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**	**A7.1.1.1.1/01:** Sxxxx Mxxxx, Txxxx Jxxxx (2003) Hydrolysis of 14C-BAS 322 I in aqueous media. Bxxxx Axxxx Rxxxx, Rxxxx Txxxx Pxxxx, Nxxxx, Uxxxx, Report No. 130739, December 19, 2003 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD 111, EC method C.7 (92/69/EEC)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1.1	Test material	As given in Section A2.	
3.1.2	Lot/Batch number	792-1012	
3.1.3	Specification	As given in Section A2. Radiolabelled compound (^{14}C) Specific activity: 349170 dpm/µg	
3.1.4	Purity	Radiochemical purity: 99.4 % Chemical purity: 98.2 %	X
3.1.5	Further relevant properties	The labelling position is shown in Figure A7.1.1.1.1- 1	
3.2	**Reference substance**	No	
3.2.1	Initial concentration reference substance	Not applicable	
3.3	**Test solution**	Data on the test solutions are given in Table A7.1.1.1.1–1.	X
3.4	**Testing procedure**		
3.4.1	Test system	See Table A7.1.1.1.1–2	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products
Annex Point IIA7.6.2.1

3.4.2	Temperature	50 ± 5 °C	X
3.4.3	pH	pH 4, 7 and 9	
3.4.4	Duration of the test	5 days	
3.4.5	Number of replicates	Two samples at each observation point.	
3.4.6	Sampling	pH 4: 0, 1, 2, 3, 4 and 5 days after test initiation. pH 7: 0, 1, 2, 4 and 5 days after test initiation. pH 9: 0, 1, 2, 4 and 5 days after test initiation. Analysis was performed immediately after taking the samples.	
3.4.7	Analytical methods	HPLC system with radio-detection and UV-detection (254 nm). LSC – Beckman LS 6000 Series, to establish the material balance. The test samples were extracted with ethyl-acetate and dichloromethane, the extract concentrated in on a rotary evaporator, dissolved acetonitrile and subjected to LSC and HPLC (175–200 µl aliquot). Aliquots of the aqueous fraction were assayed by LSC and the measured radioactivity added to the material balance.	X
3.5	**Preliminary test**	Yes According to EC method C.7. All methods and results presented refer to the preliminary test, because the respective results indicated that no further testing was necessary.	

4 RESULTS

4.1	**Concentration and hydrolysis values**	At each pH, Flocoumafen was the only product in the 5-day sample, as confirmed by HPLC and MS. Thus, the entire radioactivity found can be attributed to unchanged Flocoumafen. Recovery data at different pH values are presented in Table A7.1.1.1.1–3. No anomalies or problems occurred.	X
4.2	**Hydrolysis rate**	Not applicable because the test results indicate that the substance is hydrolytically stable.	
4.3	**Dissipation time**	Not applicable because the test results indicate that the substance is hydrolytically stable.	
4.4	**Concentration-time data**	A graph is not presented in view of the hydrolytic stability of the test substance.	
4.5	**Specification of the transformation products**	Not applicable because the test results indicate that the substance is hydrolytically stable.	

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 hydrolysis rates of Flocoumafen were studied according to EC method C.7 (92/69/EEC) and OECD guideline 111. Due to the low water solubility of the test substance, a ^{14}C-labelled test compound was used. Test substance concentrations were determined using LSC. The results of the preliminary test indicated no need for further testing. Deviations from the guidelines were not recorded.	
5.2	**Results and discussion**	The test material-specific properties (e.g. solubility, stability, volatility, potential buffer effects) are not expected to have any impact on the results. Less than 10 % of the tested Flocoumafen hydrolysed in the preliminary test during five days at 50 ± 5 °C. Hence, k_H and DT_{50} or DT_{90} values could not be established. Therefore, Flocoumafen is considered to be hydrolytically stable. The half-life periods at pH 4, 7 and 9 can be expected to exceed one year at 25 °C.	X
5.3	**Conclusion**	The quality criteria claimed by the OECD guideline 111 are fulfilled. The study is therefore considered valid. Flocoumafen is hydrolytically stable under usual environmental conditions. No major degradation products were identified.	
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

07 June 2005

Materials and Methods

(3.1.4) The radiochemical purity of the stock solution used in the study was 95.04%. This is acceptable.
(3.3) (i) Table A.7.1.1.1.1-1: the molarity of the pH 9 buffer is 0.01 M (not 0.05 M). (ii) The concentration of the co-solvent (acetonitrile) was 1.1%. OECD 111 states a maximum of 1%. This deviation is considered by the RMS to be acceptable.
(3.4.2; 5.2) The temperature is 50 ± 0.5 °C (not ± 5°C).
(3.4.7) Test samples were extracted with ethyl-acetate or dichloromethane or a combination of ethyl-acetate and dichloromethane.

Results and discussion

(4.1) It is stated that "the entire radioactivity found can be attributed to unchanged Flocoumafen". This statement is not correct because (1) radioactivity remaining in the aqueous phase (up to 16.76% AR) was not analysed and (2) up to 2 unknowns were detected in the organic extracts (total of unknowns max. 13.4% AR, individually max. 7.45% AR). High amounts (>5% AR) of radioactivity remaining in the aqueous phase were only detected at day 0 and/or 1. At all other time points radioactivity remaining in the aqueous phase was <5% AR. With the exception of day 4, pH 7 (13.4% AR not identified, consisting of 2.2% AR remaining in aqueous phase and 2 unknowns of 5.5-5.6% AR), at the final three sampling points, the sum of not-analysed radioactivity and unknowns was <10% AR, indicating no significant hydrolysis of flocoumafen at all pH values tested.

The corrected (by the RMS) Table is inserted below:

Table RMS-A7.1.1.1.1- 3: Hydrolysis of ^{14}C-flocoumafen, expressed as percentage of initial concentrations, at pH 5, pH 7 and pH 9.

Compound	**Sampling times [days]**					
	0	**1**	**2**	**3**	**4**	**5**
pH 4						
Parent compound (Flocoumafen)	96.95	82.90	88.18	96.31	91.23	93.35
Total recovery [%]	100.82	93.62	92.4	100.54	95.96	97.8
pH 7						
Parent compound (Flocoumafen)	97.79	96.08	99.70	n.d.	89.28	93.81
Total recovery [%]	99.98	110.43	106.45	n.d.	102.64	102.24
pH 9						
Parent compound (Flocoumafen)	100.15	88.56	95.10	n.d.	92.42	93.77
Total recovery [%]	116.7	106.29	102.71	n.d.	101.07	102.48

n.d. = not determined

Conclusion

Flocoumafen was stable in buffered (pH 4, 7 and 9) aqueous solutions of 0.001 mg a.s./L when incubated at 50°C for 5 days. The hydrolytic half-life at pH 4, 7 and 9 can be expected to exceed one year at 25 °C. Hence, Flocoumafen is hydrolytically stable under usual environmental conditions.

Reliability

1

Acceptability	Acceptable.
Remarks	No further remarks.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.1.1.1- 1: Description of test solution.

Criteria	**Details**
Purity of water	HPLC grade water
Preparation of test medium	*Buffer solutions:* pH 4: 0.01 M Potassium hydrogen phthalate buffer pH 7: 0.01 M Trizma (pH 7 pre-set crystals, Sigma) buffer pH 9: 0.05 M Trizma (pH 9 pre-set crystals, Sigma) buffer*
Test concentrations [mg /l]	c_0 = 0.001 mg/l at pH 4, pH 7, and pH 9, respectively
Temperature [°C]	50 ± 0.5 °C
Controls	None
Identity and concentration of co-solvent	Acetonitrile, HPLC grade, 1.1 % concentrated in the final test solutions
Replicates	Duplicate sampling at any observation point

* comment RMS: the molarity was 0.01 M

Table A7.1.1.1.1- 2: Description of test system.

Glassware	500 ml screw-capped brown glass bottles
Other equipment	Thermostated incubator
Method of sterilization	30 min autoclaving at 120 °C
Method of oxygen exclusion	Not stated
Avoidance of photolytic effects	Storage in incubator

Table A7.1.1.1.1- 3: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 5, pH 7 and pH 9; Flocoumafen (parent compound) was the only substance present, as confirmed by HPLC and MS.

Compound	Sampling times [days]					
	0	**1**	**2**	**3**	**4**	**5**
pH 4						
Parent compound (Flocoumafen)	100.82	93.62	92.4	100.54	95.96	97.8
Total recovery [%]	100.82	93.62	92.4	100.54	95.96	97.8
pH 7						
Parent compound (Flocoumafen)	99.98	110.43	106.45	n.d.	102.64	102.24
Total recovery [%]	99.98	110.43	106.45	n.d.	102.64	102.24
pH 9						
Parent compound (Flocoumafen)	116.7	106.29	102.71	n.d.	101.07	102.48
Total recovery [%]	116.7	106.29	102.71	n.d.	101.07	102.48

n.d. = not determined

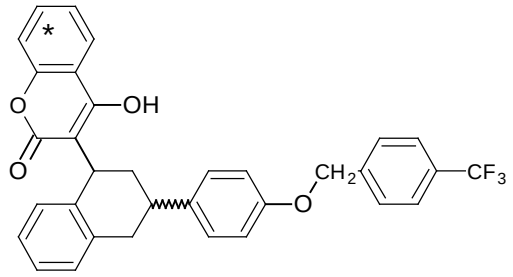

Figure A7.1.1.1.1- 1: Labelling position of ^{14}C-Flocoumafen, as marked by the asterisk (uniform labelling of the marked ring).

Section A7.1.1.1.2 Annex Point IIA7.6.2.2 Phototransformation in water including identity of transformation products

Official use only

1 REFERENCE

1.1	**Reference**	**A7.1.1.1.2/01:** Hxxxx Dxxxx (2006) Direct phototransformation of Flocoumafen in water and identity of transformation products. Fxxxx Ixxxx fxxxx Mxxxx Bxxxx axxxx Axxxx Exxxx, Sxxxx, Gxxxx, Report no. EBR-003/7-05, January 25, 2006 (unpublished). BASF DocID: 2006/1009332
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter 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 Draft OECD guideline "Phototransformation of chemicals in water, direct and indirect photolysis", August 2000. SETAC, 1995: "Procedures for assessing the environmental fate and ecotoxicology of pesticides"
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	a)	As given in Section A2.
		b)	[Coumarin-^{14}C]-Flocoumafen (= Reference item 289)
		c)	[Trifluoromethyphenyl-^{14}C]-Flocoumafen (= Reference item 290)
3.1.1	Lot/Batch number	a)	AC12140-35
		b)	792-1101
		c)	794-1101
3.1.2	Specification	a)	As given in Section A2.
		b)	Labelled substance, dissolved in a toluene / ethanol (96:4) mixture
		c)	Labelled substance, dissolved in a toluene / ethanol (96:4) mixture

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products
Annex Point IIA7.6.2.2

3.1.3	Purity	a) 99.4% b) 98.8% (radiochemical purity) c) 99.0% (radiochemical purity)
3.1.4	Radio-labelling	a) No b) Yes c) Yes Labelling positions are illustrated in Figure A7.1.1.1.2- 1.
3.1.5	UV/VIS absorption spectra and absorbance value	λ (max) = 311.2 nm (pH 6.8) ε (max) = 14 162 l × mol^{-1} × cm^{-1} (pH 6.8, 311.2 nm) (results from ref. A3.4/01); for results from the screening test in the current study please refer to Table A7.1.1.1.2- 3.
3.1.6	Further relevant properties	The extremely low water solubility (also see reference A3.5/01) necessitated the use of acetonitrile up to the guideline-compliant limit of 10% to achieve sufficient concentrations for this test (also see Table A7.1.1.1.2- 1).
3.2	**Reference substance**	No
3.3	**Test solution**	Data on the test solutions are given in Table A7.1.1.1.2- 1.
3.4	**Testing procedure**	
3.4.1	Test system	Refer to Table A7.1.1.1.2- 2.
3.4.2	Properties of light source	As described in Table A7.1.1.1.2- 2.
3.4.3	Determination of irradiance	Artificial irradiance: A p-nitroanisole / pyridine chemical actinometer was used. The intensity of irradiance was measured by the decrease of concentration of p-nitroanisole, which is proportional to the number of quanta striking the sample. Further details are presented in Table A7.1.1.1.2- 4.
3.4.4	Temperature	20°C
3.4.5	pH	Screening test (UV/VIS spectra recording): 5, 7, 9 Main test: 7
3.4.6	Duration of the test	8 hours
3.4.7	Number of replicates	3 replicates per labelled reference item per concentration.
3.4.8	Sampling	0.5, 1, 1.5, 2, 3, 4, 6 and 8h

Section A7.1.1.1.2 Annex Point IIA7.6.2.2 Phototransformation in water including identity of transformation products

3.4.9	Analytical methods	Aliquots of irradiated samples and the respective dark controls were analysed by LSC using a Packard Tri Carb liquid scintillation analyzer after mixing an aliquot of the solution of interest with an aliquot of a suitable liquid scintillation cocktail (Pico Fluor LLT). Flocoumafen and its transformation products were detected by radio-HPLC and identified by LC-MS/MS. The HPLC method was validated for unlabelled Flocumafen using 6 fortification levels ranging from 0.025 mg/l to 0.50 mg/l; the LoD was established at 0.011 mg/l, and the LoQ at 0.040 mg/l. Calibration was performed using 8 fortification levels ranging from 0.1 mg/l to 2.5 mg/l unlabelled Flocumafen; detector response was linear in the specified range, resulting in a correlation coefficient of 0.9998. For p-nitroanisole, the HPLC system was calibrated using 7 standard concentrations ranging between 0.1 and 1.5 mg/l; the LoD was established at 0.013 mg/l, and the LoQ at 0.047 mg/l. Detector response was linear in the specified range, resulting in a correlation coefficient of 0.9999.
3.5	**Transformation products**	Yes
3.5.1	Method of analysis for transformation products	By radio-HPLC and LC-MS/MS system

4 RESULTS

4.1	**Screening test**	Performed Relevant results are summarised in Table A7.1.1.1.2- 3.
4.2	**Actinometer data**	Data on the actinometry with p-nitroanisole / pyridine are presented in Table A7.1.1.1.2- 4.
4.3	**Controls**	

Coumarin-^{14}C	0.5	1.5	3.0 mg/l (nominal)
c_0 [mg/l]	0.62	0.51	1.79
c_t [mg/l]	0.669	0.241	1.566*
Trifluoromethyphenyl-^{14}C	**0.5**	**1.5**	**3.0 mg/l (nominal)**
c_0 [mg/l]	0.74	0.64	2.10
c_t [mg/l]	0.760	0.507	0.438

*) value from adjacent measurement time, since original data considered to be flawed due to analytical error; for the derivation of the initial concentrations also see Table A7.1.1.1.2- 1.

Section A7.1.1.1.2 Annex Point IIA7.6.2.2 Phototransformation in water including identity of transformation products

4.4 Photolysis data

4.4.1 Concentration values

Coumarin-^{14}C-Flocoumafen, concentrations in mol/l:

Time [h]	**c [mg/l], initial**		
	0.62	0.51	1.79
0.5	1.12×10^{-6}	8.47×10^{-7}	3.14×10^{-6}
1	9.68×10^{-7}	7.64×10^{-7}	2.79×10^{-6}
1.5	8.91×10^{-7}	6.91×10^{-7}	2.55×10^{-6}
2	9.74×10^{-7}	5.85×10^{-7}	2.35×10^{-6}
3	6.38×10^{-7}	4.77×10^{-7}	2.01×10^{-6}
4	6.86×10^{-7}	3.78×10^{-7}	1.76×10^{-6}
6	4.93×10^{-7}	2.27×10^{-7}	1.22×10^{-6}
8	3.99×10^{-7}	1.59×10^{-7}	9.22×10^{-7}

Trifluoromethyphenyl-^{14}C-Flocoumafen, concentrations in mol/l:

Time [h]	**c [mg/l], initial**		
	0.74	0.64	2.10
0.5	1.34×10^{-6}	1.07×10^{-6}	3.57×10^{-6}
1	1.18×10^{-6}	9.39×10^{-7}	3.23×10^{-6}
1.5	1.08×10^{-6}	8.02×10^{-7}	2.95×10^{-6}
2	1.02×10^{-6}	7.03×10^{-7}	2.53×10^{-6}
3	8.44×10^{-7}	5.76×10^{-7}	2.01×10^{-6}
4	7.67×10^{-7}	3.93×10^{-7}	1.81×10^{-6}
6	6.12×10^{-7}	2.74×10^{-7}	1.10×10^{-6}
8	4.42×10^{-7}	1.63×10^{-7}	8.08×10^{-7}

4.4.2 Mass balance: Please refer to Table A7.1.1.1.2- 5 and Table A7.1.1.1.2- 6.

4.4.3 kcp: 3.97×10^{-5} s^{-1} (SD = 7.23×10^{-6}, $n = 6$)
$r^2 > 0.94$ for all test substance/concentration combinations

4.4.4 Kinetic order: Pseudo first order

4.4.5 kcp/kap: 0.906

4.4.6 Reaction quantum yield (ΦcE): 8.90×10^{-4} (SD = 3.69×10^{-4})

4.4.7 kpE: Environmental degradation rates were not provided by the original report. However, recalculated photolysis rates, based on the half-lives calculated by program ABIWAS, are presented in Table A7.1.1.1.2- 7.

Section A7.1.1.1.2 Annex Point IIA7.6.2.2 Phototransformation in water including identity of transformation products

4.4.8	Half-life (t½E)	Using the program ABIWAS, half-lives in a range between 0.724 days (17.4 h) and 135 days for the months June and December were calculated, depending on solar irradiance intensity (medium values at normal climatic conditions at a latitude of 52 °N). Details are given in Table A7.1.1.1.2- 7. According to the TGD, the recommendations given in the published article of Frank and Klöpffer (1989) should be followed for the derivation of an average degradation rate to be used in the risk assessment. Frank and Klöpffer (1989) derived their reference half-life based on the solar irradiance in April. In compliance with the recommended procedures, the medium ("normal") half-life calculated for April is proposed as the reference value to be used in the risk assessment. Thus, $t_{½E}$ = 1.67 d
4.5	**Specification of the transformation products**	A total of four major transformation products (≥ 10% of applied radioactivity) were detected during the irradiation experiments. Two major transformation products could be identified as true breakdown products (see Table A7.1.1.1.2- 8 and Figure A7.1.1.1.2- 3). For the remaining two major transformation products, identification was not possible since no fragmentation in the MS analysis occurred. However, one of these compounds was also a breakdown product whereas the other showed an increased molecular weight in relation to the parent compound, suggesting that some kind of adduct was formed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The photo-transformation of Flocoumafen in water was tested according to the Draft OECD guideline "Phototransformation of chemicals in water, direct and indirect photolysis", August 2000". ^{14}C-Flocoumafen labelled at two different positions was employed in order to facilitate identification of possible transformation products. Actual test concentrations in water at pH 7 ranged between 0.51 and 2.10 mg/l. In view of the intrinsic low water solubility of the test substance, these concentrations could only be achieved by utilisation of 10% (v/v) acetonitrile as a co-solvent, which is in compliance with the guideline. Quantum yield was estimated using a temperature-controlled (20°C) irradiation apparatus, calibrated by means of a p-nitroanisole / pyridine actinometer. The environmental half-life of Flocoumafen in surface water was calculated for the geographic and climatic conditions of Central Europe at 52 °N using program ABIWAS. Due to difficulties with the identification of unknown transformation products, additional LC-MS/MS analyses were performed following irradiation in 50/50% (v/v) acetonitrile/water solution, which is not guideline-compliant.

Section A7.1.1.1.2 Annex Point IIA7.6.2.2 Phototransformation in water including identity of transformation products

5.2	**Results and discussion**	A screening test (based on UV/VIS spectra at pH 7 and 9) indicated that Flocoumafen absorbs sufficient amounts of light (peak at 309–310 nm) to potentially undergo photolytic degradation. In the irradiation experiment, Flocoumafen degraded rapidly. Four major transformation products (> 10% of the employed active substance) were formed: ▪ 4-(Trifluoromethyl)-benzoic acid (CAS-No. 455-24-3) ▪ 4-hydroxy-3-[3-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-1-naphthyl]coumarin (no CAS-No. allocated) ▪ Plus two unidentified transformation products Despite considerable analytical effort, the remaining two transformation products could not be identified since no fragmentation in the MS occurred. One of these products probably is an adduct of smaller breakdown products, in view of its molecular weight being higher than that of Flocoumafen. Nevertheless, in view of the extremely low concentrations of parent compound – consequently also of all transformation products – to be expected in surface waters even under worst case assumptions, their environmental relevance is considered to be limited.
5.2.1	kcp	$3.97 \times 10^{-5}\ s^{-1}$
5.2.2	KpE	See Table A7.1.1.1.2- 7.
5.2.3	ϕcE	8.90×10^{-4}
5.2.4	t1/2E	See Table A7.1.1.1.2- 7. The representative half-life for average conditions in Central Europe is selected as the “normal” value in April (according to Frank and Klöpffer, 1989): $t_{½E}$ = 1.67 d
5.3	**Conclusion**	Two out of four major transformation products could not be identified. Since all reasonable effort was undertaken in this respect, the non-identifiability of these transformation products may be considered as an inherent property of the investigated test substance and/or the experimental system. Taking into account these experimental difficulties, no circumstances were reported that may have affected the integrity and quality of the results. Thus, this study is considered to be valid without restrictions. On the basis of the obtained results, direct photolysis in water is considered to be a process of major importance regarding abiotic degradation of Flocoumafen in surface waters under environmentally relevant conditions. However, considering the expected extremely low environmental concentrations of both the parent compound as well as of the transformation products, the occurrence of major transformation products is nevertheless deemed to be of negligible environmental importance.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	31 October 2006
Materials and Methods	The method used to detect and identify metabolites is of low quality due to the rapid increase in solvent used.
Results and discussion	Attachment 6 is not complete. The title page is missing, despite this omission the report is accepted by the RMS.
Conclusion	Conclusions of the notifier are accepted
Reliability	2
Acceptability	
Remarks	Flocoumafen was found to be susceptible to rapid photo-transformation in water. Four major transformation products were formed, two of which could be identified
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

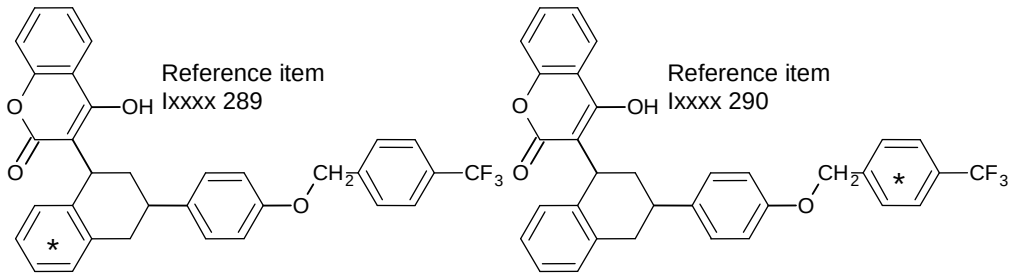

Figure A7.1.1.1.2- 1: Labelling positions of ^{14}C in Flocoumafen (marked by an asterisk).

Table A7.1.1.1.2- 1: Description of test solution and controls.

Criteria	Details
Purity of water	Deionised water, further purified using an Elga UHQ-PS purification device
Preparation of test chemical solution	Buffers: pH 5: citrate / sodium hydroxide pH 7: sodium- / potassium- phosphate pH 9: Boric acid / potassium chloride / sodium hydroxide Due to the very low water solubility of the test item, the irradiation experiments were performed with solutions of the pure radio-labelled reference items in order to improve sensitivity of the chemical analysis; An aliquot of the reference items as delivered (dissolved in toluene/ ethanol) was transferred into a 10 mL volumetric flask and the solvent removed by a gentle stream of nitrogen. The dry residue was re-dissolved and filled up to mark with acetonitrile, and samples thereof mixed with the test solutions as appropriate
Test concentrations [mg a.s./l]	Analyses of the test solutions after filtration (< 0.45 µm) showed strong variation. Following the analyses of the irradiated solutions, these initial analyses were assessed to be invalid. Initial concentrations of the solutions were therefore extrapolated retrospectively from the degradation functions. The following initial concentration were calculated: Target concentration 0.5 1.5 3.0 Reference item 289 0.62 0.51 1.79 Reference item 290 0.74 0.64 2.10
Temperature [°C]	20°C
Preparation of actinometer solution	See Table A7.1.1.1.2- 4
Controls	Concurrent dark controls, one per concentration and reference item, respectively
Identity and concentration of co-solvent	Acetonitrile, 10% (v/v)

Table A7.1.1.1.2- 2: Description of test system.

Criteria	Details
Laboratory equipment	Up to 15 cylindrical trays (21 mm in diameter, max. volume 10 mL), covered gas-tight with a quartz glass plate, stirred by magnetic stirrer, refrigerated circulator used for temperature control. Atlas SUNTEST apparatus Photometer used: Cary 1, Varian
Test apparatus	Artificial light actinometer, see above
Properties of artificial light source:	
Nature of light source	Xenon arc lamp
Emission wavelength spectrum	290–800 nm
Light intensity	Determined using the p-nitroanisole / pyridine-actinometer
Filters	Cut-off filters to ensure emission wavelength spectrum as stated above

Table A7.1.1.1.2- 3: Screening test results.

Absorption curve	See Figure A7.1.1.1.2- 2 below			
A_λ	At c =	5.57	2.58	1.10 mg/l
	A_{310}	0.161	0.077	0.032
$\varepsilon_\lambda{}^c$ [l / mol × cm]	At λ = 310 nm: 15 854			
k_{pEmax}	8.25×10^{-3} d^{-1}			
$t_{1/2Emin}$	9.7×10^{-4} d (= 84 s)			
L_λ	Not appropriate			

Table A7.1.1.1.2- 4: Actinometer data.

p-nitroanisole / pyridine concentrations	p-nitroanisole: 10^{-5} mol/l Pyridine: 5×10^{-4} mol/l
$\phi^a{}_E$	0.0005
$k^a{}_p$	4.38×10^{-5} s^{-1}, $r^2 = 0.9994$

Table A7.1.1.1.2- 5: Recovery of radioactivity, reference item Ixxxx-no. 289; DC = dark control.

Time [h]	Initial radioactivity					
	16524 Bq/mL		8262 Bq/mL		5313 Bq/mL	
	Irradiated	DC	Irradiated	DC	Irradiated	DC
	Recovery [%]					
0	141	185	146	115	104	108
0.5	86	89	100	172	108	110
1	81	86	93	60	104	106
1.5	81	80	64	55	102	106
2	88	78	69	54	101	106
3	90	81	62	57	101	103
4	84	87	69	64	101	104
6	88	87	58	45	100	105
8	84	79	59	59	102	96

Table A7.1.1.1.2- 6: Recovery of radioactivity, reference item Ixxxx-no. 290; DC = dark control.

Time [h]	Initial radioactivity					
	18109 Bq/mL		9054 Bq/mL		5453 Bq/mL	
	Irradiated	DC	Irradiated	DC	Irradiated	DC
	Recovery [%]					
0	87	108	94	93	96	102
0.5	77	72	61	66	97	103
1	78	74	57	60	94	104
1.5	78	72	57	56	88	99
2	80	Sample lost	66	62	86	95
3	76	75	54	69	88	102
4	79	82	103	76	81	97
6	89	74	50	51	72	95
8	79	67	58	53	89	93

Table A7.1.1.1.2- 7: Environmental half-lives and degradation rates of Flocoumafen due to photolysis, as calculated by program ABIWAS; environmental photolysis rates were not provided in the report but are presented here, recalculated from the half-lives.

Month	Half-life values [d]			Environmental photolysis rates [d^{-1}]		
	Minimal	**Normal**	**Maximal**	**Minimal**	**Normal**	**Maximal**
January	7.77	16.3	74.2	0.089	0.043	0.009
February	3.28	6.89	30.0	0.211	0.101	0.023
March	1.62	3.08	12.8	0.428	0.225	0.054
April	0.926	1.67	6.67	0.749	0.415	0.104
May	0.778	1.24	4.98	0.891	0.559	0.139
June	0.724	1.09	4.34	0.957	0.636	0.160
July	0.815	1.22	4.08	0.850	0.568	0.170
August	0.866	1.3	4.33	0.800	0.533	0.160
September	1.38	2.34	8.68	0.502	0.296	0.080
October	2.52	4.79	21.8	0.275	0.145	0.032
November	5.59	12.9	64.3	0.124	0.054	0.011
December	12.3	27.0	135	0.056	0.026	0.005

Table A7.1.1.1.2- 8: Specification and amount of transformation products.

CAS-Number	CAS and/or IUPAC Chemical Name(s)	Amount [%] of parent compound after 8 hours, measured at initial concentration [mg/l]:		
Parent: [Coumarin-^{14}C]-Flocoumafen				
		1.79	**0.51**	**0.62**
–	4-hydroxy-3-[3-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-1-naphthyl]coumarin (transformation product 1)	42.3	59.3	43.1
455-24-3	4-(Trifluoromethyl)-benzoic acid (transformation product 3)	–	–	–
–	Not identified (transformation product 2)	27.7	28.9	30.7
–	Not identified (transformation product 4)	–	–	–
–	Not identified (transformation product 5)	–	–	–
Parent: [Trifluoromethyphenyl-^{14}C]-Flocoumafen				
		2.10	**0.64**	**0.74**
–	4-hydroxy-3-[3-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-1-naphthyl]coumarin (transformation product 1)	–	–	–
455-24-3	4-(Trifluoromethyl)-benzoic acid (transformation product 3)	19.4	36.9	13.2
–	Not identified (transformation product 2)	–	–	–
–	Not identified (transformation product 4)	6.8	0.0	0.0
–	Not identified (transformation product 5)	22.4	30.3	11.9

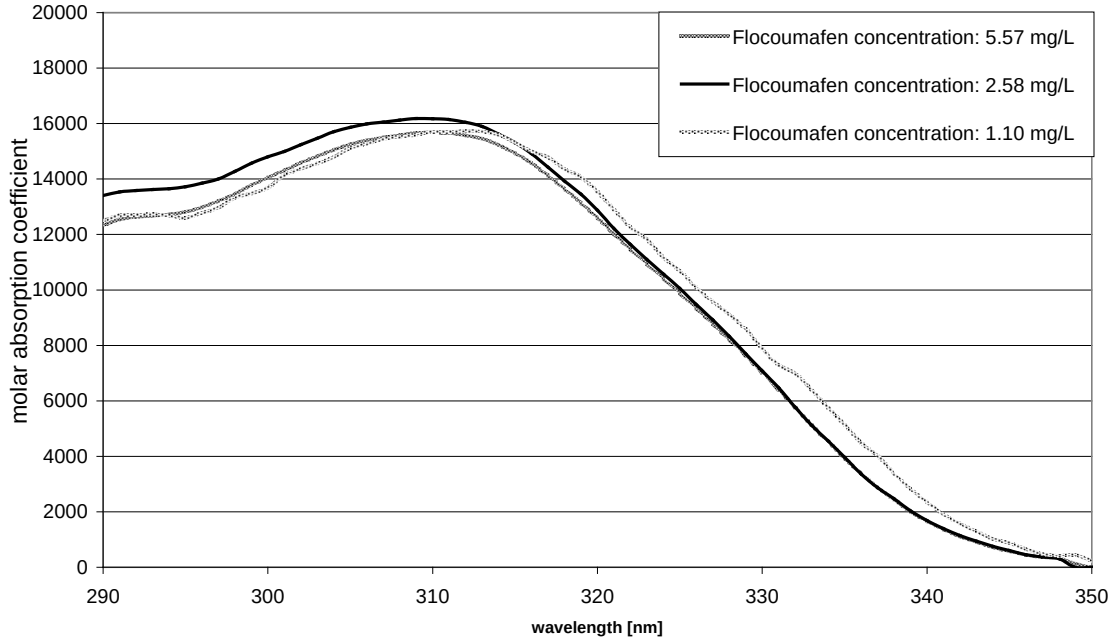

Figure A7.1.1.1.2- 2: Absorbance of Flocoumafen in aqueous solution at different pH.

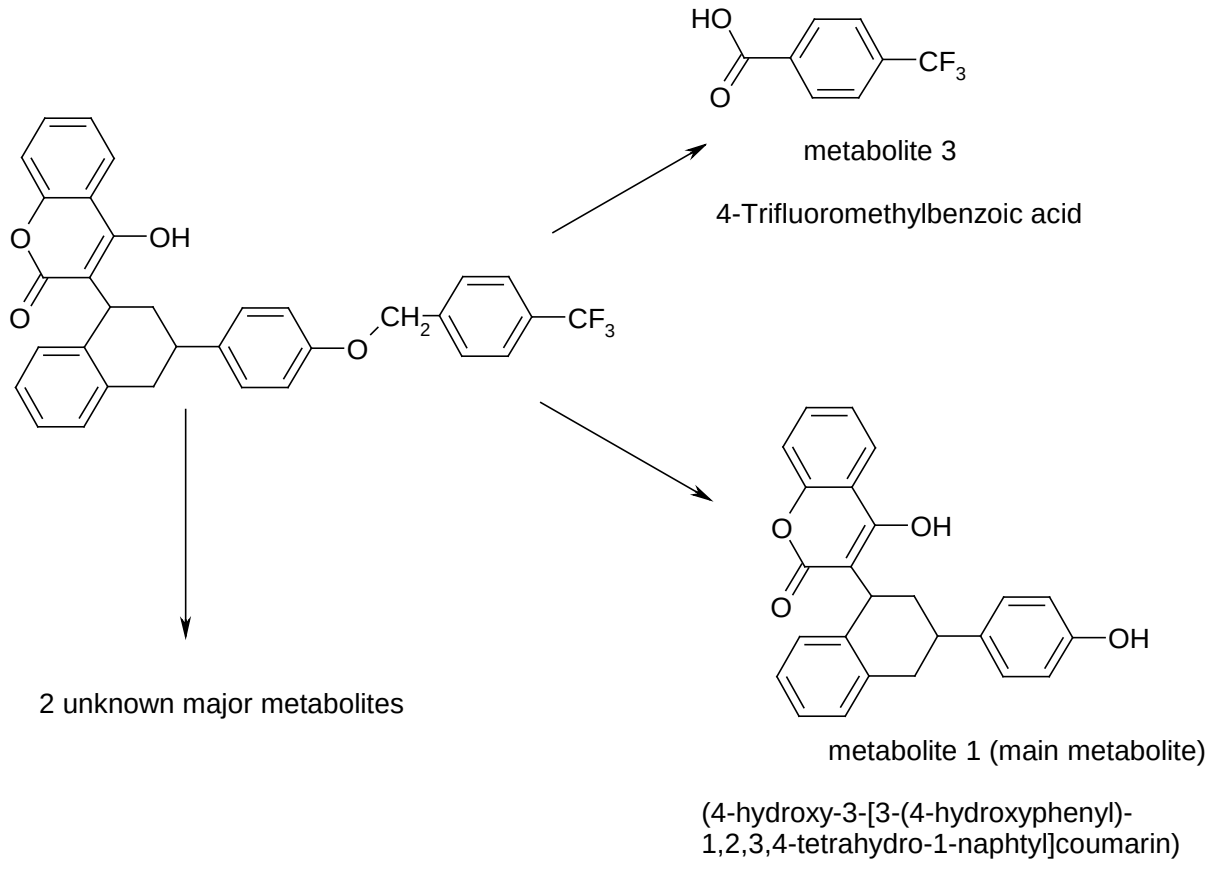

Figure A7.1.1.1.2- 3: Proposed photolytic transformation pathway of Flocoumafen in water.

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA7.6.1.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.1.1.2.1/01:** Dxxxx Dxxxx (2004) Assessment of the ready biodegradability of Flocoumafen with the closed bottle test. Axxxx Gxxxx Bxxxx Gxxxx & Ixxxx Uxxxx Gxxxx, Nxxxx, Gxxxx, Report No. 20031410/01-AACB, March 04, 2004 (unpublished). (BASF Ref.: 2004/1009182)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 301 D, EC method C.4-E
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	03
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	> 99 %
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water (see Section A3.5) and shows a tendency to adsorb to surfaces.
3.1.5	Composition of Product	Not applicable
3.1.6	TS inhibitory to micro-organisms	No (see Section A7.4.1.4)
3.1.7	Specific chemical analysis	Not applicable
3.2	**Reference substance**	Yes Benzoic acid, sodium salt

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA7.6.1.1

3.2.1	Initial concentration of reference substance	2 mg/l	
3.3	**Testing procedure**		
3.3.2	Inoculum	As given in Table A7.1.1.2.1-1.	X
3.3.3	Test system	The test system is described in Table A7.1.1.2.1-2.	
3.3.4	Test conditions	See Table A7.1.1.2.1- 3.	
3.3.5	Method of preparation of test solution	In order to deal with the low water solubility, 20 µl of a stock solution of Flocoumafen in Acetone (288 mg/10 ml) was given into each test vessel, the solvent evaporated, and then mineral medium and inoculum added.	X
3.3.6	Initial TS concentration	2 mg/l (nominal)	X
3.3.7	Duration of test	28 d	
3.3.8	Analytical parameter	Oxygen concentration	
3.3.9	Sampling	After 7, 14, 21, and 28 days, respectively.	
3.3.10	Intermediates/ degradation products	Not identified.	
3.3.11	Nitrate/nitrite measurement	No	
3.3.12	Controls	1) Control without test substance (blank inoculum) 2) Functional control: Sodium benzoate, as specified above (3.2) 3) Toxicity control: Test substance + reference substance (1 mg/l, respectively).	
3.3.13	Statistics	ThOD according to OECD 301 D and EC C.4-E guidelines. BOD according to OECD 301 D and EC C.4-E guidelines. Per cent degradation.	

4 RESULTS

4.1	**Degradation of test substance**		
4.1.1	Graph	A graphical representation of the degradation curve is given in Figure A7.1.1.2.1-1.	
4.1.2	Degradation	A plateau phase of degradation of the test substance was not reached within 28 days. At test termination, 68.1 % (mean of three replicates) of the test substance was degraded. More than 60 % degradation was reached within a 14-day window.	

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA7.6.1.1

4.1.3	Other observations	The variation among replicates upon test termination was more than 20 %. Oxygen consumption in the blank inoculum was more than 1.5 mg O_2/l (measurement mean = 1.84 mg/l).	
4.1.4	Degradation of TS in abiotic control	Not applicable; no abiotic control was performed.	
4.1.5	Degradation of reference substance	100 % within 14 days.	
4.1.6	Intermediates/ degradation products	Not determined.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The ready biodegradability of Flocoumafen, measured as per cent degradation, was tested using the closed bottle test (OECD guideline 301 D, EC C.4-E). The performance of the study was fully compliant to the stated guidelines. The low water solubility of Flocoumafen was appropriately accounted for, by applying the substance dissolved in Acetone to the test vessels dissolved in Acetone. The nominal concentration of 2 mg/l fulfils the range specified by the guidelines, but is nevertheless in excess of the water solubility of approx. 0.1 mg/l.	X
5.2	**Results and discussion**	Flocoumafen showed an average degradation of 68.1 % of ThOD. More than 60 % degradation was reached within a 14-d window. A 14-d instead of a 10-d window is acceptable according to OECD 301. The variation among replicates upon test termination was more than 20 %. However, such a large variation seems unavoidable regarding the low water solubility of Flocoumafen, although Appropriate measures to circumvent problems arising from the low solubility were taken. The criterion for oxygen consumption in the blank inoculum (< 1.5 mg/l) was marginally failed. This was explained by additional self-ingestion of the inoculum. In conclusion, the non-fulfilment of these two validity criteria can be explained by specific properties of the test substance (water solubility) and natural variation in the behaviour of the inoculum.	X
5.3	**Conclusion**	The validity criteria are only partly fulfilled (Table A7.1.1.2.1-4). Thus, the study is considered to be fully valid and reliable. Accordingly, Flocoumafen is considered to be as readily biodegradable.	X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	None	X

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	15 September 2005
Materials and Methods	(3.3.2) Table A7.1.1.2.1-1. cell concentrations were not in agreement with the report. The cel concentrations should read: Inoculum blank: 18 cells/ml Flocoumafen: 13 cells/ml Reference: 18 cells/ml Toxicity control: 24 cells/ml (3.3.5) Method of application of the test substance: the test substance was added as a solution in acetone to empty glass bottles. The acetone was evaporated off and the test medium was added. In 3.1.4 it is stated that the test substance shows a tendency to adsorb to surfaces. It is therefore possible that significant amounts of test substance were adsorbed onto the glass and did not enter the test medium. Reproducible test substance concentrations between replicates may therefore not have been obtained. This may explain the large variation in the results of replicate samples (>20%). (3.3.6) The nominal concentration of 2 mg/L is above the water solubility (~0.1 mg/L). There was no evidence in the report that the test systems were agitated in order to obtain homogeneous dispersions (although this is indicated in Table A7.1.1.2.1-4). (5.1) See remarks at 3.3.5 and 3.3.6
Results and discussion	(5.2) (i) The variation between replicates in the bottles with flocoumafen was considerable: oxygen depletion in individual bottles deviated from the mean by up to 37%, 134%, 28% and 48% after 7, 14, 21 and 28 days, respectively. The non-fulfillment of the reproducibility criteria (variation among replicates was >20%) may have been caused by the way the test substance was applied (3.3.5) and the apparent lack of agitation during the study (3.3.6).
Conclusion	The validity criteria are not fulfilled. Therefore the study is not accepted and the conclusion that Flocoumafen is readily biodegradable is considered not valid.
Reliability	3
Acceptability	Not acceptable
Remarks	The reliability was lowered to 3 because of non-fulfillment of the reproducibility criteria.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.1.2.1- 1: Description of the inoculum.

