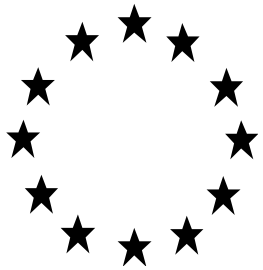
Competent Authority Report
According to Directive 98/8/EC



Bromadiolone (PT14)

The Bromadiolone Task Force

DOCUMENT III-A

Section 7: Ecotoxicological profile
including environmental fate and behaviour

Rapporteur Member State: Sweden

Final CAR, April 2011

KEMI
Kemikalieinspektionen
Swedish Chemicals Agency

Contents

Section A7.1.1.1.1 - Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

					Official use only
		1 REFERENCE			
1.1	**Reference**	Viktória Laky (2001) Draft report: Hydrolysis of Bromadiolone as a function of pH. Toxicological Research Centre Ltd. Report 01/617-336AN			X
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 111			
2.2	**GLP**	Yes			
2.3	**Deviations**	The purity of the active substance 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			
3.1.3	Purity	99.4% bromadiolone			
3.1.4	Further relevant properties	None			
3.2	**Reference substance**	No			
3.2.1	Initial concentration of reference substance				
3.3	**Test solution**	See table A7_1_1_1_1-1 See table A7_1_1_1_1-2			
3.4	**Testing procedure**				
3.4.1	Test system	See table A7_1_1_1_1-3			
3.4.2	Temperature	50ºC.			
3.4.3	pH	pH7 pH9	Start = 7.1 Start = 9.1	End = 7.0 End = 9.1	
3.4.4	Duration of the test	5 days.			X

Section A7.1.1.1.1 - Hydrolysis as a function of pH and identification of breakdown products

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3.4.5	Number of replicates	1	X
3.4.6	Sampling	Sampling at start and end of test.	
3.4.7	Analytical methods	0.5 ml samples were taken and mixed with 1 ml MeOH. The test item content was determined by reverse phase HPLC according to the conditions: Detector: UV at 260 nm Column: LiChrospher 100 RP-18 BD 250x4 mm, No.:724513 Mobil Phase: Methanol : 0.002M Phosphoric acid = 9 : 1 Flow: 0.8 ml/min Injection volume: 20 µl Retention time for Bromadiolone: 4.0 – 4.5 min	
3.5	**Preliminary test**	Yes Solubility at room temperature at 1.2, 4, 7 and 9 pH values. An over saturated solution was sonicated three times for 15 minutes and allowed to stand overnight before being filtered and analysed by HPLC. Preliminary test was also performed at 50 ± 0.5ºC. 37 ± 0.5ºC and 25 ± 0.5ºC at pH 7 and 9 to determine sampling intervals for the main study.	X
		4 RESULTS	
4.1	**Concentration and hydrolysis values**	The repeatability (CV%) of the HPLC method is ~3%. The linearity of the detector was tested between 0.05 and 5.0µg/ml. 0.05µg/ml is the limit of detection. The detector response was linear in the 0.1-5.0µgml. The recovery from water was studied on three concentrations levels: 0.0005, 0.001 and 0.1 µg/ml. The recovered amounts were 70, 74 and 83%.	X
		Compound is of extremely low water solubility (1.2ppm) and is hydrolytically stable (>1year) at pH7 and 9 at only concentration tested (2mg/l, limit of solubility).	X
		Under the acidic conditions (pH 1, 2 and 4) the solubility was found to be less than the detection limit of the HPLC determination (0.05µg/ml). Therefore the hydrolysis test was carried out only at pH 7 and 9.	
4.2	**Hydrolysis rate constant (k_h)**	Hydrolysis rate constant (k_h) =-104.3 at start and 0 at the end of the experiment	
4.3	**Dissipation time**	>1year	
4.4	**Concentration – time data**	No reaction, no degradation seen.	
4.5	**Specification of the transformation products**	No degradation and no transformation products produced.	

Section A7.1.1.1.1 - Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

		5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	**Materials and methods**	The study was conducted according to OECD 111. Hydrolysis behaviour of bromadiolone was examined at pH 7 and 9 at 50°c. Buffer solutions: pH 7.0: 147.8ml 0.2M NaOH and 250ml 0.2M Potassium-dihydrogen phosphate were diluted to 1000ml with bi-distilled water pH 9.0: 107ml 0.2M NaOH and 250ml 0.2Mboric acid-KCl solutions were diluted to 1000ml with bi-distilled water The test item was dissolved in distilled sterile water with sterile buffer medium added to it. The test concentration was 2µg/ml in both buffers. 250cm^3 sterile solutions were prepared at pH 7 and 9. 3-3 stoppered tubes containing 20cm^3 solution were stored at 50°c. The samples were analysed after 5 days. The pH of each buffer solution was checked with a calibrated pH meter.	
5.2	**Results and discussion**	Bromadiolone is hydrolytically stable under conditions tested at the limit of water solubility		
5.2.1	k_H	Start = -104.3	End = 0.0	
5.2.2	DT_{50}	>1 year		
5.2.3	r^2	Start = 1.0000	End = 0.999	
5.3	**Conclusion**	Bromadiolone is hydrolytically stable under conditions tested Half life at the limit of water solubility is >1 year		
5.3.1	Reliability	1		X
5.3.2	Deficiencies	No		

Section A7.1.1.1.1 - Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2006-03-13*
Materials and Methods	Adopt applicant's version noting the following deviations. This is a draft report, therefore the applicant need to confirm that no changes were made when the report was finished. 3.4.1 According to the OECD 111 guideline hydrolysis should be performed at three pH:s, however, the applicant have justified by a preliminary investigation, which showed a very low solubility of bromadiolone at pH 4, why a test at this pH not was included. 3.4.4 According to the OECD 111 guideline the duration of the test should be 30 days, however the applicant have shown that bromadiolone is hydrolytically stable after 5 days which justifies a shorter duration of the test. 3.4.5 Number of replicates in this test should be at least two and in the report there are standard deviations which means that the test have been performed in at least two replicates, but at 3.4.5 it is stated that it is only one replicate, this needs to be clarified. 3.5 A preliminary test of hydrolysis should be conducted with test duration of at least five days. The applicant have tested solubility at different pH:s not hydrolysis using a test duration of approximately 12 hours in their test. It is therefore suggested that this not is called a preliminary test in the report.
Results and discussion	Adopt applicant's version noting the following deviations. 4.1 The quality criteria of the OECD guideline states (OECD 111, point 11) that recoveries of the test substance should range from 90-110 %. The Recovered amounts in this test range between 70-84 %, this might be acceptable, but justification for the low recovery should, according to the OECD guideline, be given. 4.1 According to the OECD guideline (OECD 111 point 22) The concentration of the test substance should not exceed half of the saturation concentration, in this study the saturation concentration was used.
Conclusion	Adopt applicant's version.
Reliability	2
Acceptability	Acceptable Although the study do not fulfil all the demands of the OECD 111 guideline it might be acceptable after clarification of the deviations described above.
Remarks	The study is accepted after clarification of the above mentioned deviations. It was clarified that 2 mg/L is one tenth of the solubility concentration at this pH and that standard deviations not were presented because confidence intervals were used instead.

Table A7_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
5	-	-
7	-	147.8 ml 0.2 M NaOH and 250 ml 0.2M Potassium-dihydrogen phosphate diluted to 1000 ml with bi-distilled water
9	-	107 ml 0.2 M NaOH and 250 ml 0.2M boric acid-KCI solutions diluted to 1000 ml with bi-distilled water

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Ion exchanged water bi-distilled on Vitrotech distiller equipment and filtered on 0.45 µm membrane filter.
Preparation of test medium	Test substance was dissolved in distilled sterile water with sterile buffer medium added to it
Test concentrations (mg a.i./L)	Test concentration was 2 µg/ml (≈0.004 mM).
Temperature (°C)	50 ± 0.5ºC
Controls	None
Identity and concentration of co-solvent	None
Replicates	Not stated

Table A7_1_1_1_1-3: Description of test system

Glassware	Stoppered tubes containing 20 cm3 solution
Other equipment	HPLC System, Thermostat, Steriliser, Balance, Ultrasonic bath, pH Meter and Filter.
Method of sterilization	WTB Binder steriliser

Table A7_1_1_1_1-4: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 5, pH 7 and pH 9. *(one table for each pH value; adjust table size as required)*

Compound pH 7	**Sampling times (*days, hours, or other time period*)**							
	0	**5d**	***t*2**	***t*3**	***t*4**	***t*5**	***t*6**	***t*n**
Parent compound	1.92	2.08	-	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	108	-	-	-	-	-	-

Compound pH 9	**Sampling times (*days, hours, or other time period*)**							
	0	***5d***	***t*2**	***t*3**	***t*4**	***t*5**	***t*6**	***t*n**
Parent compound	2.17	2.25	-	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	104	-	-	-	-	-	-

Table A7_1_1_1_1-5: Dissipation times of parent compound, transformation products and reference compound at pH 5, pH 7 and pH 9

	pH 5		**pH 7**		**pH 9**	
	DT_{50}	**DT_{90}**	**DT_{50}**	**DT_{90}**	**DT_{50}**	**DT_{90}**
Parent compound	-	-	>1year	>1year	>1year	>1year
Transformation product 1	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-

Table A7_1_1_1_1-6: Specification and amount of transformation products *(adjust table size as required)*

CAS-Number	***CAS and/or IUPAC* Chemical Name*(s)***	**Amount [%] of parent compound measured at**		
		pH 5	**pH 7**	**pH 9**
	None – compound was stable	-	0	0

Section A7.1.1.1.2 - Phototransformation in water including identity of transformation products.

Annex Point IIA7.6.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake, RM (2005) Determination of the Direct Photolysis Rate is Water by Sunlight of Bromadiolone, Chemex Environmental International Ltd, ENV6766/080319-REISSUE, GLP	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Bromadiolone Task Force	
1.2.2	Companies with access to data	None	
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 OPPTS 835.2210	
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	Not available	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	98 %	
3.1.4	Radiolabelling	N/A	
3.1.5	UV/VIS absorption spectra and absorbance value	Bromadiolone showed three absorbance maxima in the region 190 to 340 nm only one of which was above 290nm. No absorbance was detected (above the base line) for wavelengths above 340nm.	
3.1.6	Further relevant properties	N/A	
3.2	**Reference substances**	Methanol	
3.3	**Test solution**	Bromadiolone was prepared as a 204mg/l dosing solution in acetonitrile. 1ml of the dosing solution was added to a 100ml volumetric flask and made to volume with 0.2 µm filtered deionised water (2.04mg/l-0.00000387M.) Ten test tubes (2 off pyrex and 8 off quartz) were filled with the above solution. The pyrex tubes were placed in boiling tubes and covered in aluminium foil which formed a light proof jacket (control).	
3.4	**Testing procedure**		
3.4.1	Test system	See above	

Section A7.1.1.1.2 - Phototransformation in water including identity of transformation products.

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3.4.2	Properties of light source	The quartz tubes were placed in sunlight inclined at angle of about 30°C with the tops facing magnetic north. The test was set up at 12.00 on 31 March 2004. The test site is located at latitude of 52° North.	
3.4.3	Determination of irradiance	Ranging from 1.09 E-01 to 3.61 E-06 [10^{-3} E cm^{-2} d^{-1}]	
3.4.4	Temperature	N/A	X
3.4.5	pH	The pH was not measured (page 16 section C subsection 2 of guideline states 'report the pH of all test solutions, if appropriate'). pH was not adjusted nor measured. The pH of the test solutions would have been that of deionised water (which typically is around pH5.5-6.0) as the test materials was not water soluble (introduced in a solvent) and were tested at low concentration.	X
3.4.6	Duration of the test	5 hours	
3.4.7	Number of replicates	3	
3.4.8	Sampling	Two samples were taken from the tubes every 10 minutes for the first hour and then hourly for a further 5 hours.	
3.4.9	Analytical methods	The samples were analysed using the HPLC conditions below. All samples were injected in triplicate. <u>HPLC conditions</u> The samples were analysed using HPLC which were all run in triplicate. The conditions were as follows: Chromotography System: Perkin Elmer Quaternary System Mobile phase: Methanol: distilled water: Acetic acid (850:142:8) Flow rate: 1.5ml/min Injection volume 250µl	
3.5	**Transformation products**	Not specified	
3.5.1	Method of analysis for transformation products	N/A	
		4 RESULTS	
4.1	**Screening test**	The maximum absorbance between 290 and 800 nm was at 290nm.	

4.2 Actinometer data

5.5.3 Concentration with time (PNAP)

Cumulative Fraction of day	Sample	HPLC		Conc (mol)	Adjusted for wt (average) (mol)	average Percent loss	K'_p day^{-1}	Half life (days)
		Retention time (min)	Peak area					
	Standard 0.000005 M	3.057	239334.40	0.0000050				
		3.055	237729.60	0.0000049				
		3.062	238721.60	0.0000046				
0.000	0 minutes (rep 1)	3.043	496824.51	0.0000103	0.0000105			
		3.046	497605.84	0.0000103				
		3.048	498868.04	0.0000104				
	0 minutes (rep 2)	3.042	497734.40	0.0000103	0.0000105			
		3.049	498818.91	0.0000104				
		3.046	499530.52	0.0000104				
	0 minutes (rep 3)	3.043	495321.06	0.0000103	0.0000105			
		3.042	500312.52	0.0000104				
		3.046	499564.80	0.0000104				
0.012	10 minutes (rep 1)	3.046	463751.84	0.0000096	0.0000097	9.07	7.15	0.097
		3.044	459842.09	0.0000096				
		3.043	457661.52	0.0000095				
	10 minutes (rep2)	3.049	462537.37	0.0000096	0.0000096			
		3.048	459408.57	0.0000095				
		3.043	458274.11	0.0000095				
	10 minutes (rep 3)	3.047	461503.04	0.0000096	0.0000096			
		3.044	459981.51	0.0000095				
		3.046	460474.17	0.0000096				
0.024	20 minutes (rep 1)	3.044	421589.57	0.0000088	0.0000089	16.93	7.42	0.093
		3.043	422657.11	0.0000088				
		3.043	417279.16	0.0000087				
	20 minutes (rep2)	3.042	422145.21	0.0000088	0.0000084			
		3.044	418116.22	0.0000087				
		3.047	412019.59	0.0000086				
	20 minutes (rep 3)	3.045	420923.21	0.0000088	0.0000088			
		3.045	415936.26	0.0000086				
		3.044	412444.13	0.0000086				
0.035	30 minutes (rep 1)	3.043	390721.39	0.0000081	0.0000081	23.75	7.38	0.094
		3.045	385689.71	0.0000080				
		3.046	383127.08	0.0000080				
	30 minutes (rep2)	3.047	384563.58	0.0000080	0.0000080			
		3.045	381170.41	0.0000079				
		3.046	377175.12	0.0000078				
	30 minutes (rep 3)	3.054	389445.85	0.0000081	0.0000081			
		3.045	387069.20	0.0000080				
		3.045	385359.15	0.0000080				

Cumulative Fraction of day	Sample	HPLC		Conc (mol)	Adjusted for std (average) (mol)	average Percent loss	K^{C}_{T} day^{-1}	Half life (days)
		Retention time (min)	Peak area					
0.047	40 minutes (rep 1)	3.049	350365.25	0.0000073	0.0000073	31.5	7.78	0.090
		3.042	347815.85	0.0000072				
		3.043	346201.29	0.0000072				
	40 minutes (rep 2)	3.045	347708.30	0.0000072	0.0000072			
		3.041	344421.13	0.0000072				
		3.042	341245.81	0.0000071				
	40 minutes (rep 3)	3.046	351795.38	0.0000073	0.0000073			
		3.043	348259.29	0.0000073				
		3.042	346252.94	0.0000072				
0.059	50 minutes (rep 1)	3.043	309199.03	0.0000064	0.0000064	39.40	8.34	0.083
		3.042	307104.09	0.0000064				
		3.044	303713.94	0.0000063				
	50 minutes (rep2)	3.045	303924.23	0.0000063	0.0000063			
		3.044	300943.15	0.0000062				
		3.046	298067.93	0.0000062				
	50 minutes (rep 3)	3.041	312984.35	0.0000065	0.0000065			
		3.045	309300.88	0.0000064				
		3.042	308013.25	0.0000064				
0.071	60 minutes (rep 1)	3.044	279755.15	0.0000058	0.0000058	44.55	9.21	0.084
		3.044	276857.50	0.0000057				
		3.045	273180.98	0.0000057				
	60 minutes (rep2)	3.046	286219.94	0.0000059	0.0000059			
		3.043	281946.92	0.0000059				
		3.050	280206.93	0.0000058				
	60 minutes (rep 3)	3.046	283079.56	0.0000059	0.0000059			
		3.047	280178.65	0.0000058				
		3.042	275620.23	0.0000057				
0.141	120 minutes (rep 1)	3.046	132236.40	0.0000027	0.0000027	74.4	9.80	0.072
		3.045	130432.51	0.0000027				
		3.046	130265.40	0.0000027				
	120 minutes (rep2)	3.045	132774.48	0.0000027	0.0000027			
		3.044	128136.02	0.0000027				
		3.042	127384.15	0.0000026				
	120 minutes (rep 3)	3.046	132823.96	0.0000027	0.0000027			
		3.046	128428.39	0.0000027				
		3.045	127658.59	0.0000026				
0.212	180 minutes (rep 1)	3.048	58926.20	0.0000012	0.0000012	88.58	10.25	0.068
		3.043	58134.06	0.0000012				
		3.044	57769.48	0.0000012				
	180 minutes (rep2)	3.048	57855.09	0.0000012	0.0000012			
		3.044	57356.49	0.0000012				
		3.043	56819.09	0.0000012				
	180 minutes (rep 3)	3.048	58234.40	0.0000012	0.0000012			
		3.045	57606.26	0.0000012				
		3.046	56930.06	0.0000012				

Cumulative Fraction of day	Sample	HPLC		Conc (mol)	Adjusted for std (average) (mol)	average Percent loss	K^{C}_{T} day^{-1}	Half life (days)
		Retention time (min)	Peak area					
0.282	240 minutes (rep 1)	3.044	27577.60	0.0000006	0.0000006	94.6	10.29	0.067
		3.044	27459.69	0.0000006				
		3.045	28568.60	0.0000006				
	240 minutes (rep 2)	3.049	28539.00	0.0000006	0.0000006			
		3.043	28180.60	0.0000006				
		3.049	28021.40	0.0000006				
	240 minutes (rep 3)	3.048	28656.40	0.0000006	0.0000006			
		3.043	28352.20	0.0000006				
		3.049	28054.48	0.0000006				
0.353	300 minutes (rep 1)	3.045	14893.20	0.0000003	0.0000003	97.2	10.16	0.068
		3.049	14817.20	0.0000003				
		3.048	14410.80	0.0000003				
	300 minutes (rep2)	3.049	14751.29	0.0000003	0.0000003			
		3.048	14484.60	0.0000003				
		3.046	14493.40	0.0000003				
	300 minutes (rep 3)	3.046	14621.20	0.0000003	0.0000003			
		3.047	14375.60	0.0000003				
		3.044	14204.80	0.0000003				
	Control (rep 1)	3.046	496780.45	0.0000103	0.0000105	1.3		
		3.040	499030.25	0.0000104				
		3.048	496805.46	0.0000104				
	Control (rep2)	3.044	496953.66	0.0000103	0.0000105			
		3.050	497797.35	0.0000103				
		3.048	497065.08	0.0000103				
	Control (rep 3)	3.043	495766.55	0.0000103	0.0000105			
		3.044	498957.28	0.0000104				
		3.045	497582.89	0.0000105				
	Standard 0.000005 M	3.074	241002.40	0.0000050				
		3.068	238434.00	0.0000050				
		3.066	236790.60	0.0000049				

Section A7.1.1.1.2 - Phototransformation in water including identity of transformation products.

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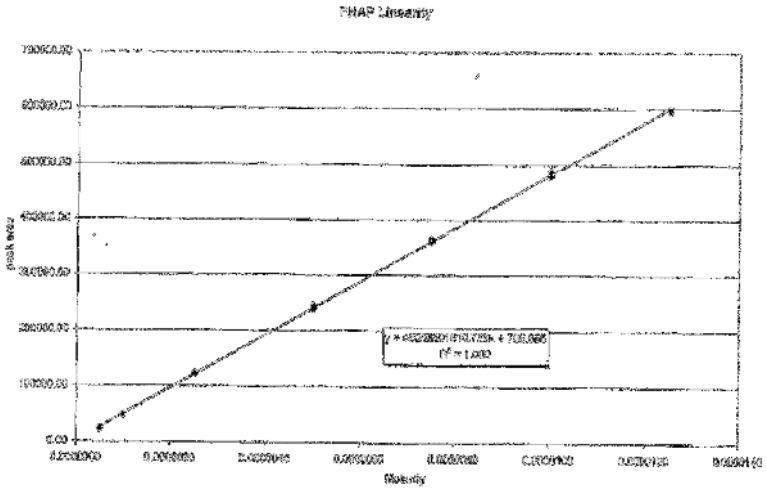
Figure 4

PNAP Linearity



4.3 Controls Control loss for Bromadiolone and the actinometer were not considered to be significant at - 3.3% and 1.3% respectively.

4.4 Photolysis data

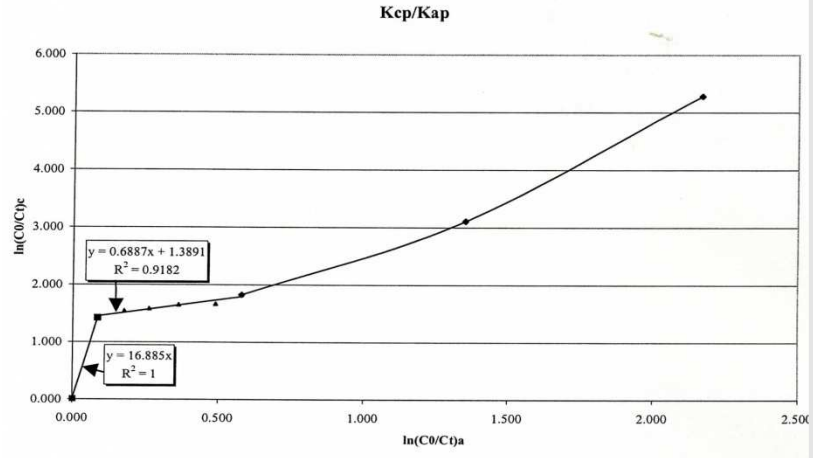
4.4.1 Concentration values

5.5.4 Concentration with time (Bromadiolone)

Cumulative Fraction of day	Sample	HPLC Retention time (min)	HPLC Peak area	Conc (mM)	Adjusted for std (average) (mol)	average Percent loss	K^{C}_{P} day^{-1}	Half life (days)
	Standard 0.00193 M	6.235	239587.60	0.0017518				
		6.213	234884.80	0.0017174				
		6.205	226261.20	0.0016543				
0.000	0 minutes (rep 1)	6.209	657798.40	0.0048113	0.00501			
		6.205	680878.80	0.0049802				
		6.214	686272.40	0.0050197				
	0 minutes (rep 2)	6.275	742232.00	0.0054291	0.00542			
		6.205	727635.60	0.0053223				
		6.217	718249.20	0.0052536				
	0 minutes (rep 3)	6.203	713038.80	0.0052155	0.00534			
		6.204	718708.00	0.0052570				
		6.228	723249.20	0.0052902				
0.012	10 minutes (rep 1)	6.224	171036.00	0.0012503	0.00123	76.14	120.64	0.081
		6.211	165853.20	0.0012123				
		6.218	158735.00	0.0011603				
	10 minutes (rep2)	6.223	160978.80	0.0011767	0.00117			
		6.218	159332.80	0.0011646				
		6.207	154082.40	0.0011262				
	10 minutes (rep 3)	6.235	194341.20	0.0014208	0.00142			
		6.216	190578.20	0.0013932				
		6.208	188830.60	0.0013804				
0.024	20 minutes (rep 1)	6.209	149382.40	0.0010918	0.00109	78.96	65.68	0.150
		6.207	147778.00	0.0010801				
		6.215	142080.40	0.0010384				
	20 minutes (rep2)	6.215	143830.60	0.0010512	0.00104			
		6.211	141753.60	0.0010360				
		6.212	136458.40	0.0009973				
	20 minutes (rep 3)	6.217	173401.20	0.0012676	0.00124			
		6.214	165433.00	0.0012093				
		6.212	161274.80	0.0011788				
0.035	30 minutes (rep 1)	6.233	136509.60	0.0009977	0.00101	79.75	44.87	0.219
		6.234	135418.00	0.0009897				
		6.288	134702.80	0.0009845				
	30 minutes (rep2)	6.211	140641.60	0.0010279	0.00101			
		6.212	137152.80	0.0010024				
		6.215	132297.20	0.0009669				
	30 minutes (rep 3)	6.235	167012.00	0.0012208	0.00122			
		6.219	165362.80	0.0012088				
		6.214	161382.80	0.0011796				

Cumulative Fraction of day	Sample	HPLC Retention time (min)	HPLC Peak area	Conc (mM)	Adjusted for std (average) (mol)	average Percent loss	K^{C}_{P} day^{-1}	Half life (days
0.047	40 minutes (rep 1)	6.218	130049.80	0.0009504	0.00095	81.03	35.04	0.281
		6.210	129557.60	0.0009468				
		6.222	124907.60	0.0009128				
	40 minutes (rep 2)	6.227	131045.60	0.0009577	0.00095			
		6.268	128038.40	0.0009357				
		6.219	126818.40	0.0009268				
	40 minutes (rep 3)	6.240	157915.80	0.0011543	0.00113			
		6.224	153289.60	0.0011204				
		6.227	146117.20	0.0010680				
0.059	50 minutes (rep 1)	6.222	129549.60	0.0009468	0.00094	81.34	28.31	0.347
		6.218	129519.60	0.0009465				
		6.227	121239.60	0.0008860				
	50 minutes (rep2)	6.225	127004.00	0.0009281	0.00092			
		6.229	124856.60	0.0009124				
		6.218	120887.20	0.0008834				
	50 minutes (rep 3)	6.226	156253.60	0.0011421	0.00112			
		6.222	152440.00	0.0011142				
		6.219	145794.40	0.0010656				
0.071	60 minutes (rep 1)	6.224	114253.20	0.0008348	0.00082	84.10	25.86	0.380
		6.219	112867.60	0.0008247				
		6.224	105724.80	0.0007725				
	60 minutes (rep2)	6.221	118244.80	0.0008640	0.00087			
		6.220	117165.00	0.0008561				
		6.225	114568.40	0.0008372				
	60 minutes (rep 3)	6.225	119387.00	0.0008724	0.00086			
		6.227	116713.60	0.0008528				
		6.222	110578.40	0.0008080				
0.141	120 minutes (rep 1)	6.218	26520.20	0.0001930	0.00020	95.59	22.02	0.446
		6.223	25886.40	0.0001884				
		6.224	26988.80	0.0001964				
	120 minutes (rep2)	6.216	32662.00	0.0002379	0.00023			
		6.229	31463.60	0.0002292				
		6.225	29579.20	0.0002154				
	120 minutes (rep 3)	6.222	38264.40	0.0002789	0.00028			
		6.221	37951.00	0.0002766				
		6.220	37184.80	0.0002710				
0.212	180 minutes (rep 1)	6.232	2817.20	0.0000196	0.00002	99.50	24.96	0.394
		6.230	2318.20	0.0000159				
		6.241	2394.60	0.0000165				
	180 minutes (rep2)	6.220	3694.00	0.0000260	0.00003			
		6.237	3695.20	0.0000260				
		6.229	3588.80	0.0000252				
	180 minutes (rep 3)	6.220	5037.00	0.0000358	0.00004			
		6.216	5143.00	0.0000366				
		6.217	5018.00	0.0000357				

Tier 2 phase 2 log of ratios



4.4.2 Mass balance N/A

4.4.3 k^{c}_{p} 30.55 day^{-1} (1 hour exposure)

4.4.4 Kinetic order N/A

Section A7.1.1.1.2 - Phototransformation in water including identity of transformation products.

