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

Annex Point IIA VII.7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Fabbrini T (1997) DIFENACOUM: Determination of abiotic degradation hydrolysis as a function of pH, ChemService S.p.A. report CH-15/96-B-DIF	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with access to data	PelGar International Ltd. Activa srl	
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**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	03940709	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.0% difenacoum	
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	x
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	Actual pH not measured. Nominal values only.	
3.4.4	Duration of the test	5 days.	
3.4.5	Number of replicates	1	
3.4.6	Sampling	Sampling at 0, 2.5 and 5 days	
3.4.7	Analytical methods	10 ml samples were taken and analysed. The test item content was determined by the following conditions:	x

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

Annex Point IIA VII.7.6.2.1

		Detector UV/V is 254 nm
		Column Lichrospher 100 RP-18, 5µm 250 x 4.6 mm
		Column temperature Room temperature
		Eluent Methanol + water + acetic acid: 94.2 + 5 + 0.8 ml
		Eluent Flow 0.8 ml/min
		Retention time for Difenacoum 6.0 min ca.
3.5	**Preliminary test**	No
		4 RESULTS
4.1	**Concentration and hydrolysis values**	See table A7_1_1_1_1-4
4.2	**Hydrolysis rate constant (k_h)**	N/A
4.3	**Dissipation time**	See table A7_1_1_1_1-5
4.4	**Concentration – time data**	.
4.5	**Specification of the transformation products**	See table A7_1_1_1_1-6
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD Guidelines 111. The pH of the buffer solutions was determined by pH/meter equipped with glass electrode. Asolution of 900 µg/ml of Difenacoum in acetone was prepared (18.0mg in 20ml). 100 µl of this solution corresponding to 90 µg of technical Difenacoum were added to 100 ml of each one of the 3 solutions at pH 4, 7 and 9. The 3 solutions were put into a constant temperature bath at 50°C. At time 0, 2.5 hours and 5 days, 10 ml of the sample was collected and analysesd for determining Difenacoum content in the following operating conditions: HPCL Column: Lichrospher 100 RPI8, 5 µM 250 X 4.6 mm Detedtor: UV/Vis 254 nm Column Temperature: room temperature Eluent: methanol+water+acetic acid: 94.2+5+0.8 ml Eluent flow: 0.8ml/min Difenacoum retention time: 6.0 min ca.
5.2	**Results and discussion**	The tests performed show that, after five days at 50°C, less than 10 % of Difenacoum added has been hydrolysed at each of the three pH values (4,7 and 9). Difenacoum can be considered stable to hyrdolysis.
5.2.1	k_H	N/A
5.2.2	DT_{50}	N/A
5.2.3	r^2	N/A

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

Annex Point IIA VII.7.6.2.1

5.3	**Conclusion**	Less than 10 % Difenacoum added had been hyrdrolysed at a 3 pH levels after 5 days at 50°C.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18.10.2006
Materials and Methods	3.3 Test concentration described in table A7_1_1_1_1-2 is 0.9 mg/l in each pH, however the solubility of difenacoum in pH 4 is considerably lower (<0.048 mg/l according to docA3), but according to the participant homogenous solutions were achieved using acetone as a solvent and 50°C temperature. 3.4.7 For the repeatability according to later submitted validation data (study CH-15/96ADIF) RSD is ≤ 20%. Sensitivity of the method can be considered to be 0.1 mg/L , the lower calibration value.
Results and discussion	The tests performed show that, after five days at 50°C, less than 10 % of Difenacoum added has been hydrolysed at each of the three pH values (4,7 and 9). Difenacoum can be considered stable to hyrdolysis.
Conclusion	Less than 10 % Difenacoum added had been hyrdrolysed at a 3 pH levels after 5 days at 50°C.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

pH	**Type of buffer (final**	**Composition**

	molarity)	
4	-	30 ml of H_3PO_4 0.1N added to 100 ml of KH_2PO_4 0.1 N (1.36 g of product in 100 ml of water) then diluted to 200 ml with water
7	-	59.00 ml of NaOH 0.1 N added to 100 ml of KH_2PO_4 0.1 N (1.36 g of product in 100 ml of water) then diluted to 200 ml with water
9	-	21 ml of NaOH 0.1 N added to 50 ml of 0.1 M H_3BO_3 (0.63 g in 100 ml) then diluted to 100 ml with water

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Freshly boiled distilled water
Preparation of test medium	A solution of 0.9 mg/ml of difenacoum in acetone was prepared (18.0 mg in 20 m/)
Test concentrations (mg a.i./L)	100µl of medium corresponding to 90µg of Difenacoum added to 100 ml of each of the 3 pH solutions. *The final concentration of 0.9mg/l is higher than the solubility in water at pH4 (<0.048mg/l). But it was chosen to have a detectable concentration that permitted also to check if a possible degradation occurred, using a direct injection in an HPLC/UV.* *The presence of a small amount of acetone co-solvent (0.1%v/v) and the 50°C temperature probably concurred to give homogeneous solutions in the three buffers and the analysis after 2.5 hours and five days demonstratin tht no degradation phenomena occurred, in accordance wht the molecular structure of the test substance.*
Temperature (°C)	50ºC
Controls	None
Identity and concentration of co-solvent	*If additive (e. g. solvents) are used to increase the solubility of the test substance, describe the additive and give the concentration (% v/v).*
Replicates	Not stated

Table A7_1_1_1_1-3: Description of test system

Glassware	Normal laboratory glassware
Other equipment	Analytical balance, Constant temperature bath with thermometer, technical balance, pH meter, HPLC system.
Method of sterilization	Not stated

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 4	Sampling times (*days, hours, or other time period*)							
	0	**2.5d**	**5d**	t_3	t_4	t_5	t_6	t_n
Parent compound mg/l	0.91	0.98	0.90	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	108	99	-	-	-	-	-

Compound pH 7	Sampling times (*days, hours, or other time period*)							
	0	**2.5d**	**5d**	t_3	t_4	t_5	t_6	t_n
Parent compound mg/l	0.82	0.86	0.86	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	105	105	-	-	-	-	-

