5.54	4.82
Total bilirubin µmol/L	28.6	22.8	23.5	18.1	13.2
ASAT U/L	34.1	32.2	20.8	15.0	12.8
ALAT U/L	65.2	54.8	44.2	35.7	40.1
Urea mmol/L	18.8	12.4	9.6	12.8	10.2
Uric acid µmol/L	78,8	64,2	96,5	118,4	128,1
Creatinine µmol/L	148	124	109	111	94
PT s	122	84	36	18	14
APTT s	86	68	24	22	10
PIVKA s	148	96	45	32	15
TT s	18	10	14	15	10
ACT s	82	64	58	75	45

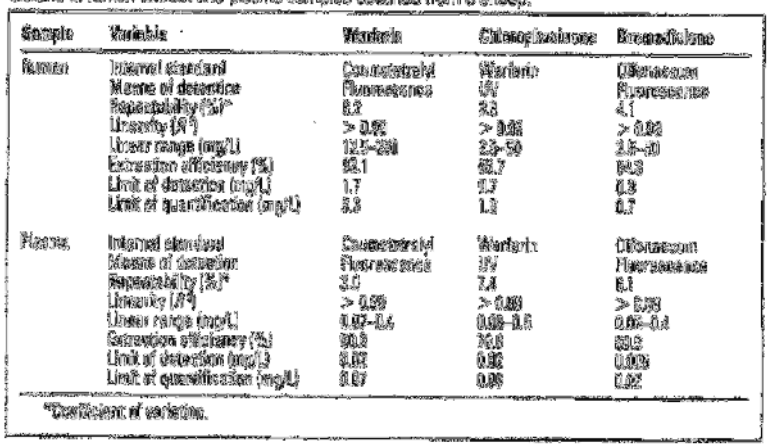
Laboratory parameters, assessed during the hospitalization period (Table 2), showed decreased HGB – 82 g/l, RBC – 5.19 T/l and HCT – 33.3% and increased WBC counts: 19.1 G/l. By the end of the observation those parameters returned to their physiological values. The differential WBC counts suggested neutrophilia with a regenerative shift to Mm (3-5%).

Section A6.13 Toxic effects on livestock and pets		
Section A6.13 (1) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Dogs - Bromadiolone*	
	Higher blood levels were measured for blood sugar, bilirubin and ALAT. They remained elevated until the end of the hospitalization, although a tendency towards normalization was observed. Significant deviations in the levels of ASAT, urea, creatinine, uric acid and ESR were not observed during the period of study. Prothrombin time (PT) was 122 s (reference range: 12-14 s), activated partial thromboplastine time (APTT) was 88 s (reference range: 12-16 s) and protein induced by vitamin K antagonism or absence (PIVKA) was 148 s (reference range: <25 s) at the beginning of the period of observation. Following treatment it was restored to within the reference range by day 12 of hospitalization. thrombin time (TT) and activated clotting time (ACT) was in reference range.	