Criteria	Details
Nature	Effluent from municipal STP
Species	Mixed species population
Strain	–
Source	Municipal STP
Sampling site	Pforzheim, Germany
Laboratory culture	No
Method of cultivation	–
Preparation of inoculum for exposure	Filtered through a coarse filter
Pre-treatment	Shaken for 1 h for starvation
Initial cell concentration	Inoculum blank: 18 cells/ml Flocoumafen: 24 cells/ml Reference: 13 cells/ml Toxicity control: 18 cells/ml

Table A7.1.1.2.1- 2: Description of the test system.

Criteria	Details
Culturing apparatus	Temperature controlled dark chamber
Number of culture flasks/ concentration	2
Aeration device	Not stated
Measuring equipment	WTW Microprocessor Oximeter OXI 340 with calibrated electrode
Test performed in closed vessels due to significant volatility of test substance	No

Table A7.1.1.2.1- 3: Description of the test conditions.

Criteria	**Details**
Composition of the medium	KH_2PO_4 8.5 mg/l K_2HPO_4 21.75 mg/l $Na_2HPO_4 \cdot 2\ H_20$ 33.4 mg/l NH_4Cl 0.5 mg/l $MgSO_4 \cdot 7\ H_20$ 22.5 mg/l $CaCl_2$ 27.5 mg/l $FeCl_3 \cdot 6\ H_20$ 0.25 mg/l
Additional substrate	No
Test temperature	20 ± 2 °C
pH	Not stated
Aeration of dilution water	Yes, 60 min strong aeration, then 24 h settling period
Suspended solids concentration	Not applicable
Other relevant criteria	None

Table A7.1.1.2.1- 4: Pass levels and validity criteria for tests on ready biodegradability.

	Fulfilled	**Not fulfilled**
Pass levels		
60% removal of ThOD or $ThCO_2$	☑	
Pass values reached within 14-d window/ 28-d test period	☑	
Criteria for validity		
Variation between replicates at the end of test < 20%		☑
Removal of reference substance reaches pass level by day 14	☑	
Criteria for poorly soluble test substances		
Selection of suitable test method (closed bottle)	☑	
Appropriate method of agitation	☑[1]	

[1] Comment by RMS: Not mentioned in the study report

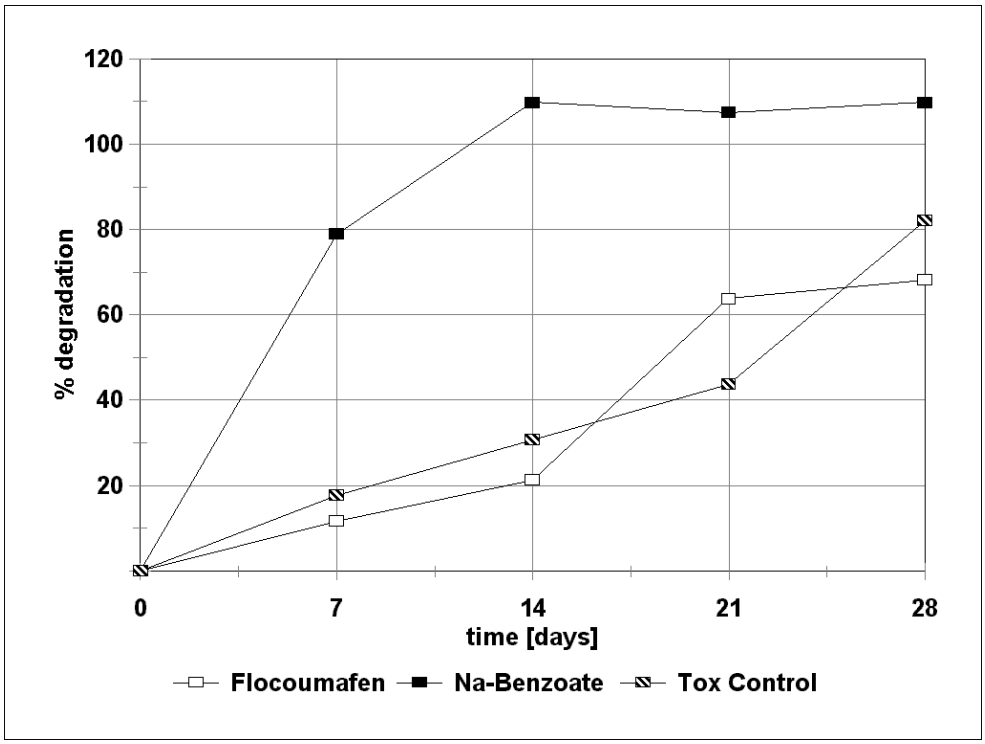

Figure A7.1.1.2.1- 1: Degradation of Flocoumafen (% ThOD) over time.

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA7.6.1.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.1.1.2.1/02:** Lxxxx Hxxxx (1995) Study on the 'ready biodegradability' of technical Flocoumafen according to OECD-test guideline 301 B (CO_2 evolution test). Ixxxx Fxxxx, Txxxx, Gxxxx, Report No. IF-95/19438-00, November 16, 1995 (unpublished). (BASF-Ref.: FL-690-004)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 301 B
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Toxicity and adsorption controls were not performed (see 3.3.11). However, to avoid adsorption problems, the solid test substance was directly placed into the test vessels instead of preparing stock solutions (see 3.3.4).

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	TP 95018
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	96.75 %
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water (see Section A3.5) and shows a tendency to adsorb to surfaces.
3.1.5	Composition of Product	Not applicable
3.1.6	TS inhibitory to micro-organisms	No (see Section A7.4.1.4)
3.1.7	Specific chemical analysis	Not applicable

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA7.6.1.1

3.2	**Reference substance**	Yes Benzoic acid, sodium salt	
3.2.1	Initial concentration of reference substance	34.3 mg/l (≡ 20 mg TOC/l)	
3.3	**Testing procedure**		
3.3.1	Inoculum	As given in Table A7.1.1.2.1- 5.	
3.3.2	Test system	The test system is described in Table A7.1.1.2.1- 6.	
3.3.3	Test conditions	See Table A7.1.1.2.1- 7.	
3.3.4	Method of preparation of test solution	In order to avoid problems with adsorption to stock solution vessels, the solid test substance was directly added to the test solution after weighing.	
3.3.5	Initial TS concentration	10 and 11 mg TOC/l, respectively. Actual test substance concentrations were 14.0 mg/l and 14.4 mg/l, respectively, exceeding its solubility by approx. the factor 10.	X
3.3.6	Duration of test	28 d	X
3.3.7	Analytical parameter	CO_2 evolution	
3.3.8	Sampling	After 4, 7, 13, 22, 28, and 29 days, respectively. On day 28, 1 ml conc. hydrochloric acid was added, to purge the system of CO_2; the corresponding titration was made on day 29.	
3.3.9	Intermediates/ degradation products	Not identified.	
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	1) Control without test substance (blank inoculum) 2) Functional control: Sodium benzoate, as specified above (3.2)	
3.3.12	Statistics	Calculation of % TCO_2 and % $ThCO_2$, as prescribed by the guideline.	

4 RESULTS

4.1	**Degradation of test substance**		
4.1.1	Graph	A graphical representation of the degradation curve is given in Figure A7.1.1.2.1- 2.	
4.1.2	Degradation	A plateau phase of degradation was not reached within 28 days. At test termination (29 d), 6.0 % (mean of two replicates) of the test substance were degraded. Replicate values = 7.9 %, 4.0 %.	
4.1.3	Other observations	No other observations were made.	

Section A7.1.1.2.1 Ready biodegradability

Annex Point IIA7.6.1.1

4.1.4	Degradation of TS in abiotic control	An abiotic control was not performed.	
4.1.5	Degradation of reference substance	Sodium benzoate was degraded to 96 % by the end of the study.	
4.1.6	Intermediates/ degradation products	No intermediates or degradation products were determined.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The ready biodegradability of flocoumafen, measured as per cent of $ThCO_2$, was tested using the CO_2 evolution test (OECD guideline 301 B). The following deviations from the guideline occurred: - no adsorption control was performed; no toxicity control was performed.	X
5.2	**Results and discussion**	After 29 d, flocoumafen was degraded on average to only 6.0 %. Solid flocoumafen was added to the test solution directly after weighing and in excess of its solubility (i.e., at approx. 14 mg/l). Whereas this procedure is recommended by the guideline for poorly soluble and adsorptive substances, it may be anticipated that the test result was negatively affected. In view of the low volatility, and the hydrolytic stability of Flocoumafen, these parameters are not expected to have affected the results.	X
5.3	**Conclusion**	The validity criteria are only partly fulfilled (Table A7.1.1.2.1- 8). The large variation between replicates, however, may be inherently linked to the low degradation rate – and thus considered unavoidable – since any test system is particularly susceptible to random effects when the processes considered are slow.	X
5.3.1	Reliability	3	
5.3.2	Deficiencies	Yes The lack of adsorption and toxicity control, are considered to represent substantial deficiencies, particularly since a test concentration was chosen that is more than 100-fold above the water solubility (0.1 mg/l). Further, the initial cell concentration of the inoculum is not reported. In conclusion, this study is not considered to be valid.	X

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	15 September 2005
Materials and Methods	(3.3.5) The solubility is exceeded by a factor 100 (not 10 as stated). (3.3.6) The study duration was 29 d. (5.1) Under 3.1.6 it was stated that the TS is not inhibitory to micro-organisms. The reported EC_{20} value was >4 mg/L (see A.7.4.1.4). The nominal TS concentration employed in this test was 14-14.4 mg/L (which could be >EC_{20}) . In study 7.1.1.2.1/01 no toxicity was observed (the latter result is however not reliable). The RMS agrees that a toxicity control should have been included (although based on the mode of action no toxicity is expected). The applicant did not clarify the term "adsorption control". Presumably an abiotic control is meant (with inoculum, which is inactivated by addition of poison).
Results and discussion	(5.2 & 5.3)) Due to the lack of a toxicity control, it is not possible to attribute the low degradation of flocoumafen to either the TS being not readiliy biodegradable or the TS being toxic to the inoculum. In the absence of an abiotic control, the contribution of adsorption to the apparent lack of degradation cannot be evaluated. (5.3.2) The fact that the initial cell concentration was not reported is considered to be a minor deviation. The amount of inoculum was sufficient to produce adequate degradation of the reference substance, and it was not in excess, considering the low CO_2 production in the blank (22 mg/L, well below the 40 mg/L limit stated in the OECD 301 guideline).
Conclusion	After 29 d, flocoumafen was degraded on average by only 6.0 %. Due to the lack of a toxicity and an abiotic control, it is not possible to attribute (with certainty) the low degradation of the TS to either the TS being not readily biodegradable, and/or the TS being toxic to the inoculum, and/or adsorption. Flocoumafen is provisionally classified as not readily biodegradable unless it can be demonstrated by the notifier that the low biodegradation was caused by toxicity of flocoumafen to the inoculum and/or adsorption.
Reliability	2
Acceptability	Acceptable. Flocoumafen is provisionally classified as not readily biodegradable unless it can be demonstrated by the notifier that the low biodegradation was caused by toxicity of flocoumafen to the inoculum and/or adsorption.
Remarks	The reliability was set at 2 because no toxicity and abiotic control were included. According to the RMS, the high flocoumafen concentration (still within the recommended range) and the lack of an adsorption (abiotic) control do not lead to a complete rejection (i.e. reliability 3 or 4) of the study.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	

Acceptability	
Remarks	

Table A7.1.1.2.1- 5: Description of the inoculum.

Criteria	**Details**
Nature	Activated sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Sewage treatment plant
Sampling site	Municipal STP at Taunusstein-Bleidenstadt, Germany
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Washed twice with mineral nutrient as used in the test; re-suspension and aeration for c. 4 hours; homogenisation for 2 min and filtering.
Pre-treatment	None
Initial cell concentration	Not reported

Table A7.1.1.2.1- 6: Description of the test system.

Criteria	**Details**
Culturing apparatus	5-l amber carboys
Number of culture flasks/concentration	2
Aeration device	Fischer & Porter “Snap-in” flowmeter
Measuring equipment	CO_2 trapping by 0.025 N $Ba(OH)_2$; Titration with 0.05 N HCl Indicator: phenolphthalein
Test performed in closed vessels due to significant volatility of test substance	No

Table A7.1.1.2.1- 7: Description of the test conditions.

Criteria	Details
Composition of the medium	KH_2PO_4 8.5 mg/l K_2HPO_4 21.75 mg/l $Na_2HPO_4 \cdot 2\ H_20$ 33.4 mg/l NH_4Cl 0.5 mg/l $MgSO_4 \cdot 7\ H_20$ 22.5 mg/l $CaCl_2 \cdot 2\ H_20$ 36.4 mg/l $FeCl_3 \cdot 2\ H_20$ 0.25 mg/l
Additional substrate	No
Test temperature	19.4 °C (range = 18.1–22.4 °C)
pH	Not reported
Aeration of dilution water	Yes 24 h aeration with CO_2-free air prior to the test; air-flow (CO_2-free) through the test system.
Suspended solids concentration	Not applicable
Other relevant criteria	Stirring of the test solution with magnetic stirrers; Inlet air passed through activated carbon filter to remove possible volatile and organic compounds.

Table A7.1.1.2.1- 8: Pass levels and validity criteria for tests on ready biodegradability.

	Fulfilled	Not fulfilled
Pass levels		
60% removal of ThOD or $ThCO_2$		☑
Pass values reached within 10-d window/ 28-d test period		☑
Criteria for validity		
Variation between replicates at the end of test < 20%		☑
Removal of reference substance reaches pass level by day 14	☑	
Criteria for poorly soluble test substances		
Selection of suitable test method (CO_2 evolution)	☑	
Appropriate method of agitation	☑	

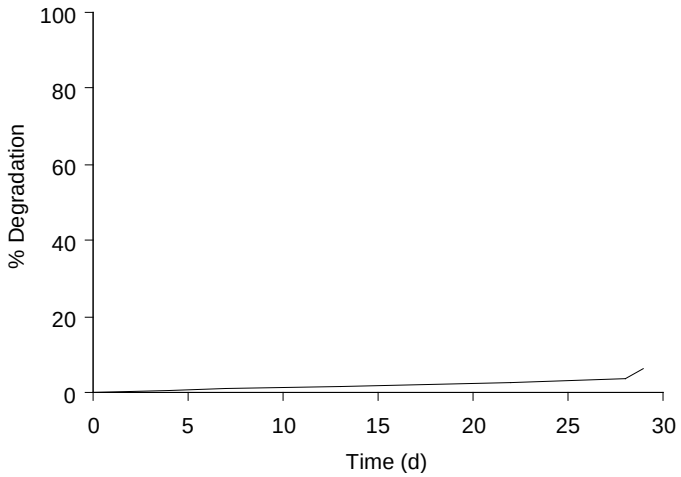

Figure A7.1.1.2.1- 2: Degradation of flocoumafen (% $ThCO_2$) over time.

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA7.6.1.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Flocoumafen was tested experimentally according to guidelines OECD 301D and EC C.4 (92/69/EEC) and was established to be "readily biodegradable". Therefore, no further studies are required for the assessment of the aerobic degradation behaviour of Flocoumafen.	X
Undertaking of intended 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 (*)
Date	15 September 2005
Evaluation of applicant's justification	Based upon the existing data and evaluation of the RMS, Flocoumafen is considered not readily biodegradable. A study on inherent biodegradability is not required because no direct release to an STP is anticipated.
Conclusion	Non-submission of data is accepted.
Remarks	-
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.1.2.3 Biodegradation in seawater

Annex Point IIIA 12.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	In view of the nature of the biocidal product – a wax bound block bait – and of the intended use pattern – rodent control in and around buildings – release to seawater must be considered absolutely unlikely. Regarding the envisaged use pattern, testing for biodegradation in seawater is not required according to the BPD, and thus no data are submitted.	
Undertaking of intended 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 (*)
Date	07 June 2005
Evaluation of applicant's justification	No comments.
Conclusion	Non-submission of data accepted.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.2
Annex Point IIIA 12.2.1

Rate and route of degradation in aquatic systems including identification of metabolites and degradation products

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements (point A7.1.2), such tests are required, if the results from paragraphs A7.1.1.2.1 or A7.1.1.2.2 indicate the need to do so, or the active substance has an overall low or absent abiotic degradation. However, studies on ready biodegradability (Section A7.1.1.2.1) and inhibition of microbial activity (Section A7.4.1.4) were conducted on Flocoumafen utilising activated sludge from a sewage treatment plant. As a result, Flocoumafen (a) is readily biodegradable and (b) does not inhibit microbial activity within its solubility limits in water. From this, it can safely be concluded that negative effects on the biological function of sewage treatment plants are not to be expected. Further, any such testing is not required according to the decision tree for biological degradation testing set forth on page 16 of chapter 3 of the TNsG on data requirements, since Flocoumafen was assessed as being "readily biodegradable" and furthermore attained > 60 % degradability within the 10-day window.	X
Undertaking of intended 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 (*)
Date	15 September 2005
Evaluation of applicant's justification	Flocoumafen is considered not biodegradable by the RMS (see A7.1.1.2.1). The studies 7.1.2.1.1, 7.1.2.1.2, 7.1.2.2.1 and 7.1.2.2.2 are however not required because no direct release to water and STPs is anticipated.
Conclusion	Non-submission of data is accepted.
Remarks	-
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.2.1.1 Aerobic biodegradation (sewage treatment)

Annex Point IIIA 12.2.1

	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:	Following the intended uses (in and around buildings only), release of significant amounts of the substance to sewage treatment plants is not anticipated. Furthermore, exposure of sewage treatment plants to flocoumafen via other routes is not expected. Thus, conduct of a study on aerobic biodegradation in STPs is not considered to be required. In addition, this test is not required in view of the ready biodegradability of the test substance.	X
Undertaking of intended 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 (*)
Date	15 September 2005
Evaluation of applicant's justification	Based on the existing data and evaluation of the RMS, Flocoumafen is considered not readily biodegradable. A study on aerobic biodegradation in STPs is however not required because no direct release to an STP is anticipated.
Conclusion	Non-submission of data is accepted.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section 7.1.2.1.2 Anaerobic biodegradation

Annex Point IIIA 12.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.1.2.1.2/01:** Sxxxx Hxxxx (2004) BAS 322 I (Flocoumafen) – Determination of the ultimate anaerobic biodegradability in the anaerobic biodegradation test. Bxxxx, Lxxxx, Gxxxx, Report No. 01/0344/40/1, February 17, 2004 (unpublished). (BASF-Ref.: 2004/1003847)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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 ISO 11734 (1995)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	AC 12140-35	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	99.4 %	
3.1.4	Further relevant properties	Poorly soluble in water (1.1 mg/l acc. to the original report)	X
3.1.5	Composition of Product	Pure active substance	
3.1.6	TS inhibitory to microorganisms	No (see Section A7.4.1.4)	X
3.1.7	Specific chemical analysis	Not applicable	
3.2	**Reference substance**	Yes Sodium benzoate	

Section 7.1.2.1.2 Anaerobic biodegradation

Annex Point IIIA 12.2.1

3.2.1	Initial concentration of reference substance	50 mg/l (given as TOC, nominal)
3.3	**Testing procedure**	
3.3.1	Inoculum/ test species	Digested sludge, see Table A7.1.2.1.2- 1.
3.3.2	Test system	The test system is described in Table A7.1.2.1.2- 2
3.3.3	Test conditions	See Table A7.1.2.1.2- 3.
3.3.4	Method of preparation of test solution	Direct addition (solid) to the test vessel.
3.3.5	Initial TS concentration	68.0–69.2 mg/l Flocoumafen 50 mg/l TOC (nominal)
3.3.6	Duration of test	60 days
3.3.7	Analytical parameter	Headspace pressure, continuously; Dissolved inorganic carbon (DIC), end of test.
3.3.8	Sampling	Daily (pressure) At test termination (DIC)
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Controls	Blank control Reference substance (see above) Toxicity control: 50 mg/l TOC of test substance and reference substance, respectively.
3.3.11	Statistics	Per cent biodegradation, according to ISO 11734

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Degradation of TS in abiotic control	Not stated
4.1.2	Degradation	There was apparent negative degradation (–9 %). Thus, no degradation occurred.
4.1.3	Graph	Please refer to the original report.
4.1.4	Other observations	None
4.1.5	Degradation of reference substance	72 %
4.1.6	Intermediates/ degradation products	Not identified

Section 7.1.2.1.2 Anaerobic biodegradation

Annex Point IIIA 12.2.1

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The anaerobic biodegradability of Flocoumafen was tested by exposing the substance at a concentration of 50 mg/l TOC to anaerobised sewage sludge according to ISO 11734 (1995).	
5.2	**Results and discussion**	The apparent biodegradation of Flocoumafen in the assay was negative (–9 %). Thus, it is concluded that no biodegradation under anaerobic conditions occurred. Anaerobic conditions were maintained (no pink colouration of Resazurin), degradation of the reference substance was > 60 %, pH values at 7 ± 1, variation among test substance replicates was < 20 %, dissolved inorganic carbon (DIS) in the inoculum stock at the start of the test was < 10 mg/l, and the pressure formation in the toxicity control was equal to or above the reference assay. Thus, the test considered to be valid.	X
5.3	**Conclusion**	It is concluded that no biodegradation under anaerobic conditions occurred.	
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	07 June 2005
Materials and Methods	(3.1.4) The water solubility at pH 7 and 20°C is 0.11 mg/L (A3 3.5). (3.1.6) The EC20 was reported as >4 mg/L and the nominal test concentration in this test was 68 mg/L. (5.2) DIS should be replaced by DIC.
Results and discussion	-
Conclusion	Flocoumafen is not biodegradable under anaerobic conditions.
Reliability	1
Acceptability	Acceptable.
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.2.1.2- 1: Inoculum/ test organism.

Criteria	**Details**
Nature	Digested sludge
Species	Mixed microbial population
Strain	Not applicable
Source	Sewage treatment plant
Sampling site	Municipal STP at Mannheim, Germany
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Not stated
Pre-treatment	7 day pre-incubation
Initial cell concentration	Suspended solids: 2 g/l

Table A7.1.2.1.2- 2: Description of the test system.

Criteria	Details
Culturing apparatus	OxiTop®-Control system
Number of culture flasks/concentration	3
Measuring equipment	Not stated
Oxidation reduction indicator	Resazurin

Table A7.1.2.1.2- 3: Description of the test conditions.

Criteria	Details
Composition of the medium	According to ISO 11743
Additional substrate	No
Solvent	No
Preparation of medium	7 days pre-incubation
Test temperature	35 ± 2 °C
pH	Start: 7.6 End: 7.0
Suspended solids concentration	2 g/l
Other relevant criteria	None

Section A7.1.2.2.1 Aerobic aquatic degradation study

Annex Point IIIA 12.2.1

	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:	According to the envisaged use pattern (rodent control in and around buildings), direct release of the active substance to aquatic systems is considered to be negligible. This is supported by the properties of the biocidal product, a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. In addition, the test substance has been shown to be readily biodegradable. Consequently, an aerobic aquatic degradation study is not required.	X
Undertaking of intended 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 (*)
Date	15 September 2005
Evaluation of applicant's justification	Based on the existing data and evaluation of the RMS, Flocoumafen is considered not readily biodegradable. An aerobic aquatic degradation study is however not required because no direct release to water and STPs is anticipated.
Conclusion	Non-submission of data is accepted.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA 12.2.1

	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:	According to the envisaged use pattern (rodent control in and around buildings), direct release of the active substance to aquatic systems is considered to be negligible. This is supported by the properties of the biocidal product, a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. In addition, the test substance has been shown to be readily biodegradable. Consequently a water/sediment degradation study is not required.	X
Undertaking of intended 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 (*)
Date	15 September 2005
Evaluation of applicant's justification	Based on the existing data and evaluation of the RMS, Flocoumafen is considered not readily biodegradable. However, as direct exposure of surface water to Flocoumafen is not expected ("Emission scenario document for biocodes used as rodenticides" (CA-Jun03-Doc.8.2-PT14)), the study is not required.
Conclusion	Non-submission of data is accepted.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

Official use only

1 REFERENCE

1.1	**Reference**	**A7.1.3/01:** Wxxxx Mxxxx (2002) BAS 322 I (flocoumafen): Estimation of the adsorption coefficient (K_{oc}) by HPLC method. Report No. 835187, Rxxxx Lxxxx, Ixxxx, Sxxxx, March 15, 2002 (unpublished). (BASF-Ref.: 2002/1016627)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 121
2.2	**GLP**	Yes
2.3	**Deviations**	Yes See 3.5.2 (mobile phase).

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	AC12140-35
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.4 % Isomer ratio: 59 % cis, 41 % trans.
3.1.4	Further relevant properties	Water solubility was stated to be 1.1 mg/l in the report (*cf.* 0.114 mg/l measured under GLP and reported in Section A3.5 of this dossier). However, this does not affect the quality and validity of the study.
3.1.5	Method of analysis	HPLC method according to OECD guideline 121.
3.2	**Degradation products**	Degradation products tested: No.
3.2.1	Method of analysis for degradation products	Not applicable.

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

3.3	**Reference substance**	Yes Six reference substances with known log K_{oc} were used to determine the calibration curve of the HPLC system, as given in Table A7.1.3- 1.		X
3.3.1	Method of analysis for reference substance	Testing method implies use of reference substances for determination of the calibration curve of the HPLC system, see above.		
3.4	**Soil types**	Not applicable.		
3.5	**Testing procedure**			
3.5.1	Test system	Apparatus:	Merck Work Station Merck Hitachi Autosampler L-7200 Merck Hitachi Pump L-7100 Merck Hitachi Detector L-7400 Jones Column Oven Mod. 7990	
		Column:	YMC CN, particle size 3 µm, 150 × 4 mm	
3.5.2	Test solution and test conditions	Mobile phase:	Acetonitrile/water, 55:45 (v/v), pH = 5.7	
		Flow rate:	1 ml/min	
		Detection:	at 254 nm (test substance) at 210 nm (reference substances and sodium nitrate)	
		Injection volume:	10 µl	
		Temperature:	25° C	
3.6	**Test performance**			
3.6.1	Preliminary test	According to ”OECD 106”: No		
3.6.2	Screening test: Adsorption	According to ”OECD 106”: No		
3.6.3	Screening test: Desorption	According to ”OECD 106”: Not performed		
3.6.4	HPLC-method	According to ”OECD 121”: Yes For details see above. Determination of dead time using Sodium nitrate.		
3.6.5	Other test	No		

4 RESULTS

4.1	**Preliminary test**	Not performed.
4.2	**Screening test: Adsorption/ desorption (HPLC)**	
4.2.1	Dead time	t_0 (± SD) = 0.73 ± 0.00 min (n = 2)
4.2.2	Retention data of reference substances	Retention times are given in Table A7.1.3–2. The calibration curve (log k’ vs. log k_{oc}) indicated satisfactory linearity and precision (details are given in Table A7.1.3–2).

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

4.2.3	Retention time of Flocoumafen	cis-isomer: t_R (± SD) = 6.41 ± 0.02 min; n = 3 trans-isomer: t_R (± SD) = 7.22 ± 0.02 min; n = 3	
4.3	**Calculations**		
4.3.1	Capacity factor	cis-isomer: log k' = 0.981 (SD = 0.001); n = 3 trans-isomer: log k' = 0.949 (SD = 0.001); n = 3	X
4.3.2	Adsorption coefficient	cis-isomer: log K_{oc} = 4.84 trans-isomer: log K_{oc} = 5.13	
4.4	**Degradation product(s)**	No degradation products tested.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The adsorption coefficient (K_{oc}) of Flocoumafen on soil and sewage sludge was estimated by the HPLC method according to OECD guideline 121. The mobile phase (Acetonitrile/water, 55:45) differed from those recommended by the guideline. However, this modification is fully justified by difficulties in achieving constant retention times with Flocoumafen in view of the high adsorption. The choice of an alternative solvent followed the recommendations given in the guideline. Thus, the described deviation is not expected to have affected the results. No other deviations from the guideline are reported.	
5.2	**Results and discussion**	The variation of measurements clearly falls within the limits set out by OECD guideline 121. Therefore, the validity criteria are considered to be fulfilled. Flocoumafen is not known to exhibit substance-specific properties that might have had an impact on the results.	
5.2.1	Adsorption coefficient	cis-isomer: log K_{oc} = 4.84 K_{oc} = 68510 trans-isomer: log K_{oc} = 5.13 K_{oc} = 134858	
5.3	**Conclusion**	The obtained adsorption coefficients suggest that both isomers of Flocoumafen are highly adsorptive to soil, sediment and/or sewage sludge.	
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	07 June 2005
Materials and Methods	(3.3) The CAS NR for phenol in Table A7.1.3- 1 is 108-95-2. (4.3.1) log *k'* for the cis-isomer = 0.891 (and not 0.981)
Results and discussion	-
Conclusion	The adsorption coefficients (Koc) for the isomers of Flocoumafen were 68510 (cis-isomer) and 134858 (trans-isomer). The obtained adsorption coefficients suggest that both isomers are highly adsorptive to soil, sediment and/or sewage sludge.
Reliability	1
Acceptability	Acceptable
Remarks	-

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.3- 1: List of reference substances used for determination of the calibration curve of the HPLC system.

Name of substance	**log k_{oc}**	**CAS-No.**
Phenol	1.32	1912-24-9
Isoproturon	1.86	34123-59-6
Triadimenol	2.40	55219-65-3
Linuron	2.59	330-55-2
α-Endosulfan	4.09	959-98-8
2,4-DDT	5.63	50-29-3

Table A7.1.3- 2: Retention times (t_r), in minutes, and capacity factors (log k') of the reference substances; n = sample size.

Name of substance	t_r **(mean)**	**log k'**	n
Phenol	1.93	0.22	6
Isoproturon	2.19	0.30	6
Triadimenol	2.47	0.38	6
Linuron	2.74	0.44	6
α-Endosulfan	5.37	0.80	6
2,4-DDT	8.41	1.02	6
Regression parameters:	Slope =	0.197	
	Intercept =	–0.060	
	r^2 =	0.989	

Section 7.1.4.1 Field study on accumulation in the sediment

Annex Point IIIA 12.2.1

	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:	According to the envisaged use pattern (use in and around buildings), direct release of the active substance to aquatic systems is considered to be negligible. This is supported by the properties of the biocidal product, a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Further, the test substance has been shown to be readily biodegradable. Consequently, major exposure of the sediment to flocoumafen is not expected and a water/sediment field accumulation study is not considered to be required.	X
Undertaking of intended 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 (*)
Date	08 June 2005
Evaluation of applicant's justification	Based on the existing data and evaluation of the RMS, Flocoumafen is considered not readily biodegradable. However, as direct (see 7.1.2.2.2) and indirect (see 7.1.2.2.1) exposure of surface water to Flocoumafen (and subsequent accumulation in sediment) is not expected, the study is not required.
Conclusion	Non-submission of data is accepted by the RMS.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

Undertaking of intended data submission [X]		Since the study summarised in the current section below is considered to be severely compromised by several deficiencies and therefore not suitable for risk assessment, the applicant intends to conduct an up-to-date soil degradation study employing environmentally relevant concentrations of Flocoumafen. A quotation has been requested at competent contract laboratories. The applicant was informed that, due to high workload, finalisation and reporting of such a study would not be possible before December 2005.	**Official use only**
		1 REFERENCE	
1.1	**Reference**	**A7.2.1/01:** Sxxxx Mxxxx (1985) The degradation of [^{14}C]WL108366 in soil under aerobic laboratory conditions. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.85.067, March 1985 (unpublished). (BASF-Ref.: FL-620-002)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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 Compliance to BBA guidelines is stated, without further specification. The procedures correspond to those described in BBA guideline, part IV (4-1) (formerly “BBA Merkblätter 36 and 56”).	
2.2	**GLP**	No GLP was not compulsory at the time the study was conducted.	
2.3	**Deviations**	Yes See 3.4	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabelled ^{14}C-Flocoumafen	
3.1.1	Lot/Batch number	Batch 1, Sample No. 719 Laboratory Book Ref.: 1932.038 Oxxxx Cxxxx Dxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx Rxxxx Lxxxx	
3.1.2	Specification	Test substance was supplied dissolved in acetone. The ^{14}C labelling position is specified in Figure A7.2.1- 1.	

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

3.1.3	Purity	Not reported	X
3.1.4	Further relevant properties	The typical properties of the test substance were not considered to have negatively influenced the test results.	
3.1.5	Analytical methods	Extraction: with water:acetonitrile (30:70 v/v) and further sequential extraction with aqueous acetonitrile, acetonitrile, and diethylether. Liquid scintillation counting (LSC): Standard routine using a Packard 460 CD counter with Packard ES 299 scintillation fluid and Packard NE 260 scintillation fluid (for alkaline solutions). Combustion analysis: Unextracted radioactivity was determined by combustion analysis of 300–500 mg subsamples of dried soil residuum; the produced CO_2 was trapped in 8 ml Carbosorb and blended with 13 ml Permafluor scintillation fluid; radioactivity was assayed by LSC as described above. Thin layer chromatography (TLC): Merck silica gel F_{254} plates; Solvent systems: acetone:hexane (35:65 v/v) ethylacetate:toluene (35:65 v/v); location and quantification of radioactive sites by a linear analyser and autoradiography. HPLC: Spherisorb S5 ODS, 25 × 4.9 mm I.D. column; mobile phase: acidified water:acetonitrile (65:35 v/v). Mass spectrometry (MS): Finnigan 4500 mass spectrometer, operated in the chemical ionisation (CI) mode.	
3.2	**Degradation products**		
3.2.1	Method of analysis for degradation products	$^{14}CO_2$ was trapped from the exhaust air by 2 M Potassium hydroxide solution and quantified by LSC as described above. Non-volatile degradation products were identified in the soil extract by HPLC and MS.	
3.3	**Reference substance**	4-hydroxycoumarin (unlabelled) was used as a reference substance in TLC and HPLC. Degradation was not tested using reference substances.	
3.3.1	Method of analysis for reference substance	TLC and HPLC, as described in 3.1.5.	
3.4	**Soil types**	The soils and their physical properties are presented in Table A7.2.1- 1. Deviating from the BBA guideline, microbial biomass was not determined. No information on the storage time of the German standard soils prior to the test is provided. The soils were received in May 1983. The date of the onset of the test is not reported. In contrast, the British soil was freshly sampled three days before the test.	X

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

3.5	**Testing procedure**		
3.5.1	Test substance concentration	Nominal dose rate = 50 mg a.i./kg soil	
3.5.2	Solvent	Acetone	X
3.5.3	Method of application	Half of the radiochemical was delivered dropwise just below the surface, the remainder randomly to the surface.	
3.5.4	Testing apparatus	Flow-through perspex chamber, to ensure radiochemical balance conditions: The inlet air was passed through (1) 2 M Potassium hydroxide to remove CO_2, and (2) through water, to moisten air; Exhaust air was passed through (1) 0.2 M sulphuric acid, to collect basic volatiles (2) 2-methoxyethan-1-ol, to collect organic volatiles (3) 2 M potassium hydroxide (2 traps) to collect acidic volatiles, specifically $^{14}CO_2$.	
3.5.5	Incubation period	217 d	
3.5.6	Incubation temperature	22 ± 2 °C	
3.5.7	Moisture	Moisture was adjusted to 40 % of maximum MHC every 2 to 3 days by addition of distilled water.	
3.5.8	Sampling	After 0, 7, 28, 56, 112, and 217 days.	

4 RESULTS

4.1	**Degradation rate**	The distribution of recovered radioactivity, transformed into Flocoumafen equivalents, is presented in Table A7.2.1- 1, and as per cent of total in Table A7.2.1- 3. At the end of the test, after 217 days, residues of Flocoumafen were: 49.6 mg/kg (soil 2.2), 48.8 mg/kg (soil 2.3), and 46.2 mg/kg (Reculver soil), and thus accounted for 92.4 to 99.2 % of the nominal concentrations. Due to the slow degradation process, the degradation rate could not be estimated.	X
4.2	**Disappearance time**	DT_{50} and DT_{90} could not be determined due to the slow degradation process. DT_{50} > 217 d	
4.3	**Degradation products**	Extractable metabolites accounted for a maximum of 28.9 % of recovered radioactivity at intermediate samples (Table A7.2.1- 3). However, it is stated that no single major metabolite was found using TLC and HPLC: Extractable metabolites were distributed between a series of products of varying polarity (not further characterised). A maximum of 2.6 mg/kg (= 5.2 % of nominal) of unextracted radioactivity was found after 217 days (also see Table A7.2.1- 2).	

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aerobic degradation of Flocoumafen was studied in two standard soils (Speyer, Germany) and a further soil from Reculver, UK. The test was conducted under radiochemical balance conditions, following BBA guidelines (the former "Merkblatt 36"), using ^{14}C-labelled Flocoumafen. Deviating from the guideline, microbial biomass of the soils was not determined.	
5.2	**Results and discussion**	The physico-chemical properties of Flocoumafen, such as solubility, hydrolytic stability, or volatility (see Section A3) are not considered to have negatively impacted the results. The recovery rates of radioactivity ranged between 98 and 104 %. At intermediate samples, a maximum of 28.9 % of metabolites was detected. These were not further characterised, since it is stated that they were distributed among a series of substances of varying polarity. At test termination (217 d), Flocoumafen was degraded by less than 8 %. Microbial biomass in the soils was not determined, as would have been required by the BBA guideline. Due to the unknown storage period of the Speyer soils, it can be assumed that microbial activity in these soils was reduced. This is underpinned by the consistently higher stability of Flocoumafen in the standard soils, compared to the Reculver soil (Table A7.2.1- 3). However, considering this difference, there was only little variation among the soils. Therefore, these deficiencies are considered to be of minor importance and the results should be comparable.	X
5.2.1	Degradation rate and half-life	The degradation process was very slow. Therefore, no degradation rate and DT_{50} could be estimated. Half-life is given as $DT_{50} > 217$ d	
5.3	**Conclusion**	The stated absence of major metabolites is not documented by corresponding data. However, this seems credible.	X
5.3.2	Reliability	3	
5.3.3	Deficiencies	Yes The study is considered to be valid with restrictions due to the uncertainty about the microbial biomass and the documentation deficiency discussed in 5.3.	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	15 September 2005
Materials and Methods	(3.1.3) The radiochemical purity was >97% (TLC) (3.4) Table A.7.2.1-1: The texture of Speyer 2.3 should read Sandy loam. (3.5.2) The volume was <1% v/v
Results and discussion	(4.1) Reference should be made to Table A7.2.1-2 (not -1) (5.2) The observed persistency of Flocoumafen may also have been caused by inhibition of microbial activity (by high initial concentrations of 50 mg/kg). (5.3) The absence of major metabolites is not proven: up to 28.9% of extractable metabolites were observed (see Table 7.2.1-3).
Conclusion	The DT50 of Flocoumafen in Speyer 2.2 (loamy sand), Speyer 2.3 (sandy loam) and Reculver (sandy loam) was >217 days. The study is considered invalid by the RMS and a new study is required.
Reliability	3 (see under remarks)
Acceptability	Not acceptable.
Remarks	Soil microbial activity was not determined at any time point during the study. The soils were received from the Speyer institute in May 1983. They were stored, tightly sealed in the dark, at ambient temperature until required. The exact date of conduct of the study was not reported, but the protocol was signed in May 1984. The prolonged storage (at least one year) of the test soils prior to use in the study, under air-locked conditions at ambient temperature, may have seriously compromised soil viability. The persistent character of Flocoumafen may have been caused by the use of non-viable soils. The study is considered to be invalid. Another possible reason for the persistent character of Flocoumafen may have been inhibition of microbial activity (test dose of 50 mg/kg is far in excess of the estimated PECsoil of 0.0087 mg/kg). The notifier indicated that a new study will be conducted, but no evidence in the form of a quotation or protocol was submitted.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

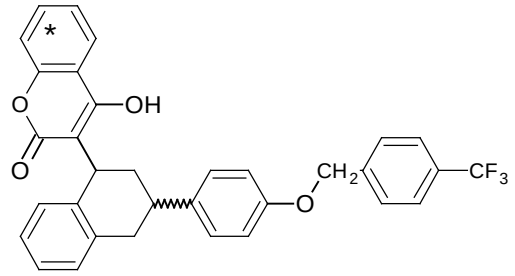

Figure A7.2.1- 1: Labelling position of ^{14}C in Flocoumafen (marked by an asterisk).

Table A7.2.1- 1: Physical properties of the soils.

Origin	**Speyer 2.2 (Germany)**	**Speyer 2.3 (Germany)**	**Reculver, Kent, UK**
Soil texture	Loamy sand	Loamy sand	Sandy loam
pH	5.5–7.5	5.5–7.5	5.5
Fraction ≤ 0.02 mm (%)	10 –20	20–30	19.6
Organic matter (% dry weight)	2–3	0.5–1.5	2.7
Moisture holding capacity (%)	65.3	39.5	44.8

2.2 and 2.3 are standard soils provided by the Agricultural Research and Testing Institute (Speyer), according to "BBA Merkblatt 37".