Compound pH 9	Sampling times (*days, hours, or other time period*)							
	0	**2.5d**	**5d**	t_3	t_4	t_5	t_6	t_n
Parent compound mg/l	0.91	0.90	0.88	-	-	-	-	-
Transformation product 1	-	-	-	-	-	-	-	-
Transformation product 2	-	-	-	-	-	-	-	-
Transformation product n	-	-	-	-	-	-	-	-
Reference compound	-	-	-	-	-	-	-	-
Volatiles *(if measured)*	-	-	-	-	-	-	-	-
Total % recovery	100	99	97	-	-	-	-	-

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	> 2.5hrs	> 2.5hrs	> 2.5hrs	> 2.5hrs	> 2.5hrs	> 2.5hrs
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 4	**pH 7**	**pH 9**
	None – compound was stable	0	0	0

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake R.M (2004) Determination of the direct photolysis rate in water by sunlight of Difenacoum. Chemex Environmental Internation Ltd. Reference ENV6767/	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with letter of access	PelGar International Ltd. Activa srl	
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 REFERENCE	
2.1	**Reference**	LETTER: Breakdown products with retention times for Difenacoum from the photolysis study, Chemex Environmental Internation Ltd., ENV6767/120139.	
2.2	**Data protection**	Yes	
2.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
2.2.2	Companies with letter of access	PelGar International Ltd. Activa srl	
2.2.3	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	
		3 REFERENCE	
3.1	**Reference**	Gomez A, 2005, Determination of the direct photolysis rate in water by sunlight of difenacoum, Proposal of Degradants, Safepharm Laboratories Ltd.	
3.2	**Data protection**	Yes	
3.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
3.2.2	Companies with letter of access	PelGar International Ltd. Activa srl	
3.2.3	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	
		4 GUIDELINES AND QUALITY ASSURANCE	
4.1	**Guideline study**	Yes OPPTS 835 2210	
4.2	**GLP**	Yes	
4.3	**Deviations**	Yes	
		5 MATERIALS AND METHODS	
5.1	**Test material**	Difenacoum	
5.1.1	Lot/Batch number	ECO120139	

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

5.1.2	Specification	As given in section 2	
5.1.3	Purity	99.7%	
5.1.4	Radiolabelling	N/A	
5.1.5	UV/VIS absorption spectra and absorbance value	Difenacoum showed three absorbance maxima in the region 190 to 340nm only one of which was above 290nm. No absorbance was detected (above the base line) for wavelengths above 340 nm	
5.1.6	Further relevant properties	N/A	
5.2	**Reference substances**	Methanol was used as a reference substance. Potassium dichromate is used as a reference chemical for absorbance confirmation	
5.3	**Test solution**	The test calculation required was calculated to be 1.50mg/L of difenacoum. Difenacoum was prepared as a 1.55mg/L dosing solution in acetonitrile. 1ml of the dosing solution was added to a 100ml volumetric flask and made to volume with 0.2µm filtered deionised water (1.55mg/l-0.00000349M)	
5.4	**Testing procedure**		
5.4.1	Test system	10 tubes were used for the first part of the test procedure (Tier 1). Two tubes were covered by aluminium foil and were the controls. The remaining tubes were placed in sunlight at an agle of 30° with the tops facing magnetic north. Two sample were taken from the tubes every hour for 6 hours. Thin walled quartz tubes were used. Exposure was performed under clear sky conditions with 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.	
5.4.2	Properties of light source	N/A	
5.4.3	Determination of irradiance	Tier 2 of the test involved using an actinomer. The preparation was prepared in the following way for Tier 2 of the test. A stock 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. 1ml of the intermediate PNAP stock was added and the flaskmade to volume with further deionised water. The test procedure was the same as in tier 1 apart from samples were taken every hour for 5 hours and additional samples were taken from the actinometer every 10 minutes for the first hour. The molar concentration of the pyridine was 2.2M. .	
5.4.4	Temperature	N/A	
5.4.5	pH	Analysis at pHs above and below the pKa is only a recommendation – see Page 8 section D of guideline. Photolysis was not determined above and below the pKa. The pH of the un-buffered test system was not measured (Page 16 section C subsection 2 – states 'report the pH of all test solutions, if appropriate').	x
5.4.6	Duration of the test	Exposure period was 6 hours for the tier 1 test and 5 hours for the tier 2 test.	
5.4.7	Number of replicates	Each sample was tested 3 times.	

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

5.4.8 Sampling N/A

5.4.9 Analytical methods 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: Aecetic acid

Flow rate: 1.5ml/min

Injection volume 250µl

Calibration data is included in the report in the form of tables and charts (for both Difenacoum and the actinometer).

The test solutions were analysed by direct aqueous injection with all samples compared to the initial concentration. It was felt that recovery data was not required under these circumstances.

Precision analysis was performed at the time of the study and is presented below in tabular form.

Precision of Difenacoum

Sample	retention time (min)	peak area	conc (mM)	Recovery %
0.78mg/l std	10.437	172842.80	0.0014165	73.40
0.78mg/l std	10.415	170288.60	0.0013984	72.46
0.78mg/l std	10.432	166210.40	0.0013694	70.96
0.78mg/l std	10.429	182737.60	0.0014868	77.03
0.78mg/l std	10.413	178701.60	0.0014581	75.55
0.78mg/l std	10.419	173513.60	0.0014213	73.64

average =	73.84
sd =	2.172847
RSD % =	2.94

Horwitz	
RSDR =	$2^{(1-0.5\log C)}$

C =	0.00000078
log C =	-6.1079054
1 - (0.5 x log C) =	4.0539527
RSDR =	16.61
proposed acceptable RSDr = *	11.13

* = RSDR x 0.67

The actual % RSD is much less than that proposed by Horwitz and is, therefore, acceptable.