Section A6.13 Toxic effects on livestock and pets		
Section A6.13 (1) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Dogs - Bromadiolone*	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	See 3.3.6 above. Additionally, therapy was administered to the dog: Throughout the period of hospitalization (days 1-15), 300 mL 20% glucose, 5 mL 10% vitamin C, 5 mL 20% coffein natricum benzoicum intravenously and 3 mg/kg body weight vitamin K1 subcutaneously were administered once daily. Medication therapy was extended by intramuscular administration of 12 mL Lincomycin-Spectinomycin 5/10 (50 mg lincomycin hydrochloride and 100 mg spectinomycin dihydrochloride in 1 mL injectable solution) (Alfasan-Woerden, Holland) every 24 hours for 10 days; 20 mg Furosemide (in 2 mL injectable solution) every 12 hours for 3 days, followed by oral application of 40 mg Furosemide tablets twice daily for 10 days, and 5 g urotropin (hexamethylentetramin) once daily for 7 days.	
5.2 Results and discussion	Anticoagulant rodenticides impair the cellular recirculation of vitamin K causing secondary coagulopathies, inhibits blood coagulation and affects blood vessels via an analogous mechanism. This explains haemocirculatory disorders manifested by accumulation of haemorrhagic transudate in thoracic (liquidothorax) and abdominal cavity (ascites) observed by us. It could be presumed that the simultaneous decrease of RBC counts, haemoglobin (normochromic anemia) and haematocrit values occurred as a consequence of blood loss. The observed leukocytosis and hyperthermia are probably caused by immunodeficiency, which in turn resulted in some kind of infection. Immunodeficiency is a consequence of long-term anaemia and hypoxia, as well as liver injury. The large amount of thoracic transudate is most likely responsible for the collapse of the compressed pulmonary parenchyma and for the signs of respiratory insufficiency: polypnea, dyspnea, and costo-abdominal type of breathing. The tachycardia is a compensatory mechanism for oligochromaemia, erythropenia and pulmonary insufficiency. Hyperglycaemia, bilirubinaemia and increased ALAT activity are other signs of the toxic effect of anticoagulant rodenticides on liver parenchyma. It could be accepted that the same toxicodynamics are also valid for bromadiolone intoxication.	
5.3 Conclusion	After a 3-week therapy, the clinical and the laboratory status of the patient was within reference ranges and the dog was discharged from the clinic as cured. Anticoagulant rodenticide toxicosis is a potentially fatal condition, but it may be treated successfully if diagnosis is made quickly and appropriate therapy is instituted.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2008	

Section A6.13 Toxic effects on livestock and pets		
Section A6.13 (1) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Dogs - Bromadiolone*	
Materials and Methods	The applicant's version is accepted	
Results and discussion	The applicant's version is accepted	
Conclusion	The applicant's version is accepted	
Reliability	2	
Acceptability	Acceptable	
Remarks		

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	
	1 REFERENCE	**Official use only**
1.1 Reference	Berney, JP., Alves de Oliveira, L., Videmann, B., Rossi, S (2006) Assessment of Ruminal Degradation, Oral Bioavailability, and Toxic Effects if Anticoagulant Rodenticides in Sheep AJVR, Vol 67, No 2	X1
1.2 Data protection	No, published paper.	
1.2.1 Data owner	Not applicable	
1.2.2 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	The guideline study is not stated in the published paper.	
2.2 GLP	The GLP status of the study is not stated in the published paper	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Bromadiolone, warfarin and chlorophacinone	
3.1.1 Lot/Batch number	Batch numbers not stated in the published paper.	
3.1.2 Specification	Not stated in the published paper	
3.1.3 Description	Not stated in the published paper	
3.1.4 Purity	Not stated in paper	
3.1.5 Stability	A specific statement on stability is not provided within the paper.	
3.1.6 Radio labelling	No	
3.2 Test Animals		
3.2.1 Species	Sheep	
3.2.2 Strain	Texel sheep	
3.2.3 Source	Not stated in the published paper	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	Not stated in paper	
3.2.6 Number of animals per group	3	
3.2.7 Control animals	No	
3.3 Administration/ Exposure	4 Oral exposures and 1 IV exposure (as a positive control)	
3.3.1 Post exposure period	2 month washout period	X2
3.3.2 Preparation of test	The area around the jugular vein was shaved in each sheep	

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	
site		
3.3.3 Concentration of test substance	5 mg/kg of warfarin, 1 mg/kg bromadaiolone and 1 mg/kg of chlorophacinone	
3.3.4 Vehicle	Dissolved in 1 ml ethanol and 4 ml dimethyl sulfoxide.	
3.3.5 Specific activity of test substance	Anticoagulant	
3.3.6 Sampling time	Blood samples were obtained immediately before exposure to rodenticide (time 0) and at intervals during the 14 days after exposure. Samples were collected at 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 240 and 360 hours. Following IV administration additional sampling time points were selected during the first day after exposure (ie 0.25, 0.5, 1, 2, 4 and 6 hours)	
3.3.7 Samples	The prothrombin time was assessed immediately after collection of the blood sample.	
	4 RESULTS AND DISCUSSION	
4.1 Result of study	Table 1—Results of the validation procedures for assays of warfarin, chlorophacinone, and bromadiolone in rumen extract and plasma samples obtained from 3 sheep.  "Coefficient of variation. The validation procedure gave satisfactory results.	