Table A7.2.1- 2: Distribution of recovered radioactivity from soils treated with [^{14}C]-Flocoumafen, expressed as Flocoumafen equivalents (mg/kg soil).

	Days after treatment					
	0	7	28	56	112	217
Soil 2.2						
Flocoumafen	50.8	47.9	45.4	51.2	47.2	49.6
Extractable metabolites	2.7	7.8	10.0	3.8	4.1	2.9
CO_2	0.0	0.1	0.2	4.1	0.8	1.2
Unextracted	1.2	1.1	0.9	0.8	1.5	2.1
Total	54.7	56.9	56.5	56.5	53.6	55.8
Recovery (%)	100	104	103	103	98	102
Soil 2.3						
Flocoumafen	52.7	47.4	45.3	52.0	47.3	48.8
Extractable metabolites	2.2	7.1	9.3	3.4	5.2	3.0
CO_2	0.0	0.1	0.2	0.4	0.8	1.5
Unextracted	0.2	0.3	0.4	0.5	1.1	1.5
Total	55.1	54.9	55.2	56.3	54.4	54.8
Recovery (%)	100	100	100	102	99	99
Reculver soil						
Flocoumafen	50.5	40.4	39.6	50.1	46.5	46.2
Extractable metabolites	4.4	16.6	14.7	5.6	4.6	4.2
CO_2	0.0	0.1	0.2	0.6	1.2	2.3
Unextracted	0.3	0.4	0.8	1.0	1.8	2.6
Total	55.2	57.5	55.3	57.3	54.1	55.3
Recovery (%)	100	104	100	104	98	100

Table A7.2.1- 3: Distribution of recovered radioactivity (% of total) from soils treated with [^{14}C]-Flocoumafen.

	Days after treatment					
	0	7	28	56	112	217
Soil 2.2						
Flocoumafen	92.9	84.2	80.4	90.6	88.1	88.9
Extractable metabolites	4.9	13.7	17.7	6.7	7.6	5.2
CO_2	0.0	0.2	0.4	7.3	1.5	2.2
Unextracted	2.2	1.9	1.6	1.4	2.8	3.8
Total	100	100	100	100	100	100
Soil 2.3						
Flocoumafen	95.6	86.3	82.1	92.4	86.9	89.1
Extractable metabolites	4.0	12.9	16.8	6.0	9.6	5.5
CO_2	0.0	0.2	0.4	0.7	1.5	2.7
Unextracted	0.4	0.5	0.7	0.9	2.0	2.7
Total	100	100	100	100	100	100
Reculver soil						
Flocoumafen	91.5	70.3	71.6	87.4	86.0	83.5
Extractable metabolites	8.0	28.9	26.6	9.8	8.5	7.6
CO_2	0.0	0.2	0.4	1.0	2.2	4.2
Unextracted	0.5	0.7	1.4	1.7	3.3	4.7
Total	100	100	100	100	100	100

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.2.1/02:** Dxxxx Kxxxx (2006) Metabolism of Flocoumafen in soil. Fxxxx Ixxxx fxxxx Mxxxx Bxxxx axxxx Axxxx Exxxx, Sxxxx, Gxxxx, Report no. EBR-003/7-90, February 01, 2006 (unpublished). (BASF DocID: 2006/1008092)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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 307 “Aerobic and anaerobic transformation in soil” (2002)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes See 3.4 below.	

3 MATERIALS AND METHODS

3.1	**Test material**	(1) As given in Section A2. (2) Coumarin labelled ^{14}C-Flocoumafen and (3) Trifluoromethylphenyl labelled ^{14}C-Flocoumafen	
3.1.1	Lot/Batch number	(1) AC12140-35 (2) 792-1101 (3) 794-1101	
3.1.2	Specification	(1) As given in Section A2 (2) + (3) supplied dissolved in toluene/ethanol 96:4 (v/v) The ^{14}C labelling position is specified in Figure A7.2.1- 2.	
3.1.3	Purity	(1) 99.4 % (2) 98.8 % (3) 99.0%	X
3.1.4	Further relevant properties	The typical properties of the test substance were not considered to have negatively influenced the test results.	

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

3.1.5	Analytical methods	Extraction: Twice with acetonitrile by shaking for 90 min. A third extraction was conducted overnight. Additionally, silty clay loam samples were Soxhlet extracted with acetone followed by four times extraction with sodium hydroxide. Liquid scintillation counting (LSC): Standard routine using a Packard Tri-Carb liquid scintillation analyser using liquid scintillation cocktail (Pico-Fluor LLT, Pico-Fluor 40 or Ultima Gold). Combustion analysis: Unextracted radioactivity was determined by combustion analysis of approx. 500 mg sub-samples of air dried soil residuum; the produced CO_2 was trapped in Oxysolve C-400 and radioactivity was assayed by LSC as described above. Thin layer chromatography (TLC): Merck silica gel 60 F254 plates; Solvent systems: Ethylacetate:toluol:formic acid:water (50:20:2:2 (v:v:v:v); location and quantification of radioactivity by Fuji BAS 1000 BioImager and evaluation by a software integration. Radio-HPLC-UV: Column: 250 mm x 4.6 mm with pre-column 10 x 4.6 mm; mobile phase: 100 % acetonitrile (eluent A) and 100 % citric acid (eluent B, pH 7).
3.2	**Degradation products**	
3.2.1	Method of analysis for degradation products	$^{14}CO_2$ was trapped from the exhaust air by 1 M sodium hydroxide solution and quantified by LSC as described above. Non-volatile degradation products were identified in the soil extract by HPLC.
3.3	**Reference substance**	Trifluoromethylphenyl labelled [^{14}C]-Flocoumafen, coumarin labelled [^{14}C]-Flocoumafen and 4-Trifluoromethylbenzoic acid.
3.3.1	Method of analysis for reference substance	LSC, TLC and HPLC, as described in 3.1.5.
3.4	**Soil types**	The physical-chemical properties of the test soils are presented in Table A7.2.1- 4. No information in terms of the history of the soils' field sites was provided.
3.5	**Testing procedure**	
3.5.1	Test substance concentration	Nominal dose rate = 500 µg a.i./kg soil
3.5.2	Solvent	Acetonitrile
3.5.3	Method of application	Pipetted onto soil surface followed by an evaporation period of 30–45 min. After this, soil samples were homogenously stirred with small sticks of stainless steel.

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

3.5.4	Testing apparatus	Flow-through system placed in an incubator, to ensure radiochemical balance conditions: The inlet air was water saturated and CO_2-free; Exhaust air was passed through (1) 0.5 M sulphuric acid, to collect basic volatiles (2) ethylene glycol, to collect volatile organics (3) 1 M sodium hydroxide to collect acidic volatiles, specifically $^{14}CO_2$.
3.5.5	Incubation period	120 d
3.5.6	Incubation temperature	20°C and 10°C
3.5.7	Moisture	Adjusted to 50 % of maximum water holding capacity weekly.
3.5.8	Sampling	Non-sterile soils: Immediately after 0, 1, 3, 7, 14, 30, 50, 70 and 120 days. Sterile soils: 30 and 120 days. Biomass measurement: 3, 60 and 120 days.

4 RESULTS

4.1	**Degradation**	The distribution of recovered radioactivity is shown in Table A7.2.1- 6 and Table A7.2.1- 7. At study termination (day 120), residues of Flocoumafen (parent) were: 86.8 % (2) and 87.4 % (3) of ITR (Borstel, 10°C), 78.9 % (2) and 80.2 % (3) of ITR (Borstel, 20°C), 57.2 % (2) and 59.4 % (3) of ITR (Soest, 20°C), 26.6 % (2) and 29.2 % (3) of ITR (Marisfeld, 20°C), and 85.0 % (2) and 84.5 % (3) of ITR (Osnabrück, 20°C),
4.2	**Disappearance time**	Calculated single 1st order DT_{50} values are presented in Table A7.2.1- 8, ranging from 71 to 442 days (n = 8) at 20°C and were extrapolated to 443 and 1293 days under reduced temperature conditions (10°C). Reliable DT_{90} values could not be determined due to the slow degradation process.

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

4.3 Degradation products

Flocoumafen was mineralised to peak amounts of 4.1 % of ITR in the Borstel soil incubated at 10°C and to 7.4 % at 20°C. In the Soest soil, maximum amounts of 7.6 % were mineralised, whereas in the Marisfeld soil a maximum of 15.6 % evolved as $^{14}CO_2$. In the Osnabrück soil merely 5.7 % were mineralised at the end of the study.

No major metabolite occurred. The sum of metabolites including Trifluoromethylbenzoic acid never exceeded 2.2 % of ITR (Borstel), 2.6 % (Soest), 3.7 % (Marisfeld) and 1.4 % (Osnabrück) for both labels at any sampling date. The maximum amount of Trifluoromethylbenzoic acid was found in the Marisfeld soil accounting for 3.6 % of ITR.

Bound residues increased steadily to a maximum of ITR at study end (day 120) as follows:

9.4 % (2) and 8.3 % (3) in the Borstel soil at 10°C
13.3 % (2) and 11.1 % (3, day 30) in the Borstel soil at 20°C
24.6 % (2) and 18.2 % (3) in the Soest soil at 20°C
47.4 % (2) and 30.4 % (3) in the Marisfeld soil at 20°C, and
10.4 % (2) and 9.0 % (3) in the Osnabrück soil at 20°C (see Table A7.2.1- 6 and Table A7.2.1- 7).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The rate of degradation of [^{14}C]-Flocoumafen was investigated in two loamy sand soils, a silt loam and a silty clay loam soil incubated at 20°C and 10°C and 50 % of maxWHC in the dark according to OECD guideline 307.

A flow-through system was installed in order to maintain aerobic soil conditions and to trap volatile compounds. Following application of 500 µg a.i./kg soil samples were taken regularly on 10 occasions until 120 DAA. Soil samples were extracted with acetonitrile and additionally in a Soxhlet apparatus with acetone, followed by extraction with sodium hydroxide in the silty clay loam soil.

The radioactivity was determined by LSC and radio-HPLC-UV. The identification was performed by TLC and bound residues were determined by combustion.

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

5.2 Results and discussion

The recovery level for radioactivity for the test soils ranged predominantly between 90 and 100 % of ITR. Only for 8 of the non-sterile samples the recovery varied between 85.6 and 89.9 % of ITR. Two individual samples were out of this range, i.e. 75.4 % and 116.0 % of ITR.

The extractable radioactivity in the non-sterile soil samples decreased steadily from initially 91.8 % (mean of (2)) and 95.5 % of ITR (mean of (3)) to a minimum of 29.2 % (2) in the silty clay loam soil. In the loamy sand soils (Borstel, Osnabrück) extractability was rather high in both labels with amounts of 80 % to 88 % after 120 days. In the heavier soils merely 60 % and 30 % could be extracted from the Soest silt loam and the Marisfeld silty clay loam soil, respectively. In the sterile controls the level of extractability at day 30 and day 120 was comparable to day 0 in all soil samples.

Bound residues increased concomitantly from initially 2.2–4.2 % to 8.2–13.4 % of ITR at test end in the loamy sand soils (both labels), whereas a maximum of 24.2 % in the silt loam and 47.4 % in the silty clay loam remained unextracted with the coumarin-label (2) at day 120.

Volatile compounds with basic character never exceeded 0.4 % of ITR at any sampling date. Volatile organics evolved at peak amounts of 0.1 %, except for the Osnabrück loamy sand (2) exceeding this level on three occasions up to a maximum 1.0 % (day 70). The formation of CO_2 peaked at amounts of 4.1 % (10°C) and 7.4 % (20°C) of ITR in the Borstel loamy sand soil. The same soil type of Osnabrück revealed merely a maximum mineralisation rate of 5.7 % at test end. In the Soest silt loam maximum amounts of 7.6 % were mineralised, thus indicating a low mineralisation rate, whereas in the Marisfeld silty clay loam a peak of 15.6 % evolved as $^{14}CO_2$, showing a moderate level.

The disappearance of Flocoumafen in non-sterile samples was slow in the loamy sand soils as shown by a decrease from initial 91–96 % to 87 % (10°C) and 79–80 % (20°C) in the Borstel soil and to 85 % in the Osnabrück soil at the end of the study. In contrast, in the heavier soils the parent compound disappeared at a moderate rate from 88–91 % (day 0) in the Soest silt loam to finally 57 and 59 %. In the Marisfeld silty clay loam decreased from initial values of 90 and 95 % to 27 and 29 % at day 120. The results of the sterile controls reveal that the disappearance is due to microbial degradation.

The development of the microbial biomass over the study period is presented in Table A7.2.1- 5. In the Borstel sandy loam incubated at 20°C, a distinct decrease in biomass was observed, which could be traced back to the diminishment in nutrient supply due to the low organic carbon content. For the same reason a reduction is noticed in the Soest silt loam. However, the microbial carbon content stayed at levels clearly above 1 % of total organic carbon, especially in the Flocoumafen treated samples. It is obvious that Flocoumafen serves as an easily available carbon source in view of the enhanced microbial biomass found in each soil. Thus, no adverse effect to microbial performance could be noticed.

Section A7.2.1 Aerobic degradation in soil, initial study

Annex Points IIIA 7.4 and IIIA 12.1.1

5.2.1	Degradation rate and half-life	As outlined in Table A7.2.1- 8, single 1st order half-lives ranged from 71 to 442 days (n = 8) at 20°C and were extrapolated to 443 and 1293 days under reduced temperature conditions (10°C). The 120-d values of the Borstel trials and the Osnabrück trials were not taken into account for the estimation due to systematic errors. Reliable DT_{90} values could not be determined because of the slow degradation process.
5.3	**Conclusion**	The degradation of Flocoumafen in soil is considered to be predominantly attributable to biological processes as demonstrated by the concentrations of parent in the sterile samples remaining on the same level after 120 days compared to initial. No significant metabolite (> 5 % of applied radioactivity) occurred. The sum of metabolites never exceeded 3.7 % (both labels) at any sampling date. The maximum amount of a single metabolite accounted for 3.6 %, identified as Trifluoromethylbenzoic acid. Thus, it may be concluded that cleavage of the ether bond in the side chain was involved as a basic step. The recovery levels determined in a range of 85.6–89.9 % of ITR were assumed to be due to analytical variation in the combustion analysis. Furthermore, the recoveries in two samples of 75.4 % and 116.0 % were assumed to be due to extraction failure and application error, respectively. However, these errors were concluded not to effect the accuracy of the overall results. Mineralisation was dependent on the soil type and the activity of the microorganisms, and showed low levels in the loamy sand soils (Borstel, Osnabrück) and moderate levels in the heavier soils. The extractability of radioactivity was also related to the soil type, found to be relatively high in the loamy sand soils but reduced in the heavier soil at study termination. The amount of bound residues increased reciprocally in all soils towards study end. Since in sterile samples only a minor increase of non-extractable residues (NER) occurred with time, formation of NER was deemed to be associated with the microbial degradation. The degradation rates varied significantly depending on the soil type and temperature. The coefficients of determination show that degradation was reasonably well modelled by single 1st order kinetics. The model fit became poorer for the experiments with slow degradation, where the natural variation of the samples preponderated to a greater extent. No differences between the labels could be detected, except for the Borstel soil incubated at 10°C.
5.3.2	Reliability	1
5.3.3	Deficiencies	No The study is considered to be valid without restrictions.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	31 October 2006
Materials and Methods	(3.1.3) CIS/TRANS ratio of the parent: 59/41;
Results and discussion	No comments
Conclusion	Geometric mean DT50 value of the 4 soils at 20°C, using both labelled molecules is 213 days.
Reliability	No comments.
Acceptability	Acceptable
Remarks	DT50 values are based on 50% water hold capacity. Recalculation to standard moisture content may lead to longer or shorter DT50 values. This was found to be not necessary for underlying risk assessment. No reliable mineralization rate can be determined.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

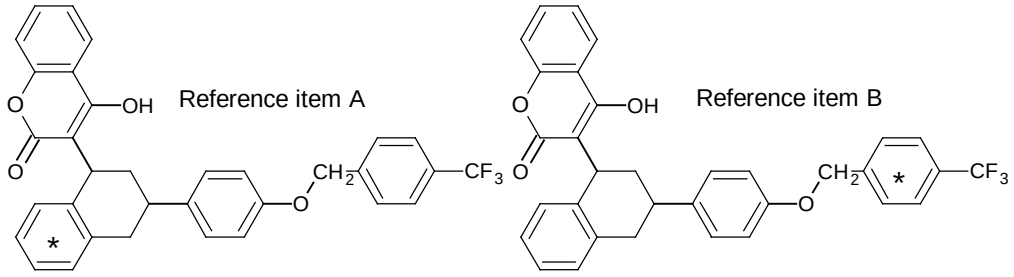

Figure A7.2.1- 2: Labelling position of ^{14}C in Flocoumafen (marked by an asterisk).

Table A7.2.1- 4: Physico-chemical properties of the test soils.

Origin	**Borstel, Germany**	**Soest, Germany**	**Marisfeld, Germany**	**Osnabrück, Germany**
Soil type	Loamy sand	Silt loam	Silty clay loam	Loamy sand
Sand (%)	70	2	13	85
Silt (%)	25	84	53	10
Clay (%)	5	14	35	5
pH (CaCl2)	5.6	6.5	5.4	6.9
Organic carbon (%)	0.95	1.37	2.84	2.75
Cation exchange capacity (mmolc/kg)	36.7	144.0	149.0	361.0
Maximum water holding capacity (%)	294	419	795	346

Table A7.2.1- 5: Results of the microbial biomass development determined by the respiration inhibition method.

Soil type	**Soil sample**	**Biomass [mg C_{mic}/kg TM]**		
		3 days	**60 days**	**120 days**
Borstel, 10°C				
	Untreated	86	65	32
	Treated with acetonitrile	863	65	43
	Treated with Flocoumafen	831	22	32
Borstel, 20°C				
	Untreated	76	76	86
	Treated with acetonitrile	169	97	65
	Treated with Flocoumafen	130	129	73
Soest, 20°C				
	Untreated	205	141	113
	Treated with acetonitrile	1630	169	129
	Treated with Flocoumafen	1342	210	113
Marisfeld, 20°C				
	Untreated	664	821	775
	Treated with acetonitrile	3177	1083	614
	Treated with Flocoumafen	2563	1115	808
Osnabrück, 20°C				
	Untreated	130	195	141
	Treated with acetonitrile	1643	151	76
	Treated with Flocoumafen	1351	141	86

Table A7.2.1- 6: Distribution of initially applied radioactivity (%) in Borstel soil treated with coumarin labelled ^{14}C-Flocoumafen (2) or trifluoromethylphenyl labelled ^{14}C-Flocoumafen (3) and incubated at 10°C and 20°C.

Days after treatment	**0**	**1**	**3**	**7**	**10**	**14**	**30**	**50**	**70**	**120**	**30**	**120**
											Sterile	
Borstel, 10°C												
Flocoumafen (2)	91.4	90.3	87.9	87.9	83.9	83.9	84.0	80.9	81.1	86.8	92.6	91.1
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	0.7	1.1	1.5	1.6	1.8	2.4	3.3	3.9	4.1	n.d.	n.d.
Unextracted	4.2	4.5	4.8	6.9	7.7	8.0	7.7	9.0	9.4	9.4	1.5	3.9
Extracted	92.8	91.7	89.2	88.3	85.2	85.5	85.2	83.1	81.7	87.1	94.0	92.3
Recovery (%)	96.9	96.9	95.0	96.7	94.4	95.3	95.3	95.2	95.0	100.6	95.5	96.3
Borstel, 10°C												
Flocoumafen (3)	94.1	93.9	94.3	91.3	88.3	87.1	89.6	88.3	88.3	87.4	96.7	96.4
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	<0.1	0.1	0.5	0.4	0.7	1.7	2.4	2.5	4	n.d.	n.d.
Unextracted	2.4	3.3	3.5	6.4	6.4	6.5	6.3	5.8	6.3	8.3	0.9	3.1
Extracted	95.8	93.9	94.3	91.3	88.9	87.2	89.6	88.3	88.3	87.4	96.9	96.4
Recovery (%)	98.2	97.2	97.8	98.2	95.7	94.5	97.6	96.5	97.1	99.7	97.9	99.6
Borstel, 20°C												
Flocoumafen (2)	92.8	89.4	87.3	84.7	85.0	83.0	80.8	97.5	75.4	78.9	91.0	87.5
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	0.1	0.3	0.6	0.6	3.4	4.8	6.3	7.4	2.8	n.d.	n.d.
Unextracted	4.2	4.8	6.6	8.6	7.5	8.4	9.4	12.2[a]	10.7	13.3	2.1	6.1[a]
Extracted	92.8	89.4	87.3	84.7	85.0	83.0	80.8	97.5[a]	75.4	78.9	91.0	87.5
Recovery (%)	96.9	94.2	94.2	93.9	93.0	94.8	95.0	116.0[a]	93.5	94.9	93.1	93.6[a]
Borstel, 20°C												
Flocoumafen (3)	94.1	93.1	88.5	85.5	83.5	80.1	78.2	78.3	79.1	80.2	92.4	95.9
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	<0.1	0.5	1.5	2.7	1.4	4.1	4.8	5.5	5.8	n.d.	n.d.
Unextracted	2.4	3.9	6.9	8.7	9.4	10.9	11.1	10.5	10.3	10.6	1.4	5.4
Extracted	95.8	93.1	88.5	85.5	84.1	80.9	78.5	78.5	79.4	80.2	92.5[a]	96.1
Recovery (%)	98.2	97.0	95.9	95.6	96.2	93.1	93.7	93.8	95.2	96.6	93.8[a]	101.5

a) analytical error

Table A7.2.1- 7: Distribution of initially applied radioactivity (%) in Soest, Marisfeld and Osnabrück soils treated with coumarin labelled ^{14}C-Flocoumafen (2) or trifluoromethylphenyl labelled ^{14}C-Flocoumafen (3) and incubated at 20°C.

Days after treatment	**0**	**1**	**3**	**7**	**10**	**14**	**30**	**50**	**70**	**120**	**30**	**120**
											Sterile	
Soest, 20°C												
Flocoumafen (2)	88.4	82.3	79.2	76.9	77.8	74.0	71.9	66.9	63.1	57.2	89.8	86.8
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	< 0.1	0.5	1.3	1.8	1.8	4.8	5.4	5.9	7.1	n.d.	n.d.
Unextracted	7.0	8.5	12.2	13.8	12.7	13.8	14.8	16.9	17.9	24.6	4.1	10.3
Extracted	89.4	85.0	80.2	77.7	78.4	76.1	73.7	68.9	65.2	59.8	91.2	88.0
Recovery (%)	96.4	93.5	92.9	92.9	92.9	91.7	93.3	91.1	89.2	91.5	95.4	98.2
Soest, 20°C												
Flocoumafen (3)	91.2	87.3	83.0	80.7	81.7	77.9	74.5	72.5	68.4	59.4	93.1	92.3
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.4	n.d.	n.d.
CO_2	n.d.	< 0.1	0.8	1.5	2.1	2.9	4.8	6.3	7.6	6.7	n.d.	n.d.
Unextracted	4.5	6.9	10.6	10.4	10.5	11.5	12.9	13.1	14.4	18.2	2.7	7.4
Extracted	92.6	88.5	84.1	81.2	82.3	78.2	74.9	72.9	68.4	60.3	93.1	92.6
Recovery (%)	97.1	95.3	95.6	93.1	94.9	92.7	92.7	92.3	90.6	85.6	95.8	100.0
Marisfeld, 20°C												
Flocoumafen (2)	90.0	82.0	76.5	71.3	68.0	63.9	49.7	41.4	38.1	26.6	94.1	86.2
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	0.1	0.7	1.9	1.9	2.9	5.1	7.8	8.7	11.9	n.d.	n.d.
Unextracted	5.4	8.9	12.7	16.5	19.0	21.4	29.6	33.9	37.7	47.4	5.8	10.5
Extracted	91.0	84.0	78.8	74.2	71.3	67.1	53.6	44.9	40.8	29.2	96.1	88.1
Recovery (%)	96.4	93.1	92.1	92.6	92.1	91.5	88.4	86.5	87.3	88.7	102.0	98.6
Marisfeld, 20°C												
Flocoumafen (3)	95.0	87.9	84.9	76.1	74.0	65.8	54.8	49.2	41.1	29.2	97.8	93.5
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	n.d.	n.d.
CO_2	n.d.	< 0.1	1.1	3.1	4.4	6.6	10.4	13.2	15.6	13.4	n.d.	n.d.
Unextracted	2.6	5.6	8.1	12.1	12.9	17.7	21.8	25.4	27.8	30.4	4.7	9.2
Extracted	96.6	91.5	89.1	79.5	76.8	68.4	57.6	51.9	43.4	31.5	98.2	94.3
Recovery (%)	99.2	97.2	98.3	94.8	94.2	92.7	89.8	90.6	86.9	75.4	102.9	103.5
Osnabrück, 20°C												
Flocoumafen (2)	91.9	88.0	88.9	86.1	86.7	87.6	83.3	81.8	80.6	85.0	94.4	92.9
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.7	1.0	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	0.2	0.2	0.2	0.3	0.7	1.3	2.3	2.8	5.7	n.d.	n.d.
Unextracted	3.8	4.0	4.9	5.8	6.1	6.4	6.3	8.4	8.5	10.4	1.6	5.6
Extracted	93.3	90.2	90.2	87.4	87.8	88.1	84.1	82.8	80.6	86.4	96.0	94.3
Recovery (%)	97.0	94.3	95.3	93.4	94.2	95.2	92.0	94.1	93.0	102.4	97.6	99.9
Osnabrück, 20°C												
Flocoumafen (3)	95.5	92.5	93.0	90.7	87.4	86.2	84.8	84.7	83.3	84.5	98.4	94.2
Volatile organics	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
Basic volatiles	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	n.d.	n.d.
CO_2	n.d.	0.2	0.4	0.7	0.8	0.9	1.3	1.9	2.3	2.5	n.d.	n.d.
Unextracted	2.2	3.5	4.8	5.9	6.1	6.0	6.8	7.4	7.7	9.0	1.0	5.4
Extracted	96.6	92.5	93.0	90.7	87.4	86.6	85.0	85.0	83.3	84.5	98.4	95.0
Recovery (%)	98.8	96.2	98.2	97.3	94.3	93.5	93.1	94.3	93.2	96.0	99.4	100.4

a) analytical error

Table A7.2.1- 8: Single 1st order DT_{50} values of Flocoumafen determined with ModelMaker 4.0

Soil	**Temperature (°C)**	**Coumarin labelled ^{14}C-Flocoumafen (2)**		**Trifluoromethylphenyl labelled ^{14}C-Flocoumafen (3)**	
		DT_{50} (days)	**r^2**	**DT_{50} (days)**	**r^2**
Borstel, loamy sand	20	281	0.869	311	0.555
Borstel, loamy sand	10	443	0.707	1293	0.403
Soest, silt loam	20	219	0.914	226	0.933
Marisfeld, silty clay loam	20	71	0.944	74	0.955
Osnabrück, loamy sand	20	442	0.846	421	0.687

r^2 = Coefficient of determination

Sections A7.2.2.1 – A7.2.2.4 Aerobic degradation in soil, further studies

Annex Points IIIA 7.4, 12.1.1, 12.1.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Exposure of soil to Flocoumafen is considered to be very limited based on the anticipated use pattern (indoor use). In the exceptional case of bait carriage by rats to outdoor areas, exposure of soil to Flocoumafen will be only punctual. Diffuse release through urine and faeces of the target species is possible, but the resulting amounts are small and temporally very limited. Overall, release of the substance to soil is considered to be negligible. Thus, the conduct of further aerobic soil degradation studies is not required.	X
Undertaking of intended 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 (*)
Date	16 September 2005
Evaluation of applicant's justification	The use pattern is "in and around buildings" (not only indoor use as stated). According to the Mackay model and the EUBEES scenario for rodenticides, soil is the major compartment for distribution of Flocoumafen residues. Point 7.2.2.1: Non-submission of data is accepted. Point 7.2.2.2: Non-submissionof data is accepted. Point 7.2.2.3: Non-submission of data is accepted. Point 7.2.2.4: Non-submissionof data is accepted.
Conclusion	Non-submission of data is accepted.
Remarks	

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.2.3.1 Adsorption/ desorption in at least three soil types
Annex Point IIIA 12.1.2 **(according to EC C.18/ OECD 106)**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Exposure of soil to flocoumafen is considered to be very limited based on the anticipated use pattern (indoor use).In the exceptional case of bait carriage by rats to outdoor areas, exposure of soil to flocoumafen will be only punctual. Overall, release of the substance to soil is considered to be negligible. Furthermore, all relevant information on the dissipation of flocoumafen in soil is provided by the following soil leaching study (A7.2.3.2/01). Thus, the conduct of an adsorption/desorption study is not required.	X
Undertaking of intended 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 (*)
Date	15 September 2005
Evaluation of applicant's justification	The use pattern is "in and around buildings" (not only indoor use as stated). Because direct exposure of soil is anticipated and based on the existing data and evaluation of the RMS, Flocoumafen is considered not readily biodegradable, a full scale adsorption test is required. Data may also have to be generated for relevant metabolites. A final assessment will be made when the announced new study under 7.2.1 has been submitted and evaluated.
Conclusion	Non-submission of data is not accepted.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
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 12.1.3

Official use only

1 REFERENCE

1.1	**Reference**	**A7.2.3.2/01:** Wxxxx Bxxxx, Exxxx Cxxxx (1984) The leaching of WL108366 in soil under laboratory conditions. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.84.205, August 1984 (unpublished). (BASF-Ref.: FL-620-001)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes BBA Merkblatt 37
2.2	**GLP**	No GLP was not compulsory at the time the study was conducted.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Radiolabelled ^{14}C-Flocoumafen
3.1.1	Lot/Batch number	Batch 1, Sample No. S 7030 Laboratory Book Ref.: 1932=046B Oxxxx Cxxxx Dxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx Rxxxx Lxxxx
3.1.2	Specification	Test substance was supplied dissolved in acetone. The ^{14}C labelling position is specified in Figure A7.2.3.2- 1. Specific activity = 18.7 µCi/mg
3.1.3	Purity	Radiochemical purity = 98.0 %
3.1.4	Further relevant properties	The typical properties of the test substance were not considered to have negatively influenced the test results.
3.1.5	Method of analysis	Liquid Scintillation Counting (LSC). Packard CD 460 liquid scintillation counter.

Section A7.2.3.2 Mobility in at least three soil types and where relevant mobility of metabolites and degradation products
Annex Point IIIA 12.1.3

3.2	**Degradation products**	Not determined	
3.2.1	Method of analysis for degradation products	Not applicable	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Soil types**	Three German standard soils (Agricultural Research and Testing Institute, Speyer) and one freshly sampled British soil from Reculver, Kent, UK, were used (Table A7.2.3.2- 1).	
3.5	**Testing procedure**		
3.5.1	Test system	Glass columns, as specified by BBA Merkblatt 37. The soils were air-dried, sieved and saturated with water as prescribed by the above guideline.	
3.5.2	Test solution and test conditions	^{14}C-flocoumafen dissolved in acetone c = 3.54 g/l	
3.6	**Test performance**		
3.6.1	Application of test substance	0.5 ml of the test solution (1.77 mg flocoumafen ≡ 33.1 µCi) were pippetted onto the centre of the soil columns.	X
3.6.2	Test conditions	After application of the TS, the soils were irrigated for 48 h with 393 ml of deionised water at a rate of 8 ml/min, as prescribed by BBA Merkblatt 37.	
3.6.3	Sampling	The leachate was collected in darkened glass containers.	
3.6.4	Analysis	By LSC; counting performed in duplicate.	

4 RESULTS

4.1	**Preliminary test**	Not performed	
4.2	**TS concentration in leachate**	The leachate samples contained 0.09–0.18 % of the applied radioactivity. Details are presented in Table A7.2.3.2- 2.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Leaching behaviour of flocoumafen in soil was studied using three standard soils and additionally a UK soil. Quantities of ^{14}C-radiolabelled flocoumafen applied to the soil surface were determined after 48 h of leaching with deionised water. The study fully complies to BBA Merkblatt 37.	

Section A7.2.3.2 Mobility in at least three soil types and where relevant mobility of metabolites and degradation products
Annex Point IIIA 12.1.3

5.2	**Results and discussion**	After 48 h, 0.09–0.18 % of the applied flocoumafen had leached through the investigated soils. Flocoumafen is poorly soluble in water (see Section A3.5). Thus, leaching through soils with water as the mobile phase is not be expected, as reflected in these results. The study does not deviate from the guideline specified by BBA Merkblatt 37. Furthermore, the physico-chemical properties are not considered to have affected the results. The study does not conform to current standards, e.g. OECD guideline 106, as recommended by the TNG on data requirements. However, neither according to the product type specific data requirements nor according to the intended uses (indoor use only; lack of exposure), the necessity of a soil mobility study is indicated. The available study is deemed sufficient to demonstrate the low tendency of flocoumafen to leach through soils. Therefore, this study is considered to be valid and acceptable.	X
5.3	**Conclusion**	Leaching through soils is not expected.	
5.3.1	Reliability	1	X
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	15 September 2005
Materials and Methods	(3.6.1) The dose of 1.77 mg was applied on a column of cross sectional area of 19 cm^2, which is equivalent to a treatment rate of 9 kg a.s./ha.
Results and discussion	(5.2) The applicant's summary states indoor use only, hence lack of exposure. The proposed use however is in and around buildings. Exposure of soil is possible through rat excreta, decaying carcasses and outdoor placement of bait.
Conclusion	The results demonstrate a low tendency of flocoumafen to leach through soils (<0.2%).
Reliability	2
Acceptability	Acceptable.
Remarks	The reliability was lowered to 2 because radioactivity in soil was not determined.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

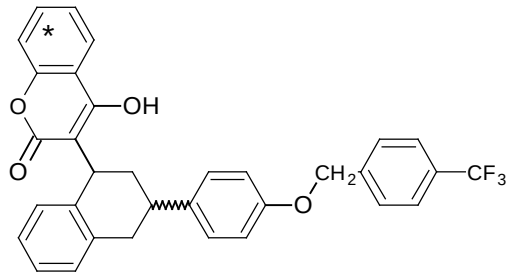

Figure A7.2.3.2- 1: Labelling position of ^{14}C in flocoumafen (marked by an asterisk).

Table A7.2.3.2- 1: Physical properties of the soils.

Origin	**Speyer 2.1**	**Speyer 2.2**	**Speyer 2.3**	**Reculver**
Soil texture	Sand	Loamy sand	Sandy loam	Sandy loam
pH	5.5–7.5	5.5–7.5	5.5–7.5	5.5
Fraction ≤ 0.02 mm [%]	< 10	10 –20	20–30	not stated
Organic matter [% dry weight]	0.25–0.75	2–3	0.5–1.5	2.4*)

*) mean of two samples

Table A7.2.3.2- 2: Recovery of radioactivity (^{14}C-flocoumafen) in the leachate from laboratory soil columns.

Soil	**Radioactivity applied [µCi]**	**Radioactivity found**	
		[µCi]	**[%]**
Speyer 2.1	33.1	0.053	0.16
Speyer 2.2	33.1	0.039	0.12
Speyer 2.3	33.1	0.058	0.18
Reculver	33.1	0.029	0.09

Section A7.3.1 Annex Point IIIA 7.5 — Phototransformation in air (estimation method), including identification of breakdown products

Official use only

1 REFERENCE

1.1	**Reference**	**A7.3.1/ 01:** Mxxxx Cxxxx (2002) BAS 322 I (Flocoumafen): Estimation of the photochemical oxidative degradation rate in the atmosphere. Bxxxx Axxxx Rxxxx, Pxxxx, Uxxxx, Report No. ENV 02-009, April 2, 2002 (unpublished). (BASF-Ref.: 2002/500383)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not applicable (no guideline available)
2.2	**GLP**	Not applicable
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	Not applicable
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	For estimation, 100 % purity was assumed.
3.1.4	Further relevant properties	Not applicable
3.1.5	Method of analysis	Not applicable
3.2	**Degradation products**	The formation of degradation products was not considered in this study.

Section A7.3.1 Phototransformation in air (estimation method), including identification of breakdown products
Annex Point IIIA 7.5

3.3	**Estimation method**	
3.3.1	Considered reactions	Reaction in the atmosphere of photochemically produced OH radicals (•OH) with organic chemicals, and ozone (O_3) with olefinic/acetylenic compounds. In the case of flocoumafen, precisely the following reactions were considered: - hydrogen abstraction - reaction with hydroxyl groups - addition to olefinic bonds - addition to aromatic rings
3.3.2	Assumptions	Atmospheric concentrations of •OH and ozone were assumed as follows: $c_{OH} = 1.5 \times 10^6$ molecules/cm^3; 12-h day for reaction with •OH. $c_{Ozone} = 7 \times 10^{11}$ molecules/cm^3 24-h day for reaction with ozone.
3.3.3	Calculations	Estimation of the rate constants k_{OH} and k_{Ozone}, based on structure-activity relationships (SAR). Calculations performed with program AOPWIN, version 1.90 (available from U.S. EPA). Atmospheric half-lives of flocoumafen: $t_{½}$ (•OH) = ln 2/($k_{OH} \times c_{OH}$) $t_{½}$ (Ozone) = ln 2/($k_{Ozone} \times c_{Ozone}$).

4 RESULTS

4.1	**Rate constants**	$k_{OH} = 86.76 \times 10^{-12}$ cm^3/molecule × s $k_{Ozone} = 13.65\ 10^{-17}$ cm^3/molecule × s The contributions of various reaction types to the total rate constant for hydroxyl radicals are listed in Table A7.3.1- 1.
4.2	**Half life**	$t_{½}$ (•OH) = 0.123 d (≡ 1.479 h) $t_{½}$ (Ozone) = 0.085 d (≡ 2.015 h)
4.3	**Specification of breakdown products**	The formation of breakdown products was not examined.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The atmospheric photo-oxidative degradation of flocoumafen by hydroxyl radicals and ozone was estimated using a structure-activity relationships (SAR), with the help of the software module AOPWIN. No guidelines for this purpose are available, but the method applied rests on generally accepted scientific principles, as also recommended by the TNsG on data requirements.

Section A7.3.1 Phototransformation in air (estimation method), including identification of breakdown products
Annex Point IIIA 7.5

5.2	**Results and discussion**	The results suggest that flocoumafen is rapidly degraded in the atmosphere by photo-oxidative processes. The numerical half-lives are given below. The TNsG on data requirements recommend an assessment of potential breakdown products, as well as an assessment of further interactions of substances with atmospheric processes. Due to the extremely low vapour pressure of flocoumafen (see Section A3.2), the potential for global warming, stratospheric ozone depletion, tropospheric ozone formation, and acidification, is considered to be negligible. Furthermore, according to the considered reactions, the formation of volatile compounds that might interact with atmospheric processes is not expected. Thus, the results from the current study are considered to be sufficient for the assessment of the fate of the substance in air. Phototransformation of flocoumafen has been estimated according to generally accepted principles. Thus, the study is considered to be valid.
5.2.1	Half life	$t_{½}$ (•OH) = 0.123 d (≡ 1.479 h) $t_{½}$ (Ozone) = 0.085 d (≡ 2.015 h)
5.3	**Conclusion**	Rapid degradation with DT_{50} << 2 days.
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	13 December 2004
Materials and Methods	No comments.
Results and discussion	No comments.
Conclusion	t½ (•OH) = 0.185 d (≡ 4.436 h) (AOPWIN v1.92 based on a 24-hour day; 0.5E6 OH/cm3) t½ (Ozone) = 0.085 d (≡ 2.015 h) (based on a 24-hour day)
Reliability	1
Acceptability	Acceptable
Remarks	-

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Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.3.1- 1: Estimated contributions of various reactions types to the total k_{OH}.

Reaction type	***k* [10^{-12} cm^3/molecule × s]**	**% of total k_{OH}**
Hydrogen abstraction	13.5805	15.7
Reaction with –OH	0.14	0.2
Addition to olefinic bonds	38.5	44.4
Addition to aromatic rings	34.5723	39.9
Overall k_{OH}	**86.7597**	

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

Annex Point IIIA 12.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Exposure of the atmosphere to Flocoumafen air is considered to be extremely unlikely: The substance is non-volatile and will not be applied as a fumigant or by spraying. Thus, exposure to air is limited and the substance is considered to cause no risks to the atmospheric environment. In view of the limited exposure, the data requirements on fate and behaviour in air are considered to be completely covered by Section A7.3.1.	
Undertaking of intended 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 (*)
Date	09 June 2005
Evaluation of applicant's justification	No comments.
Conclusion	Non-submission of data is accepted.
Remarks	-

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.1.1/01:** Zxxxx Sxxxx (2002) BAS 322 I – Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a semistatic system over 96 hours. Bxxxx Axxxx, Exxxx Txxxx axxxx Exxxx, Lxxxx, Gxxxx, Report No. 12F0344/015028, April 18, 2002 (unpublished). (BASF-Ref.: 2002/1004882)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 EC method C.1
2.2	**GLP**	Yes
2.3	**Deviations**	Yes (see 3.2)

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	AC 12140-35
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.4 %
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water, with a solubility of 0.11 mg/l at pH 7 and 14 mg/l at pH 9 (see Section A3.5); thus, the test substance was dissolved using acetone as a solubilising agent (see 3.2).
3.1.5	Method of analysis	LCMS
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Details are presented in Table A7.4.1.1-1. No vehicle control was performed. However, it is known that acetone has no adverse effects at concentrations as used in this study. This is also supported by the mortalities at the lower concentrations of test substance (see 4.2.3).