Annex Point IIA7.6.2.2

4.4.5	k^c_p / k^a_p	As Bromodialone was photolysed very rapidly (>75% in the first 10 minutes) the slope for the very first part the graph was plotted separately and gave a slope (k^c_p / k^a_p) of 16.89 The removal of Bromadiolone from 10 to 60 minutes was more gradual and describes a straight line with a slope (k^c_p / k^a_p) of 0.69. The rate of photolysis was so fast that the linear portion of the graph was observed within 10 minutes. If the sampling interval had been increased (in line with the protocol) a different graph would have been observed which would not have given two apparent removal rates but would have indicated a much lower overall rate. The control loss for tier 2 phase 2 was -3.3% and was considered to be insignificant. Precipitation is not considered to be a mechanism for the initial removal.
4.4.6	Reaction quantum yield (ϕ^c_E)	Two values were calculated for the quantum yield. At the initial rapid rate of photodegradation the value 0.25. At the slower rate the value was 0.01.

4.4.7	k_{pE}		Summer	Winter	Spring
		(ϕ_E of 0.25) The values are	335	32.8	230.5
		(ϕ_E of 0.01) The values are	13.4	1.3	9.2

4.4.8	Half-life ($t_{1/2E}$)		Summer	Winter	Spring
		(ϕ_E of 0.25) The values are (mins)	1.49	15.54	2.16
		(ϕ_E of 0.01) The values are (mins)	37.24	380.89	54.12

4.5	**Specification of the transformation products**	N/A

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	<u>Tier 2 Phase 1</u> A dilute aqueous solution of Bromadiaolone (0.0039mM) was exposed to natural sunlight in thin walled quartz tubes. Exposure was performed under clear sky conditions with the tubes inclined at around 30 ° from the horizontal (with the open ends facing magnetic north) at a latitude 52° North in the early part of spring 2004. When removal of test material is shown to be 50% (or greater) in the first day (as in this case) the protocol suggests that the test should be set up at noon (12:00) of one day and sampled once at noon of the following day. The exposure period was confined to six hours with the first hour sampled at 10 minute intervals. <u>Tier 2 Phase 2</u> From the results obtained in tier 2 phase 1, a suitable concentration of pyridine for the actinometer was determined. Both Bromadiolone and the actinometer solution were exposed to natural sunlight using the conditions employed for tier 2 phase 1.

Section A7.1.1.1.2 - Phototransformation in water including identity of transformation products.

Annex Point IIA7.6.2.2

5.2	**Results and discussion**	Tier Two Phase 1 Photolysis of Bromadiolone was particularly fast with 68% removal in the first 10 minutes of exposure. Complete photolysis was noted to have occurred by around 2 hours. Tier Two Phase 2 From the results obtained in tier 2 phase 1 a suitable concentration of pyridine for the actinometer was determined. Both Bromadiolone and the actinometer solution were exposed to natural sunlight using the conditions employed for tier 2 phase 1. The photolysis of Bromadiolone was again noted to be very fast with removal comparable to the earlier phase. Two distinct rates of removal were noted for Bromadiolone. The removal in the first 10 minutes was particularly fast, whilst the removal during the following 50 minutes was somewhat slower showing good linear correlation. Control losses for Bromadiolone and the actinometer were not considered to be significant therefore no corrections were required.	
5.2.1	k^c_p	30.55 day^{-1} (1 hour exposure)	
5.2.2	K_{pE}	Summer / Winter / Spring (ϕ_E of 0.25) The values are 335 / 32.8 / 230.5 (ϕ_E of 0.01) The values are 13.4 / 1.3 / 9.2	
5.2.3	ϕ^c_E	Two values were calculated for the quantum yield. At the initial rapid rate of photodegradation the value 0.25. At the slower rate the value was 0.01.	
5.2.4	$t_{1/2E}$	Summer / Winter / Spring (ϕ_E of 0.25) The values are (mins) 1.49 / 15.54 / 2.16 (ϕ_E of 0.01) The values are (mins) 37.24 / 380.89 / 54.12	X
5.3	**Conclusion**	Photolysis of Bromadiolone was particularly fast with 68% removal in the first 10 minutes of exposure. Complete photolysis was noted to have occurred by around 2 hours.	
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date Jan 2009

Materials and Methods Adopt applicant's version noting the following deviations.

3.4.4 The temperature should have been measured.

3.4.5 pH should have been measured according to the guideline and, following the instructions in the guideline, it should relate to pka of the substance.

Section A7.1.1.1.2 - Phototransformation in water including identity of transformation products.

Annex Point IIA7.6.2.2

Results and discussion	Adopt applicant's version noting the following deviations. 5.2.4 Half-life may be calculated using the following formula: $t_{1/2E} = Ln2/ K_{pE}$ and making the required correction for time unit. If this formula is used the corrected half-lives ($t_{1/2E}$, min)become as follows: Summer Winter Spring (ϕ_E of 0.25) 2.98 30.4 4.33 (ϕ_E of 0.01) 74.5 768 108.5 These values will be used in the CA report. In the test report the T½ values are divided by 2 assuming a 12 h day, but this is not considered relevant due to the very short half lives that measure in minutes.
Conclusion	Adopt applicant's version
Reliability	2 (lack of temperature and pH measurements render lower RI than 1)
Acceptability	acceptable Temperature and pH data have not been submitted in the revised version of the test report. However, irrespective of the result of this study, the impact of photodegradation in aquatic conditions will be considered negligible in the risk assessment.
Remarks	

Table A7_1_1_2-1: Description of test solution and controls

Criteria	**Details**
Purity of water	0.2 µm filtered deionised water
Preparation of test chemical solution	Bromadiolone was prepared as 204mg/l dosing solution in acetonitrile. 1 ml of the dosing solution was added to a 100ml volumetric flask and made to volume with 0.2µm filtered deionised water (2.04mg/l-0.00000387M)
Test concentrations	Replicate 1= 0.00501 mM Replicate 2= 0.00542 mM Replicate 3 = 0.00534 mM
Temperature (°C)	N/A
Preparation of actinometer solution	A stock solution of PNAP was prepared by making 0.165g to 100ml in acetonitrile (0.01M). An intermediate stock was prepared by diluting 10ml of this stock to 100ml with distilled water (0.001M). 17.40g of pyridine was weighed into a 100m volumetric flask and was partially filled with 0.2µm filtered deionised water. 1 ml of the intermediate PNAP stock was added and the flask made to volume with further deionised water.
Controls	Methanol was run as a control
Identity and concentration of co-solvent	N/A

Table A7_1_1_2-2: Description of test system

Criteria	**Details**
Laboratory equipment	Chromatography system: Perkin Elmer Quaternary System, HPLC gradient pump: Perkin Elmer Series 200 UV detector: Perkin Elmer 785 A UV/VIS @ 254nm (1.0V/AU) RI detector: Perkin Elmer LC-25 Interface box: 900 series and 600 Link series Computer: Boldfield Pii 350 Software: PE Nelson Turbochrom Workstation Auto sampler: Perkin Elmer Series 200 Analytical column: Phenomenex Luna, 5µm, C18, 250 by 4.6 mm. Mobile phase: Methanol distilled water, Acetic acid (850:142:8) Flow rate: 1.5ml/min Injection volume: 250µl
Test apparatus	The apparatus used for the actinometer was the same as the HPLC system used for the test substance. The only difference was that the Mobile phase involved Acetonitrile: distilled water: Acetic acid (500:475:25), the flow rate was 2.0ml/min and the analytical column was a Hypersil ODS.
Properties of artificial light source:	N/A
Nature of light source	N/A
Emission wavelenght spectrum	N/A
Light intensity	N/A
Filters	N/A
Properties of natural sunlight:	Natural light was used
Latitude	The quartz tubes were placed in sunlight inclined at angle of about 30°C with the tops facing magnetic north. The test site is located at latitude of 52° North
Hours of daylight	6 hours
Time of year	The test was set up at 12.00 on 31 March 2004.
Light intensity	N/A
Solar irradiance (L_λ)	Ranging from 1.09 E-01 to 3.61 E-06 [10^{-3} E cm^{-2} d^{-1}]

Table A7_1_1_2-3: Screening test results

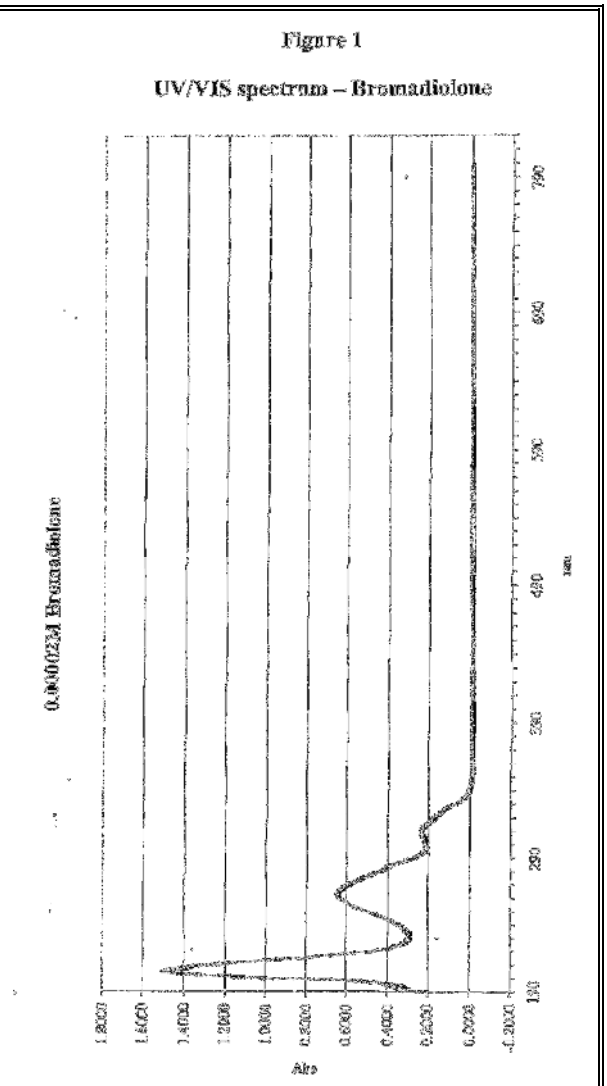
Absorption curve	
A_λ	N/A
ε_λ^c	N/A
$k_{pEmax\ (per\ day)}$	Winter = 130.87 Summer = 1340.42
$t_{1/2E\ (day)}$	Winter = 0.0053 Summer = 0.0005
L_λ	10^{-3} einsteins cm^{-2} day^{-1}

Table A7_1_1_2-4: Actinometer data

PNAP/ pyridine concentrations	Cumulative fraction of day	Concentration (mol) R1	R2	R3
	0.000	0.0000105	0.0000105	0.0000105
	0.012	0.0000097	0.0000096	0.0000096
	0.024	0.0000089	0.0000088	0.0000088
	0.035	0.0000081	0.0000080	0.0000081
	0.047	0.0000073	0.0000072	0.0000073
	0.059	0.0000064	0.0000063	0.0000065
	0.071	0.0000058	0.0000059	0.0000059
	0.141	0.0000027	0.0000027	0.0000027
	0.212	0.0000012	0.0000012	0.0000012
	0.282	0.0000006	0.0000006	0.0000006
	0.235	0.0000003	0.0000003	0.0000003
ϕ^a_E	0.037			
k^a_p	362 (At spring 50°N)			

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Szabolcs Gáty (2002) Draft report: Determination of Biodegradability of BROMADIOLONE TECHNICAL test item with Closed Bottle Test. Toxicological Research Centre Ltd. Report 01/617-322AH	X
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 301D	
2.2	**GLP**	Yes	
2.3	**Deviations**	The purity of the active substance 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	
3.1.3	Purity	99.4% bromadiolone	
3.1.4	Further relevant properties	Not applicable	
3.1.5	Composition of Product	Not Applicable	
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	None specified	
3.2	**Reference substance**	Yes – sodium acetate	
3.2.1	Initial concentration of reference substance	2mg/l i.e. limit of water solubility	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	see table A7_1_1_2-2	
3.3.2	Test system	see table A7_1_1_2-3	
3.3.3	Test conditions	see table A7_1_1_2-4	X

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

3.3.4	Method of preparation of test solution	No specific preparation
3.3.5	Initial TS concentration	TS = 2 mg/l and 8 mg/l
3.3.6	Duration of test	28 day
3.3.7	Analytical parameter	Oxygen concentrations (DOC)
3.3.8	Sampling	Start then every 7 days for 28 days
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Group C1: Salt solution Group C2: Salt solution and inoculum Group C3: salt solution, inoculum and 2mg/l Na acetate
3.3.12	Statistics	Calculations according to OECD Guideline 301 D
		4 RESULTS
4.1	**Degradation of test substance**	Non-entry field
4.1.1	Table	**Table of % biodegradability**

	Day			
Test Group	**7**	**14**	**21**	**28**
A1	7.69	8.62	8.92	9.22
A2	25.83	27.06	28.29	30.75
C3	73.67	83.28	92.89	96.09

4.1.2	Degradation	No plateau observed At the end of incubation 9.22 % degradation at 8mg/l and 30.75% at 2mg/l After 7-d window degradation was 7.69% at 8mg/l and 25.83% at 2mg/l
4.1.3	Other observations	No inhibition at limit of water solubility
4.1.4	Degradation of TS in abiotic control	No abiotic control with TS
4.1.5	Degradation of reference substance	96.09% degradation after 28 days and 73.67% degradation after 7 days
4.1.6	Intermediates/ degradation products	No intermediate or degradation product identified

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was conducted according to OECD 301. The experimental solutions were inoculated by a small number of microorganisms originated from an activated sludge plant. Two nominal concentrations levels, 2 and 8 mg/l, of the test item and 4 control groups (mineral medium without inoculum, mineral medium with inoculum, minderal medium with reference compound and inoculum) were examined in this study. Groups of parallel bottles were prepared for the determination of BOD of the test and control items in simultaneous test groups. The experimental solution was inoculated with 500µl of inoculum per litre of final volume, and the blank was inoculated similarly. The solutions were made up to volume with a hose which reached down to the bottom of the flask to achieve adequate mixing. Subsequently each prepared solutions were filled immediately into the respective group of bottles by hose from the lower quarter of the bottle. Zero time bottles were analysed fro dissolved oxygen. The remaining parallels were placed into an incubator and kept at 20°c, in the dark, and removed after 7, 14, 21 and 28 days from the incubator and analysed. Oxygen content was determined electrometrically in all bottles on days 0, 7, 15, 21 and 28.	
5.2	**Results and discussion**	The mean value of the degradation (expressed as specific BOD in the percentage of ThOD), in 8 mg/l nominal concentration of the test item was 9.22% and in the 2 mg/l nominal concentration of the test item was 30.75%. The mean value of the degradation in the 2 mg/l nominal concentration of the reference item was 96.09%	
5.3	**Conclusion**	No inhibitory effect of the Test Item was detectable on the micro-organism test system used according to the experimental data.	X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *2006-03-13*

Materials and Methods

Adopt applicant's version noting the following deviations.

5.4 The only report submitted by the applicant is a draft report; it needs to be completed with the final version of the report.

7.3.3 There are deviations between the test solution used and the test solution described in the OECD guideline, pH should have been measured.

Table A7_1_1_2-6: These results have not been shown in any report.

Results and discussion

Adopt applicant's version noting the following deviations.

8.1.3 This sentence needs to be clarified. Inhibition of what?

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

Conclusion	Adopt applicant's version noting the following deviations. 9.3 The test is performed to show if a substance is ready biodegradable, this is not stated in the conclusion. Bromadiolone is not ready biodegradable according to the test. To be classified as biodegradable 60 % should have been degraded after 28 days. Moreover the applicant states that bromadiolone have no inhibitory effect on micro-organisms, the question then is why there was a difference in biodegradability rate between the two concentrations of bromadiolone.
Reliability	2
Acceptability	acceptable After revision of the document according to the comments above.
Remarks	The applicant has responded in mail that this report is identical with the final report. The other comments are accepted by the applicant. The applicant also states that there is an inhibitory effect on micro-organisms at 8mg/L.

Table A7_1_1_2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not stated
Strain	Not stated
Source	Activated sludge plant for domestic sewage in Veszprém

Sampling site	Activated sludge plant for domestic sewage in Veszprém
Laboratory culture	No - activated sludge plant for domestic sewage
Method of cultivation	Uncultivated
Preparation of inoculum for exposure	Secondary effluent form domestic sewage filtered through a coarse filter with first 200 ml discarded.
Pretreatment	Inoculum kept aerobic until used
Initial cell concentration	500 µl per litre of final volume.

Table A7_1_1_2-3: Test system

Criteria	**Details**
Culturing apparatus	Incubator
Number of culture flasks/concentration	2
Aeration device	Compressed air
Measuring equipment	Not stated
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	**Details**
Composition of medium	Solution 1 KH_2PO_4 – 2.5 g; K_2HPO_4 – 10.88 g; NA_2HPO_4 x $12H_2O$ – 33.60 g; NH_4Cl – 0.25 g Solution 2 $CaCl_2$ x $2H_2O$ – 18.20 g Solution 3 $MgSO_4$ x $7H_2O$ – 11.25 g Solution 4 $FeCl_3$ x $6H_2O$ – 0.125 g
Additional substrate	No
Test temperature	19.7 – 20.2ºC
pH	Not measured
Aeration of dilution water	Mineral solution aerated with compressed air
Suspended solids concentration	Not stated
Other relevant criteria	-

Table A7_1_1_2-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	**not fulfilled**

Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	Yes	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	Yes	
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Yes	
Percentage of removal of reference substance reaches pass level by day 14	Yes	

Table A7_1_1_2-6: Pass levels and validity criteria for inherent biodegradability tests

	fulfilled	**not fulfilled**
Pass levels		
20% removal (DOC or COD);		
Pass values reached within 10-d window (within 28-d test period)	Yes	
Removal of reference substance (DOC or COD) > 70 % within 14 d	Yes	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)	Yes	
Percentage of DOC-removal of reference compound ≥ 40 % within 7 days and ≥ 65 % within 14 days Average residual amount of test compound in blank tests ≥ 40 % (OECD 302 C)		
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)		

Section A7.1.1.2.2 - Biodegradability (inherent)

Annex Point IIA7.6.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake RM (2005) Determination of the inherent biodegradability of Bromadiolone, Chemex Environmental International Limited, Chemex Reference no: ENV6988/080319	
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	
1.2.2	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**	Yes, OECD 302D	X
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	02478	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	97.9%	
3.1.4	Further relevant properties	n.a	
3.1.5	Composition of Product	n.a	
3.1.6	TS inhibitory to microorganisms	Yes	
3.1.7	Specific chemical analysis	Not specified	
3.2	**Reference substance**	Yes, Hexadecane	
3.2.1	Initial concentration of reference substance	2.6 mg	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	*(see table A7_1_1_2-2)*	
3.3.2	Test system	*(see table A7_1_1_2-3)*	X
3.3.3	Test conditions	*(see table A7_1_1_2-4)*	

Section A7.1.1.2.2 - Biodegradability (inherent)

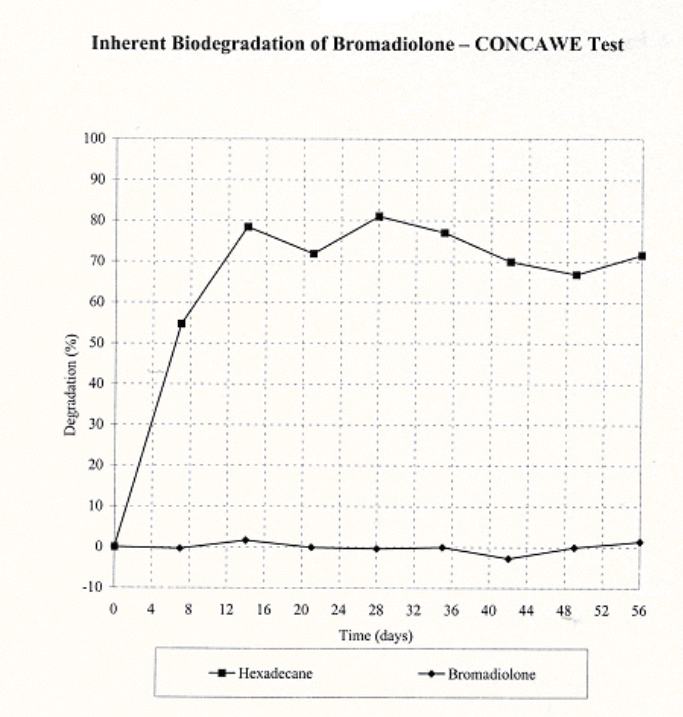
Annex Point IIA7.6.1.2

3.3.4	Method of preparation of test solution	Bromadiolone was weighed onto a microscope cover slip which was then introduced directly into a 21 conical flask containing 1000ml of inoculated mineral medium and yeast extract.	
3.3.5	Initial TS concentration	Quantity of Bromadiolone (day 0)	5.9mg/l
		Quantity of Bromadiolone added (day 7)	11.7mg/l
		Quantity of Bromadiolone added (day 11)	10.1 mg/l
3.3.6	Duration of test	56 days	
3.3.7	Analytical parameter	CO_2 evolution	
3.3.8	Sampling	Once every week, i.e days 7, 14, 21, 28, 35, 42, 49, 56	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	Yes, inoculum medium only	
3.3.12	Statistics	None performed	

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph



X

4.1.2 Degradation 1% biodegradation

4.1.3 Other observations

Section A7.1.1.2.2 - Biodegradability (inherent)

Annex Point IIA7.6.1.2

4.1.4	Degradation of TS in abiotic control	0.25mg produced, similar to the test material (0.23)	
4.1.5	Degradation of reference substance	See above	
4.1.6	Intermediates/ degradation products	Not determined	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was conducted according to OECD 302D guidelines. The test uses a composite microbial inoculum, derived from soil and a wastewater treatment plant that has been pre-exposed to the test substance. The test substance was incubated in a buffered, mineral salts medium which had been inoculated with a mixed population of micro-organisms. In order to enhance the biodegradative potential the inoculum, it is pre-exposed to the test substance for a period of 14 days. The test was performed in sealed bottles with a headspace of air that provided a reservoir of oxygen for aerobic biodegradation. CO_2 evolution from the ultimate aerobic biodegradation of the test substance is determined by measuring the inorganic carbon produced in the test bottles over that produced in blanks which only contained inoculated medium only. The final aerobic biodegradation is the breakdown of an organic chemical by micro-organisms in the presence of O_2 resulting in the production of CO_2, water and mineral salts and microbial cellular constituents. The extent of biodegradation was then expressed as a percentage of the theoretical maximum IC production (ThIC), based on the quantity of test substance added initially. CO_2 production in the bottles was determined by measuring the increase in the concentration of inorganic carbon. 1ml of 7M sodium hydroxide was injected through the septa of each bottle sampled which were then shaken for one hour at the test temperature and allowed to settle. Each bottle was opened and two 30ml samples taken for IC analysis.	
5.2	**Results and discussion**	Bromadiolone failed to meet the requirements for a pass in the test (20% degradation relative to the ThIC value) with a maximum of 2% recorded on day 14). The test was valid, because the mean percentage biodegradation of hexadecane reached 60% by the end of the test. A value of 72% was recorded. The mean amount of IC produced from the blanks at the end of the test was 15% of the organic carbon added initially as the test substance (15% of 20mg/l C-3mg). A value of 2.5 mg/l C was recorded.	
5.3	**Conclusion**	There was 1% biodegradation of Bromadiolone in the inherent test.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities
EVALUATION BY RAPPORTEUR MEMBER STATE

Section A7.1.1.2.2 - Biodegradability (inherent)

Annex Point IIA7.6.1.2

Date	*2006-03-04*
Materials and Methods	Adopt applicant's version noting the following deviations. 2.1 It is a proposal for a new guideline (draft). 3.3.2 In the draft guideline it is recommended that at least five replicates are used, in this investigation only two were used. However, since no biodegradation was found, this is of minor importance. The composition of the mineral medium is not given in the report.
Results and discussion	Adopt applicant's version noting the following deviations. 4.1.1 Since two replicates were used, standard deviations should be presented, to verify the validity of the test results.
Conclusion	Adopt applicant's version noting the following deviations. There was no biodegradation of bromadiolone in the inherent test. This should be stated since apparently biodegradability varies around 0%.
Reliability	1
Acceptability	acceptable
Remarks	

Section A7.1.1.2.3 - Biodegradation in seawater

Annex Point *IIIA XII.2.1*

	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:	Product is not used such that seawater can be contaminated in significant amounts.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	The amount of used of bromadiolone, and associated products, are so low that concentrations of these products eventually reaching seawater will be neglectible.
Conclusion	The justification presented by the applicant is acceptable.
Remarks	

Section A7.1.2.1.1 - Aerobic biodegradation

Annex Point *IIIA XI.2.1*

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Compound is of low water solubility and shown to be negligible biodegradability in ready biodegradation study. Normal rodenticide use practice is to remove product residues in order to minimise possibility of ingestion by non-target organisms. As a study into the determination of abiotic degradation and hydrolysis as a function of pH has been conducted (Section A7.1.1.1.1, Annex Point IIA VII.7.6.2.1), an aerobic biodegradadtion study is not required.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	The applicant points out that bromadiolone do not biodegrade abiotically; this can than be taken into consideration during the risk assessment process. However, it is stated in the guidance on data requirements for active substances and biocidal products page 103, point 7.1.2.1.1 that this test is required if the biocide enters a sewage treatment plant before release to the environment,. Therefore, this test should be conducted.
Conclusion	Justification is not acceptable since the biocide might enter sewage treatment plants before release into the environment.
Remarks	*Have asked for explanation or improved justification*

Section A7.1.2.1.2 - Anaerobic biodegradation

Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake RM (2005) Determination of the anaerobic biodegradability of Bromadiolone, Chemex Environmental International Ltd, Study report: ENV6989/110414	
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.	
1.2.2	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**	Yes, ISO 11734 and Method 3 of ECETOC report number 28	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Bromadiolone	
3.1.1	Lot/Batch number	02478	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.5%	
3.1.4	Further relevant properties	Not stated	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Yes	
3.1.7	Specific chemical analysis	Not stated	
3.2	**Reference substance**	Yes	X
3.2.1	Initial concentration of reference substance	2.4035	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	*(see table A7_1_2_1_2-1)*	
3.3.2	Test system	*(see table A7_1_2_1_2-2)*	

Section A7.1.2.1.2 - Anaerobic biodegradation

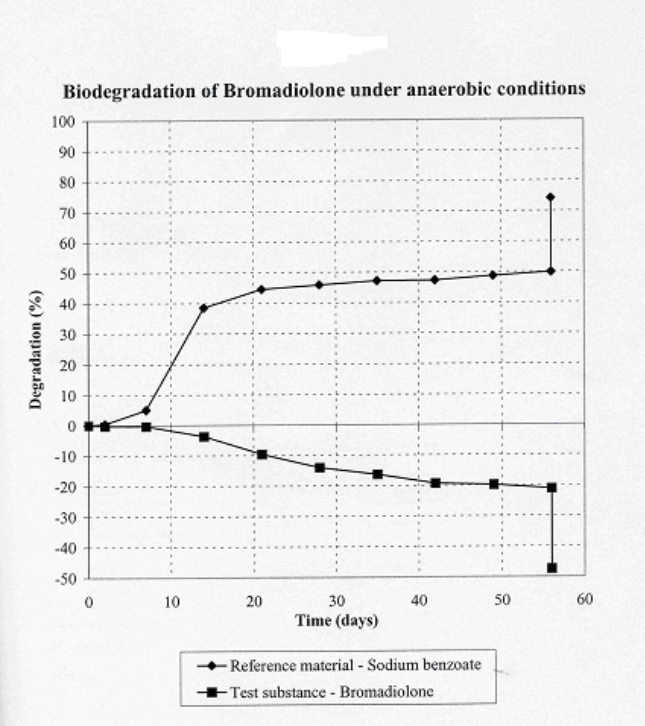
Annex Point IIIA XII 2.1

3.3.3	Test conditions	*(see table A7_1_2_1_2-3)*			X
3.3.4	Method of preparation of test solution	Not stated			
3.3.5	Initial TS concentration	10.5, 12.1, 11.2 mg sample added			
3.3.6	Duration of test	56 days			
3.3.7	Analytical parameter	CO_2 evolution			
3.3.8	Sampling	Samples were taken on day 2, 7, 14, 21, 28, 35, 42, 49 and 56			
3.3.9	Intermediates/ degradation products	Not identified			
3.3.10	Controls	Yes			
3.3.11	Statistics	Not stated			
		4 RESULTS			
4.1	**Degradation of test substance**				
4.1.1	Degradation of TS in abiotic control	-52%			
4.1.2	Degradation	Time (days)	Biodegradation (%)		*X*
			Reference material	Test substance	
		2	0	0	
		7	5	0	
		14	39	-4	
		21	45	-11	
		28	47	-15	
		35	48	-18	
		42	48	-21	
		49	49	-22	
		56	51	-23	
		Standard deviation was not carried out since only 3 replicates were used, whereas the guideline states standard deviations require at least 4 replicates			

Section A7.1.2.1.2 - Anaerobic biodegradation

Annex Point IIIA XII 2.1

4.1.3 Graph



4.1.4	Other observations	
4.1.5	Degradation of reference substance	See above
4.1.6	Intermediates/ degradation products	n.a

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A known volume of anaerobic sludge (corresponding to 10% of the sludge concentration in a real digester) suspended in an oxygen free medium was placed in a suitable vessel leaving headspace into which any gases produced may be evolved. Prior to sealing a small amount of test compound was added.