Section A7.1.1.1.2 Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products

Determination of the direct photolysis rate in water by sunlight

5.5	**Transformation products**	No
5.5.1	Method of analysis for transformation products	N/A

6 RESULTS

6.1	**Screening test**	Diafenacoum showed three abosrbance maxima in the region 190 to 340nm only one of which was above 290nm. No absorbance was detected (above the base line) for wavelengths above 340nm.
6.2	**Actinometer data**	N/A
6.3	**Controls**	Control losses for Difenacoum wasa not considered to be significant..
6.4	**Photolysis data**	*Non-entry field*

6.4.1 Concentration values

Fraction of day	Mol. Concn.Run 1	Run 2…….Run 3	
0.000	0.00499	0.00474	0.00474
0.071	0.00320	0.00309	0.00315
0.141	0.00182	0.00174	0.00161
0.212	0.00113	0.00108	0.00111
0.282	0.00094	0.00089	0.00091
0.353	0.00074	0.00079	0.00098

6.4.2	Mass balance	N/A
6.4.3	k^c_p	4.98 day $^{-1}$ (5 hours exposure)
6.4.4	Kinetic order	N/A
6.4.5	k^c_p / k^a_p	0.761
6.4.6	Reaction quantum yield (ϕ^c_E)	0.00145
6.4.7	k_{pE}	13.1day^{-1} *(summer). 2.2 (winter) and 10.2 (spring).*
6.4.8	Half-life ($t_{1/2E}$)	Half life in minutes: Summer = 38 Winter = 227 Spring = 49
6.5	**Specification of the transformation products**	N/A

7 APPLICANT'S SUMMARY AND CONCLUSION

7.1	**Materials and methods**	Test guidelines follwed were OPPTS 835 2210. The breakdown products were separated by reverse phase HPLC. The peak areas were subsequently reported in the letter (reference 2). and comparison with the known peak area of difenacoum made. The identity of degradants has been proposed by examination of the molecular structure of difenacoum, and a literature search to identify

x

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

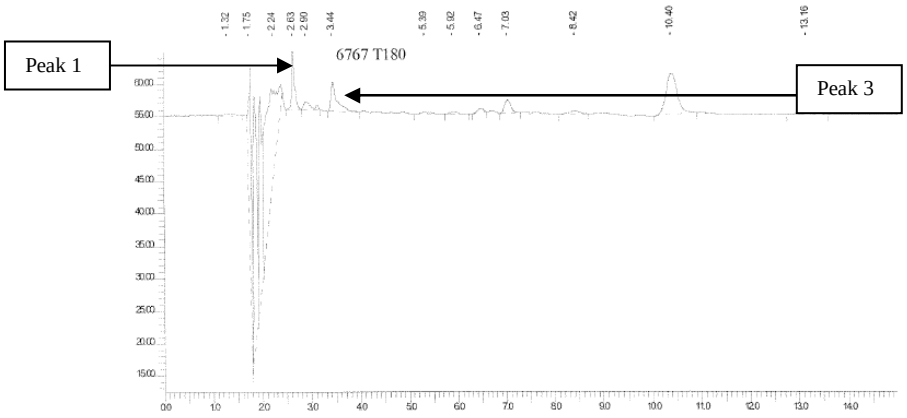
Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

likely reaction mechanisms.

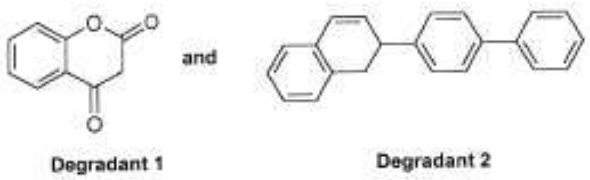
7.2 Results and discussion

By comparison of the peak areas reported in the letter with the difenacoum peak area, two breakdown products are identified as above 10% of the initial difenacoum concentration (Peaks 1 & 3). It can be observed that these peaks rapidly eluted from the reverse phase column.

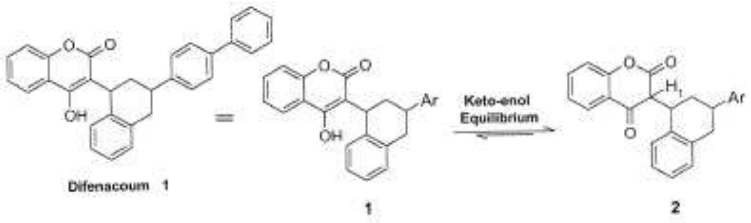


The two main degradants (1 and 2) are most likely to form via the Norrish Type II reaction. Peak 1 is likely to be degradant 1 since it is the most polar compound and therefore will elute first on reverse phase HPLC. Peak 3 is therefore proposed as degradant 2. The structure of the two degradants are tentatively suggested as the following:

Proposed Structures of Degradants

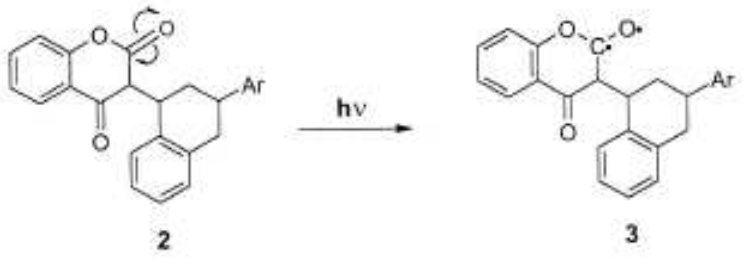


Difenacoum [1] is a polyheterocyclic compound containing an enol moiety, which is converted into the ketone functionality through a reaction tautomerisation. The presence of the enol form could be explained by the existence of the acidic C-H proton at the cycle junction (H_1). This keto-enol equilibrium lies over towards the keto form [2], whose bond energies are more favourable than the bond energies for the enol form.



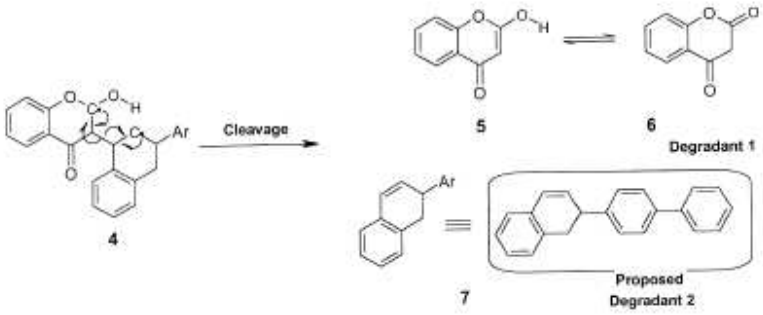
The photochemical degradation of difenacoum [1] is proposed to start with the photo-excitation of the carbonyl group by light absorption. This would yield the excited state reactant [3]. In this kind of reaction, the

triplet-excited state is usually formed following very rapid intersystem crossing (ISC) after excitation to the single state. It should be noted that the photo-excitation of either of the carbonyl moiety in the structure 3 would lead to the same final product.