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

3.3	**Reference substance**	No tests with reference substances were performed.	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Details are given in Table A7.4.1.1-2.	
3.4.2	Test organisms	Details are provided in Table A7.4.1.1-3.	
3.4.3	Test system	See Table A7.4.1.1-4.	
3.4.4	Test conditions	Test conditions are given in Tables A7.4.1.1-5 to 7.	
3.4.5	Duration of the test	96 h	
3.4.6	Test parameter	Mortality	
3.4.7	Sampling	Mortality and signs of intoxication were recorded at 1, 4, 24, 48, 72, and 96 h after start of exposure. Water parameters were measured 1, 24, 48, 72, and 96 h after start of the test.	
3.4.8	Monitoring of TS	Samples were taken at the start and at the end of each renewal interval. Analysis by LCMS was performed on the day of sampling.	
3.4.9	Statistics	LC_{50}, determined by probit analysis.	

4 RESULTS

4.1	**Limit test**	Not performed	
4.1.1	Concentration		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 0.0 (control), 0.01, 0.022. 0.05, 0.10, and 0.22 mg/l	
4.2.2	Actual concentrations of test substance	See Table A7.4.1.1-8.	
4.2.3	Effect data (Mortality)	The raw mortality data are presented in Table A7.4.1.1-9. For effect concentrations see Table A7.4.1.1-10.	X
4.2.4	Concentration/ response curve	The concentration-response curve is presented in Figure A7.4.1.1-1.	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

4.2.5	Other effects	At 0.1 mg/l, eight and ten fish in either replicate, respectively, showed erratic swimming after 48 hours. At 0.22 mg, seven and eight fish in either replicate, respectively, were lethargic.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No control animals showed adverse effects.
4.3.2	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	An acute toxicity test of Flocoumafen to freshwater fish was performed using *Oncorhynchus mykiss* according to OECD guideline 203 and EC method C.1. Acetone was used as a solubilising agent at a final concentration of 0.1 ml/l. No solvent control was performed. However, acetone is known to have no adverse effects on the test fish at the applied concentration. Thus, this deviation is assumed to have no impact on the results of the study. This conclusion is further supported by the absence of adverse effects at low test substance concentrations.
5.2	**Results and discussion**	Flocoumafen is poorly soluble in water; the tested concentrations approximately cover the range of solubility (see Section A3.5). The substance is hydrolytically stable (Section A7.1.1.1.1) and non-volatile (Section A3.2). In view of these properties, Flocoumafen is likely to remain in solution during a time period covered by the test. According to its log P_{ow} (Section A3.9), a high tendency of resorption of Flocoumafen by fish would be expected. No studies on adsorption to glass surfaces, which could potentially influence the results, are available. To prevent adsorption to the test vessels, they were saturated with the test substance prior to the tests. Furthermore, the test substance concentrations were verified analytically. Thus, a valid dose-response relationship could be established. The relevant results are listed below.
5.2.1	LC_0	0.05 mg/l
5.2.2	LC_{50}	0.07 mg/l
5.2.3	LC_{100}	0.09 mg/l

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

5.3	**Conclusion**	As summarised in Table A7.4.1.1-11, the validity criteria were fulfilled. The dose-response curve shows a very sharp rise in mortality between the 0.05 and 0.1 mg/l doses, which appears as an all-or-nothing response. For this reason, no confidence intervals could be established.	X
5.3.1	Other Conclusions		
5.3.2	Reliability	1	
5.3.3	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	11 January 2005
Materials and Methods	No comments
Results and discussion	(4.2.3) Table A.7.4.1.1-10 contains a wrong value for the 48h-LC_{50} (0.15 mg/l). This value should be 0.13 mg/l.
Conclusion	(5.3) The dose-response curve shows a very sharp rise in mortality between the 0.05 mg/l (LC_0) and 0.09-0.1 mg/l (LC_{100}, range for nominal and mean measured concentrations), which appears as an all-or-nothing response. For this reason, no confidence intervals could be established. The 96-hour LC_{50} based on (nominal and) mean measured concentrations is 0.07 mg/l.
Reliability	1
Acceptability	Acceptable
Remarks	No further comments.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.1-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes Acetone
Concentration of vehicle	0.1 ml/l (≡ 0.001 %)
Vehicle control performed	No
Other procedures	Not applicable

Table A7.4.1.1-2: Dilution water.

Criteria	Details
Source	Tap water, community of Frankenthal, Germany non-chlorinated, charcoal filtered, aerated
Alkalinity	Not reported
Hardness	2.5 mmol/l = 250 mg/l $CaCO_3$
Oxygen content	Not reported
pH	7.5 – 8.5
Conductance	Approx. 550 µS/cm (at 25 °C)
Holding water different from dilution water	No

Table A7.4.1.1-3: Test organisms.

Criteria	Details
Species/strain	*Oncorhynchus mykiss* (Rainbow trout)
Source	Forellenzucht Trostadt GbR, Dorfstr. 7, 98646 Trostadt, Germany
Wild caught	No
Age/size	3.9 – 4.5 cm (mean = 4.1 cm)
Kind of food	Standard commercial growing feed (“Forellenfutter [Zeigler]”; Provimi Kliba AG, Gossau, Switzerland); additionally on working days live *Artemia*
Amount of food	*Ad libitum* (apart from *Artemia*)
Feeding frequency	Daily
Pre-treatment	14 d of acclimatisation
Feeding of animals during test	No

Table A7.4.1.1-4: Test system.

Criteria	Details
Test type	Semistatic
Renewal of test solution	Every 24 h
Volume of test vessels	25 l
Volume/animal	2.5 l
Number of animals/vessel	10
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

Criteria	Details
Test temperature	12 °C throughout
Dissolved oxygen	Always above the required 60 % saturation; full details provided in Table A7.4.1.1-6.
pH	Details are given in Table A7.4.1.1-7.
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not stated
Photoperiod	16:8 h light/dark cycle

Table A7.4.1.1-6: Measurements of oxygen concentration [mg/l] during the test; measurements are given at the start (first value) and the end (second value) of each interval, respectively.

	Interval			
TS concentration, nominal [mg/l]	**24 h**	**48 h**	**72 h**	**96 h**
0.0	9.2–8.2	9.6–8.8	9.1–8.6	9.9–8.6
0.0	9.6–9.2	9.8–9.4	9.6–9.3	10.1–9.1
0.01	9.9–9.3	10.2–9.5	10.1–9.4	10.1–9.4
0.01	10.2–9.2	10.2–9.5	10.1–9.6	10.1–9.5
0.022	10.2–9.4	10.3–9.5	10.1–9.6	10.1–9.5
0.022	10.3–9.3	10.2–9.6	10.1–9.7	10.2–9.5
0.05	10.5–9.3	10.2–9.4	10.1–9.6	10.2–9.5
0.05	10.4–9.1	10.2–9.5	10.0–9.5	10.1–9.2
0.1	10.4–9.1	10.2–9.3	10.1–9.1	–
0.1	10.4–9.1	10.2–8.5	10.0–9.1	–
0.22	10.4–8.5	10.0–8.8	–	–
0.22	10.4–8.5	10.2–8.8	–	–

Table A7.4.1.1-7: Measurements of pH during the test; measurements are given at the start (first value) and the end (second value) of each interval, respectively.

	Interval			
TS concentration, nominal [mg/l]	**24 h**	**48 h**	**72 h**	**96 h**
0.0	8.0–7.9	7.9–7.9	7.8–7.9	7.8–7.9
0.0	8.0–8.0	8.0–8.0	7.9–7.9	7.8–8.0
0.01	7.9–8.0	8.0–8.0	7.9–7.9	7.8–8.0
0.01	7.9–8.0	8.0–8.0	7.9–7.9	7.9–8.0
0.022	8.0–8.0	8.0–8.0	7.9–7.9	7.9–8.0
0.022	8.0–8.0	8.0–8.0	7.9–7.9	7.9–8.0
0.05	8.0–8.0	8.0–8.0	7.9–7.9	7.9–7.9
0.05	8.0–8.0	8.0–8.0	7.9–7.9	7.9–7.9
0.1	8.0–8.0	8.0–8.0	7.9–7.9	–
0.1	8.0–8.0	8.0–7.9	7.9–7.9	–
0.22	8.0–8.0	8.0–8.0	–	–
0.22	8.0–8.0	8.0–8.0	–	–

Table A7.4.1.1-8: Analytical determinations of the test substance concentration [mg/l] by LCMS, at the start (immediately following renewal of the test water) and at the end of each observation interval.

	Measured concentrations [mg/l]								
TS concentration, nominal [mg/l]	**24 h**		**48 h**		**72 h**		**96 h**		**mean**
	Start	*End*	*Start*	*End*	*Start*	*End*	*Start*	*End*	
0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.01	0.00993	0.00979	0.01271	0.00909	0.01001	0.00883	0.01219	0.00906	0.01020
0.01	0.01077	0.00955	0.01153	0.01008	0.01053	0.00947	0.01146	0.00964	0.01038
0.022	0.02017	0.02226	0.02456	0.02114	0.02350	0.02077	0.02151	0.02157	0.02194
0.022	0.02177	0.02147	0.02631	0.02004	0.02166	0.02168	0.02361	0.02214	0.02234
0.05	0.05031	0.04634	0.05303	0.04255	0.05323	0.04923	0.05314	0.05324	0.05013
0.05	0.04986	0.04351	0.05252	0.04455	0.05640	0.05018	0.05686	0.04657	0.05006
0.1	0.09209	0.08585	0.09098	0.08378	0.09494	0.08380	–	–	0.08857
0.1	0.09942	0.07550	0.09316	0.08942	0.09180	0.08263	–	–	0.08866
0.22	0.17914	0.16888	0.18193	0.18986	–	–	–	–	0.17995
0.22	0.18854	0.15320	0.19014	0.20885	–	–	–	–	0.18518

n.d. = not detected

Table A7.4.1.1-9: Mortality data; tests were conducted with 10 individuals per replicate per treatment (two replicates); if mortalities coincide between replicates, only a single value is given, otherwise the replicates are separated by a slash.

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**
0.0	0	0	0	0	0	0	0	0
0.01	0	0	0	0	0	0	0	0
0.022	0	0	0	0	0	0	0	0
0.05	0	0	0	0	0	0	0	0
0.1	0	0	10	10	0	0	100	100
0.22	2/3	10	10	10	20/30	100	100	100

Temperature (°C)	12 °C
pH	7.8 – 8.0 (see Table A7.4.1.1-7)
Oxygen [mg/l]	8.2 – 10.5 mg/l (see Table A7.4.1.1-6)

Table A7.4.1.1-10: Effect data, based on the measured concentrations.

	48 h [mg/l]	95 % CI	96 h [mg/l]	95 % CI
LC_0	0.09	-	0.05	-
LC_{50}	0.15	-	0.07	-
LC_{100}	0.18	-	0.09	-

Table A7.4.1.1-11: Validity criteria for acute fish test according to OECD guideline 203.

	Fulfilled	Not fulfilled
Mortality of control animals <10%	☑	
Concentration of dissolved oxygen in all test vessels > 60% saturation	☑	
Concentration of test substance ≥80% of initial concentration during test	☑	
Criteria for poorly soluble test substances	☑	

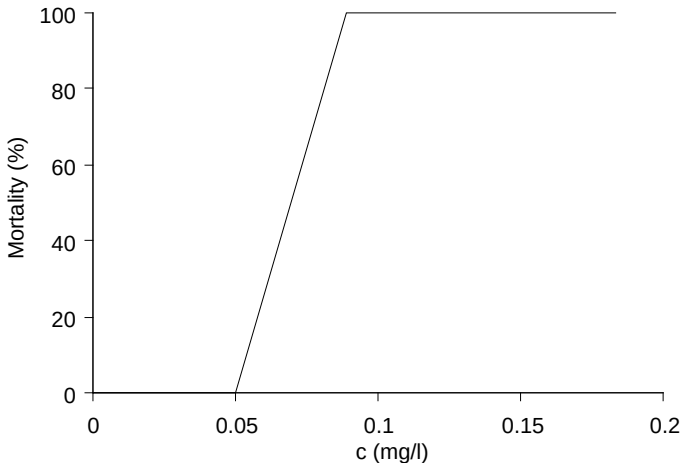

Figure A7.4.1.1-1: Concentration-response curve from the acute fish toxicity test of Flocoumafen in *Oncorhynchus mykiss*.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.1.1/02:** Zxxxx Sxxxx (2002) BAS 322 I – Acute toxicity study on the bluegill sunfish (*Lepomis macrochirus*) in a semistatic system over 96 hours. Bxxxx Axxxx, Exxxx Txxxx axxxx Exxxx, Lxxxx, Gxxxx, Report No. 14F0344/015029, April 18, 2002 (unpublished). (BASF-Ref.: 2002/1004881)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 EC method C.1
2.2	**GLP**	Yes
2.3	**Deviations**	Yes (see 3.2)

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	AC 12140-35
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.4 %
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water, with a solubility of 0.11 mg/l at pH 7 and 14 mg/l at pH 9 (see Section A3.5); thus, the test substance was dissolved using acetone as a solubilising agent (see 3.2).
3.1.5	Method of analysis	LCMS
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Details are presented in Table A7.4.1.1-12. No vehicle control was performed. However, it is known that acetone has no adverse effects at concentrations as used in this study. This is also supported by the mortalities at the lower concentrations of test substance (see 4.2.3).

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

3.3	**Reference substance**	No tests with reference substances were performed.	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Details are given in Table A7.4.1.1-13.	
3.4.2	Test organisms	Details are provided in Table A7.4.1.1-14.	
3.4.3	Test system	See Table A7.4.1.1-15.	
3.4.4	Test conditions	Test conditions are given in Tables A7.4.1.1-16 through 18.	
3.4.5	Duration of the test	96 h	
3.4.6	Test parameter	Mortality	
3.4.7	Sampling	Mortality and signs of intoxication were recorded at 1, 4, 24, 48, 72, and 96 h after start of exposure. Water parameters were measured 1, 24, 48, 72, and 96 h after start of the test.	
3.4.8	Monitoring of TS	Samples were taken at the start and at the end of each renewal interval. Analysis by LCMS was performed on the day of sampling.	
3.4.9	Statistics	LC_{50}, determined by probit analysis.	

4 RESULTS

4.1	**Limit test**	Not performed	
4.1.1	Concentration		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse		
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 0.0 (control), 0.05, 0.1. 0.22, 0.5, and 1.0 mg/l	
4.2.2	Actual concentrations of test substance	See Table A7.4.1.1-19.	
4.2.3	Effect data (Mortality)	The raw mortality data are presented in Table A7.4.1.1-20. For effect concentrations see Table A7.4.1.1-21.	X
4.2.4	Concentration/ response curve	The concentration-response curve is presented in Figure A7.4.1.1-2.	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

4.2.5	Other effects	At 0.1 mg/l, one individual showed erratic swimming after 72 hours. At 0.22 mg/l, three fish showed erratic swimming after 48 and 72 hours, respectively, and one individual swam at the surface after 72 hours. At 0.5 mg/l, two individuals showed erratic swimming after 24 hours. At 1 mg/l, seven fish showed erratic swimming after 4 hours.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No control animals showed adverse effects.
4.3.2	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	An acute toxicity test of Flocoumafen to freshwater fish was performed using *Lepomis macrochirus* according to OECD guideline 203 and EC method C.1. Acetone was used as a solubilising agent at a final concentration of 0.1 ml/l. No solvent control was performed. However, acetone is known to have no adverse effects on the test fish at the applied concentration. Thus, this deviation is assumed to have no impact on the results of the study. This conclusion is further supported by the absence of adverse effects at low test substance concentrations.
5.2	**Results and discussion**	Flocoumafen is poorly soluble in water; the tested concentrations approximately cover the range of solubility (see Section A3.5). The substance is hydrolytically stable (Section A7.1.1.1.1) and non-volatile (Section A3.2). In view of these properties, Flocoumafen is likely to remain in solution during the time period covered by the test. According to its log P_{ow} (Section A3.9), a high tendency of resorption of Flocoumafen by fish would be expected. No studies on adsorption to glass surfaces, which could potentially influence the results, are available. To prevent adsorption to the test vessels, they were saturated with the test substance prior to the tests. Furthermore, the test substance concentrations were verified analytically. Thus, a valid dose-response relationship could be established. The relevant results are listed below.
5.2.1	LC_0	0.043 mg/l
5.2.2	LC_{50}	0.112 mg/l
5.2.3	LC_{100}	0.177 mg/l

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.7.1

5.3	**Conclusion**	As summarized in Table A7.4.1.1-22, the validity criteria were fulfilled. The concentration-response curve showed an abrupt increase of mortality from 5 % at 0.1 mg/l to100 % at concentrations ≥ 0.22 mg/l. Because of the steep slope of the dose-response curve, confidence limits could not be estimated.	X
5.3.1	Other Conclusions		
5.3.2	Reliability	1	
5.3.3	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	11 January 2005
Materials and Methods	No comments
Results and discussion	(4.2.3) Table A7.4.1.1-20 contains wrong values for the percentage mortality at 0.22 mg/l: 10/0, 90/80 and 100 after 24, 48 and 72 h should be 0, 10/0 and 90/80. The maximum oxygen concentration in the footnote under Table A7.4.1.1-20 should be 9.4 in stead of 9.2 mg/l.
Conclusion	(5.3) The concentration-response curve showed an abrupt increase of mortality from 5 % at nominal and mean measured concentrations, respectively, of 0.1 and 0.081 mg/l (LC_0 0.05 and 0.043 mg/l) to100 % at ≥ 0.22 and ≥ 0.18 mg/l (LC_{100}). Because of the steep slope of the dose-response curve, confidence limits could not be estimated. The 96-hour LC_{50} based on mean measured concentrations is 0.11 mg/l.
Reliability	1
Acceptability	Acceptable
Remarks	No further comments.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.1-12: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes Acetone
Concentration of vehicle	0.1 ml/l (≡ 0.001 %)
Vehicle control performed	No
Other procedures	Not applicable

Table A7.4.1.1-13: Dilution water.

Criteria	Details
Source	Tap water, community of Frankenthal, Germany non-chlorinated, charcoal filtered, aerated
Alkalinity	Not reported
Hardness	2.5 mmol/l = 250 mg/l $CaCO_3$
Oxygen content	Not reported
pH	7.5 – 8.5
Conductance	Approx. 550 µS/cm (at 25 °C)
Holding water different from dilution water	No

Table A7.4.1.1-14: Test organisms.

Criteria	Details
Species/strain	*Lepomis macrochirus* (Bluegill sunfish)
Source	Osage Catfisheries Inc., Osage Beach, MO 65065, USA
Wild caught	No
Age/size	1.7 – 2.5 cm (mean = 2.0 cm)
Kind of food	Commercial fish diet “Tetramin”, Tetra-Werke, Melle, Germany Commercial growing feed (“Forellenfutter [Zeigler]”; Provimi Kliba AG, Gossau, Switzerland); additionally on working days live *Artemia*; once a week live *Daphnia*
Amount of food	Commercial diets *ad libitum*
Feeding frequency	Commercial diets daily
Pre-treatment	14 d of acclimatisation
Feeding of animals during test	No

Table A7.4.1.1-15: Test system.

Criteria	Details
Test type	Semistatic
Renewal of test solution	Every 24 h
Volume of test vessels	50 l
Volume/animal	5 l
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1-16: Test conditions

Criteria	Details
Test temperature	22 – 24 °C throughout
Dissolved oxygen	Always above the required 60 % saturation; full details provided in Table A7.4.1.1-17.
PH	Details are given in Table A7.4.1.1-18.
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not stated
Photoperiod	16:8 h light/dark cycle

Table A7.4.1.1-17: Measurements of oxygen concentration [mg/l] during the test; measurements are given at the start (first value) and the end (second value) of each interval, respectively.

TS concentration, nominal [mg/l]	Measured concentrations [mg/l]			
	24 h	48 h	72 h	96 h
0.0	9.0–7.7	8.4–7.8	9.4–8.5	9.3–9.1
0.0	8.7–7.3	8.7–8.4	9.1–8.2	9.3–9.1
0.05	8.7–7.6	8.7–7.9	9.1–6.3	9.0–5.9
0.05	9.0–7.7	8.8–8.2	9.2–6.4	9.1–6.3
0.1	9.0–7.9	8.8–8.3	9.2–6.2	9.1–6.3
0.1	8.9–7.2	8.8–8.2	9.0–6.8	9.0–6.1
0.22	9.0–7.4	8.8–7.8	9.2–6.8	9.1–6.3
0.22	9.1–7.5	8.7–7.4	9.1–6.9	9.1–6.8
0.5	8.7–7.0	–	–	–
0.5	8.8–6.9	8.3–6.1	–	–
1.0	9.0–7.7	–	–	–
1.0	8.8–7.7	–	–	–

Table A7.4.1.1-18: Measurements of pH during the test; measurements are given at the start (first value) and the end (second value) of each interval, respectively.

TS concentration, nominal [mg/l]	Interval			
	24 h	48 h	72 h	96 h
0.0	7.8–8.0	7.9–7.9	7.8–7.8	7.7–7.7
0.0	7.9–8.0	7.8–7.7	7.7–7.7	7.7–7.7
0.05	7.9–7.9	7.7–7.6	7.7–7.6	7.6–7.6
0.05	7.8–7.9	7.7–7.6	7.6–7.6	7.6–7.6
0.1	7.7–7.9	7.7–7.6	7.6–7.6	7.6–7.6
0.1	7.8–7.9	7.7–7.6	7.6–7.6	7.6–7.5
0.22	7.8–7.9	7.6–7.6	7.6–7.6	7.6–7.5
0.22	7.8–7.9	7.6–7.6	7.6–7.6	7.6–7.5
0.5	7.8–7.8	–	–	–
0.5	7.8–7.9	7.8–7.7	–	–
1.0	7.9–8.0	–	–	–
1.0	7.9–8.0	–	–	–

Table A7.4.1.1-19: Analytical determinations of the test substance concentration [mg/l] by LCMS, at the start (immediately following renewal of the test water) and at the end of each observation interval.

TS concentration, nominal [mg/l]	Interval								
	24 h		48 h		72 h		96 h		Mean
	Start	*End*	*Start*	*End*	*Start*	*End*	*Start*	*End*	
0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.05	0.04531	0.03001	0.04409	0.04759	0.04360	0.03630	0.04247	0.04158	0.04137
0.05	0.04645	0.03479	0.05053	0.04567	0.04962	0.03819	0.04362	0.04569	0.04432
0.1	0.08606	0.05857	0.09129	0.09417	0.09774	0.07107	0.08070	0.07782	0.08218
0.1	0.08343	0.05795	0.07782	0.08971	0.10641	0.06974	0.07274	0.07933	0.07964
0.22	0.21390	0.15727	0.18023	0.18176	0.21183	0.14951	0.15870	0.16826	0.17768
0.22	0.22544	0.14784	0.17181	0.14954	0.20982	0.14493	0.18127	0.17395	0.17558
0.5	0.31442	0.35466	–	–	–	–	–	–	0.33454
0.5	0.36330	0.35359	0.38681	0.35311	–	–	–	–	0.36420
1.0	0.80857	0.64453	–	–	–	–	–	–	0.72655
1.0	0.77180	0.68080	–	–	–	–	–	–	0.72630

n.d. = not detected

Table A7.4.1.1-20: data; tests were conducted with 10 individuals per replicate per treatment (two replicates); if mortalities coincide between replicates, only a single value is given, otherwise the replicates are separated by a slash.

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
0.0	0	0	0	0	0	0	0	0
0.05	0	0	0	0	0	0	0	0
0.1	0	0	0	0/1	0	0	0	0/10
0.22	0	1/0	9/8	10	10/0	90/80	100	100
0.5	10/6	10	10	10	100/60	100	100	100
1.0	10	10	10	10	100	100	100	100

Temperature (°C) 22 – 24 °C
pH 7.5 – 8.0 (see Table A7.4.1.1-18)
Oxygen [mg/l] 5.9 – 9.2 mg/l (see Table A7.4.1.1-17)

Table A7.4.1.1-21: Effect data, based on the measured concentrations.

	48 h [mg/l]	95 % CI	96 h [mg/l]	95 % CI
LC_0	0.081	-	0.043	-
LC_{50}	0.234	-	0.112	-
LC_{100}	0.349	-	0.177	-

Table A7.4.1.1-22: Validity criteria for acute fish test according to OECD guideline 203.

	Fulfilled	Not fulfilled
Mortality of control animals <10%	☑	
Concentration of dissolved oxygen in all test vessels > 60% saturation	☑	
Concentration of test substance ≥80% of initial concentration during test	☑	
Criteria for poorly soluble test substances	☑	

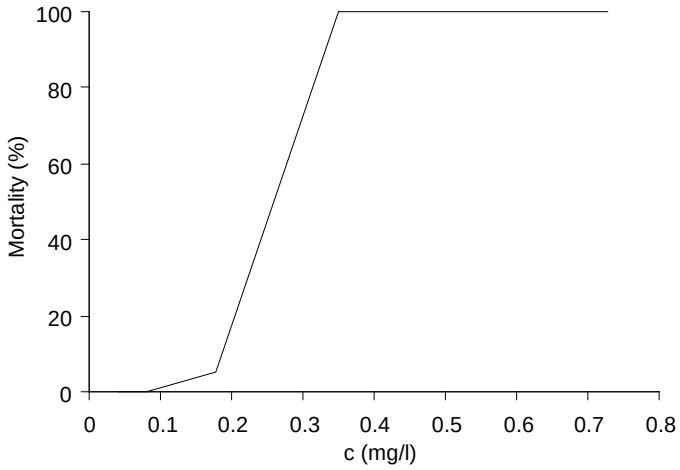

Figure A7.4.1.1-2: Concentration-response curve from the acute fish toxicity test of Flocoumafen in *Lepomis macrochirus.*

Section A7.4.1.2 Acute toxicity to invertebrates
Annex Point IIA7.2 (*Daphnia magna*)

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.4.1.2/01:** Jxxxx Jxxxx (2002) BAS 322 I – Determination of the acute effect on the swimming ability of the water flea *Daphnia magna* STRAUS. Bxxxx Axxxx, Exxxx Txxxx axxxx Exxxx, Lxxxx, Gxxxx, Report No. 01/0344/50/2, April 18, 2002 (unpublished). (BASF-Ref.: 200/1004896)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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 202 US-EPA OPPTS 850.1010	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes See 3.4.3	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	AC12140-35	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	99.4 %	
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water, with a solubility of 0.11 mg/l at pH 7 and 14 mg/l at pH 9 (see Section A3.5); thus, the test substance was dissolved using acetone as a solubilising agent (see 3.2).	
3.1.5	Method of analysis	LC/MS	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Yes Details are presented in Table A7.4.1.2-1. Furthermore, to prevent substance loss by adsorption, the test vessel walls were saturated with the test substance by flushing with diluted stock solution.	X

Section A7.4.1.2 Acute toxicity to invertebrates
Annex Point IIA7.2 (*Daphnia magna*)

3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.1.2-2.	
3.4.2	Test organisms	*Daphnia magna*, as specified in Table A7.4.1.2-3.	
3.4.3	Test system	As described in Table A7.4.1.2-4. The test was performed as a semistatic test.	
3.4.4	Test conditions	As specified in Tables A7.4.1.2-5 through 7.	X
3.4.5	Duration of the test	48 h	
3.4.6	Test parameter	Immobilisation of test organisms.	
3.4.7	Sampling	Samples for analytical verification of test substance concentrations were drawn from the fresh solutions (at 0 and 24 h), and at 24 and 48 h from the used solutions.	
3.4.8	Monitoring of TS concentration	Yes See above (3.4.7)	
3.4.9	Statistics	EC_{50} was determined graphically on log-probit paper.	

4 RESULTS

4.1	**Limit 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	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations were: 0.0 (controls), 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/l.	
4.2.2	Actual concentrations of test substance	Measured concentrations of the test substance per cent maintenance of initial concentrations are presented in Table A7.4.1.2-8.	X
4.2.3	Effect data (Immobilisation)	Effect data are presented in Table A7.4.1.2-10.	X
4.2.4	Concentration-response curve	See Figure A7.4.1.2-1.	

Section A7.4.1.2 Acute toxicity to invertebrates
Annex Point IIA7.2 (*Daphnia magna*)

4.2.5	Other effects	No other effects observed	
4.3	**Results of controls**	In the blank control, no *Daphnia* were immobilised; in the solvent control, one immobile individual (5 %) after 48 h occurred (also see Table A7.4.1.2-9). No daphnids were trapped at the surface.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute toxicity of flocoumafen to aquatic invertebrates was tested by the *Daphnia* immobilisation test according to OECD guideline 202. Acetone was used as a solubilising agent at a final concentration of 0.01 %. In the solvent control, one individual (5 %) was immobile, which is below the 10 % criterion for validity. The test was performed using a semistatic design, with renewal of the test solution after 24 hours. Actual TS concentrations were monitored, and concentrations corrected accordingly for the estimation of EC values. The failure of the ≥ 80 % concentration maintenance criterion was accounted for by taking the mean of the measurements after 0, 24, and 48 h as a basis for estimation.	
5.2	**Results and discussion**	Discrepancies between nominal and measured TS concentrations were attributed to the poor water solubility of flocoumafen in the analytical report. Indeed, at least the higher concentrations are beyond the range of solubility (cf. Section A3.5). The substance is hydrolytically stable (Section A7.1.1.1.1) and non-volatile (Section A3.2). Substance loss by adsorption was attempted to be prevented by rinsing the test vessels with flocoumafen stock solution. The validity criteria are only partly fulfilled (Table A7.4.1.2-11). Despite the semistatic design, chosen in order to counterbalance potential substance loss, it was not possible to maintain TS concentrations within 80 % of the initial levels. To account for this problem, the mean of the 0 h, 24 h, and 48 h measurements was applied in parameter estimation. Since the estimated EC values are based on measured concentrations, they can be considered valid in regard of the potential solubility problems discussed above. The corresponding results are summarised below.	X
5.2.1	EC_0	0.11 mg/l	X
5.2.2	EC_{50}	0.17 mg/l	X
5.2.3	EC_{100}	0.28 mg/l	
5.3	**Conclusion**	The dose-response curve shows a steep response, which is why calculation of confidence intervals for the EC_{50} estimate was not feasible.	X

Section A7.4.1.2 Acute toxicity to invertebrates
Annex Point IIA7.2 (*Daphnia magna*)

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	11 January 2005
Materials and Methods	(2.3) “See 3.4.3” should be “See 5.2” (3.2) The test vessels were saturated with the respective test solutions for 20 hours. (3.4.4) Table A7.4.1.2-6 contains two incorrect values for pH. The values for 0.5 and 1.0 mg/l at 48 hours should be 8.0 and 8.0. (4.2.2) Mean measured concentrations were not reported, and were calculated by RMS as the arithmetic mean of the measured concentrations in the 0-h, 24-h aged, 24-h new and 48-h aged solutions (excluding the measured concentrations at nominal 0.125 mg/l during the first 24-hour interval, which is in agreement with the recommendation by the author of the report). The mean measured concentrations calculated in this way by the RMS were 0.025, 0.068, 0.12, 0.28 and 0.62 mg/L. (4.2.3) The reported 24h- and 48h-EC_0 value was 0.11 mg/l, and he reported 24h- and 48h-EC_{50} values were 0.29 and 0.17 mg/l (all based on mean measured concentrations). As the mean measured concentrations, on which reported EC-values were based, were not reported, EC_{50}-values were recalculated by the RMS based on the recalculated mean measured concentrations (see 4.2.2 above) using Toxstat (trimmed Spearman Karber). This gave 24h- and 48h-EC_{50} values of 0.28 and 0.18 mg/l, respectively. Based on the presence of an immobile daphnia at 0.120 mg/l, taken to represent the start of the dose-response curve, the EC_0 values were set at 0.068 mg/l.

Results and discussion	(5.2) The author of the report stated that "The poor recoveries of Reg. No. 4060804 in M4 water can be explained by exceeding the solubility of the test substance in water." The water solubility of flocoumafen is 0.11 mg/L at pH 7 and 14 mg/L at pH 9. The pH of the test solutions was 7.8-8.1, hence the solubility is expected to be in excess of 0.11 mg/L, in case sufficient measures are taken to maximise the solubility in test medium (e.g. sufficient stirring). Possibly all tested concentrations are below the solubility limit. The explanation by the author of the report is not sufficient in any case, since recoveries at concentrations at and below the solubility limit (i.e. nominal concentrations of 0.0625 and 0.125 mg/l) were also not in agreement with nominal concentrations in freshly prepared solutions of day 0. The low recoveries may therefore also be attributable to inadequate preparation of the test solutions at the start of the test. It is furthermore noted that, besides chromatograms, no validation was presented for the analytical method. A full description of the analytical method was not provided ("LCMS"). It appeared that samples of test solutions following defrosting were directly injected onto the HPLC system. No information was available to verify that any precipitate formed during freezing of samples was redissolved. Sorption of flocoumafen to sample containers may also have contributed to the low recoveries. (5.2.1) Based on the presence of an immobile daphnia at 0.120 mg/l, the EC_0 is 0.068 mg/l. (5.2.2) Based on mean measured concentrations, the EC_{50} is 0.18 mg/l.
Conclusion	(5.3) The dose-response curve shows a steep response, which is why calculation of confidence intervals for the EC_{50} estimate was not feasible. The EC_{50} was 0.18 mg/l.
Reliability	2 (see point 5.2 above)
Acceptability	Acceptable
Remarks	Erratic concentration measurements occurred at 0.0625 and 0.125 mg/L during the first 24-hour interval. It is considered acceptable, in agreement with the procedure followed in the report, to omit the measurements at 0.125 mg/L during the first 24-hour interval from the calculation of the overall mean (the 0-hour value being an outlyer). The results at 0.0625 mg/L are not relevant for the calculation of the EC_{50} (0% immobility at this and the next higher concentration). The calculated 48-hour EC_{50} value (0.18 mg/L) is therefore acceptable.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	

Acceptability	
Remarks	

Table A7.4.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes Acetone
Concentration of vehicle	0.01 %
Vehicle control performed	Yes
Other procedures	Not applicable

Table A7.4.1.2-2: Dilution water.

Criteria	Details
Source	Reconstituted water “M4”, according to ISO 10706
Alkalinity	0.8 – 1.0 mmol/l
Hardness	2.37 mmol/l
pH	8.2
Ca/Mg ratio	Approx. 4:1
Na/K ratio	Not reported
Oxygen content	Not reported
Conductance	626 µS/cm
Holding water different from dilution water	No

Table A7.4.1.2-3: Test organisms.

Criteria	Details
Strain	Not specified Origin: Institut National de Recherche Chimique Appliquée, France
Source	Laboratory of Experimental Toxicology and Ecology, BASF AG, Ludwigshafen
Age	2 – 24 h
Breeding method	Not reported
Kind of food	Not reported
Amount of food	Not reported
Feeding frequency	Not reported
Pre-treatment	None
Feeding of animals during test	No

Table A7.4.1.2-4: Test system.

Criteria	Details
Renewal of test solution	Yes, after 24 h
Volume of test vessels	200 ml (volume of test solution)
Volume/animal	40 ml
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

Criteria	Details
Test temperature	20.5 – 22.3 °C
Dissolved oxygen	Data are presented in Table A7.4.1.2-6
PH	Data are presented in Table A7.4.1.2-7
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Warm white artificial light approx. 1 – 8 µE/m^2 × s
Photoperiod	16:8 h light/dark cycle

Table A7.4.1.2-6: Measurements of oxygen concentrations (mg/l) in the test solution at initiation and termination of the test.

Concentration of TS, nominal (mg/l)	Oxygen concentration (mg/l)	
	at 0 h	at 48 h
0.0 (control)	8.8	9.2
0.0 (solvent control)	8.8	8.1
0.0625	8.8	8.4
0.125	8.6	8.5
0.25	8.7	8.4
0.5	8.8	8.5
1.0	8.7	8.4

Table A7.4.1.2-7: Measurements of pH in the test solution at initiation and termination of the test.

Concentration of TS, nominal (mg/l)	pH	
	at 0 h	at 48 h
0.0 (control)	8.1	8.1
0.0 (solvent control)	8.1	8.0
0.0625	8.1	7.9
0.125	8.1	7.9
0.25	8.0	7.9
0.5	8.1	7.9
1.0	8.1	7.8

Table A7.4.1.2-8: Analytical determinations of the test substance concentration (mg/l) by LCMS; each value represents the mean of two measurements; the test substance was not detected in the controls.

TS concentration, nominal (mg/l)	Measured concentrations (mg/l)					
	0 h	24 h (old)	% of initial	24 h (new)	48 h	% of initial
0.0625	0.0189	0.0177	93.6	0.0491	0.0134	27.3
0.125	0.0021*	0.0351	–	0.1107	0.0261	23.6
0.25	0.1602	0.0933	58.3	0.1766	0.0489	27.7
0.5	0.311	0.2041	65.6	0.4024	0.1997	49.6
1.0	0.7483	0.542	72.4	0.7431	0.4658	62.7

*) this value was considered an outlier and was therefore discarded for the estimation of EC-values.

Table A7.4.1.2-9: Immobilisation data of *Daphnia magna*; each value represents the cumulative number of immobile individuals across the four replicates.

TS concentration, nominal (mg/l)	Immobile *Daphnia*						
	Number		Percentage		Oxygen (mg/l)	pH	Temperature (°C)
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0.0 (control)	0	0	0	0	9.2	8.1	–*
0.0 (solvent control)	0	1	0	5	8.1	8.0	–
0.0625	0	0	0	0	8.4	7.9	–
0.125	0	0	0	0	8.5	7.9	–
0.25	1	1	5	5	8.4	7.9	–
0.5	9	20	45	100	8.0	7.9	–
1.0	20	20	100	100	8.0	7.8	–

*) Temperature data specifically for test termination were not reported

Table A7.4.1.2-10: Effect data, expressed as effective concentrations (corrected for substance loss).

	EC_{50}	95 % CI	EC_0	EC_{100}
24 h (mg/l)	0.29	–	0.11	0.63
48 h (mg/l)	0.17	–	0.11	0.28

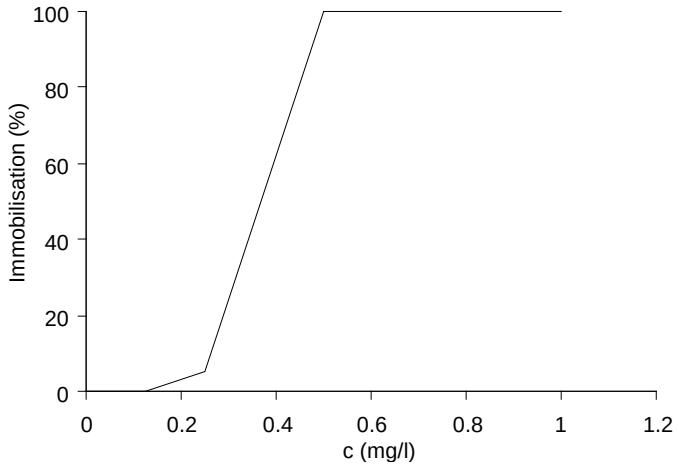

Figure A7.4.1.2-1: Concentration-response curve, based on the nominal concentrations, of the acute invertebrate toxicity test with *Daphnia magna*.

Table A7.4.1.2-11: Validity criteria for acute *Daphnia* immobilistaion test according to OECD guideline 202.

	Fulfilled	**Not fulfilled**
Immobilisation of control animals <10%	☑	
Control animals not staying at the surface	☑	
Concentration of dissolved oxygen in all test vessels >3 mg/l	☑	
Concentration of test substance ≥ 80% of initial concentration during test		☑
Criteria for poorly soluble test substances	☑	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.1.3/01:** Jxxxx Jxxxx (2002) BAS 322 I – Determination of the inhibitory effect on the cell multiplication of unicellular green algae. Bxxxx Axxxx, Exxxx Txxxx axxxx Exxxx, Lxxxx, Gxxxx, Report No. 01/0344/60/1, April 17, 2002 (unpublished). (BASF-Ref.: 2002/1004879)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 201 EC method C.3
2.2	**GLP**	Yes
2.3	**Deviations**	Yes See 3.4.7

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	AC12140-35
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.4 %
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water, with a solubility of 0.11 mg/l at pH 7 and 14 mg/l at pH 9 (see Section A3.5); thus, the test substance was dissolved using Cremophor RH 40 as a solubilising agent (see 3.2).
3.1.5	Method of analysis	LC/MS See Section A4.2 for standard methods.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Yes Details are presented in Table A7.4.1.3-1. Furthermore, to prevent substance loss by adsorption, the test vessel walls were saturated with the test substance by flushing with diluted stock solution.

X

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Culture medium	As prescribed by EC method C.3.	
3.4.2	Test organisms	*Pseudokirchneriella subcapitata*, as described in Table A7.4.1.3-2.	
3.4.3	Test system	See Table A7.4.1.3-3.	
3.4.4	Test conditions	Details are presented in Table A7.4.1.3-4.	X
3.4.5	Duration of the test	72 h	
3.4.6	Test parameter	Inhibition of growth rate and biomass accumulation.	
3.4.7	Sampling	Every 24 h: Biomass was estimated by measuring *in vivo* Chlorophyll-a fluorescence. After 72 h: Estimation of cell density using a counting chamber (Neubauer improved).	
3.4.8	Monitoring of TS concentration	Yes Prior to inoculation and at termination of the test.	
3.4.9	Statistics	Comparison of areas under the growth curve and comparison of growth rates, according to the procedures specified in EC method C.3. E_bC_{50} and E_rC_{50} estimated by linear regression.	