The vessels were incubated at a constant temperature (35 ± 1°C) and a pH for a period of 8 weeks. The headspace pressure, resulting from the production of gas, was measured. From the measured values of net gas production the extent of biodegradation was calculated. The kinetics of the degradation were followed by intermediate measurements at suitable intervals during the course of the test.

Section A7.1.2.1.2 - Anaerobic biodegradation

Annex Point IIIA XII 2.1

5.2	**Results and discussion**	Bromadiolone gave a negative result (less than 60% biodegradation based on biogas production) with a maximum value of 0% recorded. The final degradation value recorded (-52% at day 56) suggests that Bromadiolone was inhibitory to the micro-organisms. A reference material, sodium benzoate, was concurrently tested and showed biodegradation of 75% suggesting that the inoculum was viable. At the end of the test period, dissolved inorganic carbon was determined and this was added to the carbon derive from gas pressure measurements. The dissolved inorganic carbon content of the blanks was higher than that in the samples giving a lower final degradation value. All day 56 pH values were recorded in the study (pages 10-11).	X
5.3	**Conclusion**		X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2009
Materials and Methods	Adopt applicant's version noting the following deviations. 3.2 State which reference substance that have been used. 3.3.3 The temperature should have been constant and around 35°C, therefore the temperature should have been measured, at intervals.
Results and discussion	Adopt applicant's version noting the following deviations. 4.1.2 Since there were three replicates in the test standard deviations should be given in the results.
Conclusion	Adopt applicant's version noting the following deviations. 5.2 pH was recorded at the end of the test and the values are given on pages 9 and 10 in the report. All pH values lie between pH 6.5 and 6.8. 5.3 Bromadiolone was not anaerobically biodegradable.
Reliability	1
Acceptability	acceptable /
Remarks	

Table A7_1_2_1_2-1: Inoculum / Test organism

Criteria	**Details**
Nature	Primary digesting sludge
Species	Not stated
Strain	Not stated
Source	Cambridge Sewage treatment works
Sampling site	Not stated
Laboratory culture	Yes
Method of cultivation	Not stated
Preparation of inoculum for exposure	Not stated
Pretreatment	The sludge was passed through a 2000 and 500 µm sieves and then centrifuged at 3000rpm for approximately 5 minutes. The sludge was resuspended and centrifuged twice. The sludge was transferred to a 2L conical flask and placed in water bath at 35°C and a stream of nitrogen was bubbled through the sludge. This was left for two weeks. On the day of the test, the sludge was centrifuged and a sub-sample was taken for dry solids determination.
Initial cell concentration	% dry solids – 15.2

Table A7_1_2_1_2-2: Test system

Criteria	Details
Culturing apparatus	Vessel
Number of replicates/concentration	3
Measuring equipment	Pressure transducer
Oxidation reduction indicator	No

Table A7_1_2_1_2-3: Test conditions

Criteria	Details		
Composition of medium	92.28g of wet sludge was suspended in 2 litres of mineral medium. 100ml of this suspension was added to each bottle, 40ml of mineral medium (without sludge) was added to make up the final volume.		
Additional substrate	No		
Solvent	Not stated		
Preparation of medium	Not stated		
Test temperature	Day	A	B
	2	35	308.2
	7	-	-
	14	35	308.2
	21	35	308.2
	28	35	308.2
	35	35	308.2
	42	35	308.2
	49	35	308.2
	56	35	308.2
pH			
Suspended solids concentration	% dry sludge solids: 15.2% Dry sludge solids in test: 5.0 g/l		
Other relevant citeria			

Section A7.1.2.2.1 - Aerobic aquatic degradation study

Annex Point *IIIA XII.2.1*

	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:	Limited contamination of water possible due to mode of use, and low water solubility, hence therefore the test study is scientifically unjustified.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*2006-03-13*	
Evaluation of applicant's justification	The applicant states that the exposure in freshwater will be low, this must be considered since bromadiolone have a low water solubility and most likely will end up in the sediments, if it reaches fresh water.	
Conclusion	Acceptable	
Remarks		

Section A7.1.2.2.2 - Water/sediment degradation study

Annex Point *IIIA XII.2.1*

	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:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	Bromadiolone has low water solubility and will end up in sediments or in the organic layer in soils where it will be exposed to anaerobic conditions. When this is a fact a water/sediment degradation study should be conducted according to TNsG page 104 point 7.1.2.2.2. However according to the emission scenario documents the exposure will be very limited and local, therefore the justification is supported.
Conclusion	Acceptable
Remarks	

Section A7.1.3 - Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	O'Connor B.J and Woolley S.M. (2007) Bromadiolone: Determination of Adsorption Coefficient, SafePharm Laboratories Ltd., SPL Project Number: 2073/0005.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bromadiolone task force	
1.2.2	Companies with letters of access	None	
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 guidelines no. 106	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Due to test material instability under certain conditions, the contact time was reduced to 30 minutes for 2 of the soil types, see section 3.5.2.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Bromadiolone	
3.1.1	Lot/Batch number	L22678	
3.1.2	Specification	As given in section 2.7 (Appendix XI confidential information)	
3.1.3	Purity	99.9 %	
3.1.4	Further relevant properties	None stated	
3.1.5	Method of analysis	Solid phase extraction (SPE) of aqueous phases followed by HPLC separation with UV detection at 210 nm	
3.2	**Degradation products**	No	
3.2.1	Method of analysis for degradation products	N/A	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	N/A	
3.4	**Soil types**	See table A7_1 _3-1	
3.5	**Testing procedure**		

Section A7.1.3 - Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

3.5.1	Test system	Aliquots of soil and 0.01 M calcium chloride solution were taken in FEP/ETFE centrifuge tubes. Aliquots of 0.01 M calcium chloride solution required for soil-less controls and solvent blanks were also taken in test vessels.
3.5.2	Test solution and Test conditions	Preparation of stock solution (soil type 4): An aliquot of test material (0.0503 g) was dissolved in 50 mL of methanol. The resulting solution was then diluted further using methanol to generate the required stock solution. Preparation of stock solution (soil type 7): An aliquot of test material (0.0505 g) was dissolved in 50 mL of methanol. The resulting solution was then diluted further using methanol to generate the required stock solution. Preparation of stock solution (soil types 2, 3 and 5): An aliquot of test material (0.0528 g) was dissolved in 50 mL of methanol. The resulting solution was then diluted further using methanol to generate the required stock solution. Each stock solution resulted in a nominal sample concentration of 0.050 mg/l (less than half saturation water solubility) when 35 µL was spiked into 35 mL of aqueous phase, maintaining a stock solution addition below 10% v/v and a final co-solvent content below 0.01% v/v. For soil types 4 and 7, the samples at a soil to solution ratio of 1:100 were equilibrated at 25 ± 2 °C for a reduced adsorption period of 0.5 hours due to the instability of the test material at acidic pH's (procedure based on guidance document SCP/KOC/002). For soil types 2, 3 and 5, the samples at a soil to solution ratio of 1:50 were equilibrated at 25 ± 2 °C for a period of 4 hours, demonstrated to be sufficient for adsorption equilibrium to be achieved.
3.6	**Test performance**	
3.6.1	Preliminary test	According to (a)"OECD 106": Not performed
3.6.2	Screening test: Adsorption	According to (a)"OECD 106": Yes, except for soil types 2 and 7 for which a 30 minute contact time was used.
3.6.3	Screening test: Desorption	According to (a)"OECD 106": Not performed
3.6.4	HPLC-method	According to (a)" OECD-HPLC-method"[1]: Not performed
3.6.5	Other test	None
		4 RESULTS
4.1	**Preliminary test**	N/A
4.2	**Screening test: Adsorption**	See table A7_1_3-3

[1] OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{OC}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

Section A7.1.3 - Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

4.3 Screening test: Desorption N/A

4.4 Calculations

4.4.1 Ka , Kd — Distribution coefficient, Kd:

Soil Type	2	3	4	5	7
K (cm^3/g)	71.2	113	1250	153	>1190

4.4.2 Ka_{oc} , Kd_{oc} — Normalised adsorption coefficient, Kd_{oc}:

Soil Type	2	3	4	5	7
Ka_{oc} (cm^3/g)	3750	3530	41600	10200	>10400

4.5 Degradation product(s) Not assessed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods The test was performed according to OECD guideline no 106. Soil contact times were reduced to 30 minutes for 2 soils due to measured sample instability (attributed to acidic pH).

5.2 Results and discussion See tables below.

5.2.1 Adsorbed a.s. [%] N/A

5.2.2 K_a — Distribution coefficient, Kd:

Soil Type	2	3	4	5	7
K (cm^3/g)	71.2	113	1250	153	>1190

5.2.3 K_d N/A

5.2.4 Ka_{oc} — Normalised adsorption coefficient, Kd_{oc}:

Soil Type	2	3	4	5	7
Ka_{oc} (cm^3/g)	3750	3530	41600	10200	>10400

5.2.5 Ka/Kd N/A

5.2.6 Degradation products (% of a.s.) N/A

5.3 Conclusion Validity criteria are fulfilled. The overall Koc range was 3.53E03 (i.e. 3530) to at least 4.16E04 (i.e. 41600) which indicates that bromadiolone is slightly mobile to non mobile in soil, based on the SSLRC mobility classification which states that a Koc of 1000-4000 is slightly mobile and

Section A7.1.3 - Adsorption / Desorption screening test

Annex Point IIA, VII.7.7

		> 4000 is non mobile. Given the closeness to the top of the 1000-4000 range it can be concluded that mobility through soil would be predicted to either be very slow or non-existant.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Feb -09
Materials and Methods	Adopt applicant's version.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	no

Table A7_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

Appendix 2 Soil Classification and Characteristics

Parameter	Soil Type				
	2	3	4	5	7
Particle size:					
53 µm to 2 mm	37.56%	35.88%	55.72%	71.78%	85.23%
2 µm to 53 µm	29.37%	46.98%	26.81%	18.90%	11.94%
<2 µm	33.08%	17.14%	17.47%	9.32%	2.83%
pH, 1:5 soil to 0.01 M $CaCl_2$ ratio	7.5	6.4	4.5	6.4	4.2
Cation exchange capacity (mmol+/kg)	18.2	15.4	15.8	8.9	15.4
Organic carbon content (%)	1.9	3.2	3.0	1.5	11.4
Total nitrogen (mg/kg)	1693.9	1749.8	2869.7	1231.8	4367.9

Table A7_1 _3-2: Results of preliminary test:

Test substance	N/A
Sample purity	N/A
Weighed soil	N/A
Volume of $CaCl_2$ solution	N/A
Nominal concentration of a.s. final solution	N/A
Analytical concentration final of a.s. solution	N/A

Concentration of the test solution (show calculation)	N/A
Details of the analytical method used:	N/A
Method	N/A
Recovery rate	N/A
Detection limit	N/A

Table A7_1 _3-3: Results of screening test - adsorption:

	Soil 2		**Soil 3**		**Soil 4**		**Soil 5**		**Soil 7**	
Contact time with soil (hours)	4	4	4	4	0.5	0.5	4	4	0.5	0.5
Concentration of test material [mg/l]	2.36×10^{-2}	2.18×10^{-2}	-	1.72×10^{-2}	4.20×10^{-2}	4.76×10^{-2}	1.49×10^{-2}	1.18×10^{-2}	$<5.0 \times 10^{-3}$	$<5.0 \times 10^{-3}$
Correction for blank with soil	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected
Correction for blank without soil	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected	None detected
Final corrected concentration [mg/l]	2.36×10^{-2}	2.18×10^{-2}	-	1.72×10^{-2}	4.20×10^{-3}	4.76×10^{-3}	1.49×10^{-2}	1.18×10^{-2}	$<5.0 \times 10^{-3}$	$<5.0 \times 10^{-3}$
Initial concentration of test solution, nominal [mg/l]	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}	5.00×10^{-2}
Decrease in concentration [mg/l]	See % data	See % data	See % data	See % data	See % data	See % data	See % data	See % data	See % data	See % data
Quantity adsorbed [µg]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Quantity of soil [g of oven-dried equivalent]	0.6534	0.6557	-	0.6420	0.2797	0.2984	0.6820	0.6939	0.2638	0.2685
Test material adsorbed [%]	55.3	58.8	-	67.4	91.6	90.5	71.8	77.6	>90.1	>90.1
Temperature [°C]	25	25	25	25	25	25	25	25	25	25
Volume of solution recovered after centrifugation [ml]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Volume of solution not recovered [ml]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Corresponding quantity of test substance [mg]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Section A7.1.4.1 - Field study on accumulation in the sediment

Annex Point *IIIA XII.2.1*

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	X
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	It is not clarified if this study is scientifically unjustified since the water/sediment study not has been conducted. However, the study is necessary if the mineralisation of the substance is less than 5 % after 100 days, this is unlikely. In a close bottle test concentration of bromadiolone was reduced by 30 % after 28 days. Moreover, the concentrations reaching the sediments are low and only local pollution is likely to occur.
Conclusion	Acceptable
Remarks	

Section A7.2.1 - Aerobic degradation in soil, initial study

Annex Point IIIA VII.4, XII.1.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x]** **Scientifically unjustified [x]**	X
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	Although bromadiolone not degrades readily or inherently, the exposure for the soil compartment by bromadiolone will be low and photolysis of the substance will occur at the soil surface.
Conclusion	Acceptable
Remarks	

Section A7.2.2.1 - The rate and route of degradation including identification of the processes involved and identification of any metabolites and degradation products in at least three soil types under appropriate conditions.

Annex Point IIIA VII.4, XII.1.1, XII.1.4

	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:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	The applicant's reasons for justification are not supported. However, the other studies conducted on degradation have not shown the presence of any metabolites and it should be considered that the effects are local and the amount used of the substance is low.
Conclusion	Acceptable
Remarks	

Section A7.2.2.2 - Field soil dissipation and accumulation

Annex Point *IIIA XII.1.1*

	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:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	The applicants reasons for justification are not supported. However, the studies conducted above have shown that although the substance might accumulate in soil the effects are local and the amount used of the substance is low.
Conclusion	Acceptable
Remarks	

Section A7.2.2.3 - Extent and nature of bound residues

Annex Point *IIIA XII.1.4*

	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:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*2006-03-13*	
Evaluation of applicant's justification	As the applicant states the use is limited and the product is not applied to extensive areas therefore it is justified to not supply data.	
Conclusion	Acceptable	
Remarks		

Section A7.2.2.4 - Other soil degradation studies

Annex Point *IIIA XII.1.1*

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x]** **Scientifically unjustified [x]**	X
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	We agree with that the exposure to the substance will be limited and local.
Conclusion	Acceptable
Remarks	

Section A7.2.3.1 - Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant, adsorption and desorption of metabolites and degradation products

Annex Point *IIIA XII.1.2*

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x]** **Scientifically unjustified [x]**	X
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* New Study in progress at Chemex. Report expected 24-01-2005	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	~~*2006-03-13*~~ *2010-05-26*	
Evaluation of applicant's justification	CA do not accept the justification by stated by the applicant. The study is technically feasible and it depends on the outcome of the aerobic degradation test (7.2.1) if this or test 7.2.3.2 have to be conducted.	
Conclusion	~~Study might have to be conducted.~~ A study need not be performed at this stage. An acceptable adsorption/desorption study according to OECD 106 has been conducted, see Section 7.1.3.	
Remarks		

Section A7.2.3.2 - Mobility in at least three soil types and where relevant mobility of metabolites and degradation products.

Annex Point *IIIA XII.1.3*

	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:	Study not considered feasible due to low water solubility of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Plants are not sprayed with rodenticides.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*2006-03-13*	
Evaluation of applicant's justification	The applicant correctly states that the use of the substance is limited and local.	
Conclusion	The Koc value is 3530-41600, and hence there is no risk for contamination of the ground water.	
Remarks		

Section A7.3.1 - Phototransformation in air (estimation method), including identification of breakdown products

Annex Point *IIIA VII.5*

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	**Variation:** Photodegradation characteristics of the active substance have been estimated using the EPIWIN v 3.12 programme. The indirect photolysis half-life of of Bromadiolone with OH radicals is 2.090 hours (rate const. = 61.4217 x 10^{-12}cm^3/molecule/sec) and 2.015 hours (rate const. = 13.650000 x 10^{-17} cm^3/molecule/sec) with ozone. ***Atmospheric risk:*** Bromadiolone has a low volatility and emissions to the air compartment are expected to be low ***Global warming:*** Bromadiolone shows no absorption in the so-called atmospheric window (800-1200 nm) and therefore, according to the TGD on risk assessment (Part II, Section 3.7.2) is not a potential greenhouse gas. ***Stratospheric ozone:*** According to the TGD on risk assessment (Part II, Section 3.7.2) ozone depletion potential values approach zero for molecules with atmospheric halftimes less than one year. Bromadiolone has an estimated half-life of approximately 2 hours, therefore is predicted to have no effect on stratospheric ozone. ***Tropospheric ozone:*** According to the TGD on risk assessment (Part II, Section 3.7.2) there is at present no procedure available to estimate the effect on tropospheric ozone if only the basic characteristics of a substance are known. (Bromadiolone has a tropospheric half-life of approximately 2 hours). ***Acidification:*** Oxidation of Bromadiolone does not cause the formation of nitrogen containing oxides, and due to the low expected emissions to the air compartment, it is not expected that Bromadiolone will have an effect on acidification of the receiving soil or surface water. Calculation for this study: see 'references'. EPIWIN v 3.12 programme, calculation of BCF factor.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-13*
Evaluation of applicant's justification	Bromadiolone has a low volatility and is used in limited amounts. Moreover the justification shows that no negative effects are expected from the substance.
Conclusion	Acceptable
Remarks	

Section A7.3.2 - Fate and behaviour in air, further studies

Annex Point *IIIA XII.3*

	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:	Study not considered feasible due to low vapour pressure of the compound, rapid photolysis and highly limited / localised nature of use. Soil is not treated directly and the product is not applied to extensive areas. Compound is stable in air and has low v.p. Plants are not sprayed with rodenticides.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*2006-03-13*	
Evaluation of applicant's justification	CA agrees with the applicant's justification.	
Conclusion	Acceptable	
Remarks		

Section A7.4.1.1 - Acute toxicity to fish

Annex Point IIA7.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX, 2007, Bromadiolone Fish (rainbow trout), acute toxicity test, semi-static, 69h, XXXXX, Study Nº FAR113101	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Bromadiolone task force	
1.2.2	Companies with letters of access	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 203	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	L22678	
3.1.2	Specification	As given in section 2	X
3.1.3	Purity	99.9%	
3.1.4	Composition of Product	n/a	
3.1.5	Further relevant properties	Water solubility <0.5 mg/L at 20°C	
3.1.6	Method of analysis	Test material concentration and control groups were analytically verified at 0, 24, 48 and 72 h from freshly prepared media and at 24, 48, 72 and 96h from 24h old media via HPLC with a diode array detector (DAD)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_1-2	X
3.4.2	Test organisms	See table A7_4_1_1-3	X

Section A7.4.1.1 - Acute toxicity to fish

Annex Point IIA7.1

3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 h
3.4.6	Test parameter	Mortality
3.4.7	Sampling	Analytical samples taken at 0, 24, 48 and 72 h from freshly prepared media and at 24, 48, 72 and 96h from 24h old media
3.4.8	Monitoring of TS concentration	Yes
3.4.9	Statistics	The LC_{50} values after 24, 48, 72 and 96h were calculated by sigmoidal dose response regression. Confidence intervals were calculated with standard procedures according to Clopper and Pearson (1934).

4 RESULTS

If appropriate, include tables. Sample tables are given below

4.1 Limit Test Performed

4.1.1 Concentration 0, 1, 10 mg/L

4.1.2 Number/ percentage of animals showing adverse effects

Cumulative mortality

Nominal test concentration mg/L	Test duration (h)			
	24	48	72	96
10	100	-	-	-
1	0	0	0	0
0	0	0	0	0

4.1.3 Nature of adverse effects Mortality

4.2 Results test substance

4.2.1 Initial concentrations of test substance 1, 1.78, 3.16, 5.62, 10 mg/L (nominal)

4.2.2 Actual concentrations of test substance X

	New medium (day 0)	Old medium (day 1)	New medium (day 1)
Nominal concentration mg/L	Measured concentration mg/L	Measured concentration mg/L	Measured concentration mg/L
10	9.76	9.85	-
5.62	5.47	5.60	5.61
3.61	3.12	3.12	3.14

Section A7.4.1.1 - Acute toxicity to fish

Annex Point IIA7.1

1.78	1.77	1.81	1.80
1.0	0.997	1.02	1.02
Solvent control	<LOQ	<LOQ	<LOQ
Control	<LOQ	<LOQ	<LOQ

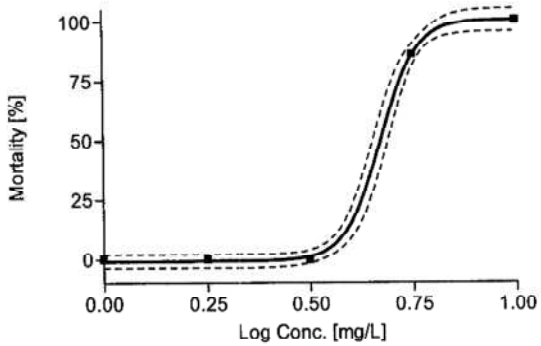
	New medium (day 2)	Old medium (day 2)	New medium (day 3)
Nominal concentration mg/L	Measured concentration mg/L	Measured concentration mg/L	Measured concentration mg/L
10	-*	-*	-*
5.62	5.45	-*	-*
3.61	3.05	3.02	3.01
1.78	1.75	1.74	1.76
1.0	1.00	0.98	0.998
Solvent control	<LOQ	<LOQ	<LOQ
Control	<LOQ	<LOQ	<LOQ

	New medium (day 3)	Old medium (day 4)
Nominal concentration mg/L	Measured concentration mg/L	Measured concentration mg/L
10	-*	-*
5.62	-*	-*
3.61	3.03	3.00
1.78	1.71	1.72
1.0	1.71	0.974
Solvent control	<LOQ	<LOQ
Control	<LOQ	<LOQ

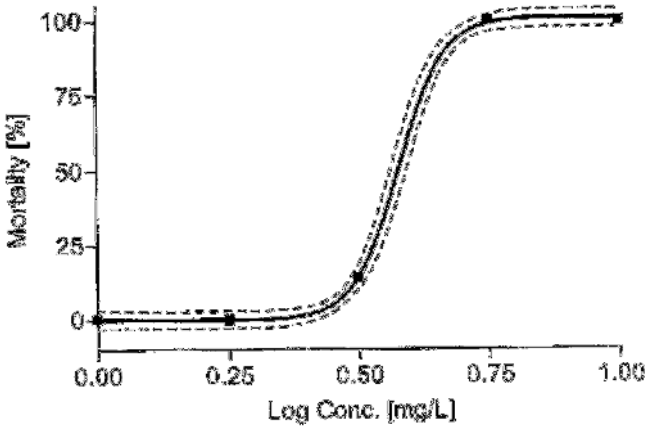
* No analytical determination was carried out due to 100% mortality

4.2.3 Effect data (Mortality) See table A7_4_1_1-7

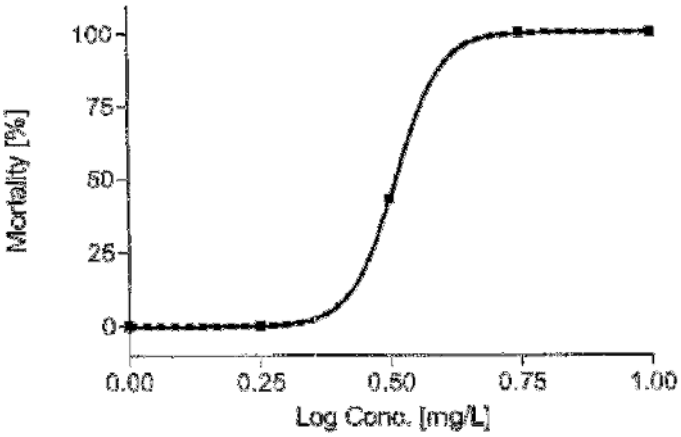
4.2.4	Concentration / response curve	Concentration effect relationship after 24h	X



Concentration effect relationship after 48h



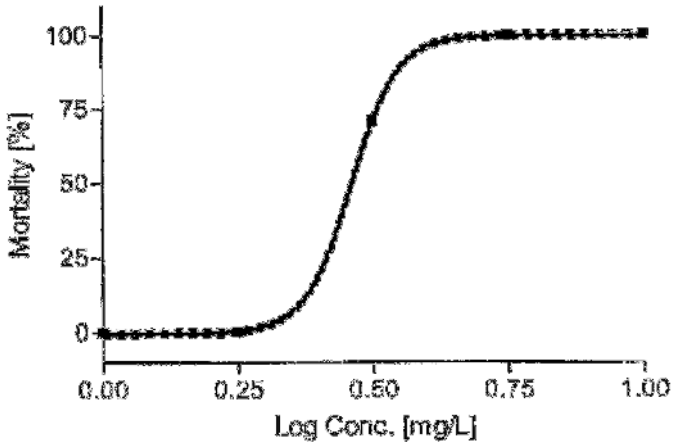
Concentration effect relationship after 72h



Concentration effect relationship after 96h

Section A7.4.1.1 - Acute toxicity to fish

Annex Point IIA7.1



4.2.5	Other effects	None stated
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were observed in either the solvent control or the control animals
4.3.2	Nature of adverse effects	n/a
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	n/a
4.4.2	Results	n/a

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	OECD 203 Three samples of test material prepared to a nominal concentration of 12.0 mg/L were sampled using no treatment, centrifuging at 40000g for 30 min and filtration through 0.45µm filter. The tests confirmed that the test material was soluble under the test conditions.
5.2	**Results and discussion**	X

Test duration (h)	LC_{50} (mg/L)	P = 95% (mg/L)
24	4.70	4.47-4.94
48	3.79	3.64-3.94
72	3.25	3.24-3.27
96	2.89	2.86-2.92

5.2.1	LC_0	1.78 mg/L
5.2.2	LC_{50}	2.86 mg/L

Section A7.4.1.1 - Acute toxicity to fish

Annex Point IIA7.1

5.2.3	LC_{100}	5.62 mg/L
5.3	**Conclusion**	All the validity criteria were met therefore the test is considered valid.
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan 2009
Materials and Methods	3.1 and 3.1.2: Unclear reference to "section 2", refers to the original study. 3.4.1: The hardness of the dilution water is given as a very wide range, and it could influence the test result depending on which value is the more common. 3.4.2: The age and size of the fish is not stated. The test guideline states that when using rainbow trout the recommended fish length is 5.0 ± 1.0 cm.
Results and discussion	4.2.2: The headings "new medium day 2" and "old medium day 2" are mixed up and should change places. Also, "new medium day 3" is reported twice with different figures. One of the two columns should be "old medium day 3". The order of the columns is not consequent. The value 1.71 for the nominal conc 1.0 day 3 (the second one) is wrong (editorial mistake), should be written 0.982. The nominal conc 3.61 is wrong, should be 3.16. All these editorial mistakes have been introduced in this summary, assuming that the original study report is correct. 4.2.4: The dose-response curves are drawn from a very limited data set and the realistic shapes of the curves are not necessarily those shown in the graphs. However, since one of the data points at 72 and 96 h is close to 50% effect, the LC50 values given may still be fairly good representations of the real situation.
Conclusion	5.2: It should be stated that the results are based on nominal concentrations and that the measured concentrations of bromadiolone were within the range 96-102% for initial concentrations and 95-102% for "old solutions".
Reliability	2, due to that neither size nor age were stated for the fish used for testing.
Acceptability	acceptable
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes Agitation
Vehicle	Yes Dimethyl sulfoxide
Concentration of vehicle	0.1mL/L
Vehicle control performed	Yes Solvent control performed with 0.1 mL DMSO/L
Other procedures	None stated

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Tap water of local origin
Alkalinity	Not stated
Hardness	10-250 mg CaCO3/L
pH	pH 6.0-8.5
Oxygen content	Not stated
Conductance	Not stated
Holding water different from dilution water	No

The water was filtered on activated charcoal and aerated for at least 24h to remove chlorine. The water is analysed biannually according to German tap water regulations.