The H-atom in position $_{\gamma}$ to the carbonyl group could be abstracted and transferred to the excited carbonyl oxygen atom, generating the 1,4-biradical intermediate $_{4}$ in the triplet state.

Finally, the Norrish Type II photo-fragmentation may occur. Radical cleavage of the C-C bond leads to the formation of the products 5 and 7. Tautomerisation of the enol 5 gives the proposed degradant product 6. Formation of the second degradant product 7 is favourable due to the resonance effect between the newly formed alkene in conjugation with the aromatic ring.



7.2.1	k^c_p	7.05 day $^{-1}$ (5 hours exposure)
7.2.2	K_{pE}	3.20 day^{-1}.
7.2.3	ϕ^c_E	0.037
7.2.4	$t_{1/2E}$	Half life in minutes: Summer = 38 Winter = 227 Spring = 49
7.3	**Conclusion**	Photolysis of Difenacoum was fast with 35 % removal in the first hour of

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

Annex Point IIA7.6.2.2

Determination of the direct photolysis rate in water by sunlight

		exposure. Greater than 80 % photolysis was noted to have occurred by around five hours. Futhermore, whatever the season the half life of difenacoum is less than a day. In the laboratory the substance completes photolyses.
7.3.1	Reliability	1
7.3.2	Deficiencies	No

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	19.10.2006
Materials and Methods	5.4.5. pH was not measured. According to the guideline at least initial pH should be reported It is also recommended the study to be carried out at pHs at least two orders of magnitude above and below the pK_a.
Results and discussion	6.4.6: Quantum yield for difenacoum was calculated in the study report to be 0.00145,first 2 hours. 6.4.8: Half-life in minutes were calculated assuming a 12 hour day (ie 12 hours of sunlight). Half-lives in days were 0.05 (summer), 0.315 (winter) and 0.068 (spring) .
Conclusion	Photolysis of Difenacoum was fast with 35 % removal in the first hour of exposure. Greater than 80 % photolysis was noted to have occurred by around five hours, half-lives were calculated to be 0.6 – 3.8 hours (summer: 0.053 d (38 min), winter: 0.315 d (227 min) and spring: 0.068 d (49 min). Two breakdown products above 10% of the initial difenacoum concentration were found and the proposal for the identification of structures was made.
Reliability	2
Acceptability	acceptable
Remarks	

COMMENTS FROM ...

Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.2.1 Biodegradability

Annex Point IIA VII.7.6.1.1

Ready biodegradability

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake R M (2003) Determination of the ready biodegradability of DIFENACOUM TECHNICAL, Chemex Environmental International Ltd report ENV5798/120139	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2		PelGar International Ltd. Activa srl	
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 301B	
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	5907101	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.70 % difenacoum	
3.1.4	Further relevant properties	Not applicable	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms		
3.1.7	Specific chemical analysis	Not specified	
3.2	**Reference substance**	Yes – sodium acetate	
3.2.1	Initial concentration of reference substance	101.9 mg reference substance is measured into mineral medium (1.5 l).	
3.3	**Test ing 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	
3.3.4	Method of preparation of test solution	The report states that 'Insoluble materials are weighed onto microscope cover slips, which are then introduced directly to the bioreactor' and the Client MSDS claims the material to be 'practically insoluble'	
3.3.5	Initial TS	12.1 mg/L	

Section A7.1.1.2.1
Annex Point IIA
VII.7.6.1.1

Biodegradability

Ready biodegradability

	concentration	
3.3.6	Duration of test	29 days
3.3.7	Analytical parameter	Carbon dioxide (determined as dissolved inorganic carbon)evolved within 28 days
3.3.8	Sampling	Sampling on days 0, 3, 6, 9, 13, 17, 22, 28 and 29
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Reference: sodium acetate 101.9 mg in 1.5l mineral medium Toxicity :sodium acetate 102.3 mg in 1.5l mineral medium and test material at 15.2 mg.
3.3.12	Statistics	Calculations according to OECD Guideline 301 B

4 RESULTS

4.1 Degradation of test substance

4.1.1 Table

Time (days)	***% Biodegradation***		
	Reference Material	**Test substance**	**Toxicity control**
0	0	0	0
3	36	7	25
6	57	10	44
9	65	12	51
13	72	15	57
17	77	18	59
22	81	21	61
28	83	27	61
29	*84*	*27*	*62*
29	*88*	*31*	*65*

4.1.2	Degradation	No plateau observed At the end of incubation 27 % degradation at 18.2mg per 1.5l
4.1.3	Other observations	
4.1.4	Degradation of TS in abiotic control	No abiotic control with TS
4.1.5	Degradation of reference substance	83% degradation after 28 days and 65% degradation after 9 days
4.1.6	Intermediates/ degradation products	No intermediate or degradation product identified

Section A7.1.1.2.1
Annex Point IIA VII.7.6.1.1

Biodegradability

Ready biodegradability

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to OECD 301 B guidelines. A measured volume of inoculated mineral medium, containing a known concentration of the test substance (10 to 20 mg/l total organic carbon) as the nominal source of organic, is aerated by the passage of carbon dioxide free air at a controlled rate in the dark at 22 ± 2°C. Degradation is followed over 28 days by determining the carbon dioxide produced. The carbon dioxide is trapped in sodium hydroxide and is measured as dissolved Inorganic Carbon (DIC) using a Tekmar-Dohrmann Phoenix 8000. The amount of carbon dioxide produced is usually expressed as a percentage of the theoretical carbon dioxide ($THCO_2$). Test and reference values were corrected for inoculum blank.

Insoluble materials were weighed onto microscope cover slips, which were then introduced directly to the bioreactor. Soluble and emulsifiable materials were washed in directly using approximately 10 ml of distilled water.