4 RESULTS

4.1	**Limit test**	Not performed	
4.1.1	Concentration		
4.1.2	Number/ percentage of animals showing adverse effects		
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	The measured initial concentrations, as determined by LCMS, are given in Table A7.4.1.3-6.	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

4.2.2	Actual concentrations of test substance	The measured concentrations at test termination, as determined by LCMS, are given in Table A7.4.1.3-6. It should be noted that a considerable increase of test substance concentrations during the course of the test was found, except for the two highest concentration levels, where concentrations apparently decreased.	
4.2.3	Growth curves	Graphical figures of the growth curves are provided in the original study.	
4.2.4	Concentration-response curve	Concentration-response curves are presented in Figure A7.4.1.3-1.	
4.2.5	Cell concentration data	Data are given in Table A7.4.1.3-7.	X
4.2.6	Effect data (cell multiplication inhibition)		X

	$c_{nominal}$ [mg/l]	$c_{corrected}$ [mg/l]
E_bC_{10}	6.63	1.21
E_bC_{50}	> 100	> 100
E_bC_{90}	> 100	> 100
E_rC_{10}	> 100	> 100
E_rC_{50}	> 100	> 100
E_rC_{90}	> 100	> 100

4.2.7	Other observed effects	None	
4.3	**Results of controls**	Data are included in Table A7.4.1.3-7.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The inhibitory effect of Flocoumafen on the growth of green algae was tested using *Pseudokirchneriella subcapitata*, according to OECD guideline 201 and EC method C.3. Cell densities were estimated indirectly by measuring Chlorophyll-a fluorescence. Concentrations of the test substance were monitored by LCMS. Test vessel walls were saturated with Flocoumafen by rinsing with stock solution, as a preventive measure against substance loss by adsorption.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

5.2	**Results and discussion**	Flocoumafen is hydrolytically stable (Section A7.1.1.1.1) and non-volatile (Section A3.2), but potential adsorption has to be considered. However, Flocoumafen is poorly soluble in water (see Section A3.5): The upper limit of the tested concentration range exceeds the solubility at pH 9 almost by the factor 10, and solubility at pH 7 approximately by 1000. The corresponding test concentration was realised by using Cremophor RH 40 as solubilising agent. Inadvertently, nominal concentrations were not maintained. The discrepancy between nominal and measured concentrations was taken into account as follows: statements were made about the variation of measured concentrations at the start and the end of the test. Then, the mean (not median, as stated in the report) percentage measured concentration (18.2 %) at 100 mg/l was applied to all other concentration levels as a correction factor. This procedure of establishing a correction factor appears acceptable in terms of risk assessment since it intrinsically underestimates EC-values (i.e. overestimates toxicity) and thereby entails a safety margin of unknown order. The results summarised below are ascribed to the aforementioned correction factor of 18.2 %. While the above procedure seems scientifically somewhat questionable, its impact on the interpretability of the results is only marginal: Even if the stated effective concentration of > 18.2 mg/l is a rather inaccurate and negatively biased estimate, it is evident that any concentration that could produce adverse effects in green algae lies beyond the solubility of Flocoumafen.	
5.2.1	NOE_rC (0 – 72 h)	> 18.2 mg/l	X
5.2.2	E_rC_{50} (0 – 72 h)	> 18.2 mg/l	
5.2.3	E_bC_{50} (0 – 72 h)	> 18.2 mg/l	
5.3	**Conclusion**	The discussed deficiencies are considered to result in an overestimate of toxicity, which is deemed uncritical for risk assessment. The dose-response relationship shows that only minor growth inhibition occurred within the tested concentration range.	X
5.3.1	Reliability	2	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

5.3.2	Deficiencies	Yes The derivation of a correction factor form nominal to measured concentrations may be questioned, but is nevertheless considered acceptable in view of the demonstrated low toxicity to algae and the adsorption of the test substance. Furthermore, there are several reporting deficiencies: - The cultivation method is described insufficiently - No information on the culturing apparatus is given - Light quality is not specified in detail (e.g. colour temperature) - The procedure to keep the algae suspended is not reported - No information on aeration of the test suspension is given - Validation data for fluorescence measurements (based on cell densities estimated using a counting chamber) are not reported - Description of the equipment for fluorescence measurement and justification of the method would be desirable - Insufficient description of statistical methods - Table 3 of the report is rather cryptic (e.g. untraceable references to “after 72 hours” and “uninoculated”) In consequence, this study is considered valid with restrictions.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	14 January 2005
Materials and Methods	(3.2) Table A7.4.1.3-1 “Dispersion – No” should be “Dispersion – Yes”, since the test solution with 100 mg/l was turbid and the solutions at 12.5-50 mg/l were slightly turbid. (3.4.4) Table A7.4.1.3-5, pH at 0 h, concentrations 0.39, 0.78 and 1.56 should read 7.8, 7.8 and 7.8.
Results and discussion	(4.2.5) Table A7.4.1.3-7, footnote pH should read 7.8-8.7. (4.2.6) $c_{corrected}$ should be $c_{measured}$. EbC_{50}, EbC_{90}, ErC_{10}, ErC_{50} and ErC_{90}, $c_{measured}$ should read >18.2 mg/l. (5.2.1) NOErC (0-72 h) should read ≥18.2 mg/l instead of >18.2 mg/l. The NOEbC is 1.7 mg/l (calculated by RMS using Toxstat, Bonferroni t-test and Tukey test, based on mean measured concentrations).
Conclusion	(5.3) The dose-response relationship shows that only minor growth inhibition occurred within the tested concentration range. The ErC_{50} and EbC_{50} are >18.2 mg/l, the72-h NOErC is ≥18.2 mg/l and the 72-h NOEbC is 1.7 mg/l.
Reliability	2 (see applicant's summary 5.3.2)
Acceptability	Acceptable
Remarks	(1) The increase in test concentrations during the test period, which was recorded at nominal concentrations of 6.25 mg/L and below, may be associated with increased solubility of flocoumafen due to the rise in pH during the test. (2) The procedure to correct all nominal test concentrations by 18.2% (percentage measured at the highest test concentration of 100 mg/L) is considered to be scientifically incorrect. However, for the key results (ErC_{50} and EbC_{50}) consideration of the results for the control and the highest tested concentration alone is sufficient.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.3-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes Cremophor RH 40
Concentration of vehicle	100 mg/l
Vehicle control performed	Yes
Other procedures	Not applicable

Table A7.4.1.3-2: Test organisms.

Criteria	Details
Species	*Pseudokirchneriella subcapitata*
Strain	SAG 61.81
Source	Collection of algal cultures, University of Göttingen, Germany
Laboratory culture	Yes
Method of cultivation	Liquid culture, not further specified
Pre-treatment	1) Preparation of a seed culture: incubation for 7 d at 23 ± 2 °C final cell density = 390×10^4 ml^{-1} 2) Pre-culture: incubation for 3 d at 23 ± 2 °C final cell density = 65×10^4 ml^{-1}
Initial cell concentration	10^4 ml^{-1}

Table A7.4.1.3-3: Test system.

Criteria	Details
Volume of culture flasks	250 ml
Volume of test solution	100 ml
Culturing apparatus	Not reported
Light quality	Artificial illumination, type universal white (Osram® L 25)
Procedure for suspending algae	Not reported
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	23 ± 2 °C
pH	Details are given in Table A7.4.1.3-5.
Aeration of dilution water	Not reported
Light intensity	60 – 120 μE × m^{-2} × s^{-1}
Photoperiod	Permanent

Table A7.4.1.3-5: Measurements of pH in the test solution at initiation and termination of the test.

Concentration of TS, nominal (mg/l)	pH	
	at 0 h	at 72 h
0.0 (control)	8.0	8.0
0.0 (solvent control)	8.0	8.0
0.39	8.0	8.0
0.78	7.9	8.0
1.56	7.9	8.7
3.13	7.9	8.5
6.25	7.9	8.4
12.5	7.9	8.5
25	7.9	8.5
50	7.9	8.4
100	7.9	8.4

Table A7.4.1.3-6: Analytical determinations of the test substance concentration (mg/l) by LCMS; each value represents the mean of two measurements; the test substance was not detected in the controls.

TS concentration, nominal (mg/l)	Measured concentrations				
	Start (mg/l)	Start (% of nominal)	End (mg/l)	End (% of nominal)	% of initial
0.39	0.094	24.0	0.23	57.8	244
0.78	0.13	16.1	0.25	32.2	192
1.56	0.29	18.8	0.46	29.2	159
3.13	0.70	22.5	1.25	40.1	179
6.25	1.56	25.0	1.82	29.1	117
12.5	3.14	25.1	3.22	25.7	103
25	4.61	18.4	4.02	16.1	87
50	10.67	21.3	7.53	15.1	71
100	24.51	24.5	11.87	11.9	48

Table A7.4.1.3-7: Cell density data; data are given as relative units (means of three replicates) from the measurement of Clorophyll-a fluorescence; percent values are given relative to the solvent control.

Test substance concentration, nominal (mg/l)	Cell concentrations							
	Measured				Percent of control			
	0 h	*24 h*	*48 h*	*72 h*	*0 h*	*24 h*	*48 h*	*72 h*
0.0 (solvent control)	32	188	1348	5386	100	100	100	100
0.0 (blank control)	32	172	1049	4753	98	92	78	88
0.39	33	176	1427	5735	102	94	106	106
0.78	32	174	1296	5140	100	93	96	95
1.56	33	175	1436	5250	101	93	107	97
3.13	32	170	1330	4893	99	91	99	91
6.25	33	172	1314	4699	101	92	97	87
12.5	32	147	1179	4525	98	79	87	84
25	34	129	1115	3892	106	69	83	72
50	34	140	1027	3658	105	75	76	68
100	32	156	1099	3731	100	83	82	69
Temperature (°C)	23 ± 2 °C							
pH	7.9 – 8.7 (see Table A7.4.1.3-5)							

Table A7.4.1.3-8: Validity criteria for algal growth inhibition test according to OECD guideline 201.

	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	☑	
Concentration of test substance ≥ 80 % of initial concentration during test		☑
Criteria for poorly soluble test substances		☑

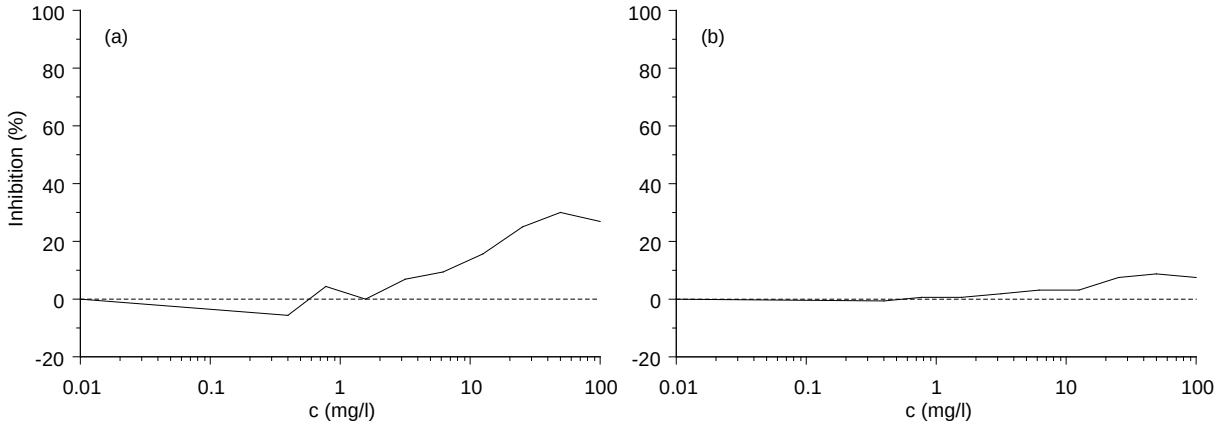

Figure A7.4.1.3-1: Concentration-response-curves of growth inhibition of the green alga *Pseudokirchneriella subcapitata* by Flocoumafen within 72 h: a) inhibition of biomass accumulation; b) inhibition of growth rate.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.1.4/01:** Hxxxx Sxxxx, Cxxxx Vxxxx (2002) BAS 322 I (Flocoumafen): activated sludge, respiration inhibition test. Axxxx Lxxxx, Ixxxx, Cxxxx, Uxxxx, Report No. 46797, February 4, 2002 (unpublished). (BASF-Ref.: FL-590-002)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 209
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Number of test concentrations (see 4.2.1.).

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	AC 12140-35
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.4 %
3.1.4	Further relevant properties	Flocoumafen is poorly soluble in water (see Section A3.5). Methods to cope with this property are described under 3.2.
3.1.5	Method of analysis	Not appropriate.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Instead of preparing a stock solution, the test substance was weighed to a cover slip and directly added to the test system. Controls received a blank cover slip.
3.3	**Reference substance**	3,5-dichlorophenol
3.3.1	Method of analysis for reference substance	Not appropriate

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic sewage feed, prepared in compliance with OECD 209.	
3.4.2	Inoculum/ test organism	See Table A7.4.1.4-1.	
3.4.3	Test system	See Table A7.4.1.4-2.	
3.4.4	Test conditions	Test conditions are presented in Table A7.4.1.4-3.	X
3.4.5	Duration of the test	3 h	
3.4.6	Test parameter	Inhibition of respiration	
3.4.7	Analytical parameter	Oxygen concentration	
3.4.8	Sampling	Continuous recording of oxygen concentration over 6 – 10 min, to achieve a section of linear response.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Two blank controls, abiotic control	
3.4.11	Statistics	Per cent respiration inhibition, as described in OECD guideline 209.	

4 RESULTS

4.1	**Preliminary test**	Not performed	
4.1.1	Concentration		
4.1.2	Effect data		
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 0, 0.5, 1.0, 2.0, and 4.0 mg/l	
4.2.2	Actual concentrations of test substance	No analytical monitoring performed.	
4.2.3	Growth curves	Not appropriate	
4.2.4	Oxygen consumption data	Respiration rates are presented in Table A7.4.1.4-5.	
4.2.5	Concentration/ response curve	Not appropriate There was no uniform response to treatment with Flocoumafen.	
4.2.6	Effect data	No inhibitory effect observed within the tested concentration range. EC_{50} > 4.0 mg/l	
4.2.7	Other observed effects	Increased respiration rates at three of the four tested concentrations (also see Table A7.4.1.4-5).	
4.3	**Results of controls**	See Table A7.4.1.4-5. Respiration rates of controls varied by < 15 %.	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	3.2, 10.0, and 32.0 mg/l	
4.4.2	Results	For respiration rates and inhibition values see Table A7.4.1.4-5. EC_{50} = 13 mg/l (95 % CI = 9 – 19)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Inhibitory effects of Flocoumafen on microbial activity were tested by the activated sludge respiration inhibition test, following OECD guideline 209. Deviating from the guideline, four instead of "at least five" concentrations were tested. However, this may be considered appropriate in view of the poor water solubility of the test substance, and does not affect the validity of the test.	
5.2	**Results and discussion**	Microbial respiration was not inhibited by Flocoumafen up to the maximum tested concentration of 4.0 mg/l. Flocoumafen is poorly soluble in water (see Section A3.5). Thus, it seems likely that realisation of the higher nominal concentrations failed. Nevertheless, the results are considered valid without any restrictions, since the study convincingly demonstrated the lack of inhibitory effects within the range of the water solubility of the test substance.	
5.2.1	EC_{20}	EC_{20} was indeterminable (> 4.0 mg/l).	
5.2.2	EC_{50}	EC_{50} was indeterminable (> 4.0 mg/l).	
5.2.3	EC_{80}	EC_{80} was indeterminable (> 4.0 mg/l).	
5.3	**Conclusion**	The abiotic control indicated absence of chemical oxygen demand. The respiration rates of the controls varied by less than 15 %. The EC_{50} of the reference substance was within the acceptable range of 5 to 30 mg/l. Thus, the test fulfils all validity criteria. Establishment of a dose-response relationship is not appropriate.	X
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	14 January 2005
Materials and Methods	(3.4.4) During the 3-hour contact time, test solutions were not only aerated but also continuously stirred using a magnetic stirring rod.
Results and discussion	No comments
Conclusion	(5.3) Establishment of a dose-response relationship is not appropriate. The EC_{50} is >4.0 mg/l
Reliability	1
Acceptability	Acceptable
Remarks	No remarks.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.4-1: Inoculum/Test organism.

Criteria	**Details**
Nature	Activated sludge
Species	Mixed species population
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Aeration basin STP of Columbia, Missouri, USA
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Threefold washing with tap water and centrifugation; resuspended in the laboratory's well water and aerated.
Pre-treatment	No
Initial cell concentration	4.0 g/l suspended solids (inoculum)

Table A7.4.1.4-2: Test system.

Criteria	Details
Culturing apparatus	1000 ml glass flasks
Number of culture flasks/concentration	1
Aeration device	in-house oil-free compressed air system
Measuring equipment	300 ml BOD flasks YSI model 58 dissolved oxygen meter; Cole-Parmer model 2020 chart recorder; Accumet model 50 pH meter
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4-3: Test conditions.

Criteria	Details
Test temperature	20 ± 2 °C
pH	see separate Table A7.4.1.4-4
Aeration of dilution water	Yes at 0.8 – 1.0 l/min
Suspended solids concentration	1.6 g/l

Table A7.4.1.4-4: pH values in the test flasks, determined at test termination.

c (mg/l)	pH
Test substance	
0.0 (Control 1)	8.46
0.0 (Control 2)	8.40
0.5	8.27
1.0	8.44
2.0	8.28
4.0	8.42
Abiotic control	8.74
Reference substance	
3.2	8.46
10	8.51
32	8.55

Table A7.4.1.4-5: Respiration rates and percent inhibition values for Flocoumafen, controls and the reference substance 3,5-dichlorophenol.

c (mg/l)	Respiration rate (mg O_2 / l × h)	% inhibition*)
Test substance		
0.0 (Control 1)	41	—
0.0 (Control 2)	42	—
0.5	72	–74
1.0	40	5
2.0	53	–28
4.0	43	–4
Abiotic control	0	not applicable
Reference substance		
3.2	38	10
10	24	43
32	10	76

*) negative values indicate stimulating effects

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.2/01:** Sxxxx Txxxx (2003) Estimation of the bioconcentration factor (BCF) of Flocoumafen. Exxxx Cxxxx Gxxxx, Hxxxx, Gxxxx, Report dated July 31, 2003 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
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/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not applicable
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	Not applicable
3.1.2	Specification	Not applicable
3.1.3	Purity	Not applicable
3.1.4	Further relevant properties	Not applicable
3.1.5	Method of analysis	Not applicable
3.2	**Reference substance**	Not applicable
3.2.1	Method of analysis for reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Test system/ performance	Not applicable

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

3.3.2	Estimation of bioconcentration	On the basis of log P_{ow}, as specified in the TGD on risk assessment. Experimentally determined log P_{ow} values are reported in reference A3.9/01. log P_{ow} (pH 7) = 6.12 log P_{ow} (pH 9) = 5.11	
		4 RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/ behaviour	Not applicable	
4.1.2	Lipid content	Not applicable	
4.1.3	Concentrations of test material during test	Not applicable	
4.1.4	Bioconcentration factor (BCF)	Not applicable	
4.1.5	Uptake and depuration rate constants	Not applicable	
4.1.6	Depuration time	Not applicable	
4.1.7	Metabolites	Not applicable	
4.1.8	Other Observations	Not applicable	
4.2	**Estimation of bioconcentration**	pH 7: log *BCF* = 4.5 pH 9: log *BCF* = 3.6	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Estimation of the bioconcentration factor (BCF) based on log P_{ow}, as specified by the TGD on risk assessment.	
5.2	**Results and discussion**	Based on experimentally determined partition coefficients (6.12 for pH = 7, 5.11 for pH = 9), bioconcentration factors were estimated at log *BCF* = 4.5 (pH 7) log *BCF* = 3.6 (pH 9).	X
5.3	**Conclusion**	Since the estimation was performed using an officially recommended method, based on measured values determined by fully valid experimental procedures, this calculation is considered valid without restrictions.	X
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	9 December 2004
Materials and Methods	No comments.
Results and discussion	(4.2 & 5.2) The formula used in the report was log BCF_{fish} = 0.85 x logKow – 0.70. The TGD part II specifies under point 3.8.3.2 that this formula applies to substances with logPow in the range 2-6. For substances with logPow >6 the following formula applies: log BCF_{fish} = -0.20 x $logKow^2$ + 2.74 x logKow – 4.72. The former formula should be used for pH 9 (logPow = 5.11), the latter for pH 7 (logPow = 6.12). The overall results (recalculated for pH 7 by RMS) are as follows: pH 7: log BCF = 4.6 (BCF = 36134) pH 9: log BCF = 3.6 (BCF = 4400).
Conclusion	pH 7: log BCF = 4.6 (BCF = 36134) pH 9: log BCF = 3.6 (BCF = 4400)
Reliability	No comments.
Acceptability	Acceptable taking into consideration above comments.
Remarks	No comments.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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

Annex Point IIIA 13.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to the intended use pattern (application in and around buildings only) and the properties of the biocidal product, significant exposure of the aquatic environment seems unlikely. The product is a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Consequently, long-term exposure of the aquatic environment to flocoumafen is not expected. A prolonged toxicity study in fish is not considered to be required.	
Undertaking of intended 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 (*)
Date	30 June 2005
Evaluation of applicant's justification	Long-term exposure is not expected during the use and waste phase of the product. Non-submission is acceptable.
Conclusion	The study is not necessary.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

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

Annex Point IIIA 13.2.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to the intended use pattern (application in and around buildings only) and the properties of the biocidal product, significant exposure of the aquatic environment seems unlikely. The product is a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Long-term exposure of the aquatic environment to flocoumafen is not expected. A study on effects on reproduction and growth rate in fish is not considered to be required.	
Undertaking of intended 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 (*)
Date	30 June 2005
Evaluation of applicant's justification	Long-term exposure is not expected during the use and waste phase of the product. Non-submission is acceptable.
Conclusion	The study is not necessary.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.3.1 Bioaccumulation in an appropriate species of fish

Annex Point IIIA 13.2.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The bioconcentration potential has been estimated based on log P_{ow} (Section A7.4.2).. According to the envisaged use pattern (use in and around buildings), release of the active substance to surface waters is very unlikely. This is supported by the properties of the biocidal product, a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Thus, there appears to exist no risk for secondary poisoning in the aquatic environment. A bioaccumulation study in fish is not considered to be required.	
Undertaking of intended 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 (*)
Date	2 September 2008
Evaluation of applicant's justification	Release to surface water, and hence exposure due to secondary poisoning via the aquatic food chain, can be assumed to be negligible. In the PBT analysis it appears that flocoumafen fulfils the all three criteria. For refinement of the analysis a bioaccumulation study is considered optional..
Conclusion	A bioaccumulation study in fish is considered optional to refine the PBT assessment.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.3.2 Bioaccumulation in an appropriate invertebrate species

Annex Point IIIA 13.2.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The bioconcentration potential has been estimated based on log P_{ow} (Section A7.4.2). An experimental study would only be appropriate if direct release to marine or brackish water was likely, as outlined in the TNG on data requirements. However, according to the envisaged use pattern (use in and around buildings), release of the active substance to surface waters is very unlikely. This is supported by the properties of the biocidal product, a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Thus, direct release to marine or brackish water is not expected and a bioaccumulation study in invertebrates is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	Any significant direct release to marine or brackish water is considered to be unlikely. Therefore the waiver is accepted.
Conclusion	The waiver is accepted.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

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

Annex Point IIIA 13.2.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to the intended use pattern (application in and around buildings only) and the properties of the biocidal product, significant exposure of the aquatic environment seems unlikely. The product is a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Long-term exposure of the aquatic environment to flocoumafen is not expected. Testing of effects on reproduction and growth in invertebrates is therefore not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	Long-term exposure is not expected during the use and waste phase of the product. Non-submission is acceptable.
Conclusion	The study is not necessary.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The acute toxicity of Flocoumafen in fish (A7.4.1.1 and A7.4.3.1), daphnia (A7.4.1.2 and A7.4.3.4) and algae (A7.4.1.3) was investigated in detail, so that sufficient data are available to allow classification and labelling of the active ingredient Flocoumafen according to the requirements of Annex VI of directive 67/548/EEC. Whereas the use in sewers or cleaning operations in and around buildings may be considered to lead to minor entries into public sewage systems, any subsequent relevant exposure of surface waters is not to be expected, since the passage of Flocoumafen through an STP can safely be predicted to lead to an effective elimination in view of the "readily biodegradability" of Flocoumafen and its lack of any inhibitory effect on sewage sludge micro-organisms. Consequently, the potential exposure of sediments can safely be deemed to be negligible. Further, according to the intended use pattern (application in and around buildings only) and the properties of the biocidal product, significant exposure of the aquatic environment seems unlikely. The product is a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Therefore, further testing of effects on sediment dwelling organisms is not considered to be required predominantly due to a lack of exposure.	
Undertaking of intended 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 (*)
Date	1 July 2005
Evaluation of applicant's justification	Long-term exposure is not expected during the use and waste phase of the product. In addition, the risk for sediment dwelling organisms was low (PEC/PNEC <1) based on PNEC calculated using the equilibrium partitioning method. Therefore testing of effects on sediment dwelling organisms is not considered to be required
Conclusion	The waiver is accepted.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.5.2 Aquatic plant toxicity

Annex Point IIIA 13.3.4

	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:	The algal growth inhibition test (Section A7.4.1.3) indicates that Flocoumafen is not toxic to unicellular algae within its solubility range. The need for further testing of aquatic plant toxicity is therefore not triggered. Furthermore, according to the intended use pattern (application in and around buildings only) and the properties of the biocidal product, significant exposure of the aquatic environment seems unlikely. The product is a wax-bound bait block, which represents a lipophilic matrix from which partitioning of the active substance to water should occur only to an extremely small extent. Thus, an aquatic plant toxicity study is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The waiver is accepted.
Conclusion	The waiver is accepted.
Remarks	No further comments.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.1.1 Inhibition to microbiological activity (terrestrial)

Annex Point IIA 7.7.4

	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:	Exposure of soil to flocoumafen is considered to be very limited. Due to the anticipated use pattern (in and around buildings), release of flocoumafen to soil is not expected. In the exceptional case of bait carriage by rats to outdoor areas, exposure of soil to flocoumafen will be only punctual and sporadic. Diffuse release through urine and faeces of the target species is possible but the resulting amounts are small and temporally very limited. Overall, release of the substance to soil is considered to be negligible. Additionally, the results from the activated sludge respiration inhibition test (Section A7.4.1.4) indicate that Flocoumafen is of low toxicity to microorganisms. Thus, the conduct of a terrestrial study on inhibition of microbial activity is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The risk for soil dwelling organisms was low (PEC/PNEC <1) based on PNEC calculated using the equilibrium partitioning method. Significant chronic exposure of soil around buildings during the use phase for application using bait boxes is not expected (but would occur when bait is also placed in holes). Hence the waiver is accepted for application which is limited to placement of bait in bait boxes. Long-term exposure of soil is also possible when sludge from the STP is spread on agricultural soil, but this is not practised in all EU countries and the issue should therefore be dealt with at member state level.
Conclusion	A study on inhibition of soil microbial activity is not required for application which is limited to placement of bait in bait boxes.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

Annex Point IIIA 13.3.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test on acute toxicity to earthworms or other soil non-target macro-organisms is required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. (i) The testing for effects on earth worms or other soil non-target organisms is not considered to be required for lack of exposure, the justification being as follows: The recommended uses of Flocoumafen as a rodenticide will involve either indoor use, or use around closed buildings. Since this use does not involve direct application of products containing Flocoumafen to soil, large area soil contamination can be excluded. In the exceptional case of bait carriage by rats to outdoor areas, exposure of soil to flocoumafen will be only punctual. Diffuse release through urine and faeces of the target species is possible but the resulting amounts are small and temporally very limited. Overall, release of the substance to soil is considered to be negligible. Therefore, any quantitatively relevant exposure of earthworms is not conceivable. (ii) It is further stated that for some product types, these tests will be required with the core data set. However, for product type 14, (cf. Chapter 2.5), the conduct of these tests is explicitly not required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The risk for soil dwelling organisms was low (PEC/PNEC <1) based on PNEC calculated using the equilibrium partitioning method. Significant chronic exposure of soil around buildings during the use phase for application using bait boxes is not expected (but would occur when bait is also placed in holes). Hence the waiver is accepted for application which is limited to placement of bait in bait boxes. Long-term exposure of soil is also possible when sludge from the STP is spread on agricultural soil, but this is not practised in all EU countries and the issue should therefore be dealt with at member state level.
Conclusion	An acute toxicity test with earthworms is not required for application which is limited to placement of bait in bait boxes.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.1.3 Acute toxicity to plants

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test on acute toxicity to plants is required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. (i) The testing for effects on plants is not considered to be required for lack of exposure, the justification being as follows: The recommended uses of Flocoumafen as a rodenticide will involve only indoor use or around closed buildings. Since this use does not involve direct application of products containing Flocoumafen to soil, large area soil contamination can be excluded. Finally, minor contamination that may be caused by contact of soil with Flocoumafen containing bait will, if any, be strictly isolated to the contact surface and only a very small fraction of the Flocoumafen contained in the bait will be released. Therefore, any quantitatively relevant exposure of plants is not conceivable. In the exceptional case of bait carriage by rats to outdoor areas, exposure of soil to flocoumafen will be only punctual and absorption of Flocoumafen by plants is not expected. Diffuse release through urine and faeces of the target species is possible but the resulting amounts are small and temporally and spatially limited, leading to the conclusion that significant exposure of plants is not expected. (ii) It is further stated that for some product types, these tests will be required with the core data set. However, for product type 14, (cf. Chapter 2.5), the conduct of these tests is explicitly not required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The risk for soil dwelling organisms was low (PEC/PNEC <1) based on PNEC calculated using the equilibrium partitioning method. Significant chronic exposure of soil around buildings during the use phase for application using bait boxes is not expected (but would occur when bait is also placed in holes). Hence the waiver is accepted for application which is limited to placement of bait in bait boxes. Long-term exposure of soil is also possible when sludge from the STP is spread on agricultural soil, but this is not practised in all EU countries and the issue should therefore be dealt with at member state level.
Conclusion	A terrestrial plant growth test is not required for application which is limited to placement of bait in bait boxes.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

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

Annex Point IIIA 13.3.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test on reproductive effects with soil non-target macro-organisms is required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. (i) The testing for effects on reproductive effects with soil non-target macro-organisms is not considered to be required for lack of exposure, the justification being as follows: The recommended uses of Flocoumafen as a rodenticide will involve either (a) indoor use, or use around closed buildings, in the form of baits containing very low concentrations of Flocoumafen. Since this use does not involve direct application of products containing Flocoumafen to soil, large area soil contamination can be excluded. Further, minor contamination that may be caused by contact of soil with Flocoumafen containing bait will, if any, be strictly isolated to the contact surface and only a very small fraction of the Flocoumafen contained in the bait will be released. Diffuse release through urine and faeces of the target species is possible but the resulting amounts are small and temporally very limited. Finally, the ready biodegradability and the rapid degradation in soil without formation of any major metabolites precludes any long-term exposure to soil organisms. Therefore, any quantitatively relevant or long-term exposure of soil non-target macro-organisms is not conceivable. (ii) It is further stated that for some product types, these tests will be required with the core data set. However, for product type 14, (cf. Chapter 2.5), the conduct of these tests is explicitly <u>not</u> required. Thus, the conduct of an earthworm (or other soil organisms) reproduction study is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The risk for soil dwelling organisms was low (PEC/PNEC <1) based on PNEC calculated using the equilibrium partitioning method. Significant chronic exposure of soil around buildings during the use phase for application using bait boxes is not expected (but would occur when bait is also placed in holes, and in that case the most sensitive species from acute testing should be used in the chronic test). Hence the waiver is accepted for application which is limited to placement of bait in bait boxes. Long-term exposure of soil is also possible when sludge from the STP is spread on agricultural soil, but this is not practised in all EU countries and the issue should therefore be dealt with at member state level.
Conclusion	A reproduction study with earthworms is not required for application which is limited to placement of bait in bait boxes.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.2.2 Long-term test with terrestrial plants

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test for long-term effects on terrestrial plants is required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. (i) The testing for long-term effects on terrestrial plants is not considered to be required for lack of exposure, the justification being as follows: The recommended uses of Flocoumafen as a rodenticide will involve either indoor use, or use around closed buildings, in the form of baits containing very low concentrations of Flocoumafen. Since the outdoor use does not involve direct application of products containing Flocoumafen to soil, large area soil contamination can be excluded. Further, minor contamination that may be caused by contact of soil with Flocoumafen containing bait will, if any, be strictly isolated to the contact surface and only a very small fraction of the Flocoumafen contained in the bait will be released. Diffuse release through urine and faeces of the target species is possible but the resulting amounts are small and temporally and spatially limited, leading to the conclusion that significant exposure of plants is not expected Finally, the ready biodegradability precludes any long-term exposure of the soil compartment. Therefore, any quantitatively relevant or long-term exposure of plants is not conceivable. (ii) It is further stated that for some product types, these tests will be required with the core data set. However, for product type 14, (cf. Chapter 2.5), the conduct of these tests is explicitly not required. Thus, the conduct of a plant toxicity study is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The risk for soil dwelling organisms was low (PEC/PNEC <1) based on PNEC calculated using the equilibrium partitioning method. Significant chronic exposure of soil around buildings during the use phase for application using bait boxes is not expected (but would occur when bait is also placed in holes, and in that case the most sensitive species from acute testing should be used in the chronic test). Hence the waiver is accepted for application which is limited to placement of bait in bait boxes. Long-term exposure of soil is also possible when sludge from the STP is spread on agricultural soil, but this is not practised in all EU countries and the issue should therefore be dealt with at member state level.
Conclusion	A long-term test with terrestrial plants is not required for application which is limited to placement of bait in bait boxes.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.5.3.1.1/01:** Mxxxx Jxxxx, Txxxx Rxxxx, Axxxx Sxxxx (2001) Avian acute oral toxicity test with BAS 322 I (Flocoumafen) in the mallard duck (*Anas platyrhynchos*). Gxxxx Lxxxx, Ixxxx, Wxxxx, Uxxxx, Report No. 67330, December 3, 2001 (unpublished). (BASF-Ref.: FL-505-026)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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 SETAC (1995) US-EPA OPPTS 850.2100 (Avian Acute Oral Toxicity Test)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Spacing of dose levels (see 3.4.4 and 4.1.1).	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	AC12140-35	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	99.4 %	
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to affect the test performance.	
3.1.5	Method of analysis in the dosing suspensions	No analysis of dosing suspensions performed. Correctness of nominal concentrations was assumed. Analysis of dosing suspensions is not required according to US-EPA OPPTS 850.2100.	
3.2	**Administration of the test substance**	By oral gavage (details on the vehicle given in Table A7.5.3.1.1–1).	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Mallard ducks, as described in Table A7.5.3.1.1–2.	X
3.4.2	Test system	See Table A7.5.3.1.1–3.	X
3.4.3	Diet	Diet is described in Table A7.5.3.1.1–3; due to the method of administration, further data are not appropriate.	
3.4.4	Test conditions	Test conditions are provided in Tables A7.5.3.1.1–4 and 5.	
3.4.5	Duration of the test	21 d	
3.4.6	Test parameter	Mortality	
3.4.7	Examination/ observation	See Table A7.5.3.1.1–3.	
3.4.8	Statistics	Body weight analysed by one-way ANOVA; Feed consumption analysed by one-way ANOVA; Normality and homogeneity of variance of these two variables tested by a chi-squared normality test and Bartlett's test.	

4 RESULTS

4.1	**Range finding test**	Performed	
4.1.1	Concentration	15, 30, 60, 120, and 240 mg/kg b.w.	
4.1.2	Number/ percentage of animals showing adverse effects	Mortality data are presented in Table A7.5.3.1.1–6.	
4.1.3	Nature of adverse effects	Apart from mortality, the following effects were observed: 15 mg/kg: None 30 mg/kg: None 60 mg/kg: None 120 mg/kg: Ataxic behaviour in a female Signs of low body carriage in a male 240 mg/kg: Signs of low body carriage in a female. In contrast to the tabulated data, deaths in the 60 mg/kg group were reported in the text, but this is probably a typing error.	
4.2	**Results test substance**		
4.2.1	Applied concentrations	5, 14, 38, 104 and 286mg/kg b.w. (also see Table A7.5.3.1.1–7).	
4.2.2	Effect data (mortality)	Mortalities are provided in Table A7.5.3.1.1–7. LD_{50} = 286 mg/kg a confidence interval could not be estimated (also see 4.2.5).	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

4.2.3	Body weight	Average body weights at each observation point are presented in Table A7.5.3.1.1–8. Results of ANOVA indicated that there were no dosage related differences in body weight at either observation point.	X
4.2.4	Feed consumption	Mean feed consumption at each observation point is presented in Table A7.5.3.1.1–9. Results of ANOVA indicated that there were no dosage related differences in feed consumption during either observation period.	
4.2.5	Concentration-response curve	The slope of the dose-response curve could not be determined since mortalities increased from 0 % to 50 % between the consecutive dose levels of 104 mg/kg and 286 mg/kg (the top level).	
4.2.6	Other effects	Macroscopic pathological findings of birds that died after administration of the top-level dose, as well as survivors from all other dose levels, included discoloured liver, heart, bile duct and spleen. Furthermore, enlarged bile duct, haemorrhages in the mouth, nares, oesophagus, lungs, heart, pericardium, liver, thoracic cavity, abdominal cavity, kidneys, bile duct, proventriculus, and gizzard, occurred. Control birds were free of symptoms.	X
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	None of the control animals showed any adverse effects.	
4.3.2	Nature of adverse effects	Not appropriate (see 4.3.1).	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Acute oral toxicity of Flocoumafen to mallard ducks was tested according to SETAC (1995) and US-EPA OPPTS 850.2100. EPA recommends spacing of dose levels by a factor of < 1.67. The study deviated from this recommendation by setting the ratio between dose levels to 2.75.	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

5.2	**Results and discussion**	The physico-chemical properties of Flocoumafen (see Section A3) are not considered to have affected the test results. Mortality of control animals was 0 %. In this and all other respects, the validity criteria of US-EPA OPPTS 850.2100 are fulfilled. The dose-response relationship did not allow estimation of a slope, since a rise from 0 % to 50 % mortality occurred between the second-highest and the highest dose level. At the highest dose level of 286 mg/kg, 50 % of the individuals died. Birds exposed to lower concentrations survived to 100 %, but showed typical symptoms of internal haemorrhages upon necropsy. Hence, the lowest lethal dose (LLD) was determined at 286 mg/kg, and the NOEL at 104 mg/kg.	X
5.2.1	LD_{50}	LD_{50} = 286 mg/kg	
5.3	**Conclusion**	The discussed spacing of dose levels is not considered to be a deficiency. The study is considered to be valid.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	Yes	

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 January 2005
Materials and Methods	(2.3) "see 3.4.4" should be "see 5.1". (3.4.1) Table A7.5.3.1.1-2 states "43 males, 44 females". This should be "30 males, 30 females", since 5 males and 5 females were exposed to five concentrations and a control. (3.4.2) Table A7.5.3.1.1-3, number of animals should be 60 instead of 50. Replicate/dosage states "Not appropriate"; this should be "5 pens with 1 male and 1 female bird per dose level".
Results and discussion	(4.2.3) Table A7.5.3.1.1-8, mean body weight at day 0, 5 mg/kg, should be 1213 instead of 1212. (4.2.6) Daily observations revealed signs of test substance related moribundity and intoxication, including ataxia, loss of righting reflex, loss of balance, low carriage and hemorrhages, at 38, 104 and 286 mg/kg b.w.. Not mentioned in the summary were the following: (i) Macroscopic pathological findings showed that the gizzard lining appeared to be necrotic. (ii) Adverse treatment related effects were noted during gross necropsy in birds of all dose levels. (5.2) Based on typical symptoms of internal haemorrhages effects at the lowest tested dose of 5 mg/kg b.w., the NOEL is set at <5 mg/kg b.w.
Conclusion	The acute NOEL is <5 mg/kg b.w. and the LD_{50} is 286 mg/kg b.w..
Reliability	1
Acceptability	Acceptable
Remarks	No further comments.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
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	Not applicable (administration by oral gavage)
Other vehicle	No
Function of the carrier/ vehicle	Solvent for test substance

Table A7.5.3.1.1–2: Test organisms.

Criteria	Details
Species/strain	*Anas platyrhynchos* (Mallard duck)
Source	Whistling Wings Inc., 113 Washington Street, Hanover, IL 61041, USA
Age	14 weeks upon arrival
Sex	43 males, 44 females
Initial body mass	922 – 1420 g (at the time of dosing)
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of dosing	22 weeks
Health condition/medication	All birds were healthy; Birds were not medicated

Table A7.5.3.1.1–3: Test system.