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss* (rainbow trout)
Source	Forellenzucht Trostadt Gbr
Wild caught	No
Age/size	Not stated
Kind of food	Not stated
Amount of food	4% of the fish body weight per feeding day.
Feeding frequency	Three times per week
Pretreatment	Acclimatisation for at least 12 days
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	**Details**
Test type	Semi-static
Renewal of test solution	Water renewed daily
Volume of test vessels	20 L
Volume/animal	<1g of fish per litre
Number of animals/vessel	7
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

	Nominal concentration (mg/L)	Oxygen saturation %		pH value		Temperature (°C)		Total hardness (mg/L as $CaCO_3$)
		New	Old	New	Old	New	Old	
Day 0	10	100	-	7.30	-	15.0	-	56
	5.62	100	-	7.31	-	15.0	-	
	3.16	100	-	7.32	-	15.1	-	
	1.78	100	-	7.30	-	14.8	-	
	1	100	-	7.35	-	15.2	-	
	Solvent	94	-	7.30	-	15.2	-	
	Control	95	-	7.28	-	14.4	-	
Day 1	10	-	96	-	7.19	-	15.0	56
	5.62	100	91	7.30	7.22	14.7	15.0	
	3.16	100	90	7.35	7.22	14.8	15.1	
	1.78	100	85	7.37	7.23	14.6	14.8	
	1	100	86	7.37	7.23	14.6	14.9	
	Solvent	100	83	7.34	7.25	15.2	15.0	
	Control	98	75	7.41	7.27	15.4	15.2	
Day 2	10	-	-	-	-	-	-	58
	5.62	-	93	-	6.88	-	14.9	
	3.16	100	93	7.07	6.87	15.0	14.9	
	1.78	100	93	7.09	6.88	15.1	14.7	
	1	100	94	7.11	6.87	15.4	14.8	
	Solvent	100	87	7.17	6.78	14.8	15.1	
	Control	100	85	7.25	6.89	15.4	15.1	
Day 3	10	-	-	-	-	-	-	55

	Nominal concentration (mg/L)	Oxygen saturation %		pH value		Temperature (°C)		Total hardness (mg/L as $CaCO_3$)
		New	Old	New	Old	New	Old	
	5.62	-	-	-	-	-	-	
	3.16	100	90	6.99	6.97	14.8	14.9	
	1.78	100	89	7.01	6.96	14.8	14.9	
	1	100	87	6.98	6.95	14.7	15.1	
	Solvent	100	89	6.95	6.97	15.0	14.8	
	Control	100	90	6.97	6.99	15.1	15.1	
Day 4	10	-	-	-	-	-	-	
	5.62	-	-	-	-	-	-	
	3.16	-	93	-	7.11	-	14.9	
	1.78	-	92	-	7.10	-	14.8	
	1	-	90	-	7.12	-	14.8	
	Solvent	-	87	-	7.11	-	15.1	
	Control	-	86	-	7.18	-	15.1	

Aeration of dilution water	Yes
Intensity of irradiation	0.1-10µmol photons/m^2/s
Photoperiod	12 h photoperiod daily

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
10	7	-	-	-	100	-	-	-
5.62	6	1	-	-	85.7	14.3	-	-
3.16	-	1	2	1	-	14.3	28.6	14.3
1.78	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
Solvent control	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	**95 % c.l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0	-	-	-	-
LC_{50}	3.79	3.64-3.94	2.89	2.86-2.92
LC_{100}	5.62	-	-	-

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances		

Section A7.4.1.2 - Acute toxicity to invertebrates

Annex Point IIA7.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX, 2007, Bromadialone Acute immobilization test (static, 48h) *Daphnia magna*, XXXXX, Study Nº DAI113101	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Bromadiolone task force	
1.2.2	Companies with letters of access	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 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	L22678	
3.1.2	Specification	As given in section 2	X
3.1.3	Purity	99.9%	
3.1.4	Composition of Product	n/a	
3.1.5	Further relevant properties	Water solubility <0.5 mg/L at 20°C	
3.1.6	Method of analysis	Test material concentration and control groups were analytically verified at 0 and 48 h via HPLC with a diode array detector (DAD)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1	
3.3	**Reference substance**	Yes Potassium dichromate	
3.3.1	Method of analysis for reference substance	Not stated	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	X
3.4.2	Test organisms	See table A7_4_1_2-3	X
3.4.3	Test system	See table A7_4_1_2-4	X
3.4.4	Test conditions	See table A7_4_1_2-5	X

Section A7.4.1.2 - Acute toxicity to invertebrates

Annex Point IIA7.2

3.4.5	Duration of the test	48h	
3.4.6	Test parameter	Immobility	
3.4.7	Sampling	Analytical samples taken at 0 and 48 h	
3.4.8	Monitoring of TS concentration	Yes	
3.4.9	Statistics	The EC_{50} value after 24h, EC_{100} values, the NOEC and LOEC were deduced directly from the dose response relationship. There was no mathematical calculation. The EC_{10} values after 24 and 48 h and EC_{50} value after 48h were calculated by sigmoidal dose response regression. Calculation of the confidence intervals for EC_{50} were carried out using standard procedures according to Clopper and Pearson (1934)	
		4 RESULTS	
4.1	**Limit Test**	Not performed	X
4.1.1	Concentration	n/a	X
4.1.2	Number/ percentage of animals showing adverse effects	n/a	X
4.1.3	Nature of adverse effects	n/a	X
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	10, 5, 2.5, 1.25, 0.625 mg/L	
4.2.2	Actual concentrations of test substance	See table below	
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6 See table A7_4_1_2-7	

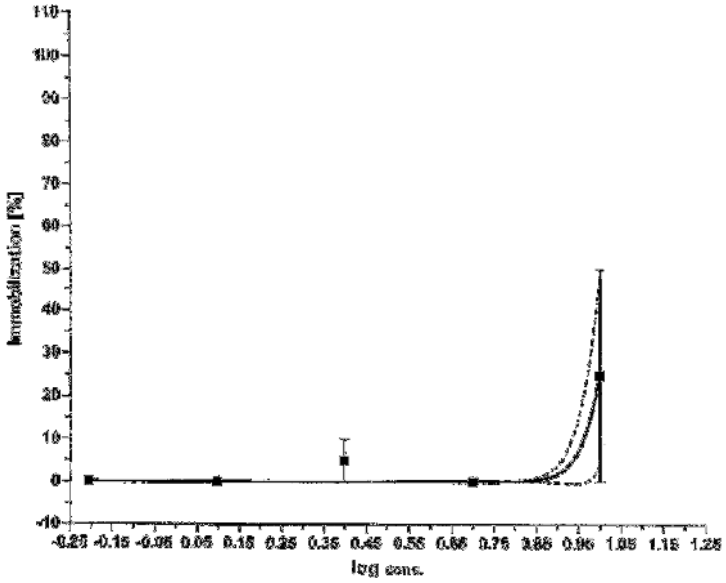
	0 h		48h	
Nominal concentration mg/L	Measured concentration mg/L	Recovery %	Measured concentration mg/L	Recovery %
10	9.90	99	10	100
5	5.31	106	5.18	104
2.5	2.69	107	2.58	103
1.25	1.33	106	1.27	101
0.625	0.656	105	0.630	101
Solvent control	<LOQ	-	<LOQ	-
Control	<LOQ	-	<LOQ	-

Section A7.4.1.2 - Acute toxicity to invertebrates

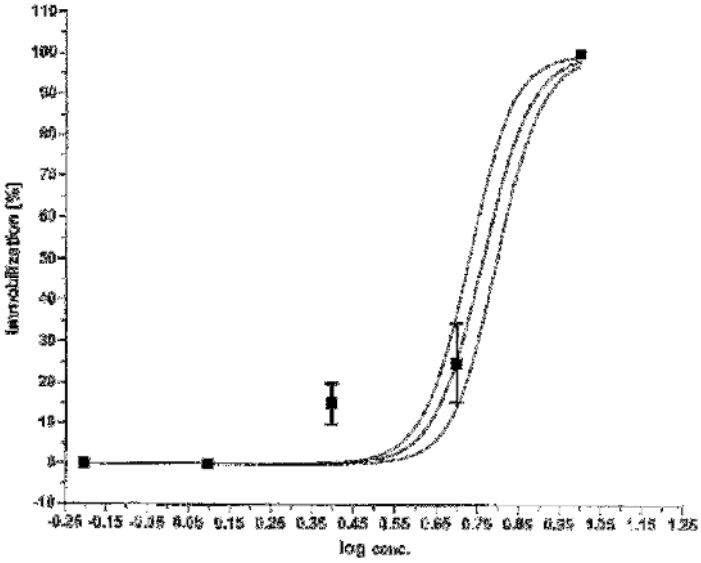
Annex Point IIA7.2

4.2.4 Concentration / response curve

Concentration effect of Bromadialone after 24h



Concentration effect of Bromadialone after 48h



4.2.5 Other effects None stated

4.3 Results of controls No difference was observed between the control and the solvent control. No immobilisation was observed at all time points.

Section A7.4.1.2 - Acute toxicity to invertebrates

Annex Point IIA7.2

4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	Not stated
4.4.2	Results	EC_{50} 1.81 mg/L
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD 202 Three samples of test material prepared to a nominal concentration of 12.0 mg/L were sampled using no treatment, centrifuging at 40000g for 30 min and filtration through 0.45µm filter. The tests confirmed that the test material was soluble under the test conditions. As the test material is subject to rapid photolysis, the test was performed in the dark.
5.2	**Results and discussion**	The highest concentration producing no immobile Daphnia was found to be 1.25 mg/L. The lowest concentration causing 100% immobilisation was found to be 10mg/L. Recovery rates were between 99-107% of the nominal concentration, results were therefore based on nominal concentrations. The 48h NOEC = 1.25 mg/L The 48h LOEC = 2.50 mg/L
5.2.1	24 h EC_{10}	9.16 mg/L
	48 h EC_{10}	4.30 mg/L
5.2.2	24 h EC_{50}	>10.0 mg/L
	48 h EC_{50}	5.79 mg/L
5.2.3	24 h EC_{100}	>10.0 mg/L
	48 h EC_{100}	10.0 mg/L
5.3	**Conclusion**	All the validity criteria were met therefore the test is considered valid.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2009

Section A7.4.1.2 - Acute toxicity to invertebrates

Annex Point IIA7.2

Materials and Methods	3.1 and 3.1.2: Unclear reference to "section 2", refers to the original study. 3.4.1: Last row not specified. According to the original study report it seems that the daphnids have been cultured in a medium different from the dilution water and that acclimation to dilution water was done for 2 h, which is *much shorter than the required 48 h* (TG 202). 3.4.2: Breeding method described in original study report page 9. 2-3 L glass vessels with appr. 1.8 L culture medium Elendt M4 adjusted to hardness 160-180 mg $CaCO_3$/L at 20 ± 2 °C, 16 h light period, light intensity max 20 µE m^{-2} s^{-1}. Pretreatment acclimatization to dilution water for 2 h. 3.4.3: Test vessel volume, medium volume and number of animals described in original study report page 11. Glass beakers 50 mL, medium volume 20 mL, 5 animals per vessel, i.e. 4 mL per animal. 3.4.4: Test temperature described in original study report page 11 as 18-22 ± 1°C.
Results and discussion	4.1: Range finding test was performed as described in original study report page 15. Immobilization was measured at 24 and 48 h at the bromadiolone concentrations 0.1, 1 and 10 mg/L. Immobilisation was found only at the highest conc. 10 mg/L and was 70% at 24 h and 100% at 48h.
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	acceptable
Remarks	-

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes Agitation
Vehicle	Yes Dimethyl sulfoxide
Concentration of vehicle	0.1mL/L
Vehicle control performed	Yes Solvent control performed with 0.1 mL DMSO/L
Other procedures	None stated

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Not stated
Alkalinity	0.8mmol/L
Hardness	262 mg $CaCO_3$/L
pH	8.04
Oxygen content	8.09 mg/L
Conductance	665 µS/cm
Holding water different from dilution water	Yes/No (If yes, specify)

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna* STRAUS (Clone 5)
Source	Bred in house (origin Institur fur Wasser, Boden und Lufthygiene
Age	2-24 hours
Breeding method	Not stated
Kind of food	*Desmodesmus subspicatus* and *Chlorella vularis*
Amount of food	Ad libnitum
Feeding frequency	5 times per week
Pretreatment	Not stated
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	Not stated
Volume/animal	Not stated
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Test temperature</td><td>Not stated</td></tr>
<tr><td>Dissolved oxygen</td><td>
<table>
<tr><th rowspan="2">Nominal concentration (mg/L)</th><th colspan="4">O_2 concentration (mg/L) Replicates</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th></tr>
<tr><td>10.0</td><td>8.53</td><td>8.55</td><td>8.44</td><td>8.41</td></tr>
<tr><td>5.00</td><td>8.44</td><td>8.42</td><td>8.44</td><td>8.46</td></tr>
<tr><td>2.50</td><td>8.39</td><td>8.37</td><td>8.39</td><td>8.41</td></tr>
<tr><td>1.25</td><td>8.39</td><td>8.44</td><td>8.41</td><td>8.48</td></tr>
<tr><td>0.625</td><td>8.28</td><td>8.46</td><td>8.37</td><td>8.42</td></tr>
<tr><td>Solvent control</td><td>8.53</td><td>8.70</td><td>8.52</td><td>8.64</td></tr>
<tr><td>Control</td><td>8.27</td><td>8.22</td><td>8.37</td><td>8.36</td></tr>
</table>
</td></tr>
<tr><td>pH</td><td>
<table>
<tr><th rowspan="2">Nominal concentration (mg/L)</th><th colspan="4">Replicates</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th></tr>
<tr><td>10.0</td><td>7.89</td><td>7.89</td><td>7.90</td><td>7.91</td></tr>
<tr><td>5.00</td><td>7.87</td><td>7.88</td><td>7.88</td><td>7.86</td></tr>
<tr><td>2.50</td><td>7.87</td><td>7.87</td><td>7.86</td><td>7.87</td></tr>
<tr><td>1.25</td><td>7.89</td><td>7.90</td><td>7.91</td><td>7.91</td></tr>
<tr><td>0.625</td><td>7.79</td><td>7.95</td><td>7.87</td><td>7.86</td></tr>
<tr><td>Solvent control</td><td>7.99</td><td>7.97</td><td>7.96</td><td>7.98</td></tr>
<tr><td>Control</td><td>8.02</td><td>7.98</td><td>7.99</td><td>7.99</td></tr>
</table>
</td></tr>
<tr><td>Adjustment of pH</td><td></td></tr>
<tr><td>Aeration of dilution water</td><td>Not stated</td></tr>
<tr><td>Quality/Intensity of irradiation</td><td>None. The test was performed in the dark due to the photosensitivity of the test material</td></tr>
<tr><td>Photoperiod</td><td>None. The test was performed in the dark due to the photosensitivity of the test material</td></tr>
</table>

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal) [1] [mg/l]	**Immobile *Daphnia***			
	Number		**Percentage**	
	24 h	**48 h**	**24 h**	**48 h**
10.0	5	20	25	100
5.00	0	5	0	25
2.50	1	3	5	15
1.25	0	0	0	0
0.625	0	0	0	0
Solvent control	0	0	0	0
Control	0	0	0	0

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	**95 % c.l.**	**EC_{10}[1]**	**EC_{100}[1]**
24 h [mg/l]	>10.0 (n)		9.16 (n)	>10.0 (n)
48 h [mg/l]	5.79 (n)		4.30 (n)	10.0 (n)

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**X**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**X**	
Concentration of test substance ≥80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances ergänzen		

Section A7.4.1.3 - Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	Scheerbaum D, 2007, Bromadiolone Alga, Growth inhibition test with *Pseudokirchneriella subcapitata*, 75 h, Dr U.Noack-Laboratorien, Study Nº SPO113101	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Bromadiolone task force	
1.2.2	Companies with letters of access	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 201	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	L2267	
3.1.2	Specification	As given in section 2	X
3.1.3	Purity	99.9%	
3.1.4	Composition of Product	n/a	
3.1.5	Further relevant properties	Water solubility <0.5 mg/L at 20°C	
3.1.6	Method of analysis	HPLC with a diode array detector (DAD)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_3-1	
3.3	**Reference substance**	No	X
3.3.1	Method of analysis for reference substance	n/a	X
3.4	**Testing procedure**		
3.4.1	Culture medium		

Component	Concentration (mg/L)
NH_4Cl	15

Section A7.4.1.3 - Growth inhibition test on algae

Annex Point IIA7.3

		$MgCl_2 \cdot 6H_2O$	12
		$CaCl_2 \cdot 2H_2O$	18
		$MgSO_4 \cdot 7H_2O$	15
		KH_2PO_4	1.6
		$FeCl_3 > 6H_2O$	0.064
		$Na_2EDTA \cdot 2H_2O$	0.1
		H_3BO_3	0.185
		$MnCl_2 \cdot 4H_2O$	0.415
		$ZnCl_2$	$3x10^{-3}$
		$Na_2MoO_4 \cdot 2\ H_2O$	$7x10^{-3}$
		$CoCl_2 \cdot 6\ H_2O$	$1.5x10^{-3}$
		$CuCl_2 \cdot 2\ H_2O$	$1x10^{-3}$
		$NaHCO_3$	50
		pH	8.2±0.2
3.4.2	Test organisms	See table A7_4_1_3-2	
3.4.3	Test system	See table A7_4_1_3-3	
3.4.4	Test conditions	See table A7_4_1_3-4	
3.4.5	Duration of the test	72h	
3.4.6	Test parameter	Growth inhibition	
3.4.7	Sampling	0, 24, 48 and 72h	
3.4.8	Monitoring of TS concentration	Yes 0 and 72 h	
3.4.9	Statistics	EC_{10}, EC_{20} and EC_{50} values of growth rate and yield inhibition after 72h were calculated by sigmoidal dose response regression. Calculation of the confidence intervals were carried out using standard procedures according to Clopper and Pearson (1934)	
		4 RESULTS	
4.1	**Limit Test**	Performed	
4.1.1	Concentration		X
4.1.2	Number/ percentage of animals showing adverse effects		
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	12, 6, 3, 1.5, 0.75 mg/L	

Section A7.4.1.3 - Growth inhibition test on algae

Annex Point IIA7.3

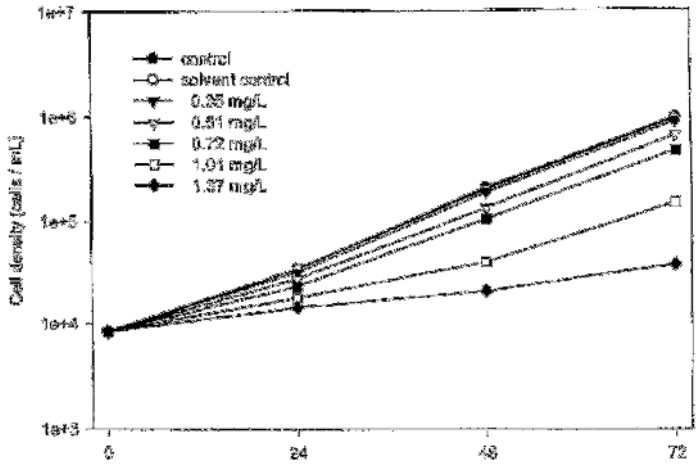
4.2.2 Actual concentrations of test substance

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

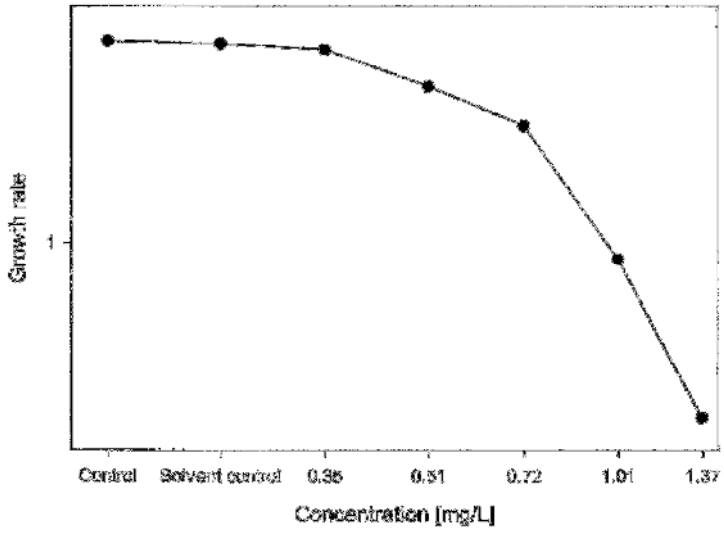
The geometric mean concentration has been calculated from the initial measurement and half of the LOQ in accordance with the OECD series on testing and assessment number 23, Guidance document on aquatic toxicity testing of difficult substances and mixtures.

4.2.3 Growth curves

Cell density for each concentration level (0-72 h)

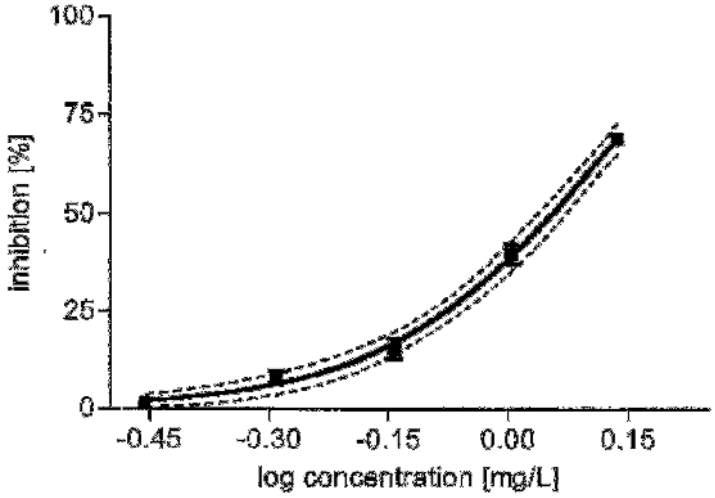


Growth rate for each concentration level (0-72h)



4.2.4 Concentration / response curve

Rate related inhibition: Dose response relationship after 72h



4.2.5 Cell concentration data

See table A7_4_1_3-5

4.2.6	Effect data (cell multiplication inhibition)	EC_{10} EC_{20} and EC_{50} values (0-72h) of Bromadiolone	

	Rate related inhibition	95% confidence interval
E_rC_{10}	0.59	0.52-0.66
E_rC_{20}	0.77	0.72-0.81
E_rC_{50}	1.14	1.08-1.19
	Yield inhibition	95% confidence interval
E_yC_{10}	0.36	<0.35-0.43
E_yC_{20}	0.45	0.40-0.51
E_yC_{50}	0.66	0.61-0.71

Based on the geometric mean

4.2.7	Other observed effects	After 72h, algae were transferred from the test material and the control to fresh untreated medium and allowed to grow for a further 4 days under test conditions. The test item effect was observed to be reversible up to the highest concentration tested	
4.3	**Results of controls**	The cell growth increased 115 fold after 72h	
4.4	**Test with reference substance**	Performed Potassium dichromate	
4.4.1	Concentrations	Not stated	X
4.4.2	Results	E_rC_{50} = 0.97 (0.93-1.01)mg/L E_yC_{50} = 0.56 (0.54-0.58) mg/L	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	OECD 201 Three samples of test material prepared to a nominal concentration of 12.0 mg/L were sampled using no treatment, centrifuging at 40000g for 30 min and filtration through 0.45µm filter. The tests confirmed that the test material was soluble under the test conditions. Due to the sensitivity of the test material to light, the results were based on the geometric mean, calculated from the initial measurement and half of the LOQ in accordance with the OECD series on testing and assessment number 23, Guidance document on aquatic toxicity testing of difficult substances and mixtures.	X
5.2	**Results and discussion**	The E_rC_{50} = 1.15 mg/L (based on the geometric mean concentration). Inhibition was reversed after 4 days growth without the test material.	
5.2.1	E_rC_{10}	0.59	
5.2.2	E_rC_{50}	1.14	
5.2.3	E_bC_{50}	0.66	
5.3	**Conclusion**	Cell concentration of the control cultures increased by a factor of 115 over 72h. The concentrations of test material measured after 72h was below the LOQ and therefore the validity criteria are not met.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	Yes	

The concentrations of test material measured after 72h was below the LOQ.

The results have been based on the geometric mean, calculated from the initial measurement and half of the LOQ, in accordance with the OECD series on testing and assessment number 23, Guidance document on aquatic toxicity testing of difficult substances and mixtures.

This deficiency has been adequately addressed and does not affect the reliability of the results.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	January 2009
Materials and Methods	3.1 and 3.1.2: Unclear reference to "section 2", refers to the original study. 3.3: Potassium dichromate was tested as reference substance. 3.3.1: Nominal concentrations were used.
Results and discussion	4.1.1: Limit test concentrations 0.01, 0.1, 1 and 10 mg/L. 4.4.1: Reference substance conc given on page 10 in the test report. 0.40, 0.59, 0.89, 1.3, 2.0 mg/L.
Conclusion	5.1: It is unclear whether it has been tested to reduce the photodegradation of the test substance during the test. This is probably possible without severely affecting the growth rate of the algae, e.g. by slightly reducing the light intensity and/or introducing extra glass panes between the light source and the test flasks in order to limit UV wavelengths.
Reliability	2, since all validity criteria were not met.
Acceptability	acceptable
Remarks	The results of the study will be used in the risk assessment.