On day 28, 1ml of concentrated hydrochloric acid is added to each bioreactor, which were aerated over night, to drive off the remaining carbon dioxide. One last analysis of evolved carbon dioxide is made on day 29.

5.2 Results and discussion

The test substance failed to meet the requirements for a pass in this test (>60% degradation relative to the $ThCO_2$ value) with a maximum of –31% recorded on day 28. However, because of the stringency of the test, this does not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates that more work would be necessary to establish biodegradability.

5.3 Conclusion

'DIFENACOUM TECHNICAL was considered not to be significantly inhibitory (degradation of the toxicity control was greater than 25% by day 14).'

5.3.1 Reliability 1

5.3.2 Deficiencies No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20.10.2006
Materials and Methods	Agree with description of the participant.
Results and discussion	Agree with description of the participant.
Conclusion	Difenacoum was not readily biodegradable under the conditions of the test The test met all the validity criteria.
Reliability	1
Acceptability	acceptable
Remarks	

Section A7.1.1.2.1
Annex Point IIA
VII.7.6.1.1

Biodegradability

Ready biodegradability

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Test	EC-method	OECD-Guideline	Test on ready biodegradability
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready

Table A7_1_1_2-2: Inoculum / Test organism

Criteria		Details
5.3.3	Nature	Activated sludge
5.3.4	Species	Not stated
5.3.5	Strain	Not stated
5.3.6	Source	Cambridge Sewage Treatment Works
5.3.7	Sampling site	Cambridge Sewage Treatment Works
5.3.8	Laboratory culture	No - activated sludge plant for domestic sewage
5.3.9	Method of cultivation	Uncultivated
5.3.10	Preparation of inoculum for exposure	Sieved (500µm) settled and decanted. Centrifuged @ 4000 rpm for 5 minutes, decanted and resuspended in mineral media. This was repeated and sludge was centrifuged and decanted.
5.3.11	Pretreatment	*e.g. adaption*
5.3.12	Initial cell concentration	30mg/l (dry sludge solids in test)

Table A7_1_1_2-3: Test system

Criteria	Details
5.3.13 Culturing apparatus	Not stated
5.3.14 Number of culture flasks/concentration	Reference =1, Test Substance = 2
5.3.15 Aeration device	Carbon Dioxide free air at a controlled rate in the dark at 22 ± 2ºC
5.3.16 Measuring equipment	UV-Persulfate Analyser
5.3.17 Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
5.3.18 Composition of medium	Not a requirement of the OECD 301 guidelines (can be provided if required)
5.3.19 Additional substrate	No
5.3.20 Test temperature	22 ± 2ºC
5.3.21 pH	Not measured
5.3.22 Aeration of dilution water	Inoculated mineral medium aerated by passage of carbon dioxide free air at controlled rate. Rate not specified.
5.3.23 Suspended solids concentration	Not stated
5.3.24 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 $ThCO_2$		X
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		X
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	

Section A7.1.1.2.2 Annex Point IIA7.6.1.2 Biodegradability (inherent)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake RM,2005,Determination of the inherent biodegradability of Difenacoum,Chemex Environmental InternationalLimited,ENV7418/120139,16 May 2005.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with access to data	PelGar International Ltd. Activa srl	
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 ; Inherent biodegradability CONCAWE test.	
2.2	**GLP**	Yes	
2.3	**Deviations**	The test material was weighed using microscopes slips, instead of GF/A (21mm diam).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Difenacoum	
3.1.1	Lot/Batch number	ECO120139.	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.7% w/w	
3.1.4	Further relevant properties	Must be kept at room temperature and in the dark	
3.1.5	Composition of Product	N/A	
3.1.6	TS inhibitory to microorganisms	Not inhibitory to microorganisms, as seen in ready biodegradability study (A7.1.1.2.1)	
3.1.7	Specific chemical analysis	N/A	
3.2	**Reference substance**	Hexadecane	
3.2.1	Initial concentration of reference substance	Give only if deviations from guideline (e.g. lower concentration due to inhibitory activity of TS)	
3.3	**Test ing 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)	
3.3.4	Method of preparation of test solution	Difenacoum was weighed onto microscope cover slips which were then introduced directly in to a flask containing 1000ml of inoculated mineral medium.	

Section A7.1.1.2.2 Annex Point IIA7.6.1.2 Biodegradability (inherent)

3.3.5	Initial TS concentration	21 to 25mg/l
3.3.6	Duration of test	56 days
3.3.7	Analytical parameter	CO_2 evolution
3.3.8	Sampling	Duplicate bottles were taken every 7 days.
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	19 bottles containing hexadecane in inoculum to ensure inoculum working(reference) and 19 bottles without anything other than inoculum to give a background(blanks)
3.3.12	Statistics	N/A

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	See report
4.1.2	Degradation	Mean values were between 0 and 1%, with a max of 3% after 35 days.
4.1.3	Other observations	None
4.1.4	Degradation of TS in abiotic control	N/A
4.1.5	Degradation of reference substance	See report. A mean of 84% was recorded after 56 days.
4.1.6	Intermediates/ degradation products	N/A

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test substance is incubated in a buffered, mineral salts medium, which has been inoculated with a mixed population of micro-organisms. In order to enhance the biodegradative potential of the inoculum, it is pre-exposed to the test substance for a period of 14 days. The pH was maintained at 7.2+/-0.25 throughout the 14 days. After this time it was filtered and used for the blank solutions, reference and test solutions. Each bottle was sealed with PTFE lined butyl rubber crimp caps and placed in the dark on an orbiting platform to keep them mixed well. Duplicate bottles were taken every 7 days and analysed. CO2 production in the bottles was determined by measuring the increase in the concentration of inorganic carbon(IC).This was achieved by using 7M sodium hydroxide to convert CO2 to carbonates which was then tested for. Each bottle was opened and two 30ml samples taken for the IC analysis. The test guideline no is OECD 302D.