Criteria	Details
Test location	Indoor in holding pens
Holding pens	Plastic coated steel wire pens 61 × 76 × 46 cm (l × w × h)
Number of animals	50
Number of animals per pen (cm²/bird)	2 individuals per pen (1 m, 1 f) 2318 cm^2/individual
Number of animals per dose	10 (5 m, 5 f)
Pre-treatment/ acclimation	Acclimation period > 15 d Environmental conditions as in the test (see Table A7.5.3.1.1–5) Feed: as in the test (see below) Feed and water available *ad libitum*
Diet during test	Dry, non-medicated "Turkey and Gamebird Grower", Ranch-Way Inc., 546 Willow, Ft. Collins, CO 80524 no analysis results reported.
Dosage levels of test substance	Single oral dose, administered by gavage; for dosage levels see, e.g. Table A7.5.3.1.1–7
Replicate/dosage level	Not appropriate
Feed dosing method	By gavage
Dosing volume per application	5 ml/kg (vol. corn oil/b.w.)
Frequency, duration and method of animal monitoring after dosing	Observation for clinical symptoms: twice daily
Time and intervals of body weight determination	At days 0, 3, 7, 14, and 21 or at death

Table A7.5.3.1.1–4: Test conditions.

Criteria	Details
Test temperature	Temperatures are listed in a separate table (A7.5.3.1.1–5)
Shielding of the animals	Not stated
Ventilation	10 – 15 air changes per hour
Relative humidity	Humidity data are listed in a separate table (A7.5.3.1.1–5)
Photoperiod and lighting	8:16 h (L:D) Full spectrum fluorescent lights, 3.9 footcandles

Table A7.5.3.1.1–5: Temperature and humidity data recorded during the test.

Date	Temperature (°C)		Relative humidity (%)	
	min.	max.	min.	max.
08/15/01	19	22	59	77
08/16/01	18	22	53	67
08/17/01	18	23	51	67
08/18/01	18	24	41	57
08/19/01	19	25	43	56
08/20/01	19	24	50	65
08/21/01	19	25	47	71
08/22/01	19	24	50	85
08/23/01	20	24	49	67
08/24/01	18	24	47	70
08/25/01	19	25	32	64
08/26/01	18	24	42	63
08/27/01	19	25	35	58
08/28/01	19	25	41	64
08/29/01	18	24	41	62
08/30/01	18	24	39	89
08/31/01	18	23	47	64
09/01/01	17	22	50	72
09/02/01	17	21	52	78
09/03/01	17	22	48	66
09/04/01	17	24	47	61
Mean	**18**	**24**	**46**	**68**
SD	**1**	**1**	**6**	**9**

Table A7.5.3.1.1–6: Mortality data from the range-finding test.

Test substance dosage level (mg/kg bw)	Mortality after test termination (21 days)	
	Number	Percent
15	1	25
30	1	25
60	0	0
120	1	25
240	3	75

Table A7.5.3.1.1–7: Mortality data from the definitive test.

Test substance dosage level (mg/kg bw)	Mortality after test termination (21 days)	
	Number	Percent
5	0	0
14	0	0
38	0	0
104	0	0
286	5	50

Table A7.5.3.1.1–8: Mean body weights of mallard ducks during the oral toxicity test of Flocoumafen (main test), including the control group.

Dose level (mg/kg)	Mean body weight (g) at day no.				
	0	**3**	**7**	**14**	**21**
0	1177	1184	1155	1166	1158
5	1212	1225	1188	1202	1212
14	1220	1224	1192	1224	1219
38	1187	1195	1153	1158	1165
104	1162	1159	1117	1141	1154
286	1107	1109	1048	1060	1072

Table A7.5.3.1.1–9: Mean daily feed consumption ($g \times individual^{-1} \times d^{-1}$) of mallard ducks during the oral toxicity test of Flocoumafen (main test), including the control group; data are presented as pooled estimates for periods as given in the table.

Dose level (mg/kg)	Mean feed consumption			
	Days 0–3	**Days 3–7**	**Days 7–14**	**Days 14–21**
0	54.6	59.2	66.0	76.0
5	59.8	56.4	70.5	87.6
14	59.8	50.9	73.0	88.9
38	66.6	49.0	58.0	80.2
104	53.9	48.3	69.0	86.1
286	56.9	34.1	43.1	92.4

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.3.1.1/02:** Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1985) The acute oral toxicity (LD_{50}) of WL 108366 to the mallard duck. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 73BT/8572, March 12, 1985 (unpublished). (BASF-Ref.: FL-505-005) **A7.5.3.1.1/03:** Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1985) The acute oral toxicity (LD_{50}) of WL 108366 to the mallard duck. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 67BT/84925, February 26, 1985 (unpublished). (BASF-Ref.: FL-505-004) Remark: Reference A7.5.3.1.1/03 is the range-finding test for the main study (A7.5.3.1.1/02). Therefore, these reports are jointly reviewed in the current study summary for convenience.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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 Conduct of the study on the basis of the UK Pesticides Safety Precautions Scheme, Revised 1979 and 1983, Working Document D5, is stated. Furthermore, the study is similar to US-EPA OPPTS 850.2100 (Avian Acute Oral Toxicity Test).
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Deviations from the Pesticides Safety Precautions Scheme cannot be assessed due to lack of availability of this document. Relevant deviations from US-EPA OPPTS 850.2001: - Photoperiod (see 3.4.4) - spacing and number of dose levels (see 3.4.4 and 4.2.1) - concurrence of testing (see 3.4.1) - age and body mass of birds at dosing (see 3.4.1)

Section A7.5.3.1.1 Acute oral toxicity to birds

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3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	> 99 %	
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to affect the test performance.	
3.1.5	Method of analysis in the dosing suspension	Standard HPLC, as described in Section A4. Analysis confirmed that the nominal concentrations were actually met.	
3.2	**Administration of the test substance**	By oral gavage (details on the vehicle given in Table A7.5.3.1.1–10).	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Mallard ducks, as described in Table A7.5.3.1.1–11.	X
3.4.2	Test system	See Table A7.5.3.1.1–12.	X
3.4.3	Diet	Diet is described in Tables A7.5.3.1.1–12 and 13.	
3.4.4	Test conditions	Test conditions are provided in Tables A7.5.3.1.1–14 and 15.	
3.4.5	Duration of the test	28 d	
3.4.6	Test parameter	Mortality	
3.4.7	Examination/ observation	See Table A7.5.3.1.1–12.	
3.4.8	Statistics	LD_{50} by probit analysis	

4 RESULTS

4.1	**Range finding test**	Range-finding was performed in a separate study (Report No. SLL 67BT784925, reference A7.5.3.1.1/ 3), which is included in the current study summary.	
4.1.1	Concentration	0, 100, and 300 mg a.i./kg bw	
4.1.2	Number/ percentage of animals showing adverse effects	Mortality data are presented in Table A7.5.3.1.1–16.	

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4.1.3	Nature of adverse effects	Apart from mortality, the following clinical signs were observed: Subdued behaviour, unsteadiness, inability to stand, lying on the floor with wings outstretched. Pathological findings of died birds indicated death by haemorrhages.	X
4.2	**Results test substance**		
4.2.1	Applied concentrations	0, 10, 30, 100, and 300 mg a.i./kg bw The control was performed in duplicate, one for each part of the study (also see Table A7.5.3.1.1–11).	
4.2.2	Effect data (mortality)	Mortalities are provided in Table A7.5.3.1.1–17. LD_{50} (m and f) = 24 mg/kg (95 % CI = 15–40) LD_{50} (m) = 34 mg/kg (95 % CI = 18–69) LD_{50} (f) = 17 mg/kg (95 % CI = 9–33)	
4.2.3	Body weight	Average body weights at each observation point are presented in Table A7.5.3.1.1–18. It is stated that body weight changes did not differ between treatment levels.	X
4.2.4	Feed consumption	Mean feed consumption at each observation point is presented in Table A7.5.3.1.1–19. It is stated that feed consumption did not differ between treatment levels.	
4.2.5	Concentration-response curve	The dose-response curve is given in Figure A7.5.3.1.1–1.	
4.2.6	Other effects	Macroscopic pathological findings of birds that died during the test were: internal and external haemorrhages, body cavity filled with fluid, and small blood clots around liver and heart. Control birds were free of symptoms.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	None of the control animals showed any adverse effects.	
4.3.2	Nature of adverse effects	Not appropriate (see 4.3.1).	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute oral toxicity of Flocoumafen to mallard ducks was tested using methods (application of test substance by oral gavage, dissolved in corn oil) similar to US-EPA OPPTS 850.2100. Relevant deviations from this guideline occurred in several respects: - dose levels were not spaced geometrically, and the spacing factor was ≥ 3 - dose levels were not tested concurrently - only 4 dose levels were tested - the photoperiod was 14:10 h (L:D) - birds in groups 1–3 were only 10 weeks of age at dosing - 25 % of the birds weighed less than 900 g at dosing	
5.2	**Results and discussion**	The physico-chemical properties of Flocoumafen (see Section A3) are not considered to have affected the test results. Flocoumafen was found to be highly toxic to the mallard duck; mortality resulted from internal haemorrhages. Mortality of control animals was 0 %. In this and all other respects, the validity criteria of US-EPA OPPTS 850.2100 are fulfilled. According to the current results, Flocoumafen is considered to be highly toxic to birds.	
5.2.1	LD_{50}	LD_{50} = 24 mg/kg	
5.3	**Conclusion**		X
5.3.1	Reliability	3	X
5.3.2	Deficiencies	Yes The deviations summarised above (5.1) can be considered to represent deficiencies of varying severity. Particularly the non-concurrent testing of different dose levels, the photoperiod, age and body mass at dosing may have affected the results. However, the degree of impact of these deficiencies is difficult to assess. Nevertheless, since the results from this study are more critical than those from the key study (Mxxxx et al., 2001, reference A7.5.3.1.1/01), they should be taken into consideration for risk assessment.	X

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 January 2005
Materials and Methods	(3.4.1) Table A7.5.3.1.1-11, age: "18 weeks at dosing (groups 4-6)" should read "17 weeks at dosing (groups 4-6)" (3.4.2) Table A7.5.3.1.1-12, replicate/dosage level states "Not appropriate"; this should be "2 (5 male or 5 female birds per replicate)".
Results and discussion	(4.1.3) Gross pathological findings were noted at all treatment levels. Clinical signs were noted at 30 mg/kg and above. (4.2.3) Table A7.5.3.1.1-18, mean body weight change 0-7 days at 30 mg/kg should be +37.5 instead of +52.5; 7-14 days at 0 mg/kg (control 1) should be +1.5 instead of +2.5 and 7-14 days at 10 mg/kg should be –5.5 instead of –6.5. (5.2) Based on pathological effects and mortality observed at the lowest tested dose of 10 mg/kg b.w., the NOEL is set at <10 mg/kg b.w..
Conclusion	The acute NOEL is <10 mg/kg. The LD_{50} is 24 mg/kg.
Reliability	1 (see remarks below)
Acceptability	Acceptable
Remarks	(5.3.2) The most relevant groups for setting the acute LD50 in this test were groups 4-6 (0, 10 and 30 mg/kg bw, 0%, 10% and 60% mortality, respectively), which were all three concurrently tested. The draft OECD guideline on avian acute oral toxicity testing of October 2002 states that birds in mature plumage should be used. Mallard duck will have acquired mature plumage between the age of 14 and 16 weeks. All the birds in groups 4-6 were 16-17 weeks of age at the start of the test (only the birds in groups 1-3, i..e control, 100 and 300 mg/kg, not concurrently tested with groups 4-6, were 10 weeks of age at the start of the test). The draft OECD guideline does not require birds to be of a minimum weight. The draft OECD guideline states that the test environment may be under controlled or ambient conditions. Hence the applicant's arguments concerning concurrency, age, weight and photoperiod are not supported. The spacing of dose levels is rather wide (preferable is a factor of 2) but given the test results, with 10% and 60% mortality at 10 and 30 mg/kg, the estimate of the LD50 is considered to be sufficiently accurate. The study result is reliable (reliability 1).
	COMMENTS FROM ...
Date	
Materials and Methods	

Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.1–10: Method of administration of the test substance.

Carrier/Vehicle	Details
Water	No
Organic carrier	Yes: corn oil
Concentration of the carrier	Not applicable (application by oral gavage)
Other vehicle	No
Function of the carrier/ vehicle	Solvent for test substance

Table A7.5.3.1.1–11: Test organisms.

Criteria	Details
Species/strain	*Anas platyrhynchos* (Mallard duck)
Source	The County Game Farm, Home Farm, Hothfield, Ashford, Kent, UK
Sex	30 males, 30 females
Age	8 weeks upon arrival 10 weeks at dosing (groups 1–3) 18 weeks at dosing (groups 4–6)
Initial body mass	715 – 1175 g (at the time of dosing) 15 birds (25 %) < 900 g
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of dosing	10 weeks (groups 1 to 3), dosed 0, 100, and 300 mg/kg 17 weeks (groups 4 to 6), dosed 0, 10, and 30 mg/kg
Health condition/medication	No information on health condition available Food was free of medicaments

Table A7.5.3.1.1–12: Test system.

Criteria	Details
Test location	Indoor in holding pens
Holding pens	Plastic coated steel wire pens 144 × 41 × 53 cm (l × w × h)
Number of animals	60
Number of animals per pen (cm²/bird)	5 individuals per pen 1180 cm^2/individual
Number of animals per dose	10 (5 m, 5 f)
Pre-treatment/ acclimation	Acclimation period > 14 d Environmental conditions as in the test (see Table A7.5.3.1.1–15 Feed: as in the test (see below) Feed and water available *ad libitum*
Diet during test	Pelleted Layer Diet, Joseph Odam Ltd., Eye Mill, Peterborough, Cambridgeshire, UK no analysis results reported composition of the diet, as specified by the supplier, is given in Table A7.5.3.1.1–13
Dosage levels of test substance	Single oral dose, administered by gavage; for dosage levels see Table A7.5.3.1.1–17
Replicate/dosage level	Not appropriate
Feed dosing method	By gavage
Dosing volume per application	10 ml/kg (vol. corn oil/b.w.)
Frequency, duration and method of animal monitoring after dosing	Observation for clinical symptoms and mortality: daily
Time and intervals of body weight determination	At days 0, 3, 7, 14, 21, and 28 or at death

Table A7.5.3.1.1–13: Composition of the commercial diet from source specified in Table A7.5.3.1.1–12.

Ingredient	Fraction (% w/w)
Ground wheat	38.25
Maize meal	30.0
Weatings (Wheat feed)	5.0
Provimi 66 fish meal	10.0
Extracted soybean meal	10.0
Limestone flour	5.5
Pantoribin 537*)	1.25

*) Mineral, vitamin and trace element supplement by B.P. Nutrition (UK) Ltd.

Table A7.5.3.1.1–14: Test conditions.

Criteria	Details
Test temperature	Temperatures are listed in a separate table (A7.5.3.1.1–15)
Shielding of the animals	Not stated
Ventilation	Not stated
Relative humidity	Humidity data are listed in a separate table (A7.5.3.1.1–15)
Photoperiod and lighting	14:10 h (L:D) type of lighting not further specified

Table A7.5.3.1.1–15: Means and ranges of daily measurements of temperatures and relative humidities during the test and the acclimation period.

	Groups 1–3		Groups 4–6	
	Mean	**Range**	**Mean**	**Range**
T (°C), max.	22	16–27	20	17–28
T (°C), min.	16	11–20	17	15–22
Rel. humidity (%)	92	70–100	81	60–100

Table A7.5.3.1.1–16: Mortality data from the range-finding test.

Test substance dosage level (mg/kg bw)	Mortality after test termination (28 days)	
	Number	**Percent**
0	0	0
100	6	60
300	10	100

Table A7.5.3.1.1–17: Mortality data from the definitive test.

Test substance dosage level (mg/kg bw)	Mortality after test termination (28 days)	
	Number	**Percent**
10	1	10
30	6	60
100	10	100
300	10	100

Table A7.5.3.1.1–18: Mean body weights changes of mallard ducks during the oral toxicity test of Flocoumafen (main test) during 7-d periods and over the entire observation period (28 d), including the control group.

Dose level (mg/kg)	Mean body weight changes (g) during sampling periods				
	0–7	7–14	14–21	21–28	0–28
0 (control 1)	+35	+2.5	+30.5	–24	+43
0 (control 2)	+30	–8	+22.5	+26.5	+71
10	+13.5	–6.5	+57	+17.5	+82.5
30	+52.5	+3	+19	+42.5	+104
100	–85	—	—	—	—
300	–92.5	—	—	—	—

Table A7.5.3.1.1–19: Mean feed consumption of mallard ducks (g × individual^{-1} × d^{-1}) during the oral toxicity test of Flocoumafen (main test) during 7-d periods, including the control group.

Dose level (mg/kg)	Mean feed consumption (g) during sampling periods			
	0–7	8–14	15–21	22–28
0 (control 1)	58.5	69.5	75	75
0 (control 2)	80.5	77.5	79	76
10	70.5	61.5	79.5	84
30	65.5	54	53.5	70
100	44.5	—	—	—
300	49	—	—	—

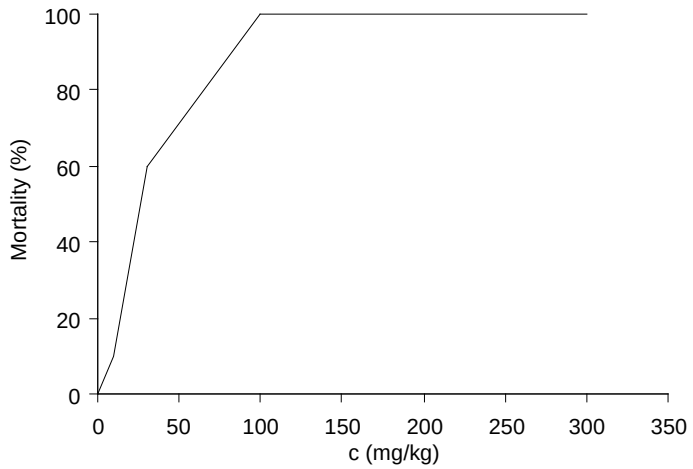

Figure A7.5.3.1.1–1: Dose-response curve from the avian acute oral toxicity test in *Anas platyrhynchos*.

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1 Supportive data

The following references are considered to contain additional information concerning acute oral toxicity to birds and are thus presented in tabular format as supportive data:

Reference	Title	Method	Results
A7.5.3.1.1/04: Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1984) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 68BT/84863, December 10, 1984 (unpublished). (BASF-Ref.: FL-505-003)	The acute oral toxicity (LD_{50}) of WL 108366 to the Japanese quail.	Test organisms: *Coturnix coturnix japonica*, age 10 weeks, body weight 142–281 g. Acute oral toxicity trial, administration of test substance by gavage, dissolved in corn oil. Two dose levels (100 and 300 mg/kg) and a control group, 5 males and 5 females per group. Post exposure observation 28 d. Compliance to Pesticides Safety Precautions Scheme, Working Document D5, is stated, but this cannot be verified since this document is not available. GLP: Yes	Mortalities: one male of the 100 mg/kg group and two males of the 300 mg/kg group died. An LD_{50} is not determinable, but the observed mortalities allow the conclusion that LD_{50} > 300 mg/kg. NOEL: not available. The deceased birds showed symptoms of anticoagulant poisoning.
A7.5.3.1.1/05: Sxxxx Rxxxx (1983) Sxxxx Lxxxx, Report (unpublished). (BASF-Ref.: FL-505-001)	The acute oral toxicity of a series of novel anticoagulants in broiler chickens.	Test organisms: *Gallus domesticus*, age 21 days, body weight 180–300 g. Acute oral toxicity trial, administration of test substance by gavage, dissolved in polyethylene glycol/ triethanolamine (9:1). Three dose levels (10.0, 31.6, and 100.0 mg/kg) and a control group. 8 unsexed individual per group. Post exposure observation 28 d. Non-guideline study. GLP: No	Mortalities: one individual of the 10 mg/kg group died. No other fatalities occurred. An LD_{50} is not determinable, but the observed mortalities allow the conclusion that LD_{50} > 100 mg/kg. NOEL: not available. The bird which died showed symptoms of anticoagulant poisoning.
A7.5.3.1.1/06: Jxxxx Jxxxx (1983) Hxxxx Lxxxx Exxxx Lxxxx, Hxxxx, Uxxxx, Report No. 3511-355/2, August 1983 (unpublished). (BASF-Ref.: FL-505-002)	WL 108366: Acute oral toxicity study in the Japanese quail.	Test organisms: *Coturnix coturnix japonica*, age not stated, body weight 135–247 g. Acute oral toxicity trial, administration of test substance by gavage, dissolved in polyethylene glycol/ triethanolamine (9:1). Four dose levels (3, 10, 30, and 100 mg/kg) and a control group, 5 males and 5 females per group. Post exposure observation 28 d. Non-guideline study. GLP: No	Mortalities: one female of the 10 mg/kg group died on day 26. No other fatalities occurred. An LD_{50} is not determinable, but the observed mortalities allow the conclusion that LD_{50} > 100 mg/kg. NOEL: not available. The bird which died did not show symptoms of anticoagulant poisoning.

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 January 2005
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	A7.5.3.1.1/04: Mortalities: all dead birds were females instead of males. The deceased birds showed haemorrhaging rather than “symptoms of anticoagulant poisoning”. A7.5.3.1.1/06: “Hxxxx” should read “Hxxxx”; body weight should read 125-234 g instead of 135-247 g.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.5.3.1.2/01:** Gxxxx Sxxxx, Gxxxx Jxxxx, Bxxxx Jxxxx, Mxxxx Jxxxx, Axxxx Sxxxx (2002) Avian dietary toxicity test with BAS 322 I (Flocoumafen) in the mallard duck (*Anas platyrhynchos*). Wxxxx Ixxxx Lxxxx, Exxxx, Uxxxx, Report No. 147-217, March 20, 2002 (unpublished). (BASF-Ref.: 2002/1013872)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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 205 US-EPA OPPTS 850.2200	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Spacing of dose levels (see 4.2.1).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	AC12140-35	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	99.4 %	
3.1.4	Further relevant properties	The physico-chemical properties of the test substance, as given in Section A3, are not considered to have affected the test performance.	
3.1.5	Method of analysis	HPLC analysis. A detailed analytical report including description of the method is appended to the original study. In the control, test substance was not detected. In the test diets, measured concentrations slightly exceeded nominal values and generally ranged between 103 and 137 % of nominal prior to administration. At the end of the dosing period, concentrations were found in the range between 80.0 and 119 % of nominal.	X

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

3.2	**Administration of the test substance**	Dietary administration; details presented in Table A7.5.3.1.2- 1.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Mallard ducks, as described in Table A7.5.3.1.2- 2.	
3.4.2	Test system	See Table A7.5.3.1.2- 3.	X
3.4.3	Diet	Basal diet is specified in Table A7.5.3.1.2- 4	X
3.4.4	Test conditions	Test conditions are provided in Table A7.5.3.1.2- 5.	
3.4.5	Duration of the test	5 days of exposure, 10 days post-exposure	
3.4.6	Test parameter	Mortality	
3.4.7	Examination/ observation	Observation of mortalities, behaviour and clinical signs at least twice daily. For determination of body weight see Table A7.5.3.1.2- 3. Feed consumption recorded for the exposure period (days 0–5), and post-exposure for the intervals day 6–8, 9–12, and 13–15. Sacrifice of all surviving birds on day 15; post-mortem examination with focus on internal and external haemorrhaging.	
3.4.8	Statistics	LC_{50} and 95 % confidence intervals by probit analysis.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentration/dose		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		
4.2	**Results test substance**		
4.2.1	Applied concentrations	0.1, 0.3, 0.9, 2.7, 8.1, 24.3, 72.9, and 219 ppm.	
4.2.2	Effect data (mortality)	Cumulative mortalities over the 15-d test period are provided in Table A7.5.3.1.2- 6. LC_0 = 0.3 ppm LC_{50} = 12 ppm (95 % CI = 5–38 ppm) LC_{100} > 219 ppm	X

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

4.2.3	Body weight	Average body weight changes for each observation period are presented in Table A7.5.3.1.2- 7.	X
4.2.4	Feed consumption	Mean feed consumption per bird per day for each observation period is presented in Table A7.5.3.1.2- 8.	
4.2.5	Concentration-response curve	The dose-response curve is given in Figure A7.5.3.1.2- 1.	
4.2.6	Other effects	Clinical signs: There were no overt signs of toxicity at 0.1 and 0.3 ppm. In the 0.9 ppm group the bird that eventually died showed signs of toxicity (not further specified) on day 8. In the 2.7 ppm groups signs of toxicity were first noted on day 2. At 8.1 ppm and all higher concentrations, signs of toxicity occurred from day 1 onwards. Typical symptoms were bleeding from the site of the wing band, loss of coordination, lower limb weakness, convulsions, swollen eyes, shallow and rapid respiration, and reduced reaction to external stimuli. At 8.1 and 24.3 ppm, clinical signs were restricted to birds that eventually died, while at 72.9 and 219 ppm also survivors transiently showed symptoms of intoxication. Necropsy: All deceased birds showed symptoms of anticoagulant poisoning, such as pale organs and musculature, haemorrhages in the heart and blood in the abdominal cavity. Treatment related lesions were sporadically recorded in the survivors from the 8.1, 72.9, and 219 ppm groups. Survivors from the 0.1, 0.3, 0.9, 2.7, and 24.3 ppm level were without pathological findings.	X
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	None (0 %).	
4.3.2	Nature of adverse effects	Not applicable.	
4.4	**Test with reference substance**	Not performed.	
4.4.1	Concentrations		
4.4.2	Results		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The short-term dietary toxicity of Flocoumafen to mallard ducks was determined according to the guidelines OECD 205 and US-EPA OPPTS 850.2200. The study deviated from the guidelines with respect to the spacing of dose levels. However, this deviation is not considered to have affected the results, as apparent from the dose-response curve.	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

5.2	**Results and discussion**	At dietary concentrations above 0.3 ppm, the test animals exhibited typical symptoms of anticoagulant poisoning. Therefore, the NOEC was established at 0.3 ppm, with respect to mortality as well as clinical and pathological findings.	
5.2.1	LC_0	LC_0 = 0.3 ppm	
5.2.2	LC_{50}	LC_{50} = 12 ppm (95 % CI = 5–38 ppm)	X
5.2.3	LC_{100}	LC_{100} > 219 ppm	
5.3	**Conclusion**	The validity criteria are considered to be fulfilled (Table A7.5.3.1.2- 9). Other circumstances that may have negatively affected the integrity and quality of the results are not reported. Thus, the study was considered to be valid.	
5.3.1	Reliability	1	X
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 January 2005
Materials and Methods	(3.1.5) 80.0-119% should read 72.6-119%. (3.4.1) Table A7.5.3.1.2-3: number of animals should read 110 instead of 130, and replicate/dosage level should read "two per test concentration and 6 for controls (5 birds per replicate)" instead of "not appropriate". (3.4.1) Table A7.5.3.1.2-4: Vitamin A should read 7,000,000 I.U. instead of 2,000,000 I.U.
Results and discussion	(4.2.3) Table A7.5.3.1.2-7: body weight change over days 8-12, 0.1 ppm group should read 128 ± 14 instead of 128 ± 18. (4.2.6) The text concerning the 0.9 ppm group should read: "In the 0.9 ppm group signs of toxicity were first noted on day 8". The toxicity signs consist of the death of one bird on that day. "At 8.1 ppm and all higher concentrations, signs of toxicity occurred from day 1 onwards" should be "At 8.1, 24.3 and 219 ppm, signs of toxicity occurred from day 1 onwards, while signs of toxicity were observed from day 2 onwards at 72.9 ppm". Other symptoms included haemorrhaging of the affected eye and lethargy (not mentioned in the list).
Conclusion	(5.2.2) The LC_{50} for mallard duck is 4.1 ppm (95% C.L. = 2.78-5.98), based on recalculation of the data using Spearman-Karber. The 5-day LC_{50} is 4.1 ppm (95% C.L. = 2.78-5.98) (equivalent to 1.9 mg/kg bw/day; calculated by RMS based on mean feed intake of 99 g/bird/day and mean body weight of 214.5 g/bird for day 0-5 period), and the 5-day NOEC is 0.3 ppm (equivalent to 0.12 mg/kg bw/day; calculated by RMS based on mean feed intake of 93 g/bird/day and mean body weight of 224.5 g/bird for day 0-5 period).
Reliability	1
Acceptability	Acceptable
Remarks	The mean procedural recoveries of the analytical method, determined at fortification levels of 0.1, 5 and 300 ppm, were 57-60 and 63-75% on days 0 and 5, respectively. Measured concentrations in samples taken from the diet were corrected for these recoveries. This is considered to be acceptable, given the fact that the fortification range encompassed the tested concentrations, and given the narrow range of recoveries obtained at each analytical day.
	COMMENTS FROM ...
Date	

Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.2- 1: Method of administration of the test substance.

Carrier/Vehicle	**Details**
Water	No
Organic carrier	Yes: Acetone
Concentration of the carrier	Acetone was removed during diet preparation by volatilisation
Other vehicle	Basal diet, as specified in Table A7.5.3.1.2- 4; the stock solution (Flocoumafen in acetone) was mixed with the diet as appropriate to achieve the desired dietary concentrations.
Function of the carrier/ vehicle	Acetone: solvent for test substance Diet: facilitation of uptake and digestion

Table A7.5.3.1.2- 2: Test organisms.

Criteria	**Details**
Species/strain	*Anas platyrhynchos* (Mallard duck)
Source	Whistling Wings, Inc., Hanover, Illinois
Age	10 days at the onset of the study
Sex	Unknown Sex is indeterminable at this age
Initial body mass	134–179 g
Age range within the test	All individuals from the same hatch
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of first dosing	10 days
Health condition/medication	Birds were not medicated; health condition were deemed appropriate for the test

Table A7.5.3.1.2- 3: Test system.

Criteria	**Details**
Test location	Wxxxx Ixxxx, Lxxxx, Exxxx, Mxxxx, Uxxxx
Holding pens	Thermostatically controlled brooding pens, constructed of vinyl-coated wire grid, measuring 72 × 90 × 23 cm (l × w × h)
Number of animals	130
Number of animals per pen [cm²/bird]	5 individuals per pen, separated by treatment group 1296 cm^2/individual
Number of animals per dose	10
Pre-treatment/ acclimation	Acclimation period: 8 d Environmental conditions as in the test Feed: basal diet as in the test, without test substance (see below) Feed and water available *ad libitum*
Diet during test	Game bird ration, formulated according to the laboratory's specification, no analysis results reported; composition of the diet is given in Table A7.5.3.1.2- 22
Dosage levels of test substance	Dietary administration; for dosage levels see, e.g. Table A7.5.3.1.2- 6.
Replicate/dosage level	Not appropriate
Dosing method	Dietary, for 5 days
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Observation for mortality and clinical signs at least twice daily.
Time and intervals of body weight determination	At days 0, 5, 8, 12, and 15

Table A7.5.3.1.2- 4: Composition of the commercial diet[1] from source specified in Table A7.5.3.1.2- 4.

Ingredient	**Fraction [% w/w]**
Fine corn meal	44.83
Soy bean meal, 48 % protein	30.65
Wheat midds	6.5
Protein base	6.0
Agway Special, 60 % protein	4.0
Alfalfa meal, 20 % protein	3.0
Dried whey	2.5
Ground limestone	0.9
Eastman CalPhos	0.6
Methionine Premix + liquid	0.35
Vitamin and mineral premix (see below)	0.32
GL Ferm (Fermatco)[2]	0.25
Salt iodised	0.1
Vitamin and mineral premix	*Amount added per ton*
Vitamin D3	2,000,000 I.C.U
Vitamin A	2,000,000 I.U.
Riboflavin	6 g
Niacin	40 g
Pantothenic acid	10 g
Vitamin B12	8 mg
Folic acid	600 mg
Biotin	64 mg
Pyridoxine	1.2 g
Thiamine	1.2 g
Vitamin E	20,000 I.U.
Manganese	102 g
Zinc	47 g
Copper	6.8 g
Iodine	1.5 g
Iron	51 g
Selenium	182 mg

1) Guaranteed analysis: min. 27 % of protein, min. 2.5 % of crude fat, max. 5 % of crude fibre

2) Fermentation by-products (source of unidentified growth factors)

Table A7.5.3.1.2- 5: Test conditions.

Criteria	**Details**
Test temperature	30.2 ± 1.3 °C (brooding compartment of the pens) 24.0 ± 0.6 °C (ambient room temperature)
Shielding of the animals	Not stated
Ventilation	15 air volume changes per hour
Relative humidity	45 ± 7 % RH
Photoperiod and lighting	8:16 h (L:D) fluorescent lights with wavelength spectrum close to noon-day sunlight, 203 lux

Table A7.5.3.1.2- 6: Treatment-related mortality data after test termination.

Dietary test substance concentration [ppm]	Cumulative mortality (15 days)	
	Number	Percent
0.1	0	0
0.3	0	0
0.9	1	10
2.7	5	50
8.1	6	60
24.3	8	80
72.9	6	60
219	7	70

For temperature and humidity data see Table A7.5.3.1.2- 5

Table A7.5.3.1.2- 7: Body weight changes of ducklings during the avian dietary toxicity test during the dosing period (days 0–5) and post-exposure (days 5–15).

TS concentration [ppm]	Initial body mass [g]	Body weight changes [g], mean ± SD				
		0–5	5–8	8–12	12–15	Total
0 (control)	154 ± 14	139 ± 18	99 ± 11	124 ± 19	73 ± 14	435 ± 49
0.1	153 ± 11	149 ± 19	94 ± 16	128 ± 18	88 ± 15	460 ± 49
0.3	153 ± 12	143 ± 18	100 ± 13	125 ± 12	77 ± 17	446 ± 51
0.9	151 ± 12	143 ± 17	90 ± 15	123 ± 18	77 ± 16	435 ± 45
2.7	153 ± 14	124 ± 18	84 ± 35	144 ± 34	82 ± 18	436 ± 65
8.1	156 ± 12	72 ± 44	45 ± 48	121 ± 21	85 ± 8	325 ± 72
24.3	152 ± 14	92 ± 30	61 ± 26	120 ± 26	69 ± 13	308 ± 66
72.9	155 ± 14	101 ± 39	34 ± 25	132 ± 12	102 ± 28	368 ± 47
219	155 ± 14	101 ± 36	38 ± 35	59 ± 30	76 ± 52	315 ± 46

Table A7.5.3.1.2- 8: Feed consumption of the ducklings used in the avian dietary toxicity test during the dosing period (days 0–5) and post-exposure (days 6–15).

TS concentration [ppm]	Feed consumption [g/bird/day], mean ± SD[1]			
	0–5	6–8	9–12	13–15
0 (control)	104 ± 13	141 ± 15	179 ± 29	195 ± 36
0.1	96	130	156	156
0.3	93	129	153	146
0.9	101	128	163	156
2.7	99	140	249	231
8.1	89	108	191	189
24.3	94	105	151	180
72.9	82	102	152	178
219	79	90	107	139

1) Standard deviations are only given for the control group

Table A7.5.3.1.2- 9: Validity criteria for short-term toxicity test according to OECD 205.

	Fulfilled	Not fulfilled
Mortality of control animals ≤10%	☑	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	☑	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	☑	

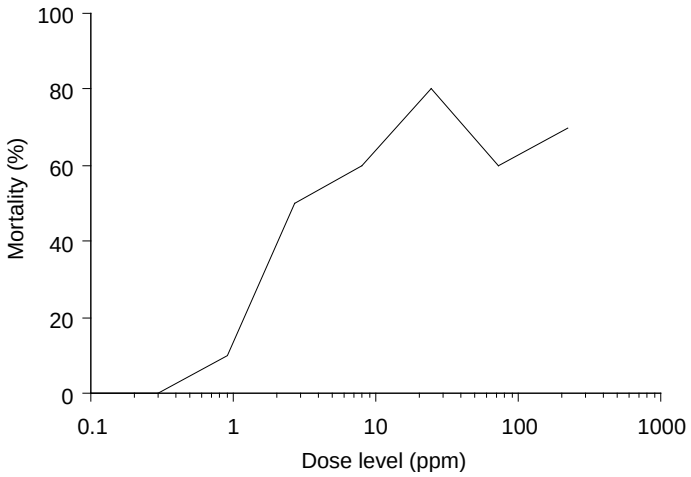

Figure A7.5.3.1.2- 1: Dose-response curve from the avian dietary toxicity test with mallard ducks.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.5.3.1.2/02:** Gxxxx Sxxxx, Gxxxx Jxxxx, Bxxxx Jxxxx, Mxxxx Jxxxx, Axxxx Sxxxx (2002) Avian dietary toxicity test with BAS 322 I (Flocoumafen) in the northern bobwhite (*Colinus virginianus*). Wxxxx Ixxxx, Lxxxx, Exxxx, Uxxxx, Report No. 147-216, March 7, 2002 (unpublished). (BASF-Ref.: 2002/1013873)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	No	
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/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US-EPA OPPTS 850.2200	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Spacing of dose levels (see 4.2.1).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	AC12140-35	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	99.4 %	
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to affect the test performance.	
3.1.5	Method of analysis	HPLC analysis. A detailed analytical report including description of the method is appended to the original study. In the control, test substance was not detected. In the test diets, measured concentrations slightly exceeded nominal values and generally ranged between 103 and 137 % of nominal prior to administration. At the end of the dosing period, concentrations were found in the range of 84.6 to 108 % of nominal.	
3.2	**Administration of the test substance**	Dietary administration; details presented in Table A7.5.3.1.2- 10.	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Northern bobwhite quails, as described in Table A7.5.3.1.2- 11.	
3.4.2	Test system	See Table A7.5.3.1.2- 12.	X
3.4.3	Diet	Basal diet is specified in Table A7.5.3.1.2- 14.	X
3.4.4	Test conditions	Test conditions are provided in Table A7.5.3.1.2- 13.	X
3.4.5	Duration of the test	5 days of exposure, 7 days post-exposure.	
3.4.6	Test parameter	Mortality	
3.4.7	Examination/ observation	Observation for mortalities, behaviour and clinical signs at least twice daily. For the timing of body weight determination see Table A7.5.3.1.2- 12. Feed consumption recorded for the exposure period (days 0–5), and post-exposure for the intervals day 6–8, 9–12, and 13–15. Sacrifice of all surviving birds on day 12; post-mortem examination with focus on internal and external haemorrhaging.	X
3.4.8	Statistics	LC_{50} and 95 % confidence intervals by probit analysis.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentration/dose		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		
4.2	**Results test substance**		
4.2.1	Applied concentrations	0.1, 0.3, 0.9, 2.7, 8.1, 24.3, 72.9, and 219 ppm.	
4.2.2	Effect data (mortality)	Mortalities are provided in Table A7.5.3.1.2- 15. LC_0 = 2.7 ppm LC_{50} = 62 ppm (95 % CI = 27–238 ppm) LC_{100} > 219 ppm	X

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

4.2.3	Body weight	Average body weight changes for each observation period are presented in Table A7.5.3.1.2- 16. There were no apparent treatment related effects on body weight gain up to the 72.9 ppm dose level. Body weight gain in the 219 ppm group was slightly reduced during the dosing period, and this effect persisted in the survivors.	
4.2.4	Feed consumption	Mean feed consumption per bird per day for each observation period is presented in Table A7.5.3.1.2- 17. Feed consumption was apparently not affected by treatment.	X
4.2.5	Concentration-response curve	The dose-response curve is given in Figure A7.5.3.1.2- 2.	
4.2.6	Other effects	<u>Clinical signs:</u> No clinical signs of intoxication were observed up to the 2.7 ppm group. At 8.1 ppm and higher doses, some birds were lethargic or showed a ruffled appearance. These effects were earliest observed on the morning of day 3. <u>Necropsy:</u> In all deceased birds, symptoms of subcutaneous and/or internal haemorrhaging were found. One or more surviving individuals from the dose levels ≥ 0.9 ppm, respectively, showed symptoms of internal and subcutaneous haemorrhaging. Birds from the 0.1 and 0.3 ppm group were without pathological findings. Based on clinical observations and post-mortem examination, the NOEL was established at 0.3 ppm.	X
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No controls birds showed effects that were considered treatment related. Two control individuals suffered leg injuries as the likely result of bullying.	
4.3.2	Nature of adverse effects	Not applicable.	
4.4	**Test with reference substance**	Not performed.	
4.4.1	Concentrations		
4.4.2	Results		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The short-term dietary toxicity of Flocoumafen to Northern bobwhite quails was determined according to the guidelines OECD 205 and US-EPA OPPTS 850.2200. The study deviated from the guidelines with respect to the spacing of dose levels. However, this deviation is not considered to have affected the results, as apparent from the dose-response curve.	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

5.2	**Results and discussion**	At dietary concentrations above 0.3 ppm, the test animals exhibited typical symptoms of anticoagulant poisoning. The NOEL was established at 0.3 ppm, with respect to clinical and pathological findings, and at 2.7 ppm with respect to mortality.
5.2.1	LC_0	LC_0 = 2.7 ppm
5.2.2	LC_{50}	LC_{50} = 62 ppm (95 % CI = 27–238 ppm)
5.2.3	LC_{100}	LC_{100} > 219 ppm
5.3	**Conclusion**	The validity criteria are considered to be fulfilled (Table A7.5.3.1.2- 18). Other circumstances that may have negatively affected the integrity and quality of the results are not reported. Thus, the study is considered to be valid.
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	04 February 2005
Materials and Methods	(2.1) Not mentioned in summary: OECD 205. (3.4.2) Table A7.5.3.1.2-12, number of animals should read "110" instead of "130" and replicate/dosage level should read "two per test concentration and 6 for controls (5 birds per replicate)" instead of "not appropriate". Acclimation period should read "10 d" instead of "8 d". (3.4.3) Table A7.5.3.1.2-14, Vitamin A should read 7,000,000 I.U. instead of 2,000,000 I.U. (3.4.4) Table A7.5.3.1.2-13, photoperiod and lighting should read "[…] noon-day sunlight" instead of "[…] daylight" (3.4.7) Feed consumption was recorded for the exposure period (days 0-5), and post-exposure for the intervals day 6-8 and 9-12 (summary erroneously also mentioned day 13-15).
Results and discussion	(4.2.2) Table A7.5.3.1.2-15, Footnote reference Table A7.5.3.1.2-5 should be Table A7.5.3.1.2-13. (4.2.4) Table A7.5.3.1.2-17, feed consumption during days 9-21 at 219 ppm should read 6 instead of 7. (4.2.6) Clinical signs: At 8.1 ppm, one bird was lethargic on day 5, but recovered during the observation period. At 24.3 ppm, one bird had a foot lesion. At 72.9 ppm, one bird displayed a slight wingdroop and a second bird displayed a ruffled appearance. At 219 ppm one bird had a swelling around the eye, two birds displayed a ruffled appearance and one bird was ruffled and lethargic.
Conclusion	The 5-day LC_{50} was 62 ppm (equivalent to 14 mg/kg bw/day; calculated by RMS based on mean feed intake of 6 g feed/bird/day and mean body weight of 27 g/bird for day 0-5 period), and the 5-day NOEC was 0.3 ppm (equivalent to 0.069 mg/kg bw/day; calculated by RMS based on mean feed intake of 6 g feed/bird/day and mean body weight of 26 g/bird for day 0-5 period).
Reliability	1
Acceptability	Acceptable
Remarks	The mean procedural recoveries of the analytical method, determined at fortification levels of 0.1, 5 and 300 ppm, were 57-60 and 48% on days 0 and 5, respectively. Measured concentrations in samples taken from the diet were corrected for these recoveries. This is considered to be acceptable, given the fact that the fortification range encompassed the tested concentrations, and given the narrow range of recoveries obtained at each analytical day.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.2- 10: Method of administration of the test substance.