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**
Dispersion	Yes Agitation
Vehicle	Yes Dimethyl sulfoxide
Concentration of vehicle	0.1mL/L
Vehicle control performed	Yes Solvent control performed with 0.1 mL DMSO/L
Other procedures	None stated

Table A7_4_1_3-2: Test organisms

Criteria	**Details**
Species	*Pseudokirchneriella subcapitata* (formerly know as *Selenastrum capricornutum*)
Strain	HINDAK SAG 61.81
Source	Sammlun von Algenkuturen
Laboratory culture	Yes
Method of cultivation	Fresh stock prepared monly on Z-Agar.
Pretreatment	Not stated
Initial cell concentration	$\approx 5x10^3 - 10^4$ cells/mL

Table A7_4_1_3-3: Test system

Criteria	**Details**
Volume of culture flasks	250 mL
Culturing apparatus	
Light quality	60 – 120 $\mu E/m^2/s$
Procedure for suspending algae	Rotary shaker (≈70rpm)
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	**Details**			
Test temperature	21-24°C			
pH	Geometric mean concentration (mg/L)	pH at 0h	pH at 72h	
	1.37	7.94	8.02	
	1.01	7.99	8.18	
	0.72	8.03	8.40	
	0.51	8.06	8.65	
	0.35	8.07	9.13	
	Solvent control	8.08	9.21	
	Control	8.12	9.32	
Aeration of dilution water	No			
Light intensity	Light intensity (lux)	Min	Max	Mean
	0 h	5150	5750	5487
	24 h	5210	5690	5487
	48 h	5250	5680	5452
	72 h	5210	5590	5415
	Mean (0-72 h)			5460
Photoperiod	24 light period			

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal/effective)[1] [mg/l]	**Cell concentrations (mean values) [cells/ml]**							
	measured				**Percent of control**			
	0 h	**24 h**	**48 h**	**72 h**	**0 h**	**24 h**	**48 h**	**72 h**
1.37	8414	14325	20537	37176	100	42.334	9.7972	3.8364
1.01	8414	179694	39246	148825	100	52.29	18.722	15.358
0.72	8414	22867	104306	469937	100	67.578	49.759	48.495
0.51	8414	27001	132829	656897	100	79.795	63.366	67.788
0.35	8414	31395	187994	895381	100	92.78	89.682	92.398
Solvent control	8414	33500	198859	946221	100	99.001	94.866	97.645
Control	8414	33838	209622	969044				

[1] specify, if TS concentrations were nominal or measured

3. Tables for Applicant's Summary and Conclusion

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**X**	
Concentration of test substance ≥80% of initial concentration during test		**X**

Criteria for poorly soluble test substances		

Section A7.4.1.4-01 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Draft report: Activated Sludge, Respiration Inhibition Test with BROMADIOLONE TECHNICAL Test Item. Study Director – Szabolcs Gáty – February 2002. Toxicological Research Centre Ltd. Report – 01/617-027AS	
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 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	The purity of the active substance 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	
3.1.3	Purity	99.4% bromadiolone	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	Analytical certificate supplied by Sponsor	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Yes, See table A7_4_1_4-1	
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		

Section A7.4.1.4-01 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

3.4.1	Culture medium	Synthetic Sewage Feed (Ratio of composition of culture media referring to 1000ml) Peptone 16 g Meat extract 11 g Urea 3 g NaCl 0.7g $CaCl_2$ x $2H_2O$ 0.4 g $MgSO_4$ x $7H_2O$ 0.2 g K_2HPO_4 2.8 g Distilled Water add 1000 ml	
3.4.2	Inoculum / test organism	see table A7_4_1_4-2	
3.4.3	Test system	see table A7_4_1_4-3	
3.4.4	Test conditions	see table A7_4_1_4-4	X
3.4.5	Duration of the test	3 hours	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen measurement	
3.4.8	Sampling	Every 30 seconds for a period of 10 minutes	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Blank, untreated, vehicle and reference (3,5-dichlorophenol).	
3.4.11	Statistics	Per cent inhibition was plotted against concentration on log normal paper and an EC_{50} value derived	X
		4 RESULTS	
4.1	**Preliminary test**	Performed	
4.1.1	Concentration	Control, 0.16, 0.80, 4.00, 20.00 and 100.00 mg/l	
4.1.2	Effect data	**Concentration (mg/l) Respiration rates (mgO_2/l/10mins)** Control (start) 4.6 Control (end) 4.6 0.16 4.6 0.80 5.0 4.00 4.7 20.00 4.5 100.00 3.8	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	6.3, 12.5, 25.0, 50.0 and 100.0 mg/l	

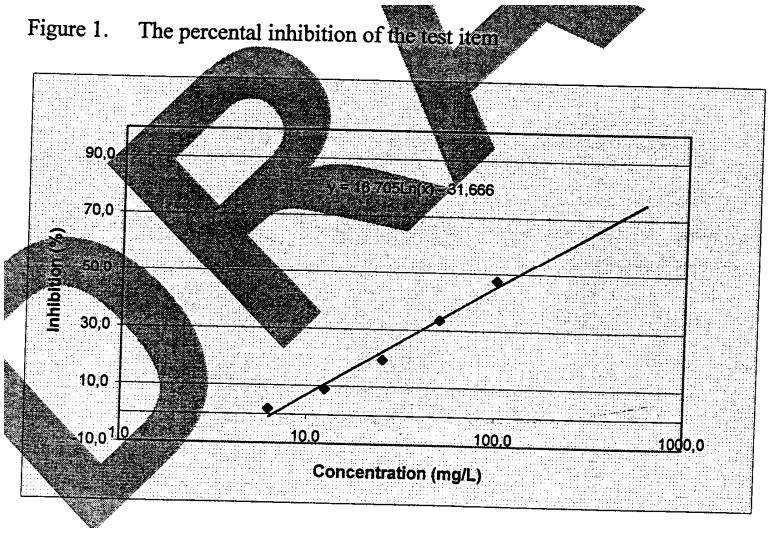
Section A7.4.1.4-01 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.2.2	Actual concentrations of test substance	Not measured
4.2.3	Growth curves	Not applicable.
4.2.4	Cell concentration data	Not reported.
4.2.5	Concentration/ response curve	

Concentration mg/l	Inhibition (%)
6.3	1.8
12.5	8.8
25.0 47.4	19.3
50.0	33.3
100.0	47.4

Figure 1. The percental inhibition of the test item



4.2.6	Effect data	EC_{50} 3 hours = 132.8 mg/l
4.2.7	Other observed effects	None reported.

4.3	**Results of controls**	Control:	Respiration Rates (mg O^2/l)	Inhibition (%)
		Blank	0.1	-
		Untreated control (Initially)	2.8	0.0
		Untreated control (finally)	2.9	0.0
		Untreated control (Mean)	2.85	0.0
		Vehicle Control	2.9	

4.4	**Test with reference substance**	Performed

Section A7.4.1.4-01 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.4.1 Concentrations: Positive control, 0.8, 4.0, 20.0 and 100.0 mg/l

4.4.2 Results:

Reference concentration (mg/l)	Respiration rate (mg O^2/l)	Inhibition (%)
0.8	2.7	5.3
4.0	1.8	36.8
20.0	1.1	61.4
100.0	0.2	93.0

EC_{50}, 3 hours = 9.4 mg/l (Validity criterion 5 to 30 mg/l)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was performed according to OECD 209 guidelines. A stock solution was prepared in DMF from the test item, and a dilution series of the test item stock solution was prepared with DMF in four steps by a factor 0.5. All of the members of this dilution series were diluted with distilled water to 20 times volume.

The diluted stock solutions of test item were diluted with synthetic sewage feed, distilled water and inoculum to 5 times volume. The final test item concentrations in the test bottles (ratio of composition of final test mixture referring to 500ml were prepared as follows: 100ml diluted test item stock solution =+ 16ml Synthetic sewage feed + distilled water added 300ml + 200 ml inoculum.

To measure the respiration rate – after three hours the content of the Erlenmeyer bottle was poured into the measuring bottle and oxygen concentration of the first test group was measured and recorded over a period up to 10 minutes and this determination was repeated on the content of each vessel at 15 minutes intervals, assuring three hours contact tine in each vessel. The respiration rate was calculated from the recorder trace over a 10 minute period.

5.2 Results and discussion

The test system was a secondary effluent of good quality, collected from a treatment plant dealing with predominantly domestic sewage. There were considerable differences between the untreated control meand (2.85 mg/O_2/L/10 min) and the test item groups. Under the conditions of this study, Bromadiolone showed low toxicity to microorganisms. The EC_{50}, 3 hours = 132.8 mg/l. The EC_{50} value of the reference item (3,5-dichlorophenol) was 9.4 mg/L. (Validity criterion 5 to 30 mg/l)

5.2.1 EC_{20} X

5.2.2 EC_{50}: 3 hours = 132.8 mg/l

5.2.3 EC_{80}

5.3 Conclusion

Under the conditions of this toxicity study, BROMADIOLONE TECHNICAL test item showed low toxicity on microorganisms. The results of the reference item met the validity criterion.

5.3.1 Reliability: 1

5.3.2 Deviation: The purity of the active substance tested is 99.4%. This will not effect the integrity of the study.

Section A7.4.1.4-01 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2006-10*
Materials and Methods	Adopt applicant's version noting the following deviations. 15.4.4 pH was not measured which should have been done according to the guideline. It would have been sufficient if the concentrations of bromadiolone would have been measured in the test solutions since the substance have such a low solubility. However this is not stated in the guideline. 15.4.11 According to the guideline EC_{20} and EC_{80} should have been calculated. It is, however, noticed that EC_{80} values can not be derived out of this investigation.
Results and discussion	Adopt applicant's version. 17.2.1 Include EC_{20} values
Conclusion	
Reliability	2
Acceptability	acceptable
Remarks	

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**	
Dispersion	Yes	
Vehicle	DMF	
Concentration of vehicle	*Test Substance Conc.*	
Vehicle control performed	Yes	
Other procedures	None	

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	**Details**
Nature	Activated sludge
Species	
Strain	
Source	Activated Sludge Plant for domestic sewage in Veszprém

Sampling site	
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Washed three times with normal (isotonic) saline solution
Pretreatment	Weighed and dried then suspended in isotonic saline
Initial cell concentration	Mixed liquor suspended solids level of 4 g/l. Concentration of 1.6 g/l of inoculum level in test mixture.

Table A7_4_1_4-3: Test system

Criteria	**Details**
Culturing apparatus	BOD bottles with special neck and grinding
Number of culture flasks/concentration	1
Aeration device	Not stated
Measuring equipment	Self stirring O_2 electrode, Oxygen meter, pH meter,
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	**Details**
Test temperature	Climatic Chamber during shaking 19.6 – 20.7ºC During oxygen Measuring 18.5 – 20.3ºC
pH	Not reported
Aeration of dilution water	No
Suspended solids concentration	4 g/l

Section A7.4.1.4-02 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Staniland, J (2004) An evaluation of the effect of Bromadiolone on the Inhibition of Activated Sludge Respiration according to OECD 209. Chemex Reference: ENV7144/110414.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The 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 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	The purity of the substance is 99.5%. 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	02478	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.5%	
3.1.4	Composition of Product	N/A	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	N/A	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		X
3.3	**Reference substance**	3,5-dichlorophenol (3,5 DCP) Purity 97%	
3.3.1	Method of analysis for reference substance	N/A	
3.4	**Testing procedure**		
3.4.1	Culture medium	Batches of synthetic medium were freshly prepared for each test as described in OECD test guideline 209.	

Section A7.4.1.4-02 - Inhibition to microbial activity (aquatic)

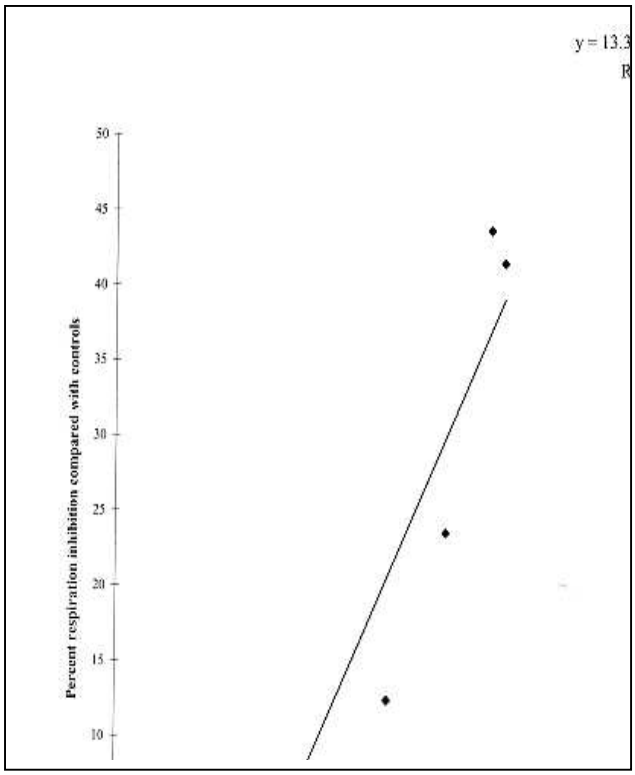
Annex Point IIA7.4

3.4.2	Inoculum / test organism	The activated sludge was collected from Cambridge Sewage Treatment Works. The sludge was centrifuged and washed with dechlorinated tap water three times before use. The pellet was re-suspended in dechlorinated tap water to give a dry weight level of 4.0/g/l in the final inoculum. A volume of 120ml of the final inoculum were added to each test vessel giving a final test concentration of 1.6g/l dry weight, in each test vessel.	
3.4.3	Test system	Apparatus: 500ml glass conical flasks 250ml (nominal) BOD bottles with ground stoppers Dissolved oxygen meter Aquarium type air pump	
3.4.4	Test conditions	Dilution water used was dechlorinated tap water held at approximately 21°C ± 2°C for at least 24 hours before use.	X
3.4.5	Duration of the test	Incubation period was 3 hours.	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen measurement	
3.4.8	Sampling	Concentration of dissolved oxygen (mg/l) was measured every minute for 10 minutes.	
3.4.9	Monitoring of TS concentration	Yes	X
3.4.10	Controls	A vessel without any test substance was set up, along with a control that was abiotic with the test substance concentration being 1002mg/l.	
		The reference substance was tested at 5, 15 and 30 mg/l.	X
3.4.11	Statistics	Linear regression analysis was carried out to describe the relationship between percentage respiration inhibition and test material concentration. In addition the computer programme of Stephan et al (SOP 256) estimated EC_{50}S with 95% confidence limits.	X
		4 RESULTS	
Preliminary test		Not performed	
4.1.1	Concentration	N/A	
4.1.2	Effect data	N/A	
Results test substance			
4.1.3	Initial concentrations of test substance	0, 62.0, 125.7 250 500, 851, 1002 mg/l	
4.1.4	Actual concentrations of test substance	N/A	
4.1.5	Growth curves	N/A	
4.1.6	Cell concentration data	N/A	

Section A7.4.1.4-02 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.1.7 Concentration/ response curve



4.1.8 Effect data

The EC_{50} value for Bromadiolone has been calculated to be greater than 1002 mg/l.

4.1.9 Other observed effects

None

Results of controls

Treatment	Respiration	Percentage inhibition
Control 1	38.95	-
Abiotic 1000mg/l	0.95	98
DCP 5mg/l	20.15	49
DCP 15mg/l	11.16	72

Test with reference substance

Performed

4.1.10 Concentrations

5, 15, 30 mg/l

4.1.11 Results

The EC_{50} for the reference substance (3,5-DCP) for the definitive test was estimated graphically at 5.1 mg/l X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

OECD guidelines 209. The appropriate volume of distilled water was measured into each test vessel and vigorously aerated. At time zero the

Section A7.4.1.4-02 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		required volume of synthetic sewage sludge and inocula was added to the dechlorinated tap water in the first tank. The appropriate amount of test material was added and the aeration restored. This process was repeated at 15 minute intervals for the remaining flasks, using the volumes previously indicated. After a 3 hour incubation period the respiration rates were measured in each vessel as follows. The contents were poured into 250ml glass BOD bottles and the oxygen electrode inserted. Air was excluded from the measuring apparatus. The contents were stirred continuously using magnetic stirrers and the concentration of dissolved oxygen (mg/l) was measured every minute in each vessel for 10 minutes.	
5.2	**Results and discussion**	The results of the abiotic control flask indicated that there were would be no reduction in oxygen concentration other than that caused by the activity of the activated sludge.	
5.2.1	EC_{20}	N/A	X
5.2.2	EC_{50}	>1002 mg/l Graphical estimation indicated the value to be 2286mg/l	X
5.2.3	EC_{80}	N/A	
5.3	**Conclusion**	The EC_{50} value for Bromadiolone has been calculated to be greater than 1002 mg/l. Extrapolation of the response curve indicates the value to be 2286 mg/l. The reference compound 3,5 DCP indicated the sensitivity of the activated sludge was within the correct range of 5 to 30 mg/l with an EC_{50} of 5.1 mg/l (estimated graphically.) The respiration rates of two blank treatments were within the 15% of the mean value.	X X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *2006-03-27*

Materials and Methods

3.2 Bromadiolone which is a poorly soluble substance was not prepared to increase it´s solubility this will affect the results of the investigations since the actual concentrations of bromadiolone in solution will be very low.

3.4.4 pH was not measured, which should have been done according to the guideline.

3.4.9 Test substance concentrations were not measured, which in this case very much will affect the result.

3.4.10 The reference substance was only prepared in three concentrations, this makes it impossible to calculate the EC_{50} of the reference in a proper way, at least five concentrations are requested for this.

Results and discussion

4.1.11 This EC_{50} is only an estimated value. Moreover, the estimated value is very close to the lowest concentration used which makes it impossible to state if it really is within the boundaries (5-30 mg/L) for a valid result.

Section A7.4.1.4-02 - Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Conclusion	5.2.1 EC_{20} was not calculated, which should have been done according to the guideline. 5.2.2 It is impossible to state if this value is correct since; the positive control failed, we do not know the actual concentations of test substance in solution and no preparations were made to increase the solubility of the test substance. 5.2.2 The calculation of EC_{50} for the reference substance is only an estimation.
Reliability	4
Acceptability	not acceptable
Remarks	

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**
Dispersion	No
Vehicle	No
Concentration of vehicle	N/A
Vehicle control performed	N/A
Other procedures	None

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	**Details**
Nature	Activated sludge
Species	Unknown
Strain	
Source	*e.g. sewage treatment plant treating predominantly domestic sewage*
Sampling site	*H*
Laboratory culture	No
Method of cultivation	
Preparation of inoculum for exposure	The activated sludge was centrifuged and washed with dechlorinated tap water 3 times before used.
Pretreatment	N/A
Initial cell concentration	1.6 g/l dry weight in each test vessel

Table A7_4_1_4-3: Test system

Criteria	**Details**
Culturing apparatus	*e.g. BOD flasks*
Number of culture flasks/concentration	
Aeration device	
Measuring equipment	*details on e.g. pH-electrode, O_2-electrode*
Test performed in closed vessels due to significant volatility of TS	Yes/No *(If yes, specify)*

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	Dechlorinated tap water was held at appox. 21 °C for at least 24 hours before use.
pH	N/A
Aeration of dilution water	Yes
Suspended solids concentration	1.6g/l of inoculum

Section A7.4.2 – Bioconcentration

Annex Point IIIA XIII.2.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	**Variation: Calculated value available.** Section 7.4.2, Chapter 2, Part A of the TGD states that an appropriate estimation of bioconcentration is needed. The test substance has low solubility in water and the estimated log octanol-water partition coefficient is 7.02. The bio-concentration factor (BCF) has been estimated using EPIwin v3.12. The BCF was calculated to be 13530, and the log value is 4.131. Calculation for this study: see 'references'. EPIwin calculation of BCF factor.	X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

2006-03-27 revised May 2010

Evaluation of applicant's justification

~~When the log Kow is above 6, as in the case of bromadiolone, there is a possibility that the BCF will be underestimated when this model for calculation of BCF is used.~~

~~If calculations are based on the formula in TGD part II page 126 equation 75, which is for substances with an log Kow higher than 6, the results gives an BCF of 45570 and a log value of 4.65.~~

~~$\text{Log BCF}_{\text{fish}} = 0.20*\log\text{Kow}^2 + 2.74*\log\text{Kow} - 4.72 \rightarrow$~~

~~$\text{Log BCF}_{\text{fish}} = 0.20*7.02^2+2.74*7.02-4.72=4.65$ the results gives a BCF of 45570.~~

The octanol-water partitioning study shows log K_{ow} = 3.8 at pH 7.1, and using this value in calculating a BCF_{fish} (equation 74 in the TGD) results in the following:

Log BCF_{fish} = 0.85 * log K_{ow} – 0.70 = 2.53

BCF_{fish} = 339

Conclusion

Justification acceptable; however, calculations were corrected above.

Remarks

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA XIII.2.2

	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:	Limited exposure of fish Product not used in or near water Product not soluble in water Product photolysis rapidly	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	The applicant states, which is confirmed by the studies performed, that bromadiolone has a low solubility and a rapid photolysis. Fish will be exposed to a limited degree. However, it is suspected due to the results of the bioaccumulation study with rainbow trout that the toxicity might have the same effect as for mammals, where accumulation time of the substance is an important factor.
Conclusion	acceptable
Remarks	

Section A7.4.3.2 - Effects on reproduction and growth rate on an appropriate species of fish

Annex Point IIIA XIII.2.2

	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:	Limited exposure of fish Product not used in or near water Product not soluble in water Product photlyses rapidly	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	The applicant states, which is confirmed by the studies performed, that bromadiolone has a low solubility and a rapid photolysis. Fish will be exposed to a limited degree and only locally.
Conclusion	Acceptable
Remarks	

Section A7.4.3.3.1 - Bioaccumulation in Rainbow trout

Annex Point IIIA XIII.2.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX, Nov 2004, The Bioconcentration potential of Bromadiolone in Rainbow Trout (*Oncorhynchus mykiss*) under flow-through conditions, XXXXX, ENV6552/080319	
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**	Yes, OECD Guidelines for Testing of Chemicals Bioconcentration: Flow-through Fish Test 305.	
2.2	**GLP**	Yes	
2.3	**Deviations**	The purity of the active substance tested is 97.9%, 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	ECO080319	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	97.9%	
3.1.4	Further relevant properties	Solubility in water is low. This was not a factor in this study.	X
3.1.5	Radiolabelling	Not radiolabelled.	

Section A7.4.3.3.1 - Bioaccumulation in Rainbow trout

Annex Point IIIA XIII.2.3

3.1.6	Method of analysis	Each sample of 4 trout was weighed and blended to a paste using a hand held food blender. 10.0 g of the trout paste was transferred to a flat bottomed dish. 20.0 g of anhydrous sodium sulphate was added and mixed. This mixture was air dried for 24 hours in the dark. The mixture was then reweighed and transferred to an accelerated solvent extraction (ASE) vial. The samples were extracted using an ASE machine with dichloromethane as the solvent (80°C, 1500psi, heat 5 min, flush 75%, cycles x 2). The collected extracts were dried over sodium sulphate and concentrated to 5.0 ml by Kuderna-Danish. 1.0 ml of the extract was removed for lipid determination, the remaining 4.0 ml was passed through a GPC column for cleanup. The GPC column was previously calibrated using a bromadialone standard. The collected GPC fraction was concentrated to 4.0 ml by Kuderna-Danish. 1.0 ml of the final extract was transferred to an HPLC vial, the remaining 3.0 ml was stored refrigerated in an amber vial. Analysis using HPLC with fluorescence detection was use to measure bromadialone in the extracts. HPLC conditions: Chromatography system: Perkin Elmer Quaternary System HPLC Gradient pump: Perkin Elmer Series 200 UV detector: Perkin Elmer 785 A UV/VIS @ 254 nm (1.0V/AU) Flourescence detector: Exλ 284 EMλ 390 Interface Box: 900 series and 600 link series Analytical column: Phenomenex Luna 5 µm C18 (2) 250 x 4.6 mm Mobile phase: Methanol: distilled water: acetic acid (850:142:8) Flow rate: 1.5 ml/min Injection volumn: 250 µl The limit of detection was determined as 0.009 µg/l.	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable.	
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	Eighty rainbow trout were placed in each of the control and test vessels. The tanks were maintained under through-flow conditions at a volume of 250 litres, one as control and the others at 0.5 µg/l and 0.05 µg/l concentrations of the test substance. The test vessels were maintained at 10.0 ± 1°C. The light source was ceiling mounted fluorescent tubes with a photo period of 16 hours light and 8 hours dark. Observations and records of mortality were made every 24 hours. The feeding rate was 1% body weight per day of Trouw (UK) Ltd Nutra Trout Fry 02 Crumb fish feed. (Quantities were recalculated daily after sampling to adjust for falling fish numbers.) Water samples were taken 24 hours before addition of trout and then at day 1, 3, 6, 10, 15, 21 and 28. The trout	X

Section A7.4.3.3.1 - Bioaccumulation in Rainbow trout

Annex Point IIIA XIII.2.3

		were also sampled as fish blanks on days: (0), 1, 3, 6, 10, 15, 21, 28, 29, 30, 33, 37 and 42. 500 ml water samples were siphoned from the middle of each tank into an amber glass bottle and stored at 4°C until extraction was completed. Additionally, 4 trout were taken randomly from each test solution, blotted dry and killed humanely and instantly. The fish samples were stored frozen (–20 to –35°C) until extraction. Temperature was measured daily to 0.5°C. pH and dissolved oxygen were measured before the addition of the trout, one day after and then weekly during the uptake phase. Measurements of pH (to 0.1) and DO (to 1% ASV) were also recorded at the beginning of the depuration phase, before the addition of the trout and then weekly. Total Organic Carbon (TOC) samples were taken 24 hours before, immediately before trout addition, on day one and then weekly.	
3.3.2	Estimation of bioconcentration	Not performed.	
		4 RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/behaviour	There was no Bromadiolone recovered from any of the fish samples. There were no mortalities recorded through the duration of the test and no abnormal behaviour of the Rainbow trout was recorded in the control or test solutions.	X
4.1.2	Lipid content	Not determined	
4.1.3	Concentrations of test material during test	A preliminary stock solution of 0.5g/l Bromadiolone in Dimethylformamide (DMF) was prepared. Secondary stock solutions were prepared daily at concentrations of 0.5 and 0.05mg/l in distilled water. A control solution of 1ml DMF per litre distilled water was also prepared. The final test concentrations of 0 (Control), 0.05 and 0.5µg/l Bromadiolone were prepared by proportional dilution of the test material to dilution water at a rate of 1 ml per litre dilution water. All records of flow rates can be found in Appendix 2.	
4.1.4	Bioconcentration factor (BCF)	As no recovery of Bromadiolone was achieved and no mortalities were recorded this may be an indication that the concentration of Bromadiolone in the water was not being maintained at a level near the nominal concentrations. Bromadiolone has been demonstrated in a separate study to suffer rapid photolytic degradation. Significant measures were taken to eliminate light from as many stages of the exposure, extraction and analytical procedures as possible, however the possibility remains that these measures were not successful. The calculation (based on prediction formula in the OECD 305 guidline) of time to 80% of steady-state for Bromadiolone is 43.2 days. The OECD guideline states that the maximum duration of the uptake phase should not exceed 60 days.	X

Section A7.4.3.3.1 - Bioaccumulation in Rainbow trout

Annex Point IIIA XIII.2.3

4.1.5	Uptake and depuration rate constants	The test was terminated after 27 days of the uptake phase as the mortalities in both concentrations had exceeded 30%. In this study Bromadiolone was successfully recovered from fish samples, however insufficient data was generated to produce uptake and depuration curves.	X
4.1.6	Depuration time	The test was terminated after 27 days of the uptake phase as the mortalities in both concentrations had exceeded 30%. In this study Bromadiolone was successfully recovered from fish samples, however insufficient data was generated to produce uptake and depuration curves.	
4.1.7	Metabolites	No metabolites identified.	
4.1.8	Other Observations	The extraction and analysis of the fish tissue samples was not completed after analysis of the maximum exposure samples failed to identify any Bromadiolone present.	
		Significant difficulties were experienced in attempting to extract and measure Bromadiolone in the water samples in this study. This is supported by the published literature on Bromadiolone that indicates that this material cannot be successfully extracted from water at low concentrations. Therefore all results from this study are derived from the nominal concentrations based on flow rates of the dilution water and test material.	X
4.2	**Estimation of bioconcentration**	Section 7.4.2, Chapter 2, Part A of the TGD states that an appropriate estimation of bioconcentration is needed. The test substance has low solubility in water and the estimated log octanol-water partition coefficient is 7.02.	
		The bio-concentration factor (BCF) has been estimated using EPIwin v3.12. The BCF was calculated to be 13530, and the log value is 4.131.	X
		Calculation for this study: see ‘references’. EPIwin calculation of BCF factor.	X

Section A7.4.3.3.1 - Bioaccumulation in Rainbow trout

Annex Point IIIA XIII.2.3

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 305 (1996) The extraction and analysis of the fish tissue samples was not completed after analysis of the maximum exposure samples failed to identify any Bromadiolone being present. The uptake phase was terminated early due to the number of mortalities, hence the steady state of bromadiolone in water and Rainbow Trout was not achieved.	
5.2	**Results and discussion**	This study has demonstrated that at the concentrations tested the test material Bromadiolone did not accumulate in the body tissues of rainbow Trout. Early termination of the uptake phase was necessary due to the number of mortalities recorded. Under these circumstances it is not possible to determine the Bioconcentration Factor (BCF_{ss}) of Bromadiolone in Rainbow trout.	X
		Significant difficulties were experienced in attempting to extract and measure Bromadiolone in the water samples in this study. This is supported by the published literature on Bromadiolone, which indicates that this material cannot be successfully extracted from water at low concentrations. Therefore all results from this study are derived from the nominal concentrations based on flow rates of the dilution water and test material and analysis of the fish tissue.	X
5.3	**Conclusion**	The BCF value was not obtained, hence the validity criteria has not been met.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	No preliminary trials were run to assess the method of analysis in water and fish. A test should have been performed using radiolabelled test material so that analysis could be conducted. The BCF is a quotient of the fish tissue concentration and the water concentration, which has not been determined in this study. The mortality levels at the higher level are unacceptable but indicate that possible accumulation has occurred since the animals have died. This implies that the animals have reached a critical body burden of the compound which has resulted in death.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *2006-03-27*

Materials and Methods Not acceptable due to the following errors in the test procedure.