Deviation relating to the weighing of material using a microscope cover slip instead of a GF/A filter paper should not cause a problem if all the

Section A7.1.1.2.2 Annex Point IIA7.6.1.2 Biodegradability (inherent)

		material was carefully transferred to the flasks.
5.2	**Results and discussion**	A maximum of 3% degradation was recorded after 35 days, but most of the results from time zero to 56 days were fairly constant at 0 to 1%. For the reference material a mean degradation of 84% was observed. For the blanks a value of 1.9mg/l Carbon was recorded. Summarize relevant results; discuss relevant test material-specific properties (e.g. solubility, stability, adsorption behaviour, volatility) and their impact on results. For BOD-tests with nitrogen containing test substances: are the results corrected for oxygen uptake through nitrification?
5.3	**Conclusion**	The 3% degradation figure for difenacoum means it has failed to pass the test and appears to indicate it is not inherently biodegradable, at least under the conditions of this test. The inoculum was working OK since the result from the reference experiment was greater than 60% and therefore valid. The blanks gave a result also supporting validity of the experiment. Validity criteria is fulfilled.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20.10.2006
Materials and Methods	Agree with description of the participant
Results and discussion	Agree with description of the participant
Conclusion	The 3% degradation figure for difenacoum indicates it is not inherently biodegradable.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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
CONCAWE test	Not stated	302D	Inherent

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Soil and activated sludge
Species	Not stated
Strain	Not stated
Source	Cambridge Sewage Treatment Plant treating predominantly domestic sewage and also soil from outside the test laboratory.
Sampling site	Cambridge Sewage Treatment Plant treating predominantly domestic sewage and also soil from outside the test laboratory.
Laboratory culture	No – activated sludge plant for domestic sewage and from soil.
Method of cultivation	Uncultivated
Preparation of inoculum for exposure	The test substance is incubated in a buffered, mineral salts medium, which has been inoculated with a mixed population of micro-organisms. In order to enhance the biodegradative potential of the inoculum, it is pre-exposed to the test substance for a period of 14 days, then coarse filtered. The pH was maintained at 7.2 +/- 0.25 throughout the 14 days.
Pretreatment	adaptation
Initial cell concentration	1g/l soil in mineral medium and 2ml/l activated sludge in the same mineral medium which contains the 1g/l soil.

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	Sealed bottles with headspace of air.
Number of culture flasks/concentration	19 bottles each for the blanks, reference and test substance.
Aeration device	Not stated
Measuring equipment	Not stated
Test performed in closed vessels due to significant volatility of TS	Yes. Sealed bottles used. Test substance is not volatile so this is not the reason the bottles are sealed. Reason unclear.

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Mineral medium as per guideline. Not required for reporting purposes, as should be same as in guideline
Additional substrate	No.
Test temperature	20+/-1°C
pH	Controlled during pre-exposure stage. Not measured during rest of study, since sealed bottles. Not measured at end of study.
Aeration of dilution water	No
Suspended solids concentration	Not stated
Other relevant criteria	Test solutions kept mobile by orbiting platform.

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake, R.M (2005) Determination of anaerobic biodegradability of Difenacoum: Chemex Environmental International Limited, Chemex Reference: EN7147/120139	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with access to data	PelGar International Ltd. Activa srl	
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**	ISO DIS 11734	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
2.4			
		3 METHOD	
3.1	**Test material**	Difenacoum	
3.2	**Lot/Batch number**	5907101	
3.3	**Specification**	As given in section 2	
3.4	**Purity**	99.7% (w/w)	
3.5	**Further relevant properties**	Must be kept at room temperature and in the dark	
3.6	**Composition of Product**	N/A	
3.7	**TS inhibitory to microorganisms**	Not inhibitory to microorganisms.	x
3.8	**Specific chemical analysis**	N/A	
3.9	**Reference substance**	Sodium benzoate	x
3.10	**Initial concentration of reference substance**	0.5 ml for each run.	x
3.11	**Test ing procedure**		
3.12	**Inoculum / test species**	See table A7_1_2_1_2-1	
3.13	**Test system**	See table A7_1_2_1_2-2)	
3.14	**Test conditions**	See table A7_1_2_1_2-3)	
3.15	**Method of preparation of test solution**	Describe if appropriate, e.g. in case of poorly soluble test substance	x
3.16	**Initial TS concentration**	The test substance amounts that were tested on were 8.5, 8.9, 8.7 mg	x

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

3.17	**Duration of test**	8 weeks
3.18	**Analytical parameter**	CH_4 and CO_2 evolution
3.19	**Sampling**	Samples were taken on Day 2, 7, 14, 21, 28, 35, 42, 49 and 56
3.20	**Intermediates/ degradation products**	Not identified
3.21	**Controls**	6 blanks were set up which had no test substance added to it but contained the same amount of wet sludge.
3.22	**Statistics**	N/A
3.23		
		4 RESULTS
4.1	**Degradation of test substance**	
4.2	**Degradation of TS in abiotic control**	N/A
4.3	**Degradation**	The final degradation value recorded was -19% at day 56.
4.4	**Graph**	See report
4.5	**Other observations**	The final degradation value (-19%) suggests that Difenacoum was inhibitory to the micro-organism population.
4.6	**Degradation of reference substance**	See report
4.7	**Intermediates/ degradation products**	N/A
4.8		
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	A known volume of anaerobic sludge suspended in an oxygen free medium was placed in a suitable vessel leaving headspace into which any gases produced may be evolve. Prior to sealing a small amount of test compound was added. The vessels were incubated at constant temperature (35 +/-1°C) and 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 can be calculated. The kinetics of the degradation are followed by intermediate measurements at suitable intervals during the course of the test.

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

5.2 Results and discussion

Time (days)	Biodegradation (%)	
	Reference material	Test material
2	0	1
7	5	0
14	39	-1
21	45	-1
28	47	-2
35	48	-3
42	48	-4
49	50	-3
56	51	-3
	75*	-19

*Additional degradation due to analysis of dissolved inorganic carbon in digesters at the end of the test.

5.3 Conclusion

A reference material, sodium benzoate, was concurrently tested and showed biodegradation of 75% (by day 56) suggesting that he inoculum was viable.
At the end of the test period, dissolved inorganic carbon was added to the carbon derived from gas pressure measurements. It was noted that the dissolved inorganic carbon content of the blanks was higher than that in the samples giving a lower final degradation value.