Carrier/Vehicle	**Details**
Water	No
Organic carrier	Yes: Acetone
Concentration of the carrier	Acetone was removed during diet preparation by volatilisation
Other vehicle	Basal diet, as specified in Table A7.5.3.1.2- 14 and : the stock solution (Flocoumafen in acetone) was mixed with the diet as appropriate.
Function of the carrier/ vehicle	Acetone: solvent for test substance Diet: facilitation of uptake and digestion

Table A7.5.3.1.2- 11: Test organisms.

Criteria	**Details**
Species/strain	*Colinus virginianus* (Northern bobwhite quail)
Source	Own breeding at Wildlife International
Age	10 days at the onset of the study
Sex	Unknown Sex is indeterminable at this age
Initial body mass	18–25 g
Age range within the test	All individuals from the same hatch
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of first dosing	10 days
Health condition/medication	Birds were not medicated; they were in good health condition at test initiation

Table A7.5.3.1.2- 12: Test system.

Criteria	Details
Test location	Wxxxx Ixxxx, Lxxxx, Exxxx, Mxxxx, Uxxxx
Holding pens	Thermostatically controlled brooding pens constructed of galvanised steel wiring and sheeting, Model No. B735Q by Beacon Steel Products Co., measuring 72 × 90 × 23 cm (l × w × h)
Number of animals	130
Number of animals per pen [cm²/bird]	5 individuals per pen
Number of animals per dose	10
Pre-treatment/ acclimation	Acclimation period: 8 d Environmental conditions as in the test Feed: basal diet as in the test, without test substance (see below) Feed and water available *ad libitum*
Diet during test	Game bird ration, formulated according to the laboratory's specification, no analysis results reported; composition of the diet is given in Table A7.5.3.1.2- 14
Dosage levels of test substance	Dietary administration; for dosage levels see, e.g. Table A7.5.3.1.2- 15.
Replicate/dosage level	Not appropriate
Dosing method	Dietary, for 5 days
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Observation for mortality and clinical signs at least twice daily.
Time and intervals of body weight determination	At days 0, 5, 8, and 12

Table A7.5.3.1.2- 13: Test conditions.

Criteria	Details
Test temperature	28.8 ± 0.6°C (SD)
Shielding of the animals	Not reported
Ventilation	Yes, turnover of 15 room air volumes per hour
Relative humidity	14 ± 3 % (SD)
Photoperiod and lighting	8:16 h (L:D) c. 137 lux from fluorescent lights with approximate daylight spectrum

Table A7.5.3.1.2- 14: Composition of the commercial diet[1] from source specified in Table A7.5.3.1.2- 12.

Ingredient	**Fraction [% w/w]**
Fine corn meal	44.83
Soy bean meal, 48 % protein	30.65
Wheat midds	6.5
Protein base	6.0
Agway Special, 60 % protein	4.0
Alfalfa meal, 20 % protein	3.0
Dried whey	2.5
Ground limestone	0.9
Eastman CalPhos	0.6
Methionine Premix + liquid	0.35
Vitamin and mineral premix (see below)	0.32
GL Ferm (Fermatco)[2]	0.25
Salt iodised	0.1
Vitamin and mineral premix	*Amount added per ton*
Vitamin D3	2,000,000 I.C.U
Vitamin A	2,000,000 I.U.
Riboflavin	6 g
Niacin	40 g
Pantothenic acid	10 g
Vitamin B12	8 mg
Folic acid	600 mg
Biotin	64 mg
Pyridoxine	1.2 g
Thiamine	1.2 g
Vitamin E	20,000 I.U.
Manganese	102 g
Zinc	47 g
Copper	6.8 g
Iodine	1.5 g
Iron	51 g
Selenium	182 mg

1) Guaranteed analysis: min. 27 % of protein, min. 2.5 % of crude fat, max. 5 % of crude fibre

2) Fermentation by-products (source of unidentified growth factors)

Table A7.5.3.1.2- 15: Treatment-related mortality data after test termination.

Dietary test substance concentration [ppm]	**Cumulative mortality (12 days)**	
	Number	**Percent**
0.1	0	0
0.3	0	0
0.9	0	0
2.7	0	0
8.1	4	40
24.3	4	40
72.9	4	40
219	7	70

For temperature and humidity data see Table A7.5.3.1.2- 5

Table A7.5.3.1.2- 16: Body weight changes of bobwhite quails during the avian dietary toxicity test during the

dosing period (days 0–5) and post-exposure (days 5–15).

TS concentration [ppm]	Initial body mass [g]	Body weight changes [g], mean ± SD			
		0–5	5–8	8–12	Total
0 (control)	22 ± 2	11 ± 3	8 ± 2	12 ± 3	31 ± 7
0.1	21 ± 2	12 ± 2	8 ± 2	13 ± 2	33 ± 4
0.3	21 ± 2	10 ± 2	7 ± 2	11 ± 3	28 ± 6
0.9	22 ± 2	11 ± 3	7 ± 4	12 ± 2	29 ± 9
2.7	22 ± 2	10 ± 2	8 ± 2	11 ± 4	29 ± 6
8.1	22 ± 2	11 ± 3	7 ± 3	11 ± 2	29 ± 8
24.3	22 ± 2	10 ± 3	8 ± 4	11 ± 5	29 ± 12
72.9	22 ± 2	10 ± 1	5 ± 4	10 ± 3	25 ± 6
219	22 ± 2	8 ± 3	7 ± 1	9 ± 5	22 ± 8

Table A7.5.3.1.2- 17: Feed consumption of bobwhite quails used in the avian dietary toxicity test during the dosing period (days 0–5) and post-exposure (days 6–15).

TS concentration [ppm]	Feed consumption [g/bird/day], mean ± SD[1]		
	0–5	6–8	9–12
0 (control)	7 ± 1	9 ± 1	8 ± 1
0.1	6	10	9
0.3	6	10	10
0.9	7	13	8
2.7	6	9	7
8.1	5	11	7
24.3	6	11	7
72.9	6	9	7
219	5	10	7

1) Standard deviations are only given for the control group

Table A7.5.3.1.2- 18: Validity criteria for short-term toxicity test according to OECD 205.

	Fulfilled	Not fulfilled
Mortality of control animals ≤10%	☑	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	☑	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	☑	

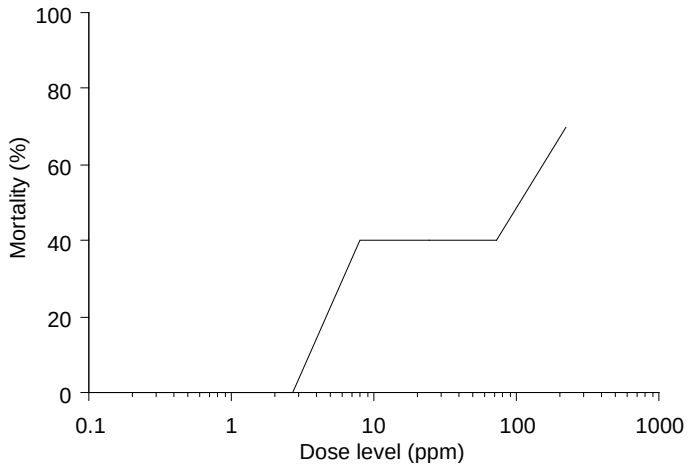

Figure A7.5.3.1.2- 2: Dose-response curve from the avian dietary toxicity test with bobwhite quails.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.3.1.2/03:** Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1985) The short-term cumulative dietary toxicity of WL 108366 to the Japanese quail. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 75BT/85111, March 12, 1985 (unpublished). (BASF-Ref.: FL-505-007) **A7.5.3.1.2/04:** Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1985) The short-term cumulative dietary toxicity of WL 108366 to the Japanese quail. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 70BT/8593, March 1, 1985 (unpublished). (BASF-Ref.: FL-505-006) Remark: Reference A7.5.3.1.2/04 was designed as a range-finding test for the main study (A7.5.3.1.2/03). Therefore, these reports are jointly reviewed in the current study summary for convenience.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
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/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No Nevertheless, the method applied is consistent to OECD 205 in all important aspects.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Spacing of dose levels (see 4.2.1); number of treatment levels (see 4.2.1).

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	Not stated
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	> 99 %

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to affect the test performance.	
3.1.5	Method of analysis	Standard HPLC, as described in Section A4. Test substance concentrations in the diet were ≥ 81.5 % of nominal.	
3.2	**Administration of the test substance**	Dietary administration; details presented in Table A7.5.3.1.2- 19.	
3.3	**Reference substance**	No reference substance examined.	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Japanese quails, as described in Table A7.5.3.1.2- 20.	X
3.4.2	Test system	See Table A7.5.3.1.2- 21.	X
3.4.3	Diet	Basal diet is specified in Table A7.5.3.1.2- 21 and Table A7.5.3.1.2- 22; the test diet was prepared as described in Table A7.5.3.1.2- 19.	
3.4.4	Test conditions	Test conditions are provided in Table A7.5.3.1.2- 23 and Table A7.5.3.1.2- 24.	
3.4.5	Duration of the test	33 d (5 d administration, 28 d post-treatment observation).	
3.4.6	Test parameter	Mortality	
3.4.7	Examination/ observation	See Table A7.5.3.1.2- 21.	
3.4.8	Statistics	LC_{50} by probit analysis.	

4 RESULTS

4.1	**Range finding test**	Range-finding was performed in a separate study (Report No. SLL 70BT/8593, reference A7.5.3.1.2/ 4), which is included in the current study summary.	
4.1.1	Concentration/dose	0, 50, and 200 ppm	
4.1.2	Number/ percentage of animals showing adverse effects	Mortality data are presented in Table A7.5.3.1.2- 25.	
4.1.3	Nature of adverse effects	Apart from mortality, the following clinical signs were observed: Apparent nausea, signs of haemorrhaging, individuals subdued and inability to move.	
4.2	**Results test substance**		
4.2.1	Applied concentrations	Nominal dietary concentrations of 1.5, 5.0, 15.0, and 50.0 ppm.	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

4.2.2	Effect data (mortality)	Mortalities are provided in Table A7.5.3.1.2- 26. LC_0 = 1.5 ppm LC_{50} = 37 ppm (95 % CI = 16–770 ppm) LC_{100} > 50 ppm	
4.2.3	Body weight	Average body weight changes in each pen (identical with sex) at each observation point are presented in Table A7.5.3.1.2- 27.	
4.2.4	Feed consumption	Mean feed consumption in each pen (identical with sex) at each observation point is presented in Table A7.5.3.1.2- 28.	
4.2.5	Concentration-response curve	A graphical representation of the dose-response curve is given in Figure A7.5.3.1.2- 3. The slope estimate of the probit line (log-transformed concentrations vs. mortality) was $\hat{\beta}_1 = 1.353 \pm 0.525$ (SE).	
4.2.6	Other effects	<u>Clinical:</u> Three individuals from the 5.0 and 50.0 ppm groups were subdued or unsteady on various days of/after treatment. <u>Necropsy:</u> Internal haemorrhaging was noted in the fatalities from the 5.0 ppm, 15.0 ppm, and 50.0 ppm level. One survivor of the 1.5 ppm and the 15.0 ppm group, respectively, also showed slight symptoms of haemorrhaging. Several events of (sometimes severe) bullying were not considered to be treatment-related, since this behaviour tends to occur in group housing of quails.	X
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	One male control bird died on day 19 (14 d after termination of treatment).	
4.3.2	Nature of adverse effects	Death of unknown cause.	
4.4	**Test with reference substance**	Not performed.	
4.4.1	Concentrations		
4.4.2	Results		

Section A7.5.3.1.2 Short-term toxicity on birds

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The short-term cumulative toxicity of Flocoumafen to birds was tested using Japanese quails (*Coturnix coturnix japonica)*. Although guideline compliance was not stated, the method used was consistent to OECD 205 in all important aspects. The study deviated from this guideline in the spacing factors between dose levels applied. They varied among each other and were larger than 2.0. Furthermore, only four treatment levels were applied. These deviations are not considered to have affected the results.	
5.2	**Results and discussion**	The physical-chemical properties of Flocoumafen (see Section A3) are not considered to have affected the test results. Analysis results demonstrated that the test substance was homogeneously mixed in the test diet and that concentrations of > 80 % of nominal were maintained.	
5.2.1	LC_0	LC_0 < 1.5 ppm	X
5.2.2	LC_{50}	LC_{50} = 37 ppm (95 % CI = 16–770)	X
5.2.3	LC_{100}	LC_{100} > 50 ppm	
5.3	**Conclusion**	This study fulfils the validity criteria of the OECD guideline 205 (Table A7.5.3.1.2- 29), and is thus considered acceptable. The dietary short-term toxicity of Flocoumafen has been estimated at LC_{50} = 37 ppm.	
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	04 February 2005
Materials and Methods	(3.4.1) Table A7.5.3.1.2-20, sex should read 25 males and 25 females instead of 35 males and 35 females. Age at time of first dosing should read 10 weeks instead of c. 13 weeks. (3.4.2) Table A7.5.3.1.2-21, Replicate/dosage level should read two per test concentration (5 male or 5 female birds per replicate).
Results and discussion	(4.2.6) Clinical: At 5.0 ppm, one bird was unsteady. At 50.0 ppm, one bird was unsteady and one bird was subdued. (5.2.1) LC_0 should read 1.5 instead of <1.5. (5.2.2) The 5-day LC50 of 37 ppm was equivalent to 19 mg/kg bw/day (reported and acceptable value).
Conclusion	Based on treatment related pathological findings, the 5-day NOEC was <1.5 ppm. The 5-day LC50 was 37 ppm (equivalent to 19 mg/kg bw/day).
Reliability	2 (see remarks)
Acceptability	Acceptable
Remarks	The recommended age of the birds is 10-17 days (OECD 205). The age of the birds used in this test was 10 weeks. The feed intake rate per kg body weight (and hence the test substance intake rate) is higher for younger birds. The reliability of the study is lowered to 2.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.2- 19: Method of administration of the test substance.

Carrier/Vehicle	**Details**
Water	No
Organic carrier	Yes: acetone
Concentration of the carrier	0.042 % w/w in the final diet after mixing acetone was removed by treatment in a rotary evaporator at 40 °C for 20 min
Other vehicle	Basal diet, as specified in Table A7.5.3.1.2- 21 and Table A7.5.3.1.2- 22: the stock solution (Flocoumafen dissolved in acetone) was mixed with the diet as appropriate.
Function of the carrier/ vehicle	Acetone: solvent for test substance Diet: facilitation of uptake and digestion

Table A7.5.3.1.2- 20: Test organisms.

Criteria	**Details**
Species/strain	*Coturnix coturnix japonica* (Japanese quail)
Source	Lincolnshire Pheasantries, Boston, Lincolnshire, UK
Age	8 weeks upon arrival
Sex	35 males, 35 females
Initial body mass	189–294 g (start of dosing period)
Age range within the test	All individuals of the same age
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of first dosing	c. 13 weeks
Health condition/medication	Signs of bad health condition and prophylactic medication were not reported

Table A7.5.3.1.2- 21: Test system.

Criteria	Details
Test location	Indoor holding pens
Holding pens	Galvanised steel cages 65 × 50 × 44 cm (l × w × h)
Number of animals	50
Number of animals per pen [cm²/bird]	5 individuals per pen, by sex and treatment; 650 cm^2/individual
Number of animals per dose	10 (5 m, 5 f)
Pre-treatment/ acclimation	Acclimation period > 14 d Environmental conditions as in the test (see Table A7.5.3.1.2- 23) Feed: basal diet as specified below Feed and water available *ad libitum*
Diet during test	Standard HRC Layer Diet, with added test substance, as appropriate; manufactured by Joseph Odam Ltd., Eye Mill, Peterborough, Cambridgeshire, UK; no analysis results reported; composition of the diet, as specified by the supplier, is given in Table A7.5.3.1.2- 22.
Dosage levels of test substance	Dietary administration; for dosage levels see, e.g. Table A7.5.3.1.2- 26.
Replicate/dosage level	Not appropriate
Dosing method	Dietary, for 5 days
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Daily observation for mortality and clinical signs.
Time and intervals of body weight determination	At days 0, 5, 12, 19, 26, and 33

Table A7.5.3.1.2- 22: Composition of the commercial diet from source specified in Table A7.5.3.1.2- 21.

Ingredient	Fraction [% w/w]
Ground wheat	38.25
Maize meal	30.0
Weatings (Wheat feed)	5.0
Provimi 66 fish meal	10.0
Extracted soybean meal	10.0
Limestone flour	5.5
Pantoribin 537*)	1.25

*) Mineral, vitamin and trace element supplement by B.P. Nutrition (UK) Ltd.

Table A7.5.3.1.2- 23: Means and ranges of daily measurements of temperatures and relative humidities during the test and the acclimation period.

	Mean	Range
T [°C], max.	22	16–27
T [°C], min.	16	11–20
Rel. humidity [%]	93	70–100

Table A7.5.3.1.2- 24: Test conditions.

Criteria	Details
Test temperature	Temperatures are separately listed in Table A7.5.3.1.2- 23
Shielding of the animals	Not stated
Ventilation	Ventilation ensured; no air change rates reported
Relative humidity	Humidity data are listed in a separate table (Table A7.5.3.1.2- 23)
Photoperiod and lighting	14:10 h (L:D) type of lighting not further specified

Table A7.5.3.1.2- 25: Mortality data from the range-finding test.

Dietary test substance concentration [ppm]	Mortality after test termination (21 days)	
	Number	Percent
0	0	0
50	9	90
200	9	90

Table A7.5.3.1.2- 26: Treatment-related mortality data after test termination.

Dietary test substance concentration [ppm]	Mortality after test termination (33 days)			
	Total number		Percentage	
	males	*females*	*males*	*females*
1.5	0	0	0	0
5.0	0	1	0	20
15.0	2	2	40	40
50.0	3	2	60	40

For temperature and humidity data see Table A7.5.3.1.2- 23

Table A7.5.3.1.2- 27: Mean body weights changes of Japanese quails during the dietary toxicity test of Flocoumafen (main test), including the control group.

TS concentration [ppm]	Sex	Mean body weight changes [g] during sampling periods				
		0–5	**5–12**	**12–19**	**19–26**	**26–33**
0	m	+4	–7	+10	+10	–11
0	f	–8	–26	+31	–16	0
1.5	m	+3	–11	+3	–3	–8
1.5	f	–39	–6	+53	–12	–15
5.0	m	+1	–12	–15	+11	+4
5.0	f	–26	–6	+47	–38	+20
15.0	m	–6	0	+10	–8	+1
15.0	f	–9	+15	+2	–9	–5
50.0	m	–18	–32	+20	+1	+2
50.0	f	–21	–32	+57	+5	–13

Table A7.5.3.1.2- 28: Mean feed consumption of Japanese quails [g / individual × d] during the dietary toxicity test of Flocoumafen (main test), including the control group.

TS concentration [ppm]	Sex	Mean feed consumption [g] during days of study								
		1	**2**	**3**	**4**	**5**	**6–12**	**13–19**	**20–26**	**27–33**
0	m	28	19	32	28	25	24	27	28	26
0	f	25	34	38	32	10	23	33	24	18
1.5	m	19	23	22	21	23	19	28	24	21
1.5	f	16	57	50	29	17	25	43	31	18
5.0	m	32	15	29	30	26	21	23	23	29
5.0	f	20	38	41	38	28	23	43	24	23
15.0	m	21	26	28	17	21	26	30	25	31
15.0	f	36	26	39	32	19	31	34	33	31
50.0	m	25	26	32	24	12	18	30	32	26
50.0	f	27	29	23	21	26	18	43	39	26

Table A7.5.3.1.2- 29: Validity criteria for short-term toxicity test according to OECD 205.

	Fulfilled	**Not fulfilled**
Mortality of control animals ≤10%	☑	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	☑	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	☑	

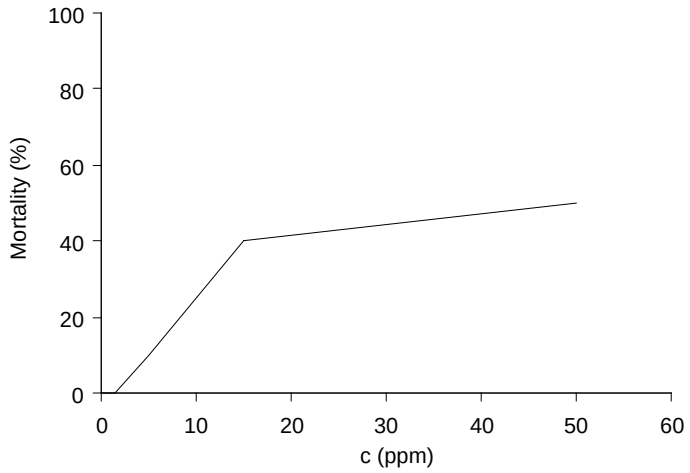

Figure A7.5.3.1.2- 3: Dose-response curve (dietary concentration vs. mortality) of Flocoumafen from the avian dietary toxicity test in *Coturnix coturnix japonica*.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.5.3.1.2/05:** Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1986) The short-term cumulative dietary toxicity of WL108366 to the mallard duck. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 74BT/841259, March 12, 1985 (unpublished). (BASF-Ref.: FL-505-009)	X
		A7.5.3.1.2/06: Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1986) The short-term cumulative dietary toxicity of WL108366 to the mallard duck. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 69BT/841085, March 5, 1985 (unpublished). (BASF-Ref.: FL-505-008) Remark: Reference A7.5.3.1.2/06 was designed as a range-finding test for the main study (A7.5.3.1.2/05). Therefore, these reports are jointly reviewed in the current study summary for convenience.	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	No	
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/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Nevertheless, the method applied is consistent to OECD 205 in all important aspects.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes The spacing of dose levels (see 4.2.1) exceeds the factor 2.0 as suggested by OECD 205. The number of treatment levels (see 4.2.1) is less than 5, as suggested by OECD 205.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	As given in Section A2.	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

3.1.3	Purity	> 99 %	
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to have affected the test performance.	
3.1.5	Method of analysis	Standard HPLC, as described in Section A4. Test substance concentrations in the diet were ≥ 81.5 % of nominal.	
3.2	**Administration of the test substance**	Dietary administration; details presented in Table A7.5.3.1.2- 30.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Mallard ducks, as described in Table A7.5.3.1.2- 31.	
3.4.2	Test system	See Table A7.5.3.1.2- 32.	X
3.4.3	Diet	Basal diet is specified in Table A7.5.3.1.2- 32 and Table A7.5.3.1.2- 33; the test diet was prepared as described in Table A7.5.3.1.2- 30.	
3.4.4	Test conditions	Test conditions are provided in Table A7.5.3.1.2- 34 and Table A7.5.3.1.2- 35.	
3.4.5	Duration of the test	33 d (5 d administration, 28 d post-treatment observation)	
3.4.6	Test parameter	Mortality	
3.4.7	Examination/ observation	See Table A7.5.3.1.2- 32.	
3.4.8	Statistics	LC_{50} by graphical procedures.	

4 RESULTS

4.1	**Range finding test**	Range-finding was performed in a separate study (Report No. SLL 69BT/841085, reference A7.5.3.1.2/ 6), which is included in the current study summary.	
4.1.1	Concentration/dose	0, 50, and 200 ppm	
4.1.2	Number/ percentage of animals showing adverse effects	Mortality data are presented in Table A7.5.3.1.2- 36.	
4.1.3	Nature of adverse effects	Apart from mortality, the following clinical signs were observed: subdued behaviour, weakness and unsteadiness over days 5–12; food consumption in the females of the 200 ppm group declined during the exposure period and remained reduced in the post-treatment period.	X

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

4.2	**Results test substance**		
4.2.1	Applied concentrations	Nominal dietary concentrations of 1.5, 5.0, 15.0, and 50.0 ppm.	
4.2.2	Effect data (mortality)	Mortalities are provided in Table A7.5.3.1.2- 37. LC_0 < 1.5 ppm LC_{50} = 1.7 ppm (no confidence interval provided) LC_{100} = 5.0 ppm	X
4.2.3	Body weight	Average body weight changes in each pen (identical with sex) at each observation point are presented in Table A7.5.3.1.2- 38.	X
4.2.4	Feed consumption	Mean feed consumption in each pen (identical with sex) at each observation point is presented in Table A7.5.3.1.2- 39.	
4.2.5	Concentration-response curve	A graphical representation of the dose-response curve is given in Figure A7.5.3.1.2- 4. A slope estimate cannot be given due to the application of graphical methods.	
4.2.6	Other effects	In the dose groups of 5.0 to 50.0 ppm, several birds showed subdued and unsteady behaviour.	X
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were observed in the control group.	
4.3.2	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The short-term cumulative toxicity of Flocoumafen to birds was tested using mallard ducks (*Anas platyrhynchos)*. Although not a guideline study, the method used was consistent to OECD 205 in all important aspects. The study deviated from this guideline in the spacing factors between dose levels applied. They varied among each other and were larger than 2.0. Furthermore, only four treatment levels were applied. These deviations are not considered to have affected the results.
5.2	**Results and discussion**	The physical-chemical properties of Flocoumafen (see Section A3) are not considered to have affected the test results. Analysis results demonstrated that the test substance was homogeneously mixed in the test diet and that concentrations of > 80 % of nominal were maintained.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

5.2.1	LC_0	LC_0 < 1.5 ppm	
5.2.2	LC_{50}	LC_{50} = 1.7 ppm	X
5.2.3	LC_{100}	LC_{100} = 5.0 ppm	
5.3	**Conclusion**	The validity criteria of the OECD guideline 205 are partly fulfilled (Table A7.5.3.1.2- 40). In addition the occurrence of adverse effects in the lowest treatment level is not considered a major deficiency since this prevents the estimation of a NOEL. The number and spacing of dose-levels, deviating from current guideline specifications (2.3) are considered to be major deficiencies. The failure to determine a NOEL is likely to be related to these dose-level settings. Thus, the study is considered to be of limited acceptability. The observed mortality can be interpreted as the result of high susceptibility of the mallard duck to repeated administration of Flocoumafen. The dietary short-term toxicity of Flocoumafen has been estimated at LC_{50} = 1.7 ppm.	X
5.3.1	Reliability	3	X
5.3.2	Deficiencies	Yes As discussed under 5.3, the study is considered to suffer from major methodological deficiencies according to current guidelines, which compromises its acceptability.	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	07 February 2005
Materials and Methods	(1.1) Both references, (1986) should read (1985) (3.4.2) Table A7.5.3.1.2-32, Pre-treatment/ acclimation, "Acclimation period >14 d" should read "Acclimation period 14 d". Replicate/dosage level should read "2 (5 male or 5 female birds per replicate)" instead of "Not applicable".
Results and discussion	(4.1.3) Mean food consumption was reduced in female birds at 200 ppm during the 5-day exposure period, and in all surviving birds at 50 and 200 ppm during the post-exposure observation period. (4.2.2) Table A7.5.3.1.2-37, percentages for males and females should respectively read 40 and 20 instead of 0 and 0 at 1.5 ppm, and 100 and 100 instead of 40 and 20 at 5.0 ppm. No mortality occurred in the control. (4.2.3) Table A7.5.3.1.2-38, body weight changes for males and females at 0 ppm should read –7 and –66, +14 and +53, and +11 and +16 for days 0-5, 5-12 and 12-19, respectively. (4.2.6) All birds that died (1.5-50.0 ppm) showed signs of haemorrhaging in the body cavity, and two surviving birds at 1.5 ppm showed signs of haemorrhaging on the legs.
Conclusion	"(5.2.2) LC50 = 6.1 ppm (mortality data recalculated with Spearman-Karber). 95% CI=4.4-8.6 ppm. The 5-day LC50 of 6.1 ppm was equivalent to 2.73 mg/kg bw/day. (5.2.3) LC100 > 10.0 ppm (estimation from graph)and the The 5-day LC_{50} was 6.1ppm (equivalent to 2.73 mg/kg bw/day) and the 5-day NOEC was <1.5 ppm.
Reliability	2 (see remarks)
Acceptability	Acceptable
Remarks	The LC_{50} is the primary end point in this test. The OECD 205 guideline does not prescribe the determination of a NOEL. Even though effects occurred at the lowest dose, the data were sufficient to determine the LC_{50}. The spacing of dose levels is rather wide (preferable is a factor of 2) but given the test results, with 30% and 100% mortality at 1.5 and 5.0 mg/kg, the estimate of the LC_{50} is considered to be sufficiently accurate. The recommended age of the birds is 10-17 days (OECD 205). The age of the birds used in this test was 10 weeks. The feed in take rate per kg body weight (and hence the test substance intake rate) is higher for younger birds. The reliability of the study is lowered to 2.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.2- 30: Method of administration of the test substance.

Carrier/Vehicle	Details
Water	No
Organic carrier	Yes: Acetone
Concentration of the carrier	0.042 % w/w in the final diet after mixing acetone was removed by treatment in a rotary evaporator at 40 °C for 20 min
Other vehicle	Basal diet, as specified in Table A7.5.3.1.2- 32 and Table A7.5.3.1.2- 33: the stock solution (Flocoumafen in acetone) was mixed with the diet as appropriate.
Function of the carrier/ vehicle	Acetone: solvent for test substance

Table A7.5.3.1.2- 31: Test organisms.

Criteria	Details
Species/strain	*Anas platyrhynchos* (Mallard duck)
Source	The County Game Farms, Home Farm, Hothfield, Ashford, Kent, UK.
Age	8 weeks upon arrival
Sex	25 males, 25 females
Initial body mass	860–1265 g (start of dosing period)
Age range within the test	All individuals of the same age
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of first dosing	c. 10–11 weeks
Health condition/medication	Signs of bad health condition or prophylactic medication were not reported

Table A7.5.3.1.2- 32: Test system.

Criteria	Details
Test location	Indoor in holding pens
Holding pens	Plastic coated steel wire, tiered cages 144 × 41 × 53 cm (l × w × h)
Number of animals	50
Number of animals per pen [cm²/bird]	5 individuals per pen, by sex and treatment 1181 cm^2/individual
Number of animals per dose	10 (5 m, 5 f)
Pre-treatment/ acclimation	Acclimation period > 14 d Environmental conditions as in the test (see Table A7.5.3.1.2-34) Feed: basal diet as specified below Feed and water available *ad libitum*
Diet during test	Standard HRC Layer Diet, Joseph Odam Ltd., Eye Mill, Peterborough, Cambridgeshire, UK, with added test substance, as appropriate; no analysis results reported; composition of the diet, as specified by the supplier, is given in Table A7.5.3.1.2- 33.
Dosage levels of test substance	Dietary administration; for dosage levels see, e.g. Table A7.5.3.1.2- 37.
Replicate/dosage level	Not appropriate
Dosing method	Dietary, for 5 days
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Daily observation for mortality and clinical signs.
Time and intervals of body weight determination	At days 0, 5, 12, 19, 26, and 33

Table A7.5.3.1.2- 33: Composition of the commercial diet from source specified in Table A7.5.3.1.2- 32.

Ingredient	Fraction [% w/w]
Ground wheat	38.25
Maize meal	30.0
Weatings (Wheat feed)	5.0
Provimi 66 fish meal	10.0
Extracted soybean meal	10.0
Limestone flour	5.5
Pantoribin 537*)	1.25

*) Mineral, vitamin and trace element supplement by B.P. Nutrition (UK) Ltd.

Table A7.5.3.1.2- 34: Means and ranges of daily measurements of temperatures and relative humidities during the test and the acclimation period.

	Mean	Range
T [°C], max.	22	16–27
T [°C], min.	16	11–20
Rel. humidity [%]	93	70–100

Table A7.5.3.1.2- 35: Test conditions.

Criteria	Details
Test temperature	Temperatures are listed in a separate table (Table A7.5.3.1.2- 34)
Shielding of the animals	Not stated
Ventilation	Ventilation ensured; no air change rates reported
Relative humidity	Humidity data are listed in a separate table (Table A7.5.3.1.2- 34)
Photoperiod and lighting	14:10 h (L:D) type of lighting not further specified

Table A7.5.3.1.2- 36: Mortality data from the range-finding test.

Dietary test substance concentration [ppm]	Mortality after test termination (21 days)	
	Number	Percent
0	0	0
50	10	100
200	10	100

Table A7.5.3.1.2- 37: Treatment-related mortality data after test termination.

Dietary test substance concentration [ppm]	Mortality after test termination (33 days)			
	Total number		Percentage	
	males	*females*	*males*	*females*
1.5	0	0	0	0
5.0	2	1	40	20
15.0	5	5	100	100
50.0	5	5	100	100

For temperature and humidity data see Table A7.5.3.1.2- 34

Table A7.5.3.1.2- 38: Mean body weights changes of mallard ducks during the dietary toxicity test of Flocoumafen (main test), including the control group.

TS concentration [ppm]	Sex	Mean body weight changes [g] during sampling periods				
		0–5	5–12	12–19	19–26	26–33
0	m	+14	+11	+10	–6	–46
0	f	+53	+16	+31	–4	–28
1.5	m	–82	–50	+65	+28	–11
1.5	f	–18	–37	+72	–11	+12
5.0	m	–81	—	—	—	—
5.0	f	–115	—	—	—	—
15.0	m	–54	—	—	—	—
15.0	f	–55	—	—	—	—
50.0	m	–106	—	—	—	—
50.0	f	–175	—	—	—	—

Table A7.5.3.1.2- 39: Mean feed consumption of mallard ducks [g × individual^{-1} × d^{-1}] during the dietary toxicity test of Flocoumafen (main test), including the control group.

TS concentration [ppm]	Sex	Mean feed consumption [g] during days of study								
		1	2	3	4	5	6–12	13–19	20–26	27–33
0	m	112	128	86	129	100	82	99	79	84
0	f	75	109	107	52	50	77	95	73	83
1.5	m	95	252	113	84	54	67	157	129	116
1.5	f	79	109	88	107	88	61	118	28	86
5.0	m	253	76	80	80	20	0	—	—	—
5.0	f	117	132	100	80	8	31	—	—	—
15.0	m	126	140	138	99	32	11	—	—	—
15.0	f	109	110	100	92	13	49	—	—	—
50.0	m	103	96	89	82	28	6	—	—	—
50.0	f	104	115	85	23	22	72	—	—	—

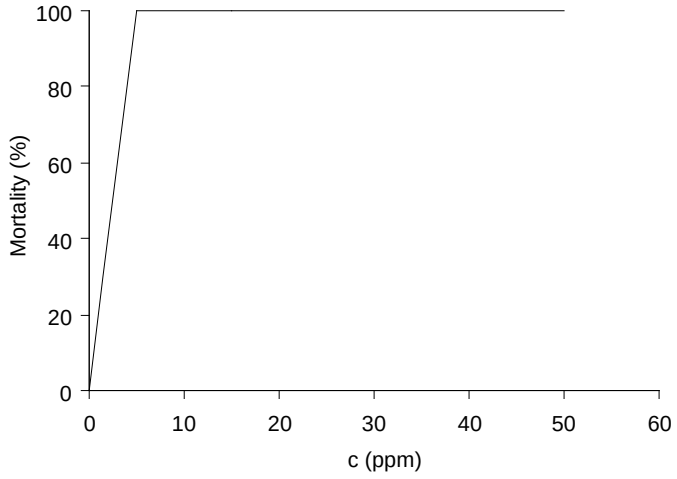

Figure A7.5.3.1.2- 4: Dose-response curve (dietary concentration vs. mortality) of Flocoumafen from the avian dietary toxicity test in *Anas platyrhynchos*.

Table A7.5.3.1.2- 40: Validity criteria for short-term toxicity test according to OECD 205.

	Fulfilled	**Not fulfilled**
Mortality of control animals ≤10%	☑	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	☑	
Lowest treatment level causing no compound-related mortality or other observable toxic effects		☑

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

The following references are considered to contain additional information concerning short-term toxicity on birds and are thus presented in tabular format as supportive data:

Reference	Title	Method	Results
A7.5.3.1.2/07: Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1986) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 79BT/851436, February 6, 1986 (unpublished). (BASF Ref.: FL-505-011)	The short-term cumulative dietary toxicity of WL108366 to the house sparrow.	Wild-caught house sparrows (*Passer domesticus*) were subjected to a short-term dietary toxicity test. 5 days of treatment at dose levels 0 (control), 1.5, 5.0, 15.0, and 50.0 ppm. 5 male and 5 female birds per dose level. 21-d post-treatment observation. Post-mortem examination after termination of the test. Although guideline compliance was not stated, the method used was consistent to OECD 205 in all important aspects. GLP: Yes	The study is considered invalid due to unintentional mortalities in the control group. Control mortalities were obviously stress-related. Thus, reliable discrimination of stress- and treatment-related mortalities was not possible, although this was attempted via necropsy findings. The LC_{50} estimate considering birds with haemorrhages only is given at 185 ppm. The LC_{50} estimate taking into account all birds is given at 17 ppm. Meaningful confidence intervals could not be obtained.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

A7.5.3.1.2/08: Fxxxx Sxxxx (1988) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SBGR.87.227, April 28, 1988 (unpublished). (BASF Ref.: FL-505-013)	The effect of a repeated oral dose of WL108366 (Storm) on the liver residue in Japanese quail.	Japanese quail (*Coturnix coturnix japonica*) were administered Flocoumafen via the diet once a week (for 24 hours) over 20 weeks at dietary concentrations of 5, 15 and 50 ppm, plus a control group. Standard clinical examinations were conducted daily. Post-exposure monitoring of up to 9 weeks was included. Intermediate sacrifices of sub-groups were done after week 5, 9, 13, 17, 21, 25 and 29 (the latter two correspond to post-exposure monitoring). Sacrificed birds were subjected to necropsy and analysis of Flocoumafen residues in livers following method SAMS 419-3, which is equivalent to that reported in A4.2/05.	*Mortality:* Control: 1/7 5 ppm: 1/21 15 ppm: 2/21 50 ppm: 2/21 Thus, there was no clear dose-response regarding mortality. *Body weight, food consumption:* No treatment-related effect on food consumption; body weights were generally within normal limits although the control group gained slightly more weight. *Clinical observations:* Faeces contained traces of blood at 15 and 50 ppm; external injuries were not considered to be treatement-related. *Necropsy:* Evidence of haemorrhaging in a small number of the 15 and 50 ppm birds, but the majority of birds showed no abnormality. *Liver residues:* Overall mean Flocoumafen residues were 55 mg/kg; residue levels were neither related to the dietary concentration nor to the number of exposures; in week 25, residues fell to 0.2 mg/kg, and in week 29 to 0.11 mg/kg. *Conclusion:* Repeated exposure of quails at weekly intervals did not result in cumulative liver residues in excess of 55 mg/kg (mean), irrespective of the dose; at such a dosing regime, birds are apparently capable of readily metabolising and eliminating Flocoumafen; toxic effects are relatively mild, with no enduring toxicity at 5 ppm; 15 and 50 ppm, however, are less tolerated.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

A7.5.3.1.2/09: Hxxxx Kxxxx, Wxxxx Pxxxx (1986) Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report no SBGR.85.192., April 16, 1986 (unpublished). (BASF Ref.: FL-505-015)	WL108366: Absorption, metabolism and disposition in Japanese quail (*Coturnix coturnix japonica*) following a single dose by intraperiotneal or oral administration.	Male Japanese quail were administered a single dose of 0.14 mg/kg b.w. [^{14}C]-Flocoumafen either i.p. or by gavage. Excreta were investigated over a 7-day period and analysed for active substance and metabolites. Tissue distribution and depletion was investigated upon necropsy on days 2, 4, 7, 14, 28 and 112 following administration. Analytical techniques employed were combustion analysis, radio-HPLC, TLC and LSC, as appropriate.	74.9% of the oral dose and 90.9% of the i.p. dose were eliminated in the faeces after 7 days. In each case, the majority of radioactivity was excreted within 24 h. Tissue distribution was *liver* > *spleen* > *skin* > *intestine*; cumulative tissue residues accounted for < 1.5% after 7 days. Radioactive residues in the liver were associated with the microsomal and mitochondrial fractions, in the former case predominantly to the rough membrane. At least 12 radioactive components were detected in the faeces, less than 10% of the recovered radioactivity was identical to unchanged Flocoumafen; the other compounds were more polar, with evidence for some of them of being glucuronic conjugates; structural characterisation of the metabolites was not possible. *Conlcusion:* Flocoumafen is rapidly metabolised and eliminated in the quail.