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	

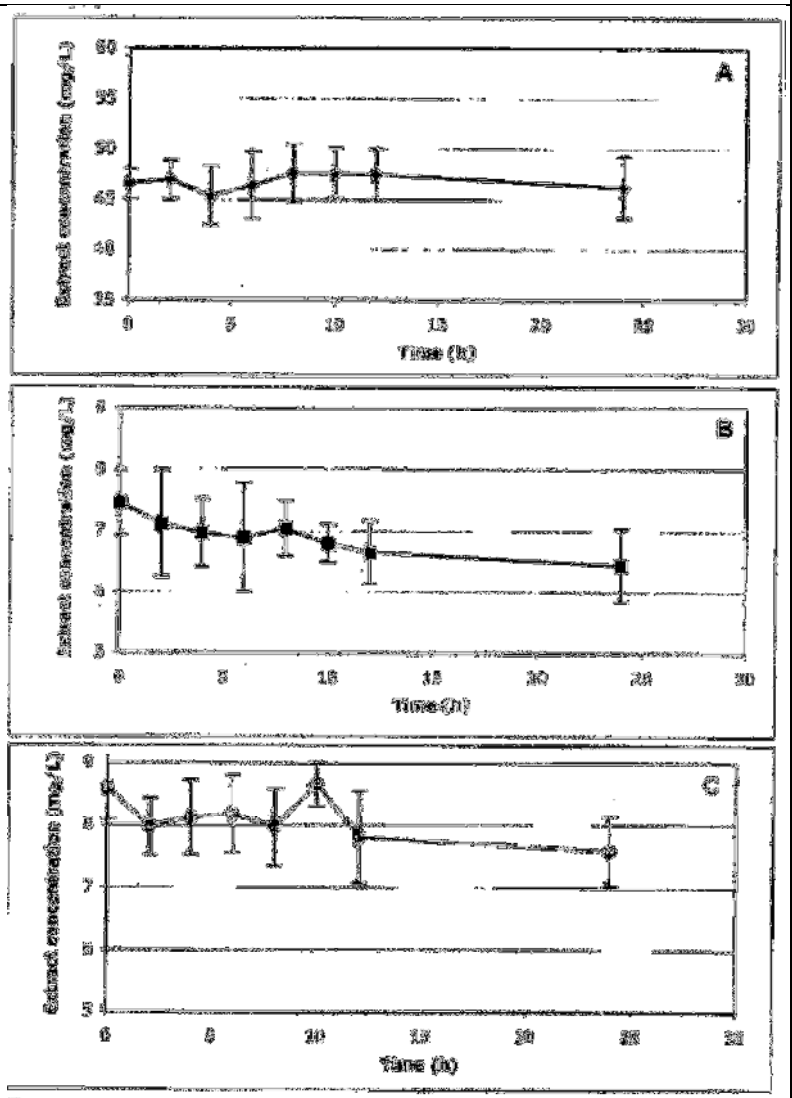
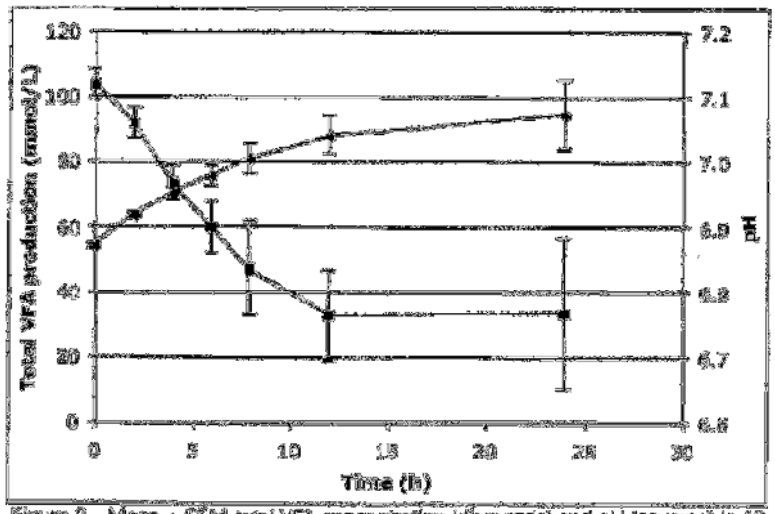


Figure 1—Mean ± SEM concentration during 24 hours of in vitro fermentation of warfarin (initial concentration, 50 mg/L; A), chlorophacinone (initial concentration, 10 mg/L; B), and bromadiolone (initial concentration, 10 mg/L; C) in rumen extract samples collected from 3 sheep. Samples from each sheep were processed in duplicate (n = 6 samples in each panel).