5.4 Reliability

2

5.5 Deficiencies

Yes
Concentration for the value for the stock solution of the test material is missing.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

20.4.2006

Materials and Methods

3.7: This is contradictory with the conclusion from the study summary A7.1.1.2.1, where it is stated that substance is considered to significantly inhibitory (in the study no inhibition control was performed) .

3.9. Proper identification of the reference substance is missing (also in the study report)

3.10. The concentration should be stated (missing also in the study report).

3.12, 3.13, 3.14: tables referred to are missing.

3.15. In the case of poorly soluble test substances, methods of preparation of test solution/suspensions should be reported, however these are missing (also in the study report).

3.16. given figures are not concentrations, these should be reported.

Results and discussion

Agree with description of the participant

Conclusion

The results for difenacoum indicates it is not biodegradable under anaerobic conditions.

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

Reliability	3
Acceptability	non acceptable, due to the several deficiencies in reporting. However, this is not regarded as data gap as it is agreed in biocides Technical Meeting that the anaerobic degradation study is not absolutely necessary (because the result can not be utilised in the risk assessment as at the moment emission scenario does not contain assessment of anaerobic conditions).
Remarks	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	Drake RM,2005,The Estimation of the adsorption coefficient (Koc) of Difenacoum, Chemex Environmental International Limited, Cambridge, Report ENV7005/120139,February 2005.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with access to data	PelGar International Ltd. Activa srl	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline for testing chemicals reference 121	
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	Sample ref ECO120139	
3.1.2	Specification	99% minimum	
3.1.3	Purity	99.7% w/w	
3.1.4	Further relevant properties	Needs to be kept out of the light during storage. Water solubility is very low based on SafePharm report 1558/011.	x
3.1.5	Method of analysis	HPLC using OECD guideline 121.	
3.2	**Degradation products**	Degradation products tested: No	
3.2.1	Method of analysis for degradation products	Only one peak (difenacoum) was detected by HPLC.	
3.3	**Reference substance**	Phenol, Atrazine, Naphthalene, 1,2,3-Trichlorobenzene, Phenanthene, DDT	
3.3.1	Method of analysis for reference substance	Reference substance was tested using HPLC. Guideline 121.	
3.4	**Soil types**	Not applicable	
3.5	**Testing procedure**	*Non-entry field*	
3.5.1	Test system	Perkin Elmer Quaternary system using a hypersil by 5micrometre CPS, 250 by 4.6mm.This is used to assess the retention of difenacoum on a	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

		column designed to simulate the characteristics of soil which in turns allows assessment of the likely retention in soil.	
3.5.2	Test solution and Test conditions	Solvent was methanol:citrate buffer(55:45). The test solution was prepared by dispersing 0.002g in 10ml of mobile phase then diluted 1 in 5.	x
3.6	**Test performance**	*Non-entry field*	
3.6.1	Preliminary test	n/a	
3.6.2	Screening test: Adsorption		
3.6.3	Screening test: Desorption	n/a	
3.6.4	HPLC-method	The HPLC method enables adsorption coefficients to be estimated in the log Koc range between 1.5 and 5.0.	x
3.6.5	Other test	None	

4 RESULTS

4.1 Preliminary test n/a

4.2 Screening test: Adsorption Test material

Sample	Detector	Retention time (mins)		k		Log k		Log Koc	
		R1	R2	R1	R2	R1	R2	R1	R2
Peak	UV	3.626	3.620	0.20	0.19	-0.71	-0.71	1.84	1.81

4.3 Screening test: Desorption

Calculations *Non-entry field*

4.3.1 Ka , Kd n/a

4.3.2 Ka_{oc} , Kd_{oc} Log Koc=1.84 in run 1 and log Koc =1.81 in run 2.

Koc =69 in run 1 and Koc=64.5 in run 2.

Degradation product(s) Degradation not detected

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods All methods are according to Chemex International's standard procedure. HPLC is performed on analytical columns with a commercially available cyanopropyl solid phase containing lipophilic and polar moieties. While passing through the column along the mobile