3.1.4 The temperature used during the test is too low, between 13-17 °C is recommended, while the temperature in the test system was 9-11 °C. TOC was too high a maximum value of 2 mg/L is recommended while the concentration were 12 mg/L occasionally in the test system. The Cu and ammonia concentrations were about 20 times higher than the recommended concentrations for both of the substances. Moreover it is recommended that the highest concentration of the test substance should be around 1 % of the EC_{50} which in this case would give a concentration of 250µg/L, instead 15µg/L was chosen, and then adjusted down to 1,5µg/L. The results of the control samples are not stated anywhere.

Section A7.4.3.3.1 - Bioaccumulation in Rainbow trout

Annex Point IIIA XIII.2.3

Results and discussion	4.1.1 and 4.1.5 The results are rather confusing when results from a initial test that was terminated is mixed with results from the main test. 4.1.4 Bromadiolone concentration, which were measured, were however not maintained anywhere close to the nominal concentrations. 4.1.5 It is noted that the fish died at measured concentrations as low as 0.14 µg/L, this will have implications on how to treat the necessity of further studies. 4.1.8 In the main study bromadiolone was not found in solution or fish, this might be because that bromadiolone was photolysed or exceeded the limits of solubility. 4.2 It has been discussed at the justification for the BCF study that this is not the correct formula for a substance with a Log Kow of 7.
Conclusion	5.2 It can not be stated that the test substance does not bioaccumulate in rainbow trout since we obviously did not have any test substance in solution. However, it can be concluded that further or revised studies on fish are needed, it seems questionable that EC_{50} for fish is anywhere near 25 mg/L when effects were found at 0.15 µg/L in this study. Although it might be that these are long-term effects and then a long term fish test might be of importance. Even though the study is found not acceptable the results are interesting.
Reliability	4
Acceptability	not acceptable
Remarks	

Section A7.4.3.3.2 - Bioaccumulation in an appropriate invertebrate species

Annex Point IIA XIII 2.3

	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:	Product is used in sewers and related localised / limited exposure areas where invertebrate populations are not of concern.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	CA agrees with applicant's justification. The only organism known to live in sewers according to the emission scenario document for rodenticides is cockroaches and they will remain there after exposure.
Conclusion	Acceptable
Remarks	

Section A7.4.3.4 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA XIII 2.4

	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:	Product is used in sewers and related localised / limited exposure areas where invertebrate populations are not of concern. It was not more than moderately toxic to *Daphnia magna*	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	
Conclusion	Justification acceptable
Remarks	

Section A7.4.3.5.1 - Effects on sediment dwelling organisms

Annex Point IIIA XIII 3.4-

	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:	Compound is of very low water solubility and is not used in situations where sediment-dwelling organisms are exposed. It is used in highly localised and limited areas such as sewers where sediment dwelling organisms do not exist, and it is not applied in a widespread fashion to extensive areas where leaching and run-off is possible.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	If the substance reach water it will rapidly adsorb to the sediments due to its low solubility. The substance will be persistent in sediments and might accumulate over time. If there is a risk for aquatic organisms, which is decided by the studies that will be conducted the study have to be performed.
Conclusion	Justification acceptable
Remarks	

Section A7.4.3.5.2 - Aquatic plant toxicity

Annex Point IIIA XIII 3.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Vryenhoef,. H., Mullee, D.M (2007) *Lemna Minor* Growth Inhibition Test, Safepharm Laboratories, SPL Report No. 2073/0006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bromadiolone	
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 authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	The study was performed in accordance with OECD Guideline Lemna Growth Inhibition Test (March 2006)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Bromadiolone	
3.1.1	Lot/Batch number	L22678	
3.1.2	Specification	As given in section 2	X
3.1.3	Purity	Not stated	
3.1.4	Composition of Product	n/a	
3.1.5	Further relevant properties	Water solubility of the test material is <0.60 mg/l at 20°C (pH 6.5)	X

Section A7.4.3.5.2 - Aquatic plant toxicity

Annex Point IIIA XIII 3.4

3.1.6	Method of analysis	Standards and samples were analysed by HPLC using the following conditions:		
		HPLC System:	Agilent Technologies 1050, incorporating autosampler and workstation	
		Column:	Prodigy ODS3, 5µ, (250 x 4.6mm id)	
		Column temperature:	40°C	
		Mobile phase:	methanol:water*(80:20, v/v) adjusted to pH 3 with phosphoric acid	
		Flow rate:	1.0 ml/min	
		UV/Vis detector wavelength:	210 nm	
		Injection volume:	100 µl	
		Retention time:	approximately 13 minutes	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_5_1_3-1		
3.3	**Reference substance**	3,5-dichlorophenol (Sigma batch no 15809K1066)		
3.3.1	Method of analysis for reference substance	Not performed		
3.4	**Testing procedure**			
3.4.1	Test plants	See table A7_5_1_3-2		
3.4.2	Test system	See table A7_5_1_3-3		
3.4.3	Test conditions	See table A7_5_1_3-4		X
3.4.4	Test duration	7 days		
3.4.5	Test parameter	Growth inhibition		
3.4.6	Examination/ Sampling	The number of fronds present in each test and control culture was recorded on days 0, 2, 5 and 7 along with observations on frond size, appearance, root length and number of colonies present. In addition the dry weight of the fronds in each control and treatment group was determined on day 7.		
3.4.7	Monitoring of TS concentration	Water samples were taken from the control (Replicate R1 –R3 pooled) and 5.5 mg/l test group (replicates R1- R3 and R4 –R6 pooled) on days 0, 2 and 5 (fresh media) and on days 2, 5 and 7 (old media) for quantitative analysis. Duplicate samples were taken on each occasion and stored at approximately -20°C for further analysis if necessary.		
3.4.8	Statistics	Statistical analysis of the average specific growth rate was carried out for the control and 0.47 mg/l test concentration using a Students t-test incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981). Statistical analysis of the yield data was carried out for the control and 0.47 mg/l test concentration using a Students t-test		

Section A7.4.3.5.2 - Aquatic plant toxicity

Annex Point IIIA XIII 3.4

		incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981).	
		4 RESULTS	
4.1	**Range finding test**	Performed	
4.1.1	Concentrations	Initial range-finding test: Nominal concentrations of 0.060 and 0.60 mg/l Second range-finding test: Nominal test concentrations of 0.44 and 4.4 mg/l	X
4.1.2	Results	Initial range-finding test: No inhibition of growth occurred at the maximum attainable concentration of 0.60 mg/l Second range-finding test: No inhibition of growth occurred at the maximum attainable concentration of 4.4 mg/l	
4.2	**Results test substance**		
4.2.1	Applied initial concentration	100 mg/l	
4.2.2	Effect data		

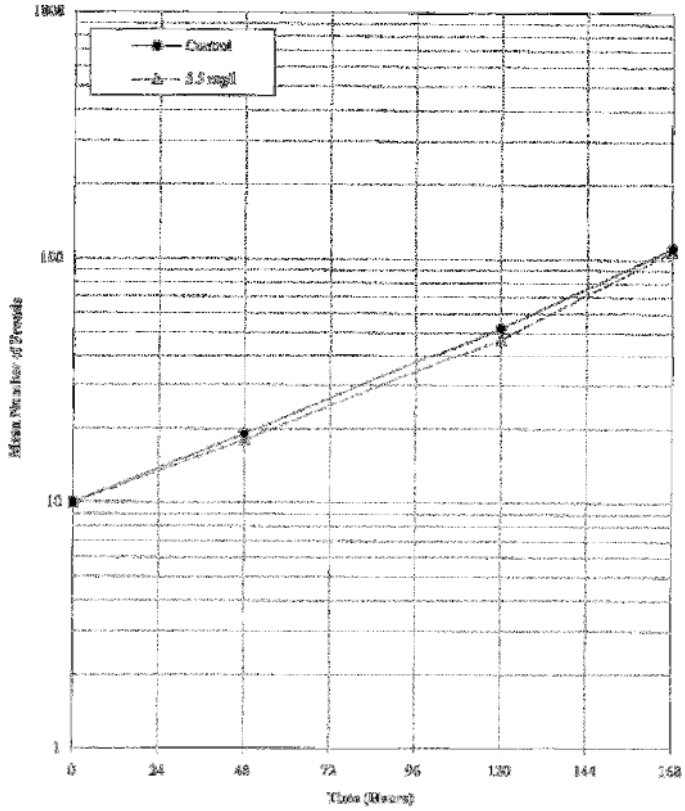
Response Variable	Measurement Variable	EC_{50} (mg/l)	No Observed Effect Concentration (NOEC) (mg/l)
Average Specific Growth Rate	Frond Number	> 0.47	0.47
	Dry Weight	> 0.47	0.47
Yield	Frond Number	> 0.47	0.47
	Dry Weight	> 0.47	0.47

Section A7.4.3.5.2 - Aquatic plant toxicity

Annex Point IIIA XIII 3.4

4.2.3 Graph

Figure 1 Mean Frond Numbers v Time for the Definitive Test



4.3	**Test with reference substance**	Performed
5.3.1	Concentrations	0.625, 1.25, 2.5, 5.0 and 10 mg/l
4.3.1	Results	

Response Variable	Measurement Variable	EC_{50} (mg/l)	95% Confidence Limits	No Observed Effect Concentration (NOEC) (mg/l)
Average Specific Growth Rate	Frond Number	3.1	2.8 – 3.4	1.25
	Dry Weight	2.3	2.2 – 2.5	1.25
Yield	Frond Number	2.4	2.1 – 2.7	1.25
	Dry Weight	2.0	*	1.25

* It was not possible to calculate 95% confidence limits for this response variable as the data generated did not fit the models available for the calculation of confidence limits.

X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was performed in accordance with OECD Guideline Lemna Growth Inhibition Test (March 2006)

Section A7.4.3.5.2 - Aquatic plant toxicity

Annex Point IIIA XIII 3.4

5.2	**Results and discussion**	Chemical analysis of the freshly prepared test samples on days 0, 2 and 5 showed measured concentrations of the saturated solution to range from 2.78 to 3.50 mg/l (50% to 64% of the predicted nominal test concentration). The slight variability in measured concentrations between the preparation periods and also between the definitive test and the media preparation trials concentrations was considered to be due to slight variances in the pH of the test media used. Analysis of the test media on days 2,5 and 7 showed a decline in measured test concentrations to less than the limit of quantification (LOQ) of the analytical method employed. The decline was considered to be due to a combination of both absorption to biomass and possible instability in light. Preliminary stability analysis conducted in the light indicated a decline of 14% over the maximum period of media renewal. Adsorption was not a factor in the stability analyses conducted since no *lemna* were present. Given the decline in measured test concentrations it was considered appropriate to base the results on the time-weighted mean measured test concentrations to give a worst cases analysis of the data.
5.2.1	EC_{10}	**Average specific growth rate:** E_rC_{10} (dry weight) > 0.47 mg/l **Yield:** E_yC_{10} (dry weight) >0.47 mg/l
5.2.2	EC_{20}	**Average specific growth rate:** E_rC_{20} (dry weight) > 0.47 mg/l **Yield:** E_yC_{20} (dry weight) >0.47 mg/l
5.2.3	EC_{50}	**Average specific growth rate:** E_rC_{50} (dry weight) > 0.47 mg/l **Yield:** E_yC_{50} (dry weight) >0.47 mg/l
5.2.4	NOEC	0.47 mg/l
5.3	**Conclusion**	EC50 = >0.47 mg/l NOEC = 0.47 mg/l
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.3.5.2 - Aquatic plant toxicity

Annex Point IIIA XIII 3.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan -09
Materials and Methods	3.1.2: Unclear reference to "section 2", refers to the original study. 3.1.5: The water solubility is surprisingly low compared to what was determined in the basal ecotoxicity tests 7.4.1.1 to 7.4.1.3. 3.4.3: It is not stated which test concentrations were tested in the definitive test. From reading the study report it becomes clear that only one test concentration is used. This is not acceptable.
Results and discussion	4.1.1: The problems with solubility are greater than what was encountered in the basal ecotoxicity tests 7.4.1.1 to 7.4.1.3, where test concentrations up to 12 mg/L were used and complete solubility up to 12 mg/L was confirmed. The reasons for this discrepancy are not understood by RMS and need an explanation. These problems have led to that it is not possible to detect any toxicity of bromadiolone towards *Lemna* in this study. The water solubility data supplied by the applicant shows a water solubility of 2.48 mg/l at 20°C, pH 6.8 and the corresponding data supplied by the other notifier LiphaTech shows a water solubility of 18.4 mg/l at 20°C, pH 7.
Conclusion	5: The resulting effect values are not reliable due to the many uncertainties in the determination of toxicity. There were no inhibition effects at all at any of the concentrations tested, neither in the range finding tests nor in the definitive test. The test concentrations used therefore gives no or very limited information regarding the actual toxicity of bromadiolone. Using time-weighted mean to obtain a "realistic" test concentration that could be used to calculate an effect value is quite meaningless in this case.
Reliability	3.
Acceptability	Not acceptable
Remarks	The problems with solubility in combination with the dissipation of bromadiolone during the study have led to that the result of the test is expressed as "greater than 0.47 mg/L", which is a low and quite unreliable value. The study will not be used in the risk assessment.

Table A7_4_3_5_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	Dimethylformamide
Vehicle control performed	No
Other procedures	Shake-flask method used to obtain required test material concentrations.

Table A7_4_3_5_2-2: Test plants

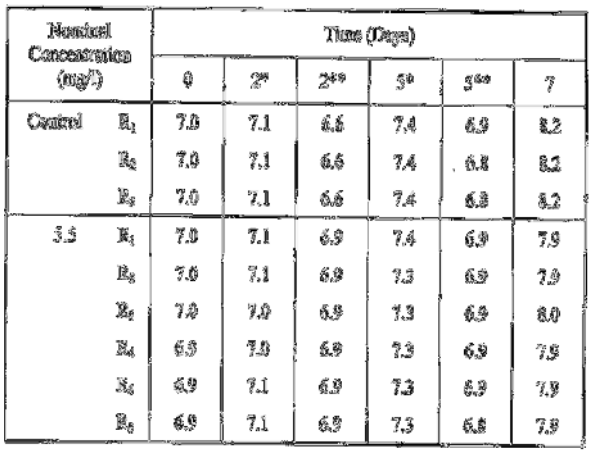
Criteria	Details
Species	*Lemna minor*
Source	University of Toronto Culture Colletion (UTCC), Toronto, Canada
Pretreatment	Culture was maintained in the laboratory at a temperature of 24 ± 1°C under continuous illumination (~7000 lux) for at least 7 days prior to the start of the test

Table A7_4_3_5_2-3: Test system

Criteria	Details
Culture medium	$NaNO_3$ 85 mg/l KH_2PO_4 13.4 mg/l $MgSO_4.7H_2O$ 75 mg/l $CaCl_2.2H_2O$ 36 mg/l Na_2CO_3 20 mg/l H_3BO_3 1.00 mg/l $MnCl_2.4H_2O$ 0.200 mg/l $Na_2MoO_4.2H_2O$ 0.010 mg/l $ZnSO_4.7H_2O$ 0.050 mg/l $CuSO_4.5H_2O$ 0.005 mg/l $Co(NO_3)_2.6H_2O$ 0.010 mg/l $FeCl_3.6H_2O$ 0.84 mg/l Na_2-EDTA.$2H_2O$ 1.40 mg/l The culture medium was prepared using reverse osmosis purified deionised water (Elga Optima 15+ or Elga Purelab Option R-15 BP) and the pH adjusted to 6.5 ± 0.2 with either 1M HCl or NaOH
Renewal of test solution	On days 2 and 5 the test solutions were renewed

Container type	Glass conical flasks
Number of replicates	Six replicate flasks
Numbers of plants per replicate per dose	3 colonies (total 10 fronds)

Table A7_4_3_5_2-4: Test conditions

Criteria	**Details**
Test temperature	24 ± 2°C
pH	
Quality/Intensity of irradiation	7000 lux

Nominal Concentration (mg/l)		Time (Days)					
		0	2*	2**	5*	5**	7
Control	R_1	7.0	7.1	6.6	7.4	6.9	8.2
	R_2	7.0	7.1	6.6	7.4	6.8	8.2
	R_3	7.0	7.1	6.6	7.4	6.8	8.2
3.5	R_1	7.0	7.1	6.9	7.4	6.9	7.9
	R_2	7.0	7.1	6.9	7.3	6.9	7.9
	R_3	7.0	7.0	6.9	7.3	6.9	8.0
	R_4	6.9	7.0	6.9	7.3	6.9	7.9
	R_5	6.9	7.1	6.9	7.3	6.9	7.9
	R_6	6.9	7.1	6.9	7.3	6.8	7.9

Section A7.5.1.1 - Inhibition to microbial activity (terrestrial)

Annex Point IIA VII7.4

	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:	Compound is of very low water solubility and is not used in situations where terrestrial microorganisms are exposed. It is used in highly localised and limited areas such as sewers, and it is not applied in a widespread fashion to extensive areas where leaching and run-off is possible. It does not inhibit microbial activity in a biodegradation study to OECD 301, or in a microbial respiration inhibition study.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	The study is technically possible and scientifically justified since the results from the biodegradation study have not clarified the absence of effect on micro-organisms. The study performed according to OECD used nominal values and the actual sensitivity for micro-organisms could thereby not be determined. There might also be long-term exposure to the soil organisms which will demand this or related studies. However, if the earthworm study is performed than there is a justification for non submission of data.
Conclusion	Acceptable.
Remarks	

Section A7.5.1.2 - Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX (2005) The toxicity to *Eisenia foetida foetida* of Bromadiolone, XXXXX, Chemex reference: ENV6987/110414	
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**	Yes, OECD 207	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	*As given in section 2*	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.5%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	Not stated	
3.2	**Reference substance**		X
3.2.1	Method of analysis for reference substance	Not stated	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	The test substance is the basic structure, which is defined as the test substance and deionised water. *(see table A7_5_1_2-1)*	
3.3.2	Application of the test substance	The test material was applied to the basic structure as a mixture of fine sand and the appropriate quantity of test material. At low test concentrations, the test solution was prepared in an organic solvent carrier (Acetone) and applied to fine sand. The solvent was allowed to vaporise and the resultant test sample/sand mixture added to the basic substrate. The test substrate was homogenised before used.	

Section A7.5.1.2 - Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

3.3.3	Test organisms	*(see table A7_5_1_2-2)*	X
3.3.4	Test system	*(see table A7_5_1_2-3)*	
3.3.5	Test conditions	*(see table A7_5_1_2-4)*	
3.3.6	Test duration	13 days	X
3.3.7	Test parameter	mortality	
3.3.8	Examination	Day 6 and 13	
3.3.9	Monitoring of test substance concentration	No	
3.3.10	Statistics	The LC_{50} value was estimated and 95% confidence limits calculated using ToxCalc version 5.0 'Comprehensive Toxicity Data Analysis and Database Software'.	X
		4 RESULTS	
4.1	**Filter paper test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	133 mg/kg dry weight	
4.2.2	Effect data (Mortality)	*(see table A7_5_1_2-5 & see table A7_5_1_2-6)*	
4.2.3	Concentration / effect curve	Not provided	
4.2.4	Other effects		
4.3	**Results of controls**		
4.3.1	Mortality	There was only one worm that was found dead at dose group 744mg/kg dry weight.	X
4.3.2	Number/ percentage of earthworms showing adverse effects	None	
4.3.3	Nature of adverse effects	Not applicable	

Section A7.5.1.2 - Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	39, 67, 120, 215, 383	
4.4.2	Results	LC_{50} = >235 mg/kg dry weight	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was conducted according to OECD guideline 207. The test vessel were square plastic containers. Each test vessel contained 750g of test substrate (wet weight). Individual worms were then selected from the stock animals. Batches of 10 animals were transferred to the vessels containing the test substrate. The study was performed in constant light (400 to 800lux), to ensure the worms stay in the medium throughout the test. The study was performed at a temperature of 20 ± 2°C. Records were made of the numbers of animals observed alive after a 6 day period and again after 13 days. Death was defined by a complete lack of reaction to gentle stimulus applied to the front end of the worm. As dead animals may have decomposed, any missing animals were counted as dead. Records were kept of any behavioural or pathological abnormalities. At the end of the 13 day exposure period, the moisture content of the test substrate was determined.	X
5.2	**Results and discussion**	The test was terminated one day early. The 6 and 13 day results show no significant change in mortality. The highest no-observed effect concentration was estimated as 1331 mg/kg dry weight. The lowest observed effect concentration was >1331 mg/kg dry weight. The lowest concentration giving 100% was not determined. 0 of the forty control earthworms died during the study.	
5.2.1	LC_0		
5.2.2	LC_{50}	> 1331 mg/kg dry weight	
5.2.3	LC_{100}		
5.3	**Conclusion**	The LC_{50} was > 1331 mg/kg, and the lowest concentration giving 100% mortality was not determined.	
5.3.1	Other Conclusions		
5.3.2	Reliability	1	
5.3.3	Deficiencies	Yes, The preliminary study was not described, only the results were shown, however this does not effect the integrity of the study	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2006-05-08*

Section A7.5.1.2 - Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Materials and Methods	Adopt applicant's version noting the following deviations. 3.2 Which reference substance that was used is not stated? 3.3.3 The correct scientific name should be *Eisenia fetida*. 3.3.6 There were minor deviations from the OECD guideline i.e. the test was run for 13 days instead of 14, this will not affect the quality of the investigation. An optimal study should, according to the OECD guideline, have one concentration with total mortality, to make it possible to calculate the EC_{50}.
Results and discussion	Adopt applicant's version noting the following deviations. 4.3.1 There was no mortality in the controls.
Conclusion	Adopt applicant's version noting the following deviations. Second paragraph, first line: The study was conducted in constant light. But was it 400 **or** 800 lux or probably something in between, please clarify this.
Reliability	1
Acceptability	Acceptable, although no effects could be stated in earthworms the concentrations used are considered to be high enough.
Remarks	

Table A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Not stated
Alkalinity / Salinity	Not stated
Hardness	Not stated
pH	Start of test = 6.4
Moisture content	Start of test = 46.1 End of test = 43.9
Conductance	Not stated
Holding water different from dilution water	No

In case of the use of an organic solvent	
Dispersion	Yes
Vehicle	Yes, Acetone
Concentration of vehicle	Not stated
Vehicle control performed	Not stated
Other procedures	

Table A7_5_1_1-2: Test organisms

Criteria	Details
Species/strain	*Eisenia foetida foetida*
Source of the initial stock	Blades Biological Ltd, Kent, UK
Culturing techniques	Not stated
Age/weight	Mean Weight: 486 Max: 598 Min: 308 Age: at least 2 months old
Pre-treatment	Temperature = 20.0°C

Table A7_5_1_1-3: Test system

Criteria	Details
Artificial soil test substrate	10% sphagnum peat 20% Kaolin clay 60% industrial fine sand 10% B&Q Organic peat free multipurpose compost About 1% calcium carbonate, pulverised, added to bring the pH between 6.0 and 6.5
Test mixture	750g of test substrate in 2 litres of test vessels containing artificial soil
Size, volume and material of test container	2 litre
Amount of artificial soil (kg)/ container	Not stated
Nominal levels of test concentrations	0, 133, 239, 426, 744, 1331mg/kg
Number of replicates/concentration	4
Number of earthworms/test concentration	10
Number of earthworms/container	10
Light source	Artificial
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	20 ± 2°C
Moisture content	Start of test = 46.1 End of test = 43.9
pH	Start of test = 6.4
Adjustment of pH	No
Light intensity / photoperiod	400 to 800 lux
Relevant degradation products	Not relevant

Table A7_5_1_2-5: Mortality data

Test Substance Concentration (nominal/measured)[1] [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	6 d	13 d	6 d	13 d
Control	0	0	0	0
133	0	0	0	0
239	0	0	0	0
426	0	0	0	0
744	1	0	2.5	0
1331	0	0	0	0
Temperature [°C]	20 ± 2°C	20 ± 2°C		
pH	-	-		
Moisture content		43.9		

[1] specify, if TS concentrations were nominal or measured

Table A7_5_1_2-6: Effect data

	13d [mg/kg soil]	95 % c.l.
LC_0		
LC_{50}	> 1331	NA
LC_{100}		

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	**x**	

Section A7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

	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 water solubility and is not used in situations where terrestrial plants are exposed. It is used in highly localised and limited areas such as sewers where plants do not exist, and it is not applied in a widespread fashion to extensive areas where leaching and run-off which might contaminate terrestrial plants is possible. It is of low vapour pressure and is not applied as a spray or vapour which might contaminate plants. Many years of use in a wide range of situations has shown no effect on plants. Plants are not treated with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	CA agrees with applicant's justification, but are there scientific proofs that no effects on plants have been found?
Conclusion	Acceptable
Remarks	

Section A7.5.2.1 - Reproduction study with other soil non-target macro-organisms

Annex Point IIIA XIII 3.2

	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 water solubility and is not used in situations where soil macrorganisms are exposed. It is used in highly localised and limited areas such as sewers where such creatures do not exist, and it is not applied in a widespread fashion to extensive soil areas where leaching and run-off might be possible.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	The result of the earthworm test show that toxicity to soil organisms is low, therefore it is scientifically justified not to perform this study.
Conclusion	Justification acceptable
Remarks	

Section A7.5.2.2 - Long-term test with terrestrial plants

Annex Point -

	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 water solubility and is not used in situations where terrestrial plants are exposed. It is used in highly localised and limited areas such as sewers where plants do not exist, and it is not applied in a widespread fashion to extensive areas where leaching and run-off which might contaminate terrestrial plants is possible. It is of low vapour pressure and is not applied as a spray or vapour which might contaminate plants. Many years of use in a wide range of situations has shown no effect on plants. Plants are not treated with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-03-27*
Evaluation of applicant's justification	The result of the earthworm test justifies this study not have to be performed.
Conclusion	Justification acceptable.
Remarks	