Concentrations of warfarin, chlorophacinone and bromadiolone in rumen extracts were assessed at intervals during a 24-hour period after exposure to each anticoagulant (Figure 1). Overall warfarin concentration in rumen extracts at time 0 was 46.5 mg/l; at 24 hours the concentration was 46.2 mg/l (a difference of -0.6%). Chlorophacinone concentration in rumen extracts at time 0 was 7.5 mg/l; at 24 hours the concentration was 6.5 mg/l (a difference of -13.3%). Bromadiolone concentration in rumen extracts at time 0 was 8.6 mg/l; at 24 hours, the concentration was 7.6 mg/l (a difference of-11.6%). Results of statistical analyses confirmed that there was no significant effect of time for warfarin concentration; however, there was a significant decrease in chlorophacinone concentration with time (P = 0.006) and a significant (albeit lesser) decrease of bromadiolone concentration with time (P = 0.02).

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	
	 Figure 2—Mean ± SEM total VFA concentration (diamonds) and pH (squares) in 18 pooled samples of rumen extract obtained from 3 sheep fermented in vitro with warfarin, chlorophacinone, or bromadiolone for 24 hours. In the rumen extract batches, pH and VFA (volatile fatty acid) concentration were monitored at each sampling time. The overall VFA production and pH variation were considered to be indicators of ruminal activity in the flasks containing ruminal fluid and artificial saliva (Figure 2). Such values are indicative of normal function.	