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

		phase the test substance interacts with the stationary phase. As a result of partitioning between the mobile and stationary phases the test substance is retarded. The dual-composition of the stationary phase having polar and non-polar sites allows for the interaction of polar and non-polar groups of a molecule in a similar way as is the case for organic mater in soil or sewage sludge matrices. This enables the relationship between the retention time on the column and the adsorption coefficient on the organic matter to be established.
5.2	**Results and discussion**	One peak was detected (in duplicate) with UV detection with log Koc values 1.84 and 1.81 respectively.
5.2.1	Adsorbed a.s. [%]	n/a
5.2.2	K_a	n/a
5.2.3	K_d	n/a
5.2.4	Ka_{oc}	1.81-1.84
5.2.5	Ka/Kd	n/a
5.2.6	Degradation products (% of a.s.)	n/a
5.3	**Conclusion**	The test material has a low Koc. Therefore Difenacoum would be classed as mobile under the SSLRC classification and would tend therefore not to adsorb onto soil
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21.4.2006
Materials and Methods	3.1.4. Information on the pKa of difenacoum is missing. Also the pH dependence of the water solubility of difenacoum should be mentioned. 3.5.2. pH of the buffer is missing (in the study report pH 7.0 is mentioned, at pH 7 water solubility of difenacoum is 0.483 mg/l according to docA3). The method of preparing the solution should be described as difenacoum has low water solubility. 3.6.4. the method used for determination of the dead time (t_0) is missing (also in the study report).
Results and discussion	5.2.4 The results for reference substances are missing in the study summary. In the study report they are reported and a graph of the regerssion line presented (log K_{oc} vs log k'), however, according to the guideline the log k' data of the reference substances should be plotted against their log K_{oc} values (log k' vs logK_{oc}).
Conclusion	Quality criteria for individual measurements to be within 0.25 log units can not be evaluated for reference substances, because individual measurements were not reported, only average of 2 values. According to the guideline as columns can vary considerably in their separation efficiency, it is recommended as a guidance that the following capacity factors to be reached when using methanol/water 55/45 % as a mobile phase: logk' >0.0 for log Koc =3 and logk'>-0.4 for log Koc=2. These values are not achieved with the column and mobile phase used in the present study. Despite of these deficiencies it is concluded that the results indicate that under pH 7, difenacoum has a low Koc. Therefore difenacoum would be quite mobile and would tend therefore not to adsorb onto soil.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.3 (2) Annex Point IIA, VII7.7	**Adsorption/Desorption screening test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	It is believed that the result from the HPLC method gives a Koc which is much lower than it should be. And hence gives a misleading mobility classification. In the Pesticide Manual (13th ed, Tomlin) it states that it is unlikely to leach and that there was no leaching in 30cm lab.columns. According to the TGD (part 3, table 4) a QSAR equation exists for calculating log Koc from Kow. The equation for 'predominantly hydrophobics' is used as difenacoum's structure and water solubility would support this. Log Koc = 0.81 log Kow +0.1 A QSAR value of log 7.62 has been calculated using EPIWIN (see section A3 provided previously) So, log Koc = (0.81 * 7.62) +0.1 = 6.27 Therefore, Koc = 1871543.8 This figure is extremely large and so far above the SSLRC value of 4000 for non-mobile in soil, that the only logical conclusion is that it is non-mobile in soil. Also the bait will be used in very localised areas in and around buildings and in sewers. Direct contact to soil will be limited due to use of bait stations. Based on the above weight-of-evidence it is believed that the risk of brodifacoum reaching groundwater is very low. Also, in the Emission Scenario Document (2003, PT14) it states on page 25 for the scenario for ' in and around buildings' that " a detailed groundwater scenario is not considered necessary due to the limited quantities of active substance , the limited frequency and the limited contamination area". On the basis of all the above arguments, a derogation to perform an adsoption/desorption study (OECD 106) is requested.	
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	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A7.1.3 (2) Annex Point IIA, VII7.7	Adsorption/Desorption screening test
Date	23.10.2006
Evaluation of applicant's justification	When taking into account the intrinsic properties of difenacoum (molecular structure, behaviour as weak acid, low water solubility which increases with increasing pH, high estimated lokKow) and the anticipated limited exposure to soil when bait stations are used, the justification of the participant is regarded reasonable by RMS. However, the statement regarding groundwater scenario is related in ESD document only to liquid concentrate.
Conclusion	Justification of the participant is regarded acceptable by RMS and an adsoption/desorption study (OECD 106) is not required.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Annex Point IIA7.6.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	SafePharm Laboratories (2004) QSAR method for estimation of phototransformation in air, EPIWIN v 3.12	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Activa / PelGar Brodifacoum and Difenacoum Task Force	
1.2.2	Companies with letters of access	PelGar International Ltd. Activa srl	
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**	Not applicable	
2.2	**GLP**	Not applicable	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Not applicable	
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Not applicable	
3.1.4	Radiolabelling	Not applicable	
3.1.5	UV/VIS absorption spectra and absorbance value	Not applicable	
3.1.6	Further relevant properties	Not applicable	
3.2	**Reference substances**	Not applicable	
3.3	**Test solution**	Not applicable	
3.4	**Testing procedure**	Not applicable	
3.4.1	Test system	EPIWIN v 3.12	
3.4.2	Properties of light source	Not applicable	
3.4.3	Determination of irradiance	Not applicable	
3.4.4	Temperature	Not applicable	
3.4.5	pH	Not applicable	
3.4.6	Duration of the test	Not applicable	
3.4.7	Number of replicates	Not applicable	
3.4.8	Sampling	Not applicable	

Section A7.3.1 Annex Point IIA7.6.2.2

Phototransformation in air (estimation method), including identification of breakdown

3.4.9	Analytical methods	Not applicable

4 RESULTS

4.1 Summary of hydroxyl radicals

Hydrogen abstraction: 7.8831 E-12 cm^3/molecule-sec

Reaction with N, S and –OH = 0.1400 E-12 cm^3/molecule-sec

Addition to triple bonds = 0.0000 E-12 cm^3/molecule-sec

Addition to Olefininc bonds = 38.500 E-12 cm^3/molecule-sec

Addition to Aromatic rings = 15.1846 E-12 cm^3/molecule-sec

Addition to fused rings = 0.0000 E-12 cm^3/molecule-sec

Overall OH rate constant = 61.7077 E-12 cm^3/molecule-sec

Half-life = 0.173 days (12-hr day; 1.5E6 OH/cm^3)

Overall Ozone rate constant = 13.65000 E-17 cm^3/molecule-sec

Half-life = 0.084 days

Half-life = 2.015 hrs

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Difenacoum structure was analysed using the QSAR programme, EPIWIN v 3.12 and the results interpreted.

5.2 Results and discussion

The indirect photolysis half-life of of Difenacoum with OH radicals is 2.08 hours (rate constant = 61.7077E-12cm3/molecule/sec) and 2.015 hours (rate const. = 61.7077E-12 cm3/molecule/sec) with ozone.

Atmospheric risk: Difenacoum has a low volatility and emissions to the air compartment are expected to be low

Global warming: Difenacoum 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. Difenacoum 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. (Difenacoum has a tropospheric half-life of approximately 2 hours).

Acidification: Oxidation of Difenacoum 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 Difenacoum will have an effect on acidification of the receiving soil or surface water.

5.3 Conclusion

The indirect photolysis half-life of of Difenacoum with OH radicals is 2.08 hours (rate constant = 61.7077E-12cm3/molecule/sec) and 2.015 hours (rate constant = 61.7077E-12 cm3/molecule/sec) with ozone.

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

Annex Point IIA7.6.2.2

5.3.1	Reliability	2
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26.4.2006
Materials and Methods	Agree with description of the participant.
Results and discussion	Agree with description of the participant.
Conclusion	Agree with description of the participant. The indirect photolysis half-life of of Difenacoum with OH radicals is 2.08 hours (rate constant = 61.7077E-12cm3/molecule/sec) and 2.015 hours (rate constant = 61.7077E-12 cm3/molecule/sec) with ozone.
Reliability	Not applicable as not an experimental study.
Acceptability	acceptable
Remarks	According to TGD concentration of OH-radicals in atmosphere of 5×10^5 molecule/cm^3 and 24-hour day should be used. With these values AopWin calculation gives first order DT_{50} of 6.24 hours for difenacoum. This does not change overall conclusions of rapid photodegradation of difenacoum.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	