Section A7.5.3.1.1-01 - Effects on birds - Acute oral toxicity

Annex Point IIIA XIII.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Paul L. Hegdal and Raymond W. Blaskiewicz (1984) Evaluation of the potential hazard to barn owls of Talon (brodifacoum bait) used to control rat sand house mice. Environmental Toxicology and Chemistry Vol 3. 167-179.	
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.	X
2.2	**GLP**	The GLP status of the study is not stated in the published paper	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
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		
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	Barn Owls	
3.2.2	Strain	*Tyto alba*	
3.2.3	Source	Wild	
3.2.4	Sex	Not stated in published paper	X
3.2.5	Age/weight at study initiation	Not stated in published paper as animals were wild.	
3.2.6	Number of animals per group	26 adults (17 female , 9 male) and 8 fledged young	X
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral (Rats and mice treated with brodifacoum)	
3.3.1	Preparation of test site	All regurgitated pellets were removed from the sites under test as well as post treatment pellets	

Section A7.5.3.1.1-01 - Effects on birds - Acute oral toxicity

Annex Point IIIA XIII.1.1

3.3.2	Concentration of test substance	Bait with 0.005% brodifacoum (50 ppm).	
3.3.3	Specific activity of test substance	Not relevant	
3.3.4	Volume applied	Unknown as owls had to catch their prey.	X
3.3.5	Sampling time	Once every two days. Efforts were made not to visit a test site on successive nights to avoid nest abandonment.	
3.3.6	Samples	Dead birds were checked for brodifacoum residues and also for cause of death.	
		4 RESULTS AND DISCUSSION	
4.1	**Result of study**	Analysis of regurgitated pellets showed that barn owls do consume rats and mice but only 3.9 and 2.2% respectively of their total diet. Voles are the most common prey and they do not tend to exist in farmsteads. During the study there was little or no evidence that the owls captured or consumed rodents that had eaten Talon bait. Only one owl analysed had a trace of brodifacoum residue at the detection limit (0.05 ppm) but this death was attributed to electrocution.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Observations of 34 barn owls feeding on poisoned rats and mice	
5.2	**Results and discussion**	From laboratory studies it was found that brodifacoum can kill barn owls secondarily if enough poisoned rodents are consumed, but brodifacoum did not appear to be involved in any of the barn owl deaths in this study.	X
		Out of 34 owls observed, only one owl analysed had a trace of brodifacoum residue at the detection limit (0.05 ppm). However this death was attributed to electrocution.	X
		At least 9 of the owls were still on treated sites up to 62 days post-treatment. None of these birds died under circumstances which would suggest secondary poisoning by brodifacoum.	X
5.3	**Conclusion**	The results of this study indicate that the potential for barn owl mortality as a result of brodifacoum rodenticide baiting around farms to be low..	X
		Out of 34 owls observed, only one owl analysed had a trace of brodifacoum residue at the detection limit (0.05 ppm). However this death was attributed to electrocution.	X
		At least 9 of the owls were still on treated sites up to 62 days post-treatment. None of these birds died under circumstances which would suggest secondary poisoning by brodifacoum.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities
EVALUATION BY RAPPORTEUR MEMBER STATE

Section A7.5.3.1.1-01 - Effects on birds - Acute oral toxicity

Annex Point IIIA XIII.1.1

Date	*2006-05-08*
Materials and Methods	Adopt applicant's version noting the following deviations. This is not an acute oral toxicity study since the dose was not known and if the owls consumed any Brodifacoum it was repeatedly, not as should be in a acute oral toxicity study one dose and then the birds should be observed for ten days. 18.1 No guideline was followed. 18.2 GLP was not used. 19.2.4 17 female, 9 male and 8 fledged young. 19.2.6 The birds were not divided into groups, i.e. forest living, living close to farms etc. 19.3.4 Total amount of brodifacoum applied in the areas should be stated.
Results and discussion	Adopt applicant's version noting the following deviations. 20.1 It is difficult to analyse the results of this since all birds were from one group the results can not be considered in a general way. The birds, in average, consumed so small amounts of rats and mice so it is hard to conclude anything from this study. How many birds died??
Conclusion	Adopt applicant's version noting the following deviations. 21.2 Was it found in this study that barn owls could be killed by secondary poisoning? How many of the owls did actually die? If it was only one and this one had brodifacoum residues in its body it should be considered seriously.
Reliability	4
Acceptability	not acceptable
Remarks	

Section A7.5.3.1.1-02 - Effects on birds - Acute oral toxicity

Annex Point IIIA XIII.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	A. Krambias and A.H. Hoppe (1987) The response of captive partridges to dosing with anticoagulant rodenticides. Control of Mammal Pests 181- 186.	
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.	X
2.2	**GLP**	The GLP status of the study is not stated in the published paper	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Bromadiolone and Difenacoum baits containing 0.005% a.i	
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	Partridges	
3.2.2	Strain	Chukar	
3.2.3	Source	Government farm in Athalassa.	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age from one to two years Mean weight – bromadiolone group – 464g Mean weight – difenacoum group – 448.6g	
3.2.6	Number of animals per group	12 (6 male and 6 female)	
3.2.7	Control animals	No	X
3.3	**Administration/ Exposure**	10-day no choice oral administration in food	
3.3.1	Preparation of test site	Not applicable	

Section A7.5.3.1.1-02 - Effects on birds - Acute oral toxicity

Annex Point IIIA XIII.1.1

3.3.2	Concentration of test substance	Bait with 0.005% bromadiolone or 0.005% difenacoum.	
3.3.3	Specific activity of test substance	Not relevant	
3.3.4	Volume applied	Free access to food.	
3.3.5	Sampling time	Daily	
3.3.6	Samples	Dead birds were taken to veterinary services to confirm cause of death due to poisoned bait.	
		4 RESULTS AND DISCUSSION	
4.1	**Result of study**	The result of the ten day no-choice tests indicated that 50% mortality for bromadiolone and 66% mortality for difenacoum. The mean bait consumption per day for bromadiolone was 27g of bait (28.9 mg of bromadiolone/kg bw). For Difenacoum this was 20 g of bait (22,3 mg of difenacoum/kg bw)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Ten day no-choice tests toxicity study in partridges using baits containing 0.005% a.i.; observations for food consumption and mortality; gross pathology of decendents	
5.2	**Results and discussion**	The highest quantity of bait consumed by an individual bird without lethal effect was 411g for bromadiolone and 326.2g for difenacoum. If placement of bait packs in holes or crevices of trees the chances of partridges reaching the bait is reduced making the consumption of lethal doses less probable. This has been supported by evidence from the field were examination of 10 partridges shot in areas heavily treated with rodenticides did not show evidence of poison in their crops..	X
5.3	**Conclusion**	If the results of these laboratory tests are considered in relation to field conditions, where the birds have free choice of food and poison bait is largely inaccessible to them, it can be concluded that the chances of lethal exposure are very small.	X
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2006-05-08*
Materials and Methods	Adopt applicant's version noting the following deviations. 2.1 Not a guideline study. 2.2 No GLP. 3.2.7 In a scientific investigation, it is necessary to have control animals. In this investigation the cause of death of the birds could be confirmed by investigations, still other effects could have been observed if compared to a control group i.e. was there a reduction in food consumption.

Section A7.5.3.1.1-02 - Effects on birds - Acute oral toxicity

Annex Point IIIA XIII.1.1

Results and discussion	Adopt applicant's version noting the following deviations. 5.2 There is no evidence from scientific investigations that rodenticides do not affect birds in field. In the paper there are no references for this statement, it has to be investigated thoroughly with replicates and controls before a statement is made whether or not there are such effects on birds of rodenticides.
Conclusion	5.3 These conclusion is an overestimation of the results, birds usually live longer than 10 days and the substance will accumulate in their bodies. This means that although the exposure might be lower there is a possibility that concentrations, after accumulation, eventually will reach toxic concentrations. The study shows that acute effects will be found when the consumed amount exceeds 20 mg a.i/kg bw.
Reliability	3
Acceptability	Not acceptable as an acute toxicity study
Remarks	It might be considered as a short-term toxicity test but there is only one dose which gives it limited reliability also as a short-term test.

Section A7.5.3.1.1-03 - Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX, 2004, Draft Report: Acute oral toxicity of Bromadiolone technical on Japanese Quail (Coturnix coturnix japonica), XXXXX, 04/916-115FU, GLP, Unpublished.	
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**	Yes, OPPTS 850.2100	
2.2	**GLP**	Yes	
2.3	**Deviations**	The purity of the test item tested is 97.6%. This will not affect the integrity of the study.	
		3 METHOD	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Fluka, 1090258	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	97.6%	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	None.	
3.1.6	Method of analysis in the diet	Not stated.	
3.2	**Administration of the test substance**	See table A7_5_3_1_2-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**	*Non-entry field*	
3.4.1	Test organisms	See table A7_5_3_1_1-2	
3.4.2	Test system	See table A7_5_3_1_1-3	
3.4.3	Diet	Test substance was administered by gavage using corn oil as a vehicle. Babolna Poultry Pullet Standard Diet was provided ad libitum during the observation period.	

Analysed component	Supplier declared content (%)
Dry matter	86

Section A7.5.3.1.1-03 - Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

		ME poultry	12.15 MJ/kg
		Crude protein	15.00
		Lysine	0.60
		Methionine	0.31
		Met. + Cystine	0.60
		Crude fat	2.50
		Crude fibre	3.85
		Sodium	0.15
		Calcium	0.82
		Phosphorous	0.58
		Vitamin A	6000.0 NE/kg
		Vitamin D3	2000.0 NE/kg
		Vitamin E	20.0 mg/kg
3.4.4	Test conditions	See table A7_5_3_1_1-4	
3.4.5	Duration of the test	14 day observation period	
3.4.6	Test parameter	Mortality	
3.4.7	Examination / Observation	Clinical observations of the animals were made for the first 60 minutes, then: 3h, 4h, 5h and then daily for the 14 days. Body weight was measured on days –14, -7, 0, 3, 7 and 14. Average estimated feed consumption was determined for each dose group and the control on days 0-3, 3-7, and 7-14.	
3.4.8	Statistics	LD50 was calculated by SPSS PC + statistical software using probit analysis and was given with 95% confidence limits. Analysis was performed on the body weight data with SPSS statistical program. The homogeneity of variance was determined by F-test. The heterogeneity of variance was checked by Bartlett's homogeneity of variance test.	
		4 RESULTS	
Limit Test / Range finding test		Performed	
4.1.1	Concentration	0.2, 2, 20, 200, 2000 mg/kg bw	

4.1.2 Number/ percentage of animals showing adverse effects

Dose (mg/kg bw)	Treated birds	Dead birds
Control	2	0
0.2	2	0
2	2	0
20	2	0
200	2	1
2000	2	2

Section A7.5.3.1.1-03 - Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

4.1.3 Nature of adverse effects

Mortality

Other effects:

No abnormal behaviour was observed in either the control or the lowest dose group (31.3 mg/kg bw).

In the 62.5 mg/kg bw dose group 5 birds cowered, and 10 birds were fluffed feather in the first 60 minutes. At 6 days after treatment 1 male bird was dead. No other symptoms were observed in this group.

In the 125 mg/kg bw group, 8 birds cowered and 10 birds were fluffed feather in the first 60 minutes. At 5 days 1 female was dead, at 6 days, a further 2 males and 2 females had died. No other symptoms were observed in this group.

In the 250 mg/kg bw group, all the birds cowered and had fluffed feather in the first 60 minutes. At 3, 4, and 5 hours fluffed feathers were still observed. At 5 days 1 male and 3 female birds were dead, at 6 days, a further 2 males and 2 females had died. No other symptoms were observed in this group.

In the 500 mg/kg bw group, all the birds cowered and had fluffed feather in the first 60 minutes. At 3, 4, and 5 hours fluffed feathers were still observed. At 5 days 3 male and 4 female birds were dead, and at 6 days, all had died.

Results test substance *Non-entry field*

4.1.4 Applied concentrations

Control, 31.3, 62.5, 125.0, 250.0, 500.0 mg/kg bw.

4.1.5 Effect data (Mortality)

See table A7_5_3_1_1-5

Male: 153 mg/kg bw (95% confidence limits: 83-299 mg/kg bw)

Female: 118 mg/kg bw (No confidence limits could be calculated)

Average: 134 mg/kg bw (95% confidence limits: 96-188 mg/kg bw)

4.1.6 Body weight

Mean body weight gain:

Dose (mg/kg bw)	Days:	Body weight gain (g)			
		3-0	7-3	14-7	SUM
Control	Male	-0.40	1.8	1.6	3.00
	Female	2.00	1.20	4.00	7.20
31.3	Male	0.00	1.00	1.80	2.80
	Female	-0.80	0.00	1.60	0.80
62.5	Male	0.00	1.50	1.25	2.75
	Female	-0.40	-1.60	4.80	2.80
125.0	Male	-0.60	-1.00	3.00	2.00
	Female	0.80	-1.00	3.50	3.50
250.0	Male	0.60	0.00	2.50	3.50
	Female	-1.20	-	-	-
500.0	Male	2.80	-	-	-
	Female	-2.40	-	-	-

4.1.7 Feed consumption

Mean daily food consumption (g/animal/day)

Section A7.5.3.1.1-03 - Acute oral toxicity on birds

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Dose (mg/kg bw)	0-3 days	3-7 days	7-14 days
Control	17.6	19.7	16.5
31.3	17.8	20.3	16.4
62.5	17.4	20.2	16.1
125.0	17.1	19.8	16.3
250.0	17.3	20.3	16.4
500.0	17.2	20.4	-

4.1.8 Concentration / response curve: Not reported.

4.1.9 Other effects: Necroscopy:

Dose Group (mg/kg bw)	Observations
62.5	Blood found in the abdominal cavity of 1 male
125.0	Blood in the abdominal cavity and subcutaneous haematoma in the craw region in 1 male. Blood in the craw in 1 male and 1 female. In 2 females, blood was in the abdominal cavity. Subcutaneous haematoooma was observed in the thoracal region in 1 female and in the left limb region in 1 animal.
250.0	Blood filled the craw of 1 male, and was observed in 2 females. Blood was in the abdominal cavity of 2 male, and filled in 3 females. Subcutaneous haematoma: in the thoracal region in 1 male and 1 female; in the neck region in 2 females; in the thoracal region in 1 male and 1 female.
500.0	Blood filled craw in 1 male and 2 female. Blood in the abdominal cavity in 4 male and 3 female. Subcutaneous haematoma: in the thoracal region in 1 male; in the neck region in 1 male and 1 female; in the thoracal region in 1 male and in the left limb region in 2 female.

In the animals sacrificed after the observation period, no macroscopic alterations were observed. In the control group, only 2 male and 1 female animals were examined.

Results of controls

4.1.10 Number/ percentage of animals showing adverse effects: None observed.

4.1.11 Nature of adverse effects: None noted.

Section A7.5.3.1.1-03 - Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Test with reference substance		Not performed	
4.1.12	Concentrations	-	
4.1.13	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OPPTS 850.2100 Clinical observations of the animals were made for the first 60 minutes, then: 3h, 4h, 5h and then daily for the 14 days. Body weight was measured on days –14, -7, 0, 3, 7 and 14. Average estimated feed consumption was determined for each dose group and the control on days 0-3, 3-7, and 7-14.	
5.2	**Results and discussion**	In the animals that died during the study, the observed macroscopic alterations: blood in the abdominal cavity, in the craw, subcutaneous haematoma in the left limb region, in the neck region, in the abdominal region and in the thoracal region, could be in connection with acute circulatory insufficiency as the cause of death.	X
5.2.1	LD_{50}	Male: 153 mg/kg bw (95% confidence limits: 83-299 mg/kg bw) Female: 118 mg/kg bw (No confidence limits could be calculated) Average: 134 mg/kg bw (95% confidence limits: 96-188 mg/kg bw)	
5.3	**Conclusion**	No clinical signs were observed in the control animal and hence the validity criteria were satisfied. (see table A7_5_3_1_1-7).	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Materials and Methods	Adopt applicants version.
Results and discussion	Adopt applicants version noting the following deviation. 5.2 Initially 50 % of the birds were affected at bromadiolone concentrations of 62.5 mg/kg bw.
Conclusion	Adopt applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	

Table A7_5_3_1_1-1: Method of administration of the test substance

Carrier / Vehicle	**Details**
Water	No
Organic carrier	Yes, corn oil.
Concentration of the carrier [% v/v]	Give the concentration
Other vehicle	
Function of the carrier / vehicle	Solvent for the test substance.

Table A7_5_3_1_1-2: Test animals

Criteria	**Details**		
Species/strain	Japanese Quail, (*Coturnix coturnix japonica*)		
Source	Dezso Rokolya, Csavoly, Hungary		
Age (in weeks), sex and initial body weight (bw)	Minimum 16 weeks old at test start.		
	Group	Sex	Initial body weight (g)
	1	Male	167, 181, 182, 185, 188
		Female	168, 183, 186, 190, 201
	2	Male	160, 171, 182, 173, 197
		Female	185, 186, 187, 208, 207
	3	Male	180, 165, 182, 192, 193
		Female	195, 178, 193, 201, 200
	4	Male	170, 172, 175, 187, 194
		Female	187, 200, 211, 201, 205
	5	Male	175, 165, 182, 183, 201
		Female	200, 193, 195, 196, 202
	6	Male	158, 166, 183, 158, 200
		Female	191, 170, 208, 179, 200
Breeding population	Pen rared.		
Amount of food	Ad libitum during acclimatisation and study observation period.		
Age at time of first dosing	Minimum 16 weeks at test start.		
Health condition / medication	All birds were in apparent good health.		

Table A7_5_3_1_1-3: Test system

Criteria	**Details**
Test location	Indoor, in cages.
Holding pens	Galvanised wire cages, indoors.
Number of animals	60 including control group.
Number of animals per pen [cm²/bird]	10, 50 cm^2/bird

Number of animals per dose	10
Pre-treatment / acclimation	15 hour starvation period before dosing. Babolna Poultry Pullet Standard Diet, provided ad libitum. Acclimatisation of 14 days.
Diet during test	Babolna Poultry Pullet Standard Diet, provided ad libitum after dosing.
Dosage levels (of test substance)	Control, 31.3, 62.5, 125.0, 250.0, 500.0 mg/kg bw.
Replicate/dosage level	10 animals per cage, 1 cage per dose group.
Feed dosing method	Gavage.
Dosing volume per application	5 ml/kg bw
Frequency, duration and method of animal monitoring after dosing	First 60 minutes, then: 3h, 4h, 5h and then daily for the 14 days.
Time and intervals of body weight determination	On days: -14, -7, 0, 3, 7 and 14.

Table A7_5_3_1_1-4: Test conditions (housing)

Criteria	**Details**
Test temperature	18.6 – 24.1°C
Shielding of the animals	Not Stated.
Ventilation	Not stated.
Relative humidity	47 – 62%
Photoperiod and lighting	8 hours of light per day.

Table A7_5_3_1_1-5: Mortality data after test termination

Test substance dosage level [mg/kg bw]	Mortality after test termination (…. days)			
	Total number per dose level		Percentage per dose level	
	Male	Female	Male	Female
Control	0/5	0/5	0	0
31.3	0/5	0/5	0	0
62.5	1/5	0/5	20%	0
125.0	2/5	3/5	40%	60%
250.0	3/5	5/5	60%	100%
500.0	5/5	5/5	100%	100%
Temperature [°C]	18.6 – 24.1°C			
Relative humidity	47 – 62%			

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	**X**	

Section A7.5.3.1.2 - Short-term toxicity on birds

Annex Point IIIA XIII 1.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The effects on birds have been shown in Section A7.5.3.1.1 and A7.5.3.1.3. The conduct of short term toxicity on birds is unnecessary and scientifically unjustified.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*Give date of action*
Evaluation of applicant's justification	Short term toxicity tests seem necessary in the light of the findings for other species. Acute oral tests for rodenticides are not necessary according to decision on technical meetings but short-term toxicity test are. However, since the acute oral test is used only in a qualitative assessment which is not used in the definitive risk assessment the requirement for a short term study may be excepted.
Conclusion	RMS considers that the justification is acceptable under the above circumstances.
Remarks	

Section A7.5.3.1.3 - Effects on reproduction of birds

Annex Point IIIA XIII 1.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	XXXXX (2004) Avian Reproduction toxicity test of Bromadiolone technical in the Japanese Quails (*Coturnix coturnix japonica)*, XXXXX XXXXX (2005) Amendment to the final report: Avian reproduction toxicity test of Bromadiolone technical in Japanese quails. XXXXX. Study number: 04/804-206FU	
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**	Yes – OECD No 206	
2.2	**GLP**	Yes	
2.3	**Deviations**	The following data has been changed from the final report and is summarised in the amendment to the final report: 1. SUMMARY Conclusion: Under the conditions of this study the reproduction NOEC is 0.4 mg/l water concentration (70 µg/kg nominal, 39 µg/kg measured) and the value of the reproduction LOEC is 0.8 mg/l water concentration (140 µg/kg nominal, 79 µg/kg measured). 5. DISCUSSION Under the conditions of this study the reproduction NOEC is 0.4 mg/l water concentration (70 µg/kg nominal, 39 µg/kg measured) and the value of the reproduction LOEC is 0.8 mg/l water concentration (140 µg/kg nominal, 79 µg/kg measured). This does not affect the validity of the study.	X
		3 METHOD	
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	Purity	99.2 %	
3.1.4	Composition of Product	Not applicable	

Section A7.5.3.1.3 - Effects on reproduction of birds

Annex Point IIIA XIII 1.3

3.1.5	Further relevant properties	Not applicable
3.1.6	Method of analysis	Bromadiolone content was determined in water samples on the start and the end of the study. One sample was taken from each test concentration and the control. The sample volume was 50 ml. Bromadiolone samples were diluted with methanol.

Nominal conc Bromadiolone (mg/l)	Sample volume (ml)	Sample vol after dilutions	Intended conc, mg/l
0.2	7	10	0.14
0.4	5	10	0.2
0.8	5	10	0.4

Control samples were directly measured The samples were analysed by HPLC according to the following conditions:
HPLC system: Merck-Hitachi LaChrom HPLC System II
Balance: BP 221S Sartorius, Germany, No. 11809117
Ultrasonic bath: TESLA, Poland, No. 302080
Chromatography conditions:
Detector: UV at 260nm
Column: LiChrospher 100 RP-18 5µ 250x4 mm, No: 316308
Mobile phase: Methanol: 0.25 g/l Phoshphoric acid = 9:1
Flow: 0.8ml/min
Injection volume: 20µl
Retention time: 4.84 min ± 10%

3.2	**Administration of the test substance**	Ethanol abs.
3.3	**Testing procedure**	
3.3.1	Test organisms	(see table A7_5_3_1_3-2)
3.3.2	Test system	(see table A7_5_3_1_3-3)
3.3.3	Diet	SSNIFF SM quail diet
3.3.4	Test conditions	(see table A7_5_3_1_2-4)
3.3.5	Duration of the test	Six weeks
3.3.6	Test parameter	Mortality, clinical signs, eggs laid, egg mass, abnormal eggs, cracked/broken eggs, eggshell thickness, fertility, viability, hatchability
3.3.7	Examination / Observation	Food consumption: weekly for adults and daily for the hatchlings Water consumption: every day for adults Organ weights: at test termination

Section A7.5.3.1.3 - Effects on reproduction of birds

Annex Point IIIA XIII 1.3

3.3.8	Statistics	Statistical analysis was done with SPSS PC+ software package for the following points: - body weight data of adults - water consumption data of adults - food consumption data of adults - organ weight data of surviving adults - egg weight data - egg-shell thickness data - body weight data of 0-day and 14 day old chicks - food consumption data of chicks Bartlett's homogeneity of variance test, Duncan's multiple range test, Kolmogorov-Smirnov test, Kruskal-Wallis One-way analysis of variance, Mann-Whitney U-test.	X
		4 RESULTS	
4.1	**Limit Test / Range finding test**	Performed	
4.1.1	Concentration	17.5, 35, 52.5µg/kg bw	
4.1.2	Number/ percentage of animals showing adverse effects	None	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Applied concentrations	35, 70 and 140 µg/kg bw	
4.2.2	Effect data (Mortality and reproductivity)	See table A7_5_3_1_3-5	

4.2.3 Body weight

Adults birds:

There was no significant effect upon body weight at any of the concentrations tested during the treatment period.

X

SUMMARY OF BODY WEIGHT OF THE ADULT QUALIS (g)

DOSE		Male Start of pre-exposure	Male Start of exposure	Male End of exposure	Female Start of pre-exposure	Female Start of exposure	Female End of exposure
Control (1)	Mean	182.25	189.50	200.00	225.42	238.33	253.83
	SD	9.07	8.01	10.20	15.28	10.50	12.72
	n	12	12	12	12	12	12
35 µg/kg (2)	Mean	182.50	191.92	211.17	218.42	232.58	252.00
	SD	9.86	11.48	13.93	10.70	8.99	13.27
	n	12	12	12	12	12	12
	±%	0	1	6	-3	-2	-1
70 µg/kg (3)	Mean	187.92	199.83	209.83	229.92	241.83	241.67
	SD	11.97	13.54	19.84	10.10	7.02	14.82
	n	12	12	12	12	12	12
	±%	3	5 *(1-3)	5	2	1	-5
140 µg/kg (4)	Mean	192.58	198.25	202.91	224.33	238.08	240.73
	SD	8.50	7.23	11.59	13.40	9.96	20.99
	n	12	12	11	12	12	11
	±%	6 *(1-4) *(2-4)	5	1	0	0	-5
		DN	DN	NS	NS	NS	NS

REMARKS :
±% = Percent Deviation Versus Control
NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test;
*(group1-group2) group mean1 < group mean2

Bodyweights of the 0-day and 14-day old survivors:

The hatchling showed no dose-related effect.

SUMMARY OF BODY WEIGHT OF CHICKS ON DAY 0 (g)

DOSE		Week -2	-1	1	2	3	4	5	6
Control (1)	MEAN	8.84	8.93	8.96	8.22	8.21	8.26	8.10	7.94
	SD	0.84	0.57	0.91	0.55	0.51	0.62	0.88	0.85
	n	45	43	47	51	49	51	45	40
		**(4-1)							
35 µg/kg (2)	MEAN	8.86	8.74	8.60	8.11	8.05	7.95	8.19	8.21
	SD	0.86	0.76	0.80	0.59	0.66	0.65	0.79	0.81
	n	56	55	63	50	51	52	47	48
	±%	0	-2	-4	-1	-2	-4	1	3
		**(4-2)		*					
70 µg/kg (3)	MEAN	9.17	9.35	9.09	8.18	8.32	7.99	8.21	8.24
	SD	0.85	0.96	1.15	0.84	1.10	1.02	1.17	1.02
	n	35	44	54	48	45	44	44	41
	±%	4	5	1	0	1	-3	1	4
		**(4-3)							
140 µg/kg (4)	MEAN	7.38	8.28	8.80	8.11	8.15	8.10	8.08	8.06
	SD	0.57	1.08	0.60	0.61	0.65	0.77	0.69	0.71
	n	25	21	38	40	46	43	31	31
	±%	-17	-7	-2	-1	-1	-2	0	1
			*						
		NS	NS	NS	NS	NS	NS	NS	NS

Remarks:
* = p < 0.05
** = p < 0.01
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

SUMMARY OF BODY WEIGHT OF CHICKS ON DAY 14 (g)

DOSE		Week -2	-1	1	2	3	4	5	6
Control (1)	MEAN	73.39	76.20	51.26	48.81	45.58	29.24	26.63	27.51
	SD	9.86	3.71	5.03	2.84	5.54	4.70	2.89	2.87
	n	44	43	46	50	47	48	42	36
							**(2-1)		
							**(3-1)		
							**(4-1)		
35 µg/kg (2)	MEAN	72.46	77.32	48.73	47.86	45.16	25.28	27.98	28.41
	SD	9.71	3.35	3.91	1.98	2.45	3.73	2.20	2.75
	n	53	55	62	48	48	49	45	42
	±%	-1	1	-5	-2	-1	-14	5	3
				*					
70 µg/kg (3)	MEAN	73.54	78.09	48.79	48.10	45.69	24.61	27.79	27.92
	SD	8.67	3.00	3.03	2.91	3.82	4.71	3.16	3.53
	n	33	43	49	44	41	39	39	35
	±%	0	2	-5	-1	0	-16	4	1
			*(1-3)	*					
140 µg/kg (4)	MEAN	72.13	77.71	49.29	47.82	45.55	26.37	27.24	28.31
	SD	9.64	3.16	4.36	2.17	2.31	4.35	2.53	1.89
	n	23	17	33	35	41	36	23	23
	±%	-2	2	-4	-2	0	-10	2	3
		NS	DN	U	NS	NS	DN	NS	NS

Remarks:
* = p < 0.05
** = p < 0.01
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

4.2.4 Food consumption Adults:

SUMMARY DAILY MEAN FOOD CONSUMPTION (g/animal/day)

DOSE GROUP		TIME (week) -2	-1	1	2	3	4	5	6
Control (1)	MEAN	35.27	28.22	26.57	30.59	30.18	30.56	30.13	30.16
	SD	5.30	5.77	2.90	4.30	1.76	1.48	1.57	1.51
	n	12	12	12	12	12	12	12	12
35 µg/kg (2)	MEAN	37.10	29.32	25.63	29.14	28.97	29.65	29.12	29.43
	SD	4.71	3.82	3.68	5.91	2.64	2.55	2.67	2.60
	n	12	12	12	12	12	12	12	12
	±%	5	4	-4	-5	-4	-3	-3	-2
70 µg/kg (3)	MEAN	35.62	30.03	27.76	33.47	31.34	31.72	31.16	31.44
	SD	5.22	4.39	4.07	6.46	3.58	3.19	3.37	3.26
	n	12	12	12	12	12	12	12	12
	±%	1	6	4	9	4	4	3	4
140 µg/kg (4)	MEAN	33.21	29.58	26.26	31.48	31.73	32.31	31.84	31.75
	SD	4.42	5.53	5.84	8.12	4.66	4.05	4.25	4.05
	n	12	12	12	12	12	11	11	11
	±%	-6	5	-1	3	5	6	6	5
		NS	NS	NS	NS	NS	NS	NS	NS

Remarks:
* = p < 0.05
** = p < 0.01
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

Statistical significant differences were not observed during the 6 weeks treatment.