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	

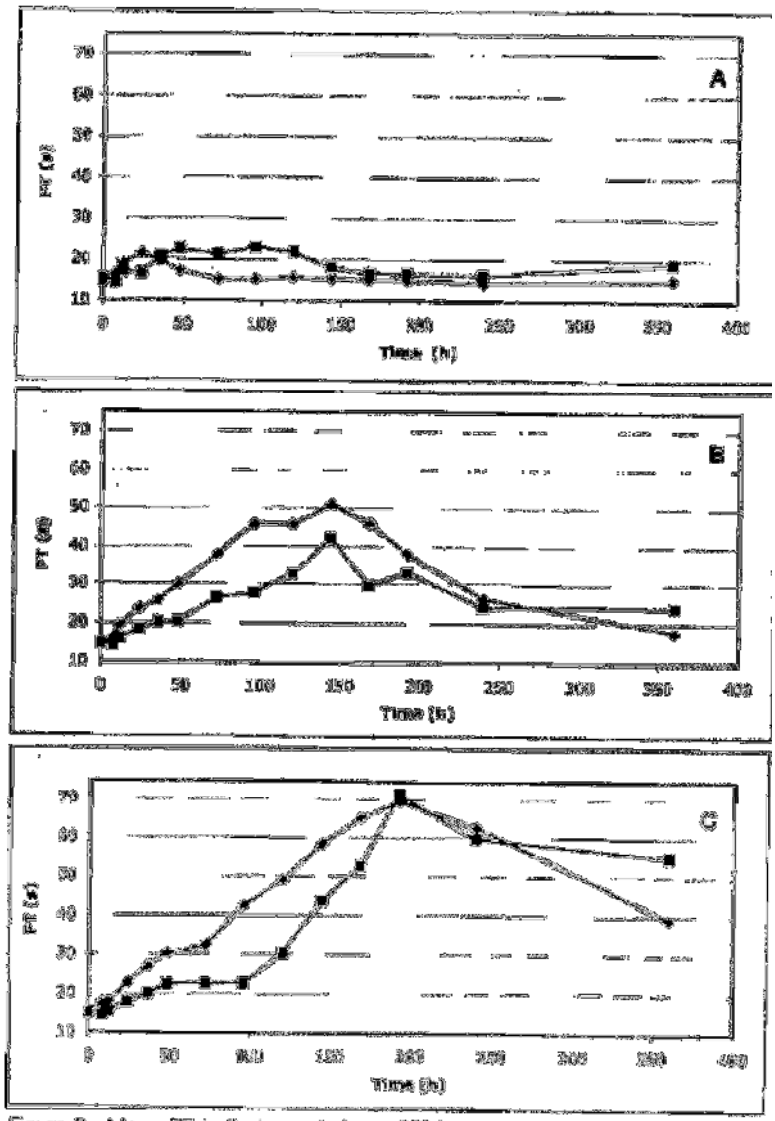


Figure 3—Mean PT in 3 sheep during a 360-hour period after IV (dimercaprol or intraruminal (approved) administration of warfarin (6 mg/kg; A), chlorophacinone (1 mg/kg; B), or bromadiolone (1 mg/kg; C). Values for each intraruminal administration represent the mean of 3 determinations (1 experiment/animal).

After IV and oral administration, PT (prothrombin time) was assessed at intervals during a 360 hour period (figure 3). Maximum PT value after oral administration of warfarin, chlorophacinone and bromadiolone was 25, 50 and 70 seconds. After oral administration of warfarin the highest PT values were detected at 2 to 3 days. After IV and oral administration of chlorophacinone the highest PT values were detected at 6 days. After IV and oral administration of chlorophacinone the highest PT values were detected at 8 days. At subsequent timepoits PT decreased to within reference range at 144 hours (6 days) after warfarin administration, at 360 hours (15 days) after chlorophacinone administration, and at 480 to 500 hours (20 to 21 days) after bromadiolone administration

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	

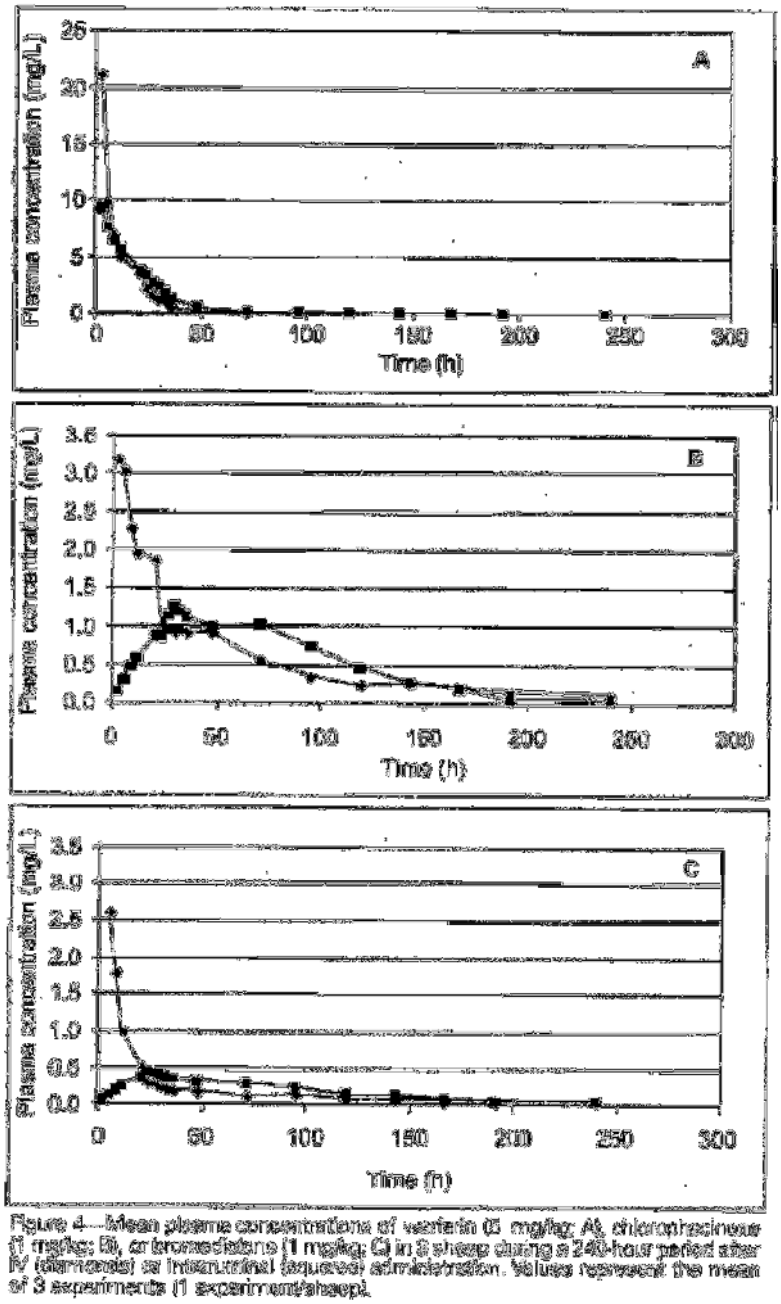


Figure 4—Mean plasma concentrations of warfarin (5 mg/kg; A), chlorophacinone (1 mg/kg; B), or bromadiolone (1 mg/kg; C) in 3 sheep during a 240-hour period after IV (dimercol) or intraruminal (aqueous) administration. Values represent the mean of 3 experiments (1 experiment/sheep).

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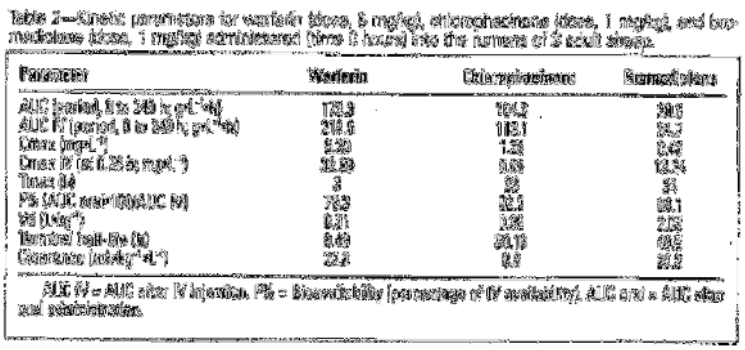
Mean plasma concentrations of warfarin, chlorophacinone and bromadiolone after IV and oral administration were determined (figure 4). Oral bioavailability and kinetic parameters were calculated (table 2).

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	
		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The objective of the study was to assess the rate and extent of ruminal degradation of warfarin, chlorophacinone and bromadiolone in vitro and determine the oral availability and clinical and hemostatic effects of each anticoagulant in sheep. Samples of ruminal fluid were incubated with each of the anticoagulants to assess the kinetics of ruminal degradation over 24 hours. To determine the plasma kinetics of the antocoagulants each sheep received each of the anticoagulants IV or via a rumen-implanted cannula at 2 month intervals (3 rodenticide exposures/sheep). At intervals during a 240- to 360- hour period after treatment, prothrombin time (PT) was measured, plasma anticoagulant concentration was assessed and clinical signs of rodenticide poisoning were monitored. In plasma and rumen extracts anticoagulant concentrations were determined by HPLC.	
5.2 Results and discussion	Results indicated that anticoagulants are poorly or not degraded by the ruminal microflora of sheep. During the 12 hour period after exposure, warfarin concentrations in rumen extracts did no decrease at all and only moderate (albeit significant) decreases in chlorophacinone and bromadiolone concentrations obtained 24 hours after exposure were almost identical. The mean digestive retention time is approximately 24 hours in sheep; therefore the compounds not degraded at that time point may be absorbed or eliminated in feces. The final anticoagulant concentrations measured in ruminal extract batches at 24 hours after exposure were still high enough to results in systemic absorption. Overall, the bioavailability of the 3 anticoagulants was high (table 2). Warfarin (79%), chlorophacinone (92%) and 88% (bromadiolone). The measurement of PT indicated that the toxic effect of bromadiolone was greatest.	X3
5.3 Conclusion	In sheep, warfarin, chlorophacinone and bromadiolone were not degraded in the rumen but their bioavailabilities were high after oral administration; the kinetics of these compounds in sheep and other mammals are quite similar, These data suggest the lack of susceptibility of ruminants to these anticoagulant rodenticides cannot be explained by either ruminal degradation or the specific toxicokinetics of these anticoagulants.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Not applicable	