Hatchlings:

Section A7.5.3.1.3 - Effects on reproduction of birds

Annex Point IIIA XIII 1.3

SUMMARY DAILY MEAN FOOD CONSUMPTION (g/animal/day)

DOSE GROUP		PERIOD (week) -2	-1	1	2	3	4	5	6
Control (1)	MEAN	7.33	6.63	5.80	5.30	6.05	5.28	6.46	6.92
	SD	1.24	1.21	1.53	1.26	1.19	1.19	1.27	1.65
	n	14	14	14	14	14	14	14	14
35 µg/kg (2)	MEAN	5.88	5.12	4.29	5.54	5.83	5.20	5.96	6.01
	SD	1.07	0.84	1.04	1.31	1.24	1.09	1.19	1.22
	n	14	14	14	14	14	14	14	14
	±%	-20	-23	-26	5	-4	-2	-8	-13
		**	**	*					
70 µg/kg (3)	MEAN	9.21	6.27	5.13	5.87	6.72	6.32	6.74	7.00
	SD	1.81	1.01	1.51	1.52	1.45	1.53	1.41	1.46
	n	14	14	14	14	14	14	14	14
	±%	26	-6	-11	11	11	20	4	1
		**					*(2-3)		
140 µg/kg (4)	MEAN	12.86	15.58	7.35	7.40	6.65	6.86	11.25	10.43
	SD	2.58	2.86	2.42	1.83	1.49	1.73	2.64	2.57
	n	14	14	14	14	14	14	14	14
	±%	75	135	27	40	10	30	74	51
		**	**		**(1-4)		**(1-4)	**	**
					**(2-4)		**(2-4)		
					**(3-4)				
		U	U	U	DN	NS	DN	U	U

Remarks:
* = p < 0.05
** = p < 0.01
U = Mann-Whitney U-Test Versus Control
DN = Duncan's Multiple Range Test

No test item related differences were observed in the treated groups

4.2.5	Results of residue analysis	Not measured	
4.2.6	Other effects		X
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	Fluffed feathers were observed in the control group.	
4.3.2	Nature of adverse effects	Not applicable	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was conducted according to OECD guidelines 206. The objective of the study was to investigate the effect of 6-week treatment of the test item on quails' reproduction. Three different nominal dose groups 35, 70 and 140 µg/kg bw and one control group were tested in the study.

Section A7.5.3.1.3 - Effects on reproduction of birds

Annex Point IIIA XIII 1.3

		Each dose group consisted of 12 male and 12 female birds, which were 9 weeks old at the beginning of the test. Test birds were housed indoors by dosage groups in pens. Each pen consisted of one male and one female. Birds were exposed to drinking water containing the test item for a period of six weeks. Effects on adult health, body weight gain, food consumption, pathological changes and reproductive parameters were monitored and evaluated. The 14-day old survivors, their body weights, food consumption and general health state were observed.	
5.2	**Results and discussion**	Mortality: No animals died in the control, in the group 35µg/kg and in the medium dose group (70µg/kg). In the high dose group only one female animal died. In all the test groups, fluffed feathers and sitting birds were observed during the study. Only one statistically significant increase of the water consumption was observed in the highest group of 140µg/kg in the first week of pre-treatment period. There was no concentration related effect. Organ weight: The mean liver and spleen weight of male birds was statistically increased in each male dose group compared to the control. The mean testes weight of male birds was statistically significant in male medium and high dose groups compared to the control group. The mean liver and spleen weight of female birds showed dose dependent increase, which was significant in female medium and high dose groups compared to the control and to the lowest dose groups. The increases of organ weights are in connection with the toxic effect of the test item. The relative egg number/female/day was similar to the control in all test groups. There were no differences in egg mass. No test item related changes were observed in the percentage number of abnormal eggs of test groups compared to the control group. There were no test item related changes to the cracked/broken eggs. The parameters such as eggshell thickness, percentage of fertile eggs and percentage of viable embryos showed no differences between test groups and the control. 14-day old survivors: The numbers decreased in the treatment period of 140µg/kg dose level compared to the control group. The percentage number of dead chicks increased in the high dose group compared to the control group at the last item treated period (week 5 and 6).	
5.2.1	NOEC	Reproduction NOEC is 0.4 mg/l water concentration (70 µg/kg nominal, 39 µg/kg measured).	
5.3	**Conclusion**	The reproduction LOEC is 0.8 mg/l water concentration (140 µg/kg nominal, 79 µg/kg measured). Based on the results of the stability pre-test, stability measurements were not performed in the main study, because the concentration of the test item was constant in the water for 4 days. The initial concentrations of the test item in drinking water were measured at the start and at the end of the treatment in each dose. There was no mortality or illness during the acclimation period. There was no mortality in the control during the test. Weekly egg production in	X

Section A7.5.3.1.3 - Effects on reproduction of birds

Annex Point IIIA XIII 1.3

		the control group during the pre-treatment period was not different from the mean weekly egg production during the treatment period. The egg production was greater than 9 eggs per hen during the two-weeks of pre-treatment.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2006-05-08*
Materials and Methods	Adopt applicant's version noting the following deviations. 11.3 The validity of the study is affected since it is stated that the measured concentrations should not be below 80 % of the nominal concentration, which it was in this study. 12.3.2 Pairs of animals which showed to be affected in two weeks before the study should be excluded from the study, this seems not to have been done. 12.3.8 More parameters were analysed; egg production, cracked eggs, viability, hatching, surviving of chicken for 14-days. 12.3.8 When body weight data are analysed (for adults) it is of importance that it is the body weight gain that is analysed, not the actual body weight. 12.3.8 Why was different statistical methods used for the same data set, i.e. organ weight where both MWU and Duncans test was used, this seems to be an incorrect way of analysing data.
Results and discussion	Adopt applicant's version noting the following deviations. 12.2.3 There might be significant effects if body weight gain is analysed. 12.2.6 Liver, testes and spleen weight was analysed.
Conclusion	Adopt applicant's version noting the following deviations. 13.3 Although the drinking water containing the test substance was changed daily, the concentration of the test substance was not kept within the validity limits (80 % of nominal). This is not surprising, given that the test system used a photoperiod with 16 h light.
Reliability	2
Acceptability	acceptable
Remarks	

Table A7_5_3_1_2-1: Method of administration of the test substance

Carrier / Vehicle	**Details**
Water	Yes Deionised water prepared by LAB on day of formulations.
Organic carrier	Yes Ethanol abs.
Concentration of the carrier [% v/v]	Stock solution: 100 mg test item in 100 ml ethanol.
Other vehicle	None
Function of the carrier / vehicle	In the preliminary study, the test item was mixed in SSNIFF SM quail diet. In the lowest dose level the first death occurred 6 days after the start of treatment. Lower dose levels were therefore necessary. At the lowest diet concentration that can be quantitatively determined with suitable precision and accuracy a large number of the birds died. The test item was therefore mixed in the drinking water and in this case the low concentrations could be measured by analytical methods.

Table A7_5_3_1_3-2: Test animals (if more than one species is used, for each species one table)

Criteria	Details
Species/strain	Japanese quail (*Coturnix coturnix japonica*)
Source	Dezsö Rokolya quail breeder, Csávoly, Szent István u. 83, H-6448, Hungary
Age (in weeks), sex and initial body weight (bw)	9 week old birds were used at the test initiation. Body weight range at test initiation: Males 170 – 211 g Females 203 – 251 g
Age range within the test	Not specified
Breeding population	Not specified
Amount of food	During acclimation/ stabilisation and the pre-treatment period untreated layer diet was administered ad libitum. During the test period 600 g/ day of food was available to the birds.
Age at time of first dosing	Not stated
Health condition / medication	No medical treatment was carried out during the entire period of the test.
Pre-treatment	Acclimation period: 3 – 19 September 2004, photoperiod was 7-8 hours/day. Pre- treatment period: 20 September – 3 October 2004 During acclimation/ stabilisation and the pre-treatment period untreated layer diet was administered ad libitum. During stabilisation the egg laying and fertility were observed in each cage.

Table A7_5_3_1_2-3: Test system

Criteria	Details
Test location	Housed indoors by pairs (1 male and 1 female) in pens.
Holding pens	Each pen has floor space that measures approximately 50 x 50 cm. Ceiling height is 40 cm. External walls, ceiling and floors are constructed of galvanised wire.
Number of animals (male/female)	96 quails in total: 48 males and 48 females.
Number of animals per pen [cm²/bird]	2 animals/ pen (1250 cm^2/bird)
Number of animals per dose	24 animals/ dose

Pre-treatment / acclimation	Acclimation period: 3 – 19 September 2004, photoperiod was 7-8 hours/day. Pre- treatment period: 20 September – 3 October 2004 During acclimation/ stabilisation and the pre-treatment period untreated layer diet was administered ad libitum. During stabilisation the egg laying and fertility were observed in each cage.
Diet during test	SSNIFF SM quail diet was used during the test. 600g of food per day was provided to the birds.
Dosage levels (of test substance)	The applied nominal concentration levels in the drinking water: 0.2, 0.4 and 0.8 mg/l (measured: 0.10, 0.26 and 0.55 mg/l). These water concentrations are equal to nominal 35, 70 and 140 µg/kg bw dose groups (measured: 15, 39 and 79 µg/kg bw).
Replicate/dosage level	There were 12 pens per dosing level, each pen housed 2 birds (one male and one female).
Dosing method	Birds received the drinking water prepared with test item ad libitum.
Dosing volume per application	The volume of the drinking water was 100 ml/cage. The drinking water was changed daily.
Frequency, duration and method of animal monitoring after dosing	Clinical observations: All adult birds and hatchlings were observed daily for mortality, signs of toxicity or abnormal behaviour following test initiation until termination. Body weights: Individual body weights of the adults were measured at the start of pre-treatment, at the start of treatment and at the end of the treatment period. Individual body weights of the hatchlings were measured by pen at hatching and at the end of the rearing period. Food consumption: Average estimated food consumption was determined for each dosage group and the control at one week intervals throughout the study. Food consumption of the young animals was determined daily from the first day to the 14th day after hatching. Water consumption: Average estimated water consumption was determined for each dosage group and the control at every changing of the drinking water throughout the study.
Time and intervals of body weight determination	Individual body weights of the adults were measured at the start of pre-treatment, at the start of treatment and at the end of the treatment period. Individual body weights of the hatchlings were measured by pen at hatching and at the end of the rearing period.

Incubation, storing and hatching	The birds were kept indoors in brooder pens, separated by dose levels. Each brooder pen has floor space that measures 1.5 x 1.0 m. Ceiling height is 40 cm. External walls, ceiling and floors are constructed of galvanised wire. Eggs were incubated in PL MASCHINE incubator and hatched in PL MASCHINE hatcher.
Test period after egg-laying	*Give the time period for continuing the test after egg-laying begins*
Turning of eggs	Yes Eggs were automatically turned during incubation.
Collection period for eggs	Eggs were collected over 7 days.

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	**Details**
Test temperature	Animal room: 15.4 – 27.0 °C Brooder pens: 30 – 38 °C
Shielding of the animals	Not specified
Ventilation	HELIOS – HS type ventilator, maximal aeration 18 times/hour.
Relative humidity	Animal room: 47 – 80 % Brooder pens: 50 – 73 %
Photoperiod and lighting	Animal room: photoperiod was 7-8 h/day at the beginning of acclimation/ stabilisation then during 2 weeks it was continuously increased to 16 – 18 h/ day. Lighting was artificial, minimum 10 lux. Brooder pens: photoperiod was 16h/day
Storing, incubation and hatching conditions for eggs	Eggs were stored not more than 7 days at a temperature of 13.3 – 16.0 °C and 52 – 64 % relative humidity. Temperature in the incubator was 37.5 – 37.7 °C and relative humidity was 51 – 65 %. Temperature in the hatcher was 37.0 – 37.4 °C and relative humidity was 71 – 72 %.
Environmental conditions for young birds	Not specified

Table A7_5_3_1_3-5: Values of reproduction ability

Parameter	Nominal test substance dosage level (µg/kg bw)	Week (-2 and -1 are pre-treatment weeks and 1-6 are treatment/exposure weeks)							
		-2	-1	1	2	3	4	5	6
Egg production (number of eggs laid per hen)	Control (0.0)	0.73	0.79	0.83	0.85	0.83	0.80	0.73	0.75
	35	0.92	0.83	0.93	0.82	0.85	0.87	0.80	0.81
	70	0.75	0.85	0.94	0.83	0.76	0.75	0.83	0.76
	140	0.92	0.95	0.95	0.85	0.87	0.81	0.77	0.69
Percentage of cracked/broken eggs	Control (0.0)	10.2		8.9					
	35	4.7		6.3					
	70	6.7		8.2					
	140	3.2		7.2					
Viability (per cent viable embryos of eggs set): Results are expressed as the % of dead embryos	Control (0.0)	13.5	20.4	16.1	12.1	19.7	15.0	13.5	24.5
	35	15.2	11.5	8.7	15.3	17.7	17.5	17.5	21.3
	70	35.2	29.0	22.9	17.2	21.1	18.5	25.4	22.6
	140	62.1	72.0	44.1	34.4	23.4	17.0	39.2	32.6
Hatchability (per cent hatching of eggs set): Results are expressed as the number of 0-day old chicks/female/day.	Control (0.0)	0.54	0.51	0.56	0.61	0.58	0.61	0.54	0.48
	35	0.67	0.65	0.75	0.60	0.61	0.62	0.56	0.57
	70	0.42	0.52	0.64	0.57	0.54	0.52	0.52	0.49
	140	0.30	0.25	0.45	0.48	0.55	0.56	0.40	0.40
Percentage of hatchings that survive to 14 days*	Control (0.0)	96.3	100	98.2	98.4	96.6	93.4	92.6	89.6
	35	94.0	100	98.7	95.0	93.4	93.5	96.4	87.7
	70	92.9	98.1	90.6	91.2	90.7	88.5	88.5	85.7
	140	90.0	80.0	86.7	87.5	89.1	83.9	75.0	75.0
Number of 14-day old survivors per hen: Results are expressed as the number of 14-day old chicks/female/day.	Control (0.0)	0.52	0.51	0.55	0.60	0.56	0.57	0.50	0.43
	35	0.63	0.65	0.74	0.57	0.57	0.58	0.54	0.50
	70	0.39	0.51	0.58	0.52	0.49	0.46	0.46	0.42
	140	0.27	0.20	0.39	0.42	0.49	0.47	0.30	0.30
Eggshell thickness (mm)	Control (0.0)	0.25	0.26	0.25	0.24	0.23	0.25	0.24	0.25
	35	0.25	0.24	0.26	0.24	0.22	0.22	0.23	0.22
	70	0.27	0.23	0.26	0.23	0.21	0.22	0.25	0.23
	140	0.30	0.26	0.28	0.26	0.22	0.25	0.25	0.25

* The percentage of hatchlings that survived to 14 days was calculated as follows:

$$\frac{\text{number of 14-day old chicks/female/day}}{\text{number of 0-day old chicks/female/day}} \times 100$$

Table A7_5_3_1_3-6: Validity criteria for bird reproduction test according to OECD 206

	Fulfilled	Not fulfilled
Mortality of control animals <10%	**X**	
Average number of 14-day-old survivors per hen in controls ≥ 24 for Japanese quail		**X**
Average eggshell thickness for the control group ≥ 0.19 mm for Japanese quail	**X**	
Concentration of the test substance in the diet ≥ 80 % of the nominal concentration throughout the test period		

Section A7.5.4.1 - Acute toxicity to honeybees and other beneficial arthropods, for example predators

Annex Point IIIA XIII 3.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	X
Detailed justification:	Compound is of very low water solubility and is not used in situations where bees or beneficial arthropods are exposed. It is used in highly localised and limited areas such as sewers where bees and beneficial arthropods do not exist, and it is not applied in a widespread fashion to extensive areas where leaching and run-off which might contaminate their habitat is possible. It is of low vapour pressure and is not applied as a spray or vapour which might contaminate their environment. Many years of use in a wide range of situations has shown no effect on bees or beneficial arthropods. Plants are not treated with rodenticides.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	CA agrees with the applicant in that honeybees and other beneficial arthropods are not exposed to the substance. Justification by limited exposure is supported by the emission scenario document for biocides used as rodenticides.
Conclusion	Acceptable
Remarks	

Section A7.5.5 - Bioconcentration, terrestrial

Annex Point IIA VII 7.5

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	X
Detailed justification:	Recommendations are to collect all dead rodents and remains of uneaten bait. Product is used in limited and localised areas such that continued exposure is minimal	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	There is a possibility of bioconcentration in soil organisms. However the bioconcentration for terrestrial organism can be based on the partitioning coefficient, therefore it is scientifically unjustified.
Conclusion	Acceptable
Remarks	

Section A7.5.5.1 - Bioconcentration, further studies

Annex Point IIA VII 7.5

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	X
Detailed justification:	Further studies may be considered in the light of findings.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	We do not know if there is a limited exposure since no calculations yet have been done on secondary poisoning of non target animals. However it have to be decided later if the study is necessary. Initial calculations have revealed that the risk of secondary poisoning will be highest following the examples in the ESD, bait-rat-predator, therefore this study is considered unnecessary.
Conclusion	Acceptable
Remarks	

Section A7.5.6 - Effects on other terrestrial non-target organisms

Annex Point IIIA XIII 3

	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 water solubility and is used in highly localised and limited areas such as sewers. It is not applied in a widespread fashion to extensive areas where leaching and run-off which might contaminate terrestrial plants is possible. It is of low vapour pressure and is not applied as a spray or vapour which might contaminate habitat. Many years of use in a wide range of situations has shown only limited effects on terrestrial non-target organisms provided product is used correctly. Rodent corpses and remains of uneaten bait should be collected, and baiting points guarded to prevent access by non-target species.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	The study is scientifically unjustified since the tests on earthworms showed only a low toxicity.
Conclusion	Acceptable
Remarks	

Section A7.5.7.1.1 Effects on mammals - Acute oral toxicity

Annex Point IIIA XIII.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	C.G. Rammell, J.J.L. Hoogenboom, M. Cotter, J.M. Williams and J. Bell (1984) Brodifacoum residues in target and non-target animals following rabbit poising trials. New Zealand Journal of Experimental Agriculture, 1984, Vol. 12: 107-111.	
1.2	**Data protection**	No, published paper.	
1.2.1	Data owner	© Crown copyright 1984	
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.	X
2.2	**GLP**	The GLP status of the study is not stated in the published paper	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
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		
3.1.4	Purity	94%	
3.1.5	Stability	A specific statement on stability is not provided within the paper.	
3.1.6	Radio labelling		
3.2	**Test Animals**		
3.2.1	Species	Target animals - rabbits	
3.2.2	Strain	Not stated in the published paper	
3.2.3	Source	Wild	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	All ages and weights	
3.2.6	Number of animals per group	Not applicable as this is a trial in open countryside	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Preparation of test site	Not applicable	

Section A7.5.7.1.1 Effects on mammals - Acute oral toxicity

Annex Point IIIA XIII.1.1

3.3.2	Concentration of test substance	Final estimated concentration in the baits of 50 mg/kg.	
3.3.3	Specific activity of test substance	Low density, cereal based 'Mapua' baits sprayed with a water/monopropylene glycol suspension (4/1, v/v) of technical brodifacoum (94%)	
3.3.4	Volume applied	1500-4000 baits/ha (Each bait 0.83 g) laid at 3 sites	
3.3.5	Sampling time	Dead rabbits and other non-target animals were collected 4-28 days after baits were laid.	
3.3.6	Samples	Liver, muscle and fat tissues taken for analysis..	
		4 RESULTS AND DISCUSSION	
4.1	**Result of study**	Animals, in which brodifacoum was detected, showed haemorrhages at necropsy typical of anticoagulant poisoning. Haemorrhage sites in rabbits were massive abdominal (52%), thoracic (17%) and the remaining (31%) were muscle, caecum, stomach, kidney, mesentery and placenta of pregnant does. Levels of brodifacoum >0.05mg/kg was detected in 41 out of 43 dead rabbits analysed and in all 14 other animals found dead in the experimental areas. High levels of brodifacoum were found in the liver, up to 11.7 mg/kg, and up to 2.1 mg/kg in fatty tissues. The mean liver level for females was 5.8 mg/kg compared to 3.2 mg/kg for males. Other dead animals found were, hare, sheep, cat, paradise duck, seagull, hawk, magpie and passerine, all having significant levels of brodifacoum in the liver.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**		
5.2	**Results and discussion**	The higher levels of brodifacoum in the liver of females may bedue to the fact that the trial was during the breeding season as 21% of females autopsied were pregnant. This means that the males would have been more active than female due to territorial displays, chasing and fighting. This in turn would increase the effect of the coagulant poison. The earlier death of the males would then account for the lower levels of brodifacoum in their liver. The published LD_{50} for cats is 25 mg/kg, ferrets is approx 9.2 mg/kg and hawks approx 10 mg/kg. For typical weights of 750g for the hawk and 3.25 kg for the cat this would give LD_{50} values of 7 mg and 80 mg. To get this dose would require them to have consumed about 175 and 2000 baits respectively. As there are predominantly carnivores this seems to be unlikely therefore the dose must have come from poisoned rabbit carcasses.	X

Section A7.5.7.1.1 Effects on mammals - Acute oral toxicity

Annex Point IIIA XIII.1.1

5.3	**Conclusion**	Brodifacoum is an effective coagulant poison for the control of rabbits. The presence of brodifacoum in the carcasses of poisoned rabbits poses hazards to rabbit predators. Although the predators do not control dense populations of rabbits they do help in some areas. It is therefore desirable that poisoning operations have minimal impact on rabbit predators. To help reduce the effect on non-target animals it may be necessary to reduce the toxicity of the bait in order to reduce the residue levels.	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2006-05-08*
Materials and Methods	Adopt applicant's version noting the following deviations. 26.1 No guideline was used. 26.2 There was no check of GLP.
Results and discussion	29.2 The published Ld 50 values must be discussed in the light of the findings of this paper. For hawk it seems as concentrations of 0.08 mg/kg muscle could be lethal. This corresponds to a dose of 0.107 mg brodifacoum.
Conclusion	29.3 The conclusions drawn from this study are not in connection with what this study is intending to be used for by the applicant. The study shows that Brodifacoum, and thereby possibly bromadiolone, was found in the highest concentration in liver. Liver concentration around 4.4 mg/kg liver was found to be mortal for rabbit, 1.2 mg/kg liver (range 0.48-3.7) for sheep. This corresponds to muscle concentrations of 0.26 and 0.16 mg/kg muscle respectively.
Reliability	3
Acceptability	The study is not considered acceptable as a study on acute oral toxicity, since it is not a dose related study. However, data will be considered in the risk evaluation and the discussion.
Remarks	

Section A7.5.7.1.1 - Effects on mammals - Acute oral toxicity

Annex Point IIIA XIII.1.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x]	**Technically not feasible [x]** **Scientifically unjustified [x]**	X
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Product is of high toxicity to a range of species as shown by literature and poisoning incidents. Product belongs to a group of closely analogous compounds which have similar properties and all are toxic to a range of mammals Further studies will be considered to be an unnecessary use of experimental animals.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	The study is technically feasible but it is not scientifically justified since the applicant has presented a report on toxicity by Brodifacoum to rabbits. Brodifacoum is classified as more toxic than bromadiolone which makes it possible to use the figures derived in the Brodifacoum study.
Conclusion	Acceptable
Remarks	

Section A7.5.7.1.2 - Effects on mammals - Short term toxicity

Annex Point IIIA XIII 3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	X
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.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	There are studies that show effects on mammals by secondary poisoning, and since there are indications that bromadiolone have a rather slow degradation rate in some mammals there is a possibility that effects will be found in this kind of a study. The study is a 28-day study not 90-day. Data have been presented in tests with rats, therefore the study is considered unnecessary.
Conclusion	Justification acceptable
Remarks	

Section A7.5.7.1.3 - Effects on mammals - Effects on reproduction

Annex Point IIIA XIII 3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
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. 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, another study in the mammals will be scientifically unnecessary.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*2006-05-08*
Evaluation of applicant's justification	Studies on effects of bromadiolone on reproduction have obviously been performed, and is presented in the toxicological part of the application.
Conclusion	Acceptable
Remarks	

Section A7.6 - Summary of ecotoxicological effects and fate and behaviour in the environment Annex Point IIA VII 7.8		
	The active substance is a large aromatic organic compound of low volatility with two polar groups, which can potentially ionise at environmental pH.	
	The active substance has a high Log Pow (> 7), a high predicted BCF of 13530, is not readily biodegradable and is of low solubility (~1 mg/l). The predicted Log Koc indicates that the active substance would not be mobile in soil and would be expected to absorb to soil particles. The substance does not undergo hydrolysis (t½ > 1yr) and undergoes rapid direct photodegradation. There are no predicted effects on the atmosphere.	X
	The active substance is very toxic to aquatic organisms (ErC50 < 1 mg/l) and is potentially bioaccumulative.	
	Determination of PNEC's	
	PNEC for aquatic organisms On the basis of acute toxicity data for fish, invertebrates and algae only, the PNEC is derived from the lowest L/EC50 value (algae ErC50 = 0.664 mg/l) with a safety factor of 1000. Therefore, **PNEC aquatic organisms = 6.64 x 10-4 mg/l**	X
	PNEC for STP micro-organisms A study for inhibition to sewage sludge micro-organisms has been performed. The EC50 = 132.8 mg/l, with a safety factor of 100. Therefore, **PNEC STP micro-organisms = 1.328 mg/l**	
	PNEC for terrestrial organisms A study on acute toxicity to earthworms is being performed. An assessment factor of 1000 applies. Until the results of the study are obtained a screening method for determination of the effects to the terrestrial compartment is possible by comparison of the soil pore water PEC against the aquatic PNEC (6.64 x 10-4 mg/l).	X
	A study on acute toxicity to earthworms is being performed. An assessment factor of 1000 applies. Until the results of the study are obtained a screening method for determination of the effects to the terrestrial compartment is possible by comparison of the soil pore water PEC against the aquatic PNEC (6.64E-04 mg/l).	