Section A6.13 (2) **Annex Point IIIA VI.2**	**Toxic effects on livestock and pets** *Sheep - Bromadiolone*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2008	
Materials and Methods	X1: AJVR should be Am J Vet Res X2: The same animals were used for all three substances with a two month washout period in between.	
Results and discussion	X3: No clinical signs were seen	
Conclusion	The applicant's version is accepted	
Reliability	2	
Acceptability	Acceptable	
Remarks		

Section A6.14 Other test(s) related to the exposure of humans

Section A6.14 **Annex Point IIIA *III-XI.2***	**Other test(s) related to the exposure of humans**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	Information regarding exposure to humans from anticoagulants are already well researched and fully elucidated, for this reason it is deemed to be scientifically unjustified to conduct a study for which the end points have been reasonable determined. Bromadialone is a well-known compound which has been used extensively for many years. The properties of Bromadialone are understood as is its mode of action. Anticoagulant rodenticides such as Bromadialone are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin K dependent post translation processing before they are converted into the respective procoagulant zymogens. The specific point of action is thought to be the inhibition of K_1 epoxide reductase. The anticoagulants accumulate and are stored in the liver until broken down. The plasma prothrombin (procoagulant factor II) concentration provides a suitable guide to the severity of acute intoxication and to the effectiveness and required duration of the antidoting therapy (vitamin K_1), this process is seen in all other mammalian species tested, including humans in therapeutic use (warfarin) and in poisoning incidents in humans and animals. The technical active ingredient has a high level of purity and there are no other substances that are of concern included as impurities or additives. There are also no other known significant toxic effects. In addition, based on animal welfare grounds other tests related to the exposure of humans is considered to be of no value as this additional animal testing would not provide any additional relevant data than is not already available.Current exposure estimates do not suggest any routes of exposure which are not already satisfactorily covered by existing data. No further studies have been conducted for this reason and no studies are planned or scheduled which might be relevant to this area	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification replaced with: X1 According to the Milan Poisons Centre Report (Davanzo F et al. (2001) Bromadiolone: Information about and toxicity of anticoagulant rat poisons: Case Histories from Milan Poisons Centre 1996-1999) the predominant route of exposure is through ingestion (87% of bromadiolone cases reported 1996-1999). Since a case report of oral bromadiolone can be found in previous sections, no further data is required.	
Conclusion	Revised justification accepted	
Remarks		

Section A.6.15 Food and feedingstuffs		
Section A6.15.1 **Annex Point *IIIA X.1.1, 1.3, 1.6***	**Food and feedingstuffs - Identification of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated foods or feedingstuffs**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Bromadialone will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	X1: Bromadiolone is not supposed to be used in areas where food and feeding stuff are prepared or stored. The formulation and use pattern of the products also prevent any large amounts of bromadiolone to be transported out of the bait station, minimizing the risk of contamination and thus the contamination of food or feedingstuff is highly unlikely. Furthermore, should rats be present in areas of food production or storage then the risk of contamination with rodent borne diseases is considered to be sufficient grounds to support destruction of the goods exposed. However, it is necessary to have an analytical method to be able to analyse for residues if contamination is suspected.	
Conclusion	Justification accepted provided that an analytical method for residues is presented and found acceptable.	
Remarks	A study has been been submitted since this evaluation was performed. The study summary can be found in Doc IIIA, section 4.3.	

Section A6.15.2 **Annex Point *IIIA XI.1.2, 1.3, 1.5, 1.6***	**Food and feedingstuffs - Behaviour of the residues of the active substance, its degradation and reaction products and where relevant, its metabolites on the treated or contaminated food or feedingstuffs including the kinetics of disappearance**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Bromadialone will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification replaced with: X1 Taking into account the use pattern of bromadiolone containing products, the risk of food or feedingstuff contamination is considered to be highly unlikely. Also, since if is recommended that any food suspected of bromadiolone or rodent borne disease contamination is destroyed, information of metabolite behaviour will in most cases not be considered relevant. Also, since bromadiolone contamination is highly unlikely, it is also hard to predict the conditions of the contamination, making a residue kinetics study hard to perform.	
Conclusion	Revised justification accepted	
Remarks		

Section A6.15.3 **Annex Point *IIIA XI..1.4***	**Food and feedingstuffs - Estimation of potential or actual exposure of the active substance to humans through diet and other means**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Bromadialone will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicant justification replaced with: X1 Exposure to bromadiolone through diet is predicted to be highly unlikely to occur since contamination is predicted to involve the presence of rodents, causing greater reasons for contamination concerns (such as rodent borne diseases) than concerns linked to rodenticide exposure. Also, this data is not required for PT14.	
Conclusion	Revised justification accepted	
Remarks		

Section A6.15.4 **Annex Point *IIIA XI.1.7***	**Food and feedingstuffs - Proposed acceptable residues and the justification of their acceptability**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Bromadialone will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification replaced with: X1 Due to the high level of acute toxicity of bromadiolone and the link between bromadiolone contamination and contamination with rodent borne diseases, no acceptable residues have been identified.	
Conclusion	Revised justification accepted	
Remarks		

Section A6.15.5 **Annex Point *IIIA XI.1.8***	**Food and feedingstuffs - Any other available information that is relevant**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Bromadialone will be used around food and feedingstuff. However, it is not yet fully elucidated as it has not been decided as to what foodstuff should be tested on. Hence, at this present time it is not possible to conduct a study to identify the residues, degradation and reaction products and on metabolites of the active substance in contaminated foods or feedingstuffs.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicants justification replaced with: X1: No other relevant data has been identified	
Conclusion	Revised justification accepted	
Remarks		

Section A6.16 Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required		
Section A6.16 **Annex Point *IIIA VI.3.5, XI.2***	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Current exposure estimates do not suggest any routes of exposure which are not already satisfactorily covered by existing data. No further studies have been conducted for this reason and no studies are planned or scheduled which might be relevant to this area. The properties of anticoagulants are already well researched and fully elucidated, for this reason it is deemed to be scientifically unjustified to conduct a study for which the end points have been reasonable determined. Bromadialone is a well-known compound which has been used extensively for many years. The properties of Bromadialone are understood as is its mode of action. Anticoagulant rodenticides such as Bromadialone are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin K dependent post translation processing before they are converted into the respective procoagulant zymogens. The specific point of action is thought to be the inhibition of K_1 epoxide reductase. The anticoagulants accumulate and are stored in the liver until broken down. The plasma prothrombin (procoagulant factor II) concentration provides a suitable guide to the severity of acute intoxication and to the effectiveness and required duration of the antidoting therapy (vitamin K_1), this process is seen in all other mammalian species tested, including humans in therapeutic use (warfarin) and in poisoning incidents in humans and animals. The technical active ingredient has a high level of purity and there are no other substances that are of concern included as impurities or additives. There are also no other known significant toxic effects. Current exposure estimates do not suggest any routes of exposure which have not already been covered by existing data. Based on this and on animal welfare grounds it is considered to be of no value to conduct any further testing.	X1
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	Applicant’s justification replaced with: X1: No further testing is required	
Conclusion	Revised justification is accepted	

Section A6.16 Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required		
Section A6.16 **Annex Point *IIIA VI.3.5, XI.2***	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**	
Remarks		

Section A6.17 If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required		
Section A6.17 **Annex Point *IIIA VI.6***	**If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [x]**	
Detailed justification:	Product is not used in products for action against plants	X1
	BROMADIOLONE IS A RAT POISON, NOT A WEED KILLER	
	RATS ARE NOT PLANTS	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2006	
Evaluation of applicant's justification	**NOTE: The heading states "IF the active substance is used in….."** Applicants justification replaced with: X1: Bromadiolon is not used in products for action against plants.	
Conclusion	Revised justification accepted	
Remarks		

Section A6.18 Summary of mammalian toxicology and conclusions		
Section 6.18 **Annex Point *IIA 6.18***	**Summary of mammalian toxicology and conclusions**	**Official use only**
	A summary of the mammalian toxicology and conclusions for the active substance bromadiolone is provided in Document IIA.	