Evaluation by Competent Authorities	
Use separate “evaluation boxes” to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	3 February 2005
Conclusion	The presentation of the above studies as supportive data is accepted.

Remarks	**A7.5.3.1.2/07**: The acclimatisation period following capture was 7 days. Concentrations of flocoumafen in the diet were confirmed by analysis. Mortality rates at 0, 1.5, 5, 15 and 15 ppm, respectively, were 20, 60, 20, 60 and 80% (males) and 20, 60, 20, 100 and 80% (females). Deaths in the control were reported to be stress related. Reported LC50 values (using method of Thompson and Weil, taking into account control mortalities) were 56 ppm (95% CI 2-18333 ppm) for males, 5 ppm (95% CI 1-178 ppm) for females, and 17 ppm (95% CI 2-161 ppm) for combined sexes. Mortality rates of birds showing signs of haemorrhaging at post-mortem at 0, 1.5, 5, 15 and 15 ppm, respectively, were 0, 0, 0, 0 and 40% (males) and 0, 40, 0, 80 and 60% (females). Reported LC50 values based on the latter data (using method of Thompson and Weil, taking into account control mortalities) were 457 ppm (95% CI 2-115143 ppm) for males, 30 ppm (95% CI 7-12522 ppm) for females, and 185 ppm (95% CI 2-22025 ppm) for combined sexes. **A7.5.3.1.2/08**: (1) This reference comprises the full report of the study summarised under part c of supportive data A7.5.3.1.3/01. (2) Clarification of study design and study results: The study was conducted under GLP. Replication: 3 birds were sacrificed per treatment level and time point (and 1 bird fed untreated control diet). Concentrations of flocoumafen in the diet were confirmed by analysis. It was demonstrated that under frozen conditions flocoumafen was stable in diet for 4 weeks. Body weight gain was reduced at all treatment levels compared to the control. The overall mean flocoumafen concentration in the liver was 0.55 mg/kg, and the range for all dose levels and time points was 0.38-0.75 mg/kg. In week 25, mean liver residues fell to 0.20 mg/kg, and in week 29 to 0.11 mg/kg. **A7.5.3.1.2/09**: (1) This reference comprises the full report of the studies summarised under parts a and b of supportive data A7.5.3.1.3/01. (2) Clarification of study design and study results: The study was conducted under GLP. Quails were 9-11 weeks old. The dose level was 14 mg/kg bw (not 0.14 mg/kg bw). Replication: 4 birds per group (oral versus i.p. dose administration), 2 birds per time point for the tissue distribution versus time test. 76% (not 74.9%) of the oral dose and 90.9% of the i.p. dose were eliminated in the faeces after 7 days. Residues in liver and spleen were 2.29 and 0.97 mg/kg after 2 days, and 0.44 and 0.13 mg/kg after 112 days (when residues in other organs investigated were ≤0.07 mg/kg). After 2 days, flocoumafen represented 69% of the TRR in liver. Radioactivity in excreta after 7 days represented 8.3% and 10.2% of the TRR in samples from i.p. and orally dosed birds.
	COMMENTS FROM ...

Date	
Conclusion	
Remarks	

Section A7.5.3.1.3 Effects on reproduction in birds

Annex Point IIA 13.1.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	If a rodenticide is intended to be used "outside buildings in the form of baits (…)", a full set of avian toxicity studies including a test of effects on reproduction is required according to Chapter 2.5 of the TNG on data requirements. Since the intended uses for the Flocoumafen-based product STORM BB are specified as "in and around buildings", this would usually trigger the conduct of an avian reproduction study. The current document summarises the arguments why the performance of such a study is not considered to be required. *Toxicity profile and mode of action* Flocoumafen, as a typical 4-hydroxycoumarin derivative, acts as an indirect anticoagulant via inhibition of the enzyme "vitamin K epoxide reductase". Recycling of vitamin K hydroquinone via this enzyme is an essential prerequisite for maintenance of the synthesis of blood clotting factors (Section A5.4). This specific inhibitory property determines the mode of action against target organisms as well as the nature of toxic effects on vertebrates in general (Section A6). In the large body of mammalian toxicity studies allocated to Section A6 of the current dossier, no other toxic effects than reduced blood clotting ability have been reported. Specifically, there are no indications for reproductive or developmental toxicity in rats and rabbits (A6.8.1). In addition, there was a complete absence of effects on organ weight, morphology and histopathology of reproductive organs in a sub-chronic (oral) study in rats (A6.4.1). In acute and short-term avian toxicity studies (A7.5.3.1.1 and A7.5.3.1.2), apart from haemorrhaging as a typical symptom of anticoagulant poisoning, no other detectable adverse effects at all were reported. A study on the dietary toxicity and pharmacokinetics of Flocoumafen in hens also considered effects on egg production (A6.13/04 and /10). Significant dose-related effects on the number of eggs and egg mass were not detected at dosages that produced mortality. Additionally, dietary exposure of Japanese quail up to 50 ppm Flocoumafen at weekly intervals up to 20 weeks (A7.5.3.1.3/01, presented as supportive data below) did not reveal any other adverse effects than typical anticoagulant poisoning symptoms. Results indicate that birds are able to metabolise Flocoumafen at a relatively high rate, which is considered to be the reason for the relatively low toxicity of the compound to birds. Thus, according to the toxicity profile, it is not expected that the conduct of a reproduction toxicity study in birds would significantly contribute to existing knowledge. *Primary exposure* A hypothetical hazard of primary exposure to rodenticide bait material is given for any seed-eating bird. However, since Flocoumafen is applied only in the form of wax-bound bait blocks, the design and size of these baits render them virtually impossible to be ingested by birds. The only possible risk of exposure arises from "crumbs" as a result of gnawing of	

Section A7.5.3.1.3 Effects on reproduction in birds

Annex Point IIA 13.1.3

bait blocks by rodents, which are similar to grains in shape and size, thus rendering the extent of such exposure as very limited, for the following reasons: (i) the blue colour of the bait makes them demonstrably unattractive to birds (B7.8.7.2/01); (ii) according to the use instructions (good baiting practice) on the label of the product, the bait must be laid out in specially designed bait boxes, rodent burrow entrances, or to be covered by boards, tiles etc. in such a manner that any relevant availability even of small bait remains to birds is excluded. Actually, the stated low primary exposure potential for birds is supported by the results from field trials on risks to non-target organisms (B7.8.7.1/05–08).

Thus, the design of the wax block formulation makes the bait virtually impossible for birds to ingest. In combination with good application practice, primary exposure hazard for birds is considered negligible.

Secondary exposure

Predatory (owls, birds of prey) and scavenging (crows, magpies, gulls) birds are potentially at risk of secondary exposure via consumption of intoxicated rodents (dead or moribund). However, the risk of consumption of dead rodents is initially greatly reduced by following good baiting practice as given in the label instruction “collect and burn or bury all rodent bodies”.

Temporal coincidence of exposure to rodenticides and reproduction seems very unlikely, since predatory birds breed during spring and summer, whereas rodent control campaigns in contrast are usually conducted during autumn and winter, when rodents become a problem in domestic areas.

The low risk of secondary exposure in general is supported by findings of toxicity studies in barn owls (*Tyto alba*) and monitoring data: Barn owls fed on Flocoumafen-poisoned mice, thus receiving doses of 0.11–0.23 mg/kg, showed no symptoms of anticoagulant poisoning and bred successfully in the subsequent season (B7.8.7.2/02, B7.8.7.2/03), which supports the assessment that there are no long-term effects on reproduction. Monitoring of exposure of barn owls via pellet analysis in areas with known Flocoumafen use resulted in no confirmed residues (B7.8.7.1/01–03). Recent data from a monitoring programme (B7.8.7.2/17, 18) indicate extremely low exposure of predatory birds to Flocoumafen.

In summary, secondary exposure of birds to Flocoumafen can be considered to be minimal. While predatory birds indeed may be potentially exposed to Flocoumafen, a review of available data suggests that significant exposure in the reproductive period is very unlikely to occur. Furthermore, the label instructions direct users to adopt measures in order to minimise any potential for exposure of birds to Flocoumafen.

Technical feasibility and its implications for risk assessment

Particularly with second-generation anticoagulants such as Flocoumafen, progressive daily doses can be expected to accumulate in the liver until the coagulation cascade is compromised and death occurs. While use of the materials as rodenticides in baits at 50 ppm is lethal after one or two exposures, it is theoretically possible to administer low, non-lethal doses in the experimental situation. However, no matter how low the dose, the compound will still accumulate with time, until lethal levels are reached. Whereas it is possible to conduct short-term avian studies provided the accumulated dose never reaches lethal levels, it is

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not foreseeable that the daily dose in a long-term study such as the avian reproduction study would be possible at a dose that elicits a toxic maternal response other than anticoagulation and subsequent haemorrhaging with lethal outcome, for the following reasons: to ensure validity of such a study, the highest dose should induce some form of toxicity, not necessarily to reproduction, but in a form that can be measured (for example, reduced bodyweight gain, or changes in organ function or histopathology). This level of toxicity (referred to as the Maximum Tolerated Dose – MTD) at the high dose level should ideally not affect the animals sufficiently to affect adversely their survival over the length of the study, and should not induce more than 10 % additional mortality compared to the control. However, by cross-reading to the sub-chronic study performed in rats, we note that prolonged exposure of this species either did not elicit any effect whatsoever, or at borderline doses that accumulated in the body, but merely induced severe haemorrhaging, with frequent lethal outcome. However, in none of the cases did the administration of the compound elicit any measurable effect on the parameters stated above for an assessment of the onset of toxicity. Violent courtship behaviour of the common test species, and the event of ovulation intrinsically provide further potential for haemorrhagic events.

Avian reproduction studies are usually conducted using a model species in order to extrapolate the results to other bird species. In fact, both the OECD and U.S. EPA guidelines recommend the mallard duck (*Anas platyrhynchos*) and the Northern bobwhite quail (*Colinus virginianus*). However, the most relevant species with respect to long-term exposure to anticoagulants are in fact raptors and owls, as discussed above, which are in fact very distant from the model species regarding habitat, diet and behaviour. Thus, it seems unreasonable to expect that the results from a study conducted on such model species allow meaningful predictions of the potential effects on reproduction in the more relevant predators. As an alternative, the performance of a reproduction study using a focal species (owl, raptor) might be considered. However, this would entail considerable difficulties: according to OECD guideline 206, at least 128 adult birds are required. Apart from the fact that it seems very unlikely that such numbers of a focal species (e.g. the barn owl) are commercially available, it is noted that many such raptors and owls are legally protected throughout the EU. Finally, as a technical aspect, the maintenance of specific and uniform Flocoumafen concentrations in the birds' test diet is considered technically unfeasible: in order to simulate potential exposure in the field, the test substance would have to be administered via rodents fed on Flocoumafen bait. Due to individual variation of metabolism and feeding behaviour, uniform body burdens of Flocoumafen in mice are impossible to maintain. A further practical obstacle would be egg production: According to OECD guideline 206, a minimum number of 28 eggs per breeding pair is a prerequisite for valid estimation of reproductive parameters. All potential focal species by far fall below this value. Thus, a guideline-conform study with an owl or raptor species cannot be conducted.

Conclusions

In conclusion, a review of mammalian and avian toxicological data, including a basic evaluation of reproductive parameters in birds, suggests that reproductive toxicity associated with Flocoumafen should not be expected. Thus, conduct of an avian reproduction study would not contribute significantly to existing knowledge. Further, primary

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	exposure of birds can be considered negligible. Whereas secondary exposure cannot be completely ruled out, practical experience shows that exposure of owls and raptors during the baiting season is indeed minimal. Compliance with good application practice should reduce the potential for secondary exposure to a sustainable minimum. In view of the EU policy to minimise animal testing and the arguments presented above in the current document, the performance of an avian reproduction study does not appear to be justified.	
Undertaking of intended 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 (*)	
Date	23 June 2005	
Evaluation of applicant's justification	The submission of an avian reproduction study is a core data requirement for rodenticides. Reference to mammalian toxicity data is considered to be of limited relevance due to the difference in physiology between birds and mammals. It should be noted however that the waiver for a 2-generation reproduction study in the rat, partly based on similar grounds (toxicological profile, practical non-feasibility) was not accepted (see doc BIII, section 6.8.2). The toxicity studies in hen were a 5-day dietary toxicity study that only investigated egg production (slight reduction at highest dose of 50 mg/kg diet) and egg weight (no effects), and a 5-day repeated dose oral gavage study that only investigated egg production (reported to be erratic in hens surviving the highest dose of 4 mg/kg bw/day). The number of parameters investigated in these studies is too small, and the duration of exposure too short, for the results to be relevant. The repeated dose study in Japanese quail (exposure through the diet once weekly for up to 20 weeks) did not investigate reproductive parameters but showed that Japanese quails can tolerate this intermittent exposure to low levels of flocoumafen (up to 50 mg/kg diet) for a long period. Use of flocoumafen is not be limited to autumn and winter, but will be performed when there is a pest problem, and treatment may even be continuous. In the toxicity studies with barn owls, only acute effects were investigated, and although one pair of owls bred successfully the season after the treatment, the other pair did not breed, although this failure may have been the result of incorrect sexing. It is not a guideline requirement that an avian reproduction study should include a dose eliciting maternal toxicity in order to be valid. There is no experimental evidence (e.g. a range-finding study) that a reproduction study with low levels of flocoumafen is not technically feasible. A protocol for a range-finding study with difenacoum however has been submitted. There is insufficient evidence that the	

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	claim that, no matter how low the dose, the compound will still accumulate with time, until lethal levels are reached, is correct for the duration of an avian reproduction study. It should be noted that the data gap for an avian reproduction study was already identified in the completeness check of September 2004, where it was concluded that a waiver and a study protocol were acceptable and that the study report would be submitted within a few months.
Conclusion	Repeated exposure of birds to flocoumafen is possible, and the risk for effects on reproduction needs to be evaluated. The arguments put forward are insufficient to waive the need for an avian reproduction study.
Remarks	No further remarks.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

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Annex Point IIA 13.1.3 Supportive data

The following reference is considered to contain further information in order to support non-submission of an avian reproduction study and is thus presented in tabular format as supportive data:

Reference	Title	Method	Results
A7.5.3.1.3/01: Huckle KR, Warburton PA, Forbes S, Logan CJ (1989), Xenobiotica 19: 51-62 (published).	Studies on the fate of Flocoumafen in the Japanese quail (*Coturnix coturnix japonica*).	a) *Absorption and elimination*: Male quail were administered single doses (14 mg/kg b.w.) of ^{14}C-Flocoumafen (50 µCi/bird) orally (*n*=4) or by intra-peritoneal injection (*n*=4). Excreta were collected every 24 h for 7 days. Samples from selected tissues were taken following sacrifice. Flocoumafen residues in tissues and excreta (sub-samples) were analysed by radio-analysis (combustion and LSC). For determination of metabolites, excreta were analysed using radio-chromatographic techniques (TLC). Partially purified metabolites were subjected to enzyme hydrolysis by sulphatase and *β*-glucoronidase to gather information on identity. b) *Time-dependent depletion from various tissues*: Quail orally dosed ^{14}C-Flocoumafen as above (*n*=12) were sacrificed at 2, 4, 14, 28, and 112 days after dosing and tissues submitted to radio-analysis as above. Birds from (a) provided a day 7 time point. c) *Effects of repeated dietary exposure on accumulation in the liver*: Non-radioactive Flocoumafen was administered to male quails (*n*=63) at dietary concentrations of 5, 15, and 50 ppm for 24 h at weekly intervals, up to 20 weeks. Three birds at each dose level were sacrificed 7 days after receiving 4, 8, 12, 16, or 20 dietary doses. Further 3 individuals, respectively, were fed off for 5 or 9 weeks before termination. Livers were removed and Flocoumafen residues determined by HPLC.	a) 69% (oral dose) and 79% (intra-peritoneal) of the administered radioactivity were eliminated after 24 h. After 7 days, these figures were 76% and 91%, respectively. ^{14}C-Flocoumafen was recovered from all investigated tissues, with the highest concentration found in the liver. The two principal components detected by radio-TLC were labile to *β*-glucoronidase and thereby yielded aglycones chromatographically identical with the Flocoumafen isomers. b) Depletion from the liver (and similarly from all other tissues) was biphasic, with a rapid decline in the first week (half life *c.* 3 to 5 days) followed by a slow terminal elimination phase (half life > 100 days). The radioactivity located in the liver was mostly (86%) unchanged Flocoumafen. c) Hepatic Flocoumafen concentrations were independent of the dietary dose (overall mean = 0.55 mg/kg or 1.0 nmol/g), indicating that the binding capacity was saturated. After removal of Flocoumafen from the diet, residues were reduced by 66% and 80% after 5 and 9 weeks, respectively, and the rate of depletion was independent of the dose. Flocoumafen treatment was survived by all birds. In conclusion, the hepatic binding capacities for Flocoumafen are similar in quail and rats. However, the apparently lower toxicity in quail obviously results from their ability to more extensively metabolise the compound than the target organism rat.

Date **Conclusion** **Remarks**	EVALUATION BY RAPPORTEUR MEMBER STATE (*) 3 February 2005 The presentation of the above study as supportive data is accepted. (**Results, part b**) The radioactivity located in the liver consisted of 69% unchanged Flocoumafen (not 86%). (**Results, part c**) The full report of this part of the study was submitted as supportive data under reference A7.5.3.1.2/08. The overall mean concentration in the liver was 0.55 mg/kg, and the range for all dose levels and time points was 0.38-0.75 mg/kg. (**...In conclusion...**) It is deemed more appropriate to state: "... the apparently lower toxicity in quail may result from ..." than "... the apparently lower toxicity in quail obviously results from ...". Further remarks: The description of study was sufficiently detailed, and the results are considered to be reliable.
Date **Conclusion** **Remarks**	COMMENTS FROM ...

Section A7.5.3.1.3 Effects on reproduction in birds

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Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.3.1.3/02:** Bxxxx Jxxxx (2005) Avian reproduction study with Difenacoum in the Japanese quail (*Coturnix coturnix japonica*). Gxxxx Lxxxx, Ixxxx, Report no. 04012 Unpublished [DF-7.5.3.1.3-0389]. Study Initiation: October 28, 2004; in-vivo experimental work carried out between 31 May 2005 and 18 September 2007.
1.2	**Data protection**	Yes
1.2.1	Data owner	Sorex Limited
1.2.2	Companies with letter of access	BASF, HENTSCHKE & SAWATZKI KG, Liphatech S.A.S., Syngenta Crop Protection AG
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Primary: OECD Test Guideline 206: Avian Reproduction Test, 1984. Secondary: Modified in places to follow OECD Test Guideline "Draft Document 1998": Avian Toxicity Test in the Japanese Quail or Japanese Quail, and US EPA Ecological Effects Guideline OPPTS 850.2300: Avian Reproduction Test
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Difenacoum
3.1.1	Lot/Batch number	H224750057
3.1.2	Specification	Technical grade active ingredient
3.1.3	Purity	96.7% w/w
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Not applicable
3.1.6	Method of analysis	The extraction of difenacoum from avian feed involved homogenisation with acetone followed by evaporation. The extract is then further cleaned up using a hexane:acetonitrile liquid-liquid partition. An aliquot of the acetonitrile phase is taken for quantification by LC-MS/MS using positive ion chemical ionisation. A validated LOQ of 0.01 mg/kg difenacoum was obtained.

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3.2	**Administration of the test substance**	Treated diets prepared and offered *ad libitum*. Refer also to Table A7.5.3.1.3- 1.
3.3	**Testing procedure**	
3.3.1	Test organisms	Japanese quail, *Coturnix coturnix japonica*. Refer also to Table A7.5.3.1.3- 2.
3.3.2	Test system	Dietary administration offered *ad libitum*. Refer also to Table A7.5.3.1.3- 3.
3.3.3	Diet	Mazuri® Exotic Gamebird Breeder was used as the basal feed to prepare all test diets. The test substance was dissolved in HPLC-grade acetone to make a stock solution. For each dietary concentration, an appropriate aliquot of the stock solution was transferred to another container and diluted with additional acetone. The total amount of vehicle added to a batch was set at two percent by weight. The final solution for each dietary level was added to the basal feed in the mixing bowl of a large Hobart mixer. The diet was mixed for 15 minutes after the vehicle was added. The Vehicle Control (VC) diet was always mixed first with neat acetone, followed by the T1, T2, T3 and T4 test diets. Fresh test diets were prepared at least every two weeks. Prepared diets were stored in a walk-in freezer for two weeks, at which time a new batch was mixed.
3.3.4	Test conditions	Please refer to Table A7.5.3.1.3- 4.
3.3.5	Duration of the test	Adult Treatment Period: 10 weeks pre-egg laying; 10 weeks egg-laying. Hatchling Observation Period: 14 days post-hatch.
3.3.6	Test parameter	Adult Parameters: Daily observations, diet consumption, body weight, necropsy including wet weights of the liver, spleen and testes. Reproductive Parameters: Eggs laid, eggshell thickness, defective and cracked eggs, viable embryos, live embryos. Hatchling Parameters: Hatching success/hatchability, hatchling survival, hatchling body weights.
3.3.7	Examination/ observation	The birds were observed daily during the 20 week exposure period. Inspections were made to monitor symptoms that may be indicative of test substance related effects. Birds that died during the treatment period were removed, weighed and necropsied. Feed consumption of each pair of birds was measured weekly during the exposure period. The body weight of each bird was measured at the initiation of the 14-day acclimation period, on day 0, at the end of week 8, and at the end of week 20. At the conclusion of the treatment period, remaining birds were euthanized and necropsied for gross pathological abnormalities. Specific examination was made on the gastro-intestinal tract, liver, kidneys, bile duct, heart, spleen, and reproductive organs. Wet weights of the liver, spleen and testes were measured at the time of necropsy. Other observations were recorded as necessary.
3.3.8	Statistics	Adult endpoints and reproductive parameters were statistically analyzed using TOXSTAT Version 3.4. The experimental unit is each pen (or

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adult pair), except in the case of adult body weight, in which case the experimental unit is each adult bird.

If a data set passed the chi-square test for normal distribution, and Bartlett's test for homogeneity of variance, it was analyzed by ANOVA. If no significant difference was identified by the ANOVA, no additional data was used. If ANOVA identified a difference, then the post hoc results generated by TOXSTAT were used. Bonferroni's test was used for pair-wise comparisons of each treatment with the control group. Bonferroni's test is appropriate when the replicates per group were not equal, as was the case in with many of the data sets.

Data sets consisting of count data which did not pass the chi-square test and/or Bartlett's test, were transformed and analyzed again. If an appropriate transformation did not succeed in normalizing the distribution, or if the variance was not homogeneous, the original, untransformed data was analyzed by Kruskal-Wallis non-parametric test (H-statistic). If a post hoc pair-wise comparison was indicated, Dunn's multiple comparison procedure was used. Dunn's procedure compares all possible pairs of means. If no significant difference was identified by the Kruskal-Wallis test, no additional data was used. If the Kruskal-Wallis test identified a significant difference, then the post hoc results were reported.

Proportional (percentage) data was analyzed following the above process, but if the untransformed data failed normality and/or homogeneity tests, the data was transformed with "anscombe arcsin" or "arcsine (square root (Y))" according to parameters in SOP CO-8.02, and the appropriate test was performed (Kruskal-Wallis or ANOVA), regardless of the results of the transformed analysis. If the data set was percent data, and the untransformed data did not pass the normality and homogeneity tests, it was transformed. Depending upon the results of the transformation, the appropriate analysis of variance procedure was performed.

Power analyses were performed for each test parameter to determine the probability of rejecting the null hypothesis of equal means (H0), when in fact the alternative hypothesis of significantly different means is true (H1). Re-stated, the power of the test is the probability of detecting a difference when there is a difference. The analysis is a pair-wise comparison of two means. In all cases, the mean values tested were the vehicle control group (VC) and the highest dietary concentration group, treatment level 4 (T4). The rationale for this comparison was that any test substance related effect would be expressed most strongly in the highest dose group. Power analyses were performed using the program XLStatistics. The test parameters were set at:

Significance Level (α): 0.05

Test Hypotheses: H0: $\mu_1 - \mu_2 = 0$

H1: $\mu_1 - \mu_2 \neq 0$ (two-tailed test)

Actual standard deviations associated with the means were used since the analyses were performed post hoc. The power statistic is expressed as $(1-\beta)$.

4 RESULTS

4.1 Limit test/ range Not performed

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	finding test		
4.1.1	Concentration		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		
4.2	**Results test substance**		
4.2.1	Applied concentrations	Nominal Dietary Concentrations: 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3), 0.100 (T4) mg/kg diet, equivalent to: 0 (VC), 0.016 (T1), 0.075 (T2), 0.317 (T3), 1.642 (T4) mg/kg bw at the conclusion of the 20 week treatment period.	X
4.2.2	Effect data (Mortality and reproductivity)	Adverse effects to adults are presented in Table A7.5.3.1.3- 5. For reproductive effects, please refer to Table A7.5.3.1.3- 6 and Table A7.5.3.1.3- 7. **NOEC:** > 0.100 mg/kg diet administered for 20 weeks **NOEL:** > 0.01138 mg/kg body weight/day (mean of males and females)	
4.2.3	Body weight	Adult body weights are presented in Table A7.5.3.1.3- 8. For body weights of hatchlings, refer to Table A7.5.3.1.3- 9.	
4.2.4	Food consumption	Food consumption was measured in adults, as presented in Table A7.5.3.1.3- 10.	
4.2.5	Results of residue analysis	T1 and T2 diets were not analysed as the concentration is below the LOQ of the validated analytical method. However, the lower dietary concentrations are verified indirectly firstly by the careful dilution of the stock solution, secondly by the consistent mixing process used to prepare all levels, and thirdly, by the analytical verification of difenacoum levels in the T3 and T4 diets, which were mixed in the same manner and at the same time. Details are presented in Table A7.5.3.1.3- 11.	
4.2.6	Other effects	Birds that died during the treatment were necropsied and the significant findings are presented in Table A7.5.3.1.3- 5. The findings of the terminal necropsies are detailed in Table A7.5.3.1.3- 12. Organ weights recorded during the terminal necropsies were as detailed in Table A7.5.3.1.3- 13.	

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4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	All data for the control group is included in the chapters above and referred tables.	
4.3.2	Nature of adverse effects	Although the listed findings are consistent with anticoagulant exposure, the observations do not form a pattern of consistent effects either within groups or across treatment groups. There were six cases of sub-lethal observations that could be related to anticoagulant exposure. The six cases were distributed among four groups: VC (n=2), T1 (n=2), T2 (n=1), T3 (n=1), and T4 (n=0) treatment groups. Two control group birds were found to have haemorrhaging in the oesophagus upon necropsy. This illustrates that the birds were incurring many forms of physical stress and tissue damage that was related to aggressive interactions among pen mates. While some of the sub-lethal conditions observed may be consistent with anticoagulant exposure, similar observations in the control group suggest that there were other causative factors at work. The lack of any systematic dose-response in physical symptoms and in any of the other parameters measured in the test support this conclusion.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Primary guideline: OECD Test Guideline 206: Avian Reproduction Test, 1984. Secondary guidelines: Modified in places to follow OECD Test Guideline "Draft Document 1998": Avian Toxicity Test in the Japanese Quail or Japanese Quail, and USEPA Ecological Effects Guideline OPPTS 850.2300: Avian Reproduction Test. No deviations. Treated diet was prepared every two weeks and offered *ad libitum* to groups of 10 male and female pairs for 10 weeks pre-egg laying and 10 weeks egg laying. Treated diets contained nominal 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3) and 0.100 (T4) mg/kg diet. Adults were observed daily and diet consumption, body weight, necropsy including wet weights of the liver, spleen and testes recorded. Eggs were collected daily for 10 weeks. The number of eggs laid, eggshell thickness, defective and cracked eggs, viable embryos, live embryos were recorded. Eggs were incubated and hatching success/hatchability, hatchling survival and hatchling body weight at day 14 were recorded. Parameters were analysed statistically.	
5.2	**Results and discussion**	Of all the parameters measured and analysed in the study, only four were declared to have significant differences, these were: adult female liver weights; the number of viable eggs; the mean body weight of 14-day old hatchlings; and, the mean number of normal hatchlings per hen. The adult female liver weights were significantly different (lower), according to ANOVA, but no significant differences were identified by pair-wise comparisons of each treatment group mean with the VC group. The ANOVA analysis of the number of viable eggs also found a significant overall difference, but again the means separation procedure did not identify differences between any of the treatment groups and the VC group.	

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		The mean body weight of 14-day old hatchlings found the T3 group to be significantly different (lower) from the VC group but this may have been due to behavioural interactions as the hatchling density in the brooders for this group was the highest. The mean number of normal hatchlings per hen in the T2 group was significantly different (lower) from the VC group. These results do not appear as part of a larger pattern and are likely to be a consequence of two pairs in this group having very low numbers of hatchlings. It is therefore considered to be an artefact of the groupings and the analysis process. Regarding the adult generation, although the listed symptoms are consistent with anticoagulant exposure, the observations do not form a pattern of consistent effects either within groups or across treatment groups. There were six cases of sub-lethal observations that could be related to anticoagulant exposure. Two control group birds were found to have haemorrhaging in the oesophagus upon necropsy. This illustrates that the birds were incurring many forms of physical stress and tissue damage that was related to aggressive interactions among pen-mates. While some of the sub-lethal conditions observed may be consistent with anticoagulant exposure, similar observations in the control group suggest that there were other causative factors at work. The lack of any systematic dose-response in physical symptoms and in any of the other parameters measured in the study support this conclusion.	X
		Dietary consumption of up to 0.100 mg/kg diet had no observed effect on the body weight, feed consumption, or reproductive performance of adult Japanese quail when administered via the diet for 20 weeks. No effects were attributed to the test substance in egg development, or hatchling observations, hatchling body weights and hatchling feed consumption for 14 days.	X
5.2.1	NOEC	NOEC > 0.100 mg/kg diet administered for 20 weeks	X
		NOEL > 0.01138 mg/kg body weight/day (mean of males and females)	X
5.3	**Conclusion**	The validity criteria can be considered to have been fulfilled. Although the control mortality was very slightly higher than the threshold it is not considered to have affected the integrity of the study. Based on the results of this study with Japanese quail, the NOEC for Difenacoum can be considered to be greater than 0.100 mg a.i./kg diet. Adult Japanese quail fed Difenacoum in the diet for 20 weeks at this level and at three lower levels, did not show any pattern of symptoms consistent with anticoagulant toxicity. There was no suggestion of a dose response at the dietary concentrations listed. All symptoms observed in birds administered Difenacoum-treated diets were also observed in the control group. Symptoms observed may have been magnified by the presence of Difenacoum in the treated birds, but the degree of interaction cannot be separated and appears to be minor.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	
5.3.3	Justification for read-across between Difenacoum and Flocoumafen	Both Difenacoum and Flocoumafen are second-generation anticoagulants. The physiological effects of the second generation anticoagulants are based on very similar biochemical properties: Both Difenacoum and Flocoumafen are coumarin derivatives, act by inhibition of the vitamin K cycle and their high toxicity is attributed to	

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	(i) the lipophilicity of their side chains and (ii) their slow (relative to first generation compounds) metabolic degradation and subsequent elimination (in mammals). Since effects other than anticoagulation have not been observed for any of these compounds in birds and in other species, potential reproductive effects (if any) are most likely linked to the anticoagulant properties. If reproductive toxicity to birds were an inherent property common to second generation anticoagulant rodenticides based on their chemical similarity, then read-across from one representative compound to another should be possible without restrictions. Any potential reproductive effects may be assumed to be elicited soonest with a compound of comparatively low anticoagulant activity: the higher the toxicity of a compound, the more likely it would result in fatal haemorrhages (from acute or cumulative anticoagulant effects), thus overriding the potential reproductive effects (if any). Out of the group of second generation anticoagulants (Brodifacoum, Bromadiolone, Difethialone, Difenacoum, Flocoumafen), Difenacoum is that with the lowest toxicity – to mammals as well as to birds. This substance therefore appeared most appropriate as a representative compound for read-across to other second generation anticoagulants. Therefore, by way of read-across and based on the above presented results obtained for the test substance Difenacoum, it is concluded that Flocoumafen is void of adverse effects to the reproduction of birds.	X

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

EVALUATION BY RAPPORTEUR MEMBER STATE FINLAND

Date	31.1.2006
Materials and Methods	Agree with the description of the participant. It is stated on p. 18, last para, that pairs in treatment groups whose egg production in week 13 was more than two standard deviations below the control group mean were eliminated from the test. What does this mean? How many pairs were eliminated for this reason? Wouldn't that procedure also eliminate effects of the test substance? In calculation of power the difference between the treatments should have been given. It would have been informative to give how big difference could have been found in this data with power of 0.8. Mortality exceeded 10% in the control. It was explained by aggressive behaviour of pen mates. The OECD draft recommends a minimum light intensity of 10 lux, no upper limit is set. However, it is mentioned that too high light intensity will encourage aggressive behaviour. In this experiment light intensity was 72.5 lux. Would lower light intensity have decreased aggressive behaviour and mortality in the controls? There were some problems to maintain the temperature and humidity in the range recommend by the OECD 206 and OECD draft. During the storage of eggs the humidity was outside the recommended range of 55-75% for most of the time. In the hatching room temperature was mostly outside the range recommended and humidity was below the OECD recommendation. In Appendix B4 the temperature should be given in Celsius degrees.
Results and discussion	Agree with the participant. NOEC > 0.1 mg/kg food, NOEL > 0.01 mg/kg bw/d
Conclusion	Difenacoum did not cause significant differences between the control and treatment groups in a dose-dependent manner.
Reliability	2
Acceptability	Acceptable
Remarks	3.3.1, Table A7_5_3_1_3-2: Age range at test start and at time of first dosing should be 7-8 weeks. 3.3.2, Table A7_5_3_1_3-3: Number of animals per dose should be 38-40. 4.2.5: The measured average concentrations of difenacoum were 81.6-103.5% for T3 (0.02 mg/kg) and 78.2-119.9% for T4 (0.1 mg/kg) for the three batches analysed. Table A7_5_3_1_3-6: Validity criteria of < 10% mortality in control animals was not fulfilled. Despite this the test is regarded as acceptable, because the mortality was explained by the aggressive behaviour between pen mates.

COMMENTS FROM RMS THE NETHERLANDS

Date	05.12.2006
Materials and Methods	Discussions among Finland and The Netherlands are included concerning the acceptability of the underlying reproduction toxicity study with Difenacoum as read across for the risk assessment of flocoumafen.

Comment of The Netherlands:

We agree with the description of the participant. The reproduction study with Difenacoum in the Japanese quail was evaluated on its usefulness for read-across to other second generation anticoagulants, in this case Flocoumafen.

Statement of GLP compliance is not signed (page 3).

The study was carried out according OECD Test Guideline 206. Therefore mortality in the control should not exceed 10% at the end of the test. According to Appendices C1 and C3 and info on page 28 in the study report, 7 out of 20 female (and male) quails had died in the control at the end of the test. The 7 partner quails were killed. Therefore 17.5%≤mortality≤35%. This renders the study not acceptable, had the study not already been accepted by the RMS Finland.

The OECD Test Guideline 206 recommends that Japanese quail be proven breeders before use in the test, so as to reduce variability with this species. In the present study variability was reduced by removing birds that had relatively low egg production until week 13.

The test protocol agreed upon (Biocides Working group on testing strategies for environmental and toxicological data, Ispra, 20-22 October 2004) states that the study (that is intended for read-across) is conducted according OECD 206 with the amendment that "all birds included in the test must be proven breeders".

It is stated on page 18, last paragraph, that pairs in treatment groups whose egg production in week 13 was more than two standard deviations below the control group mean were eliminated from the test to reduce variance in the sample sets (and thus to increase statistical power). On the basis of the information presented one can conclude that the number of female quails eliminated from the test because of extremely low egg production were one (T1), two (T2) and two (T4).

The procedure followed is unsatisfactory since the eventually most susceptible treatment groups were removed.

Furthermore, it is not clear which data were included in the statistical analysis.

On the basis of the various reproduction parameters (except egg production, that could have been influenced by the methodology) it is concluded that no statistically significant effects were observed. In summary: the study is acceptable (Ri=2). Since the study is critical, it is not the best choice for read-across.

To validate the statement on comparative ecotoxicity, the acute toxicity data of difenacoum and flocoumafen were compared. For Difenacoum two 5-day dietary studies with birds are reported (doc IIIA_7-8: Difenacoum Sorex, PT14 and Difenacoum Hentschke & Sawatzki, PT14). In both studies no dose-effect relationship was observed. The authors conclude that Reliability of the studies is 1. The data presented for the study with the Bobwhite quail are conflicting (observation RMS). This finding plus the lack of a dose-response are the reasons to ignore the calculated LD/LC50 for the Bobwhite quail. In the other study with the Mallard duck LC50(5d) = 18.9 mg a.i./kg diet (recalculation of the data: probit analysis resulted in 95% CI=7.6-97 mg a.i./kg diet).With Flocoumafen two avian dietary tests in the Mallard duck have been described. In doc IIIA_7.5.3.1.2/01 LC50(5d) was 12 mg a.i./kg diet (95% CI=5-38 mg a.i./kg diet; Ri=1). In doc IIIA_7.5.3.1.2/05 LC50(5d) was 1.7 mg. a.i./kg diet (as determined graphically; Ri=2). Recalculation of the mortality data of this study resulted in a mean LC50 (males and females combined) of 6.1 mg. a.i./kg diet (95% CI=4.4-8.6 mg a.i./kg diet; method Spearman-Karber). On the basis of the above 95% Cl values, the RMS concludes that short term dietary toxicity of Difenacoum and Flocoumafen are comparable.

Results and discussion	The Netherlands: It seems reasonable that Difenacoum is representative for evaluating the effect on reproduction for Flocoumafen. However, since the study is critical, it is not the best choice for read-across. For the time being NOEC > 0.1 mg/kg food, NOEL > 0.012 mg/kg bw/d Finland: Agree with the participant. NOEC > 0.1 mg/kg food, NOEL > 0.012 mg/kg bw/d
Conclusion	The Netherlands: It seems reasonable that Difenacoum is representative for evaluating the effect on reproduction for Flocoumafen. However, since the study is critical, it is not the best choice for read-across. For the time being this study can be used to as a read-across to other second generation anticoagulants, in this case Flocoumafen. Finland: Difenacoum did not cause significant differences between the control and treatment groups in a dose-dependent manner.
Reliability	The Netherlands: 2 Finland: 2
Acceptability	The Netherlands: Acceptable (Ri=2) Finland: Acceptable
Remarks	The Netherlands: 4.2.1 Applied concentrations T4= 1.593 mg/kg a.i./kg bw at the conclusion of the 20 week treatment period 4.2.2, Table A7.5.3.1.3-6: Nominal concentration (mg a.i./kg diet) should be VC= 0; T1=0.001; T2=0.005; T3=0.020 and T4=0.100. 4.2.2, Table A.7.5.3.1.3-7: the cumulative dose is given; the nominal concentration in feed is preferred. 4.2.5, Table A7.5.3.1.3-11: The measured average concentrations of difenacoum were 88.5-112.5% for T3 (0.02 mg/kg) and 86.9-108.4% for T4 (0.1 mg/kg) for the three batches analysed. 4.3.2 Nature of adverse effects : There were seven cases of sub-lethal observations … T2 (n=2) instead of T2 (n=1) 5.2 Results and Discussion: … seven cases of sub-lethal observations ... 5.2: Dietary consumption of up to 0.100 mg a.i./kg diet 5.2.1 NOEC > 0.100 mg a.i./kg diet 5.2.1 NOEL >0.01138 mg a.i./kg body weight/day 5.3.3 It is stated that Difenacoum is least toxic to birds out of the group of second generation anticoagulants. In difenacoum doc IIA of Sorex Limited Table 4.2.3.2.2 five studies are included. All of these studies are based on nominal concentrations, which should not be accepted at all, as stated by the RMS. Furthermore, 3 of them were considered invalid due to high mortality in controls. The key study with Mallard Duck *Anas platyrhynchos* has a LC50 of 18.9mg/kg diet. As compared with the flocoumafen 5d LC50 of 4.1 mg/kg diet (measured conc.), flocoumafen is considered more toxic (factor 4.6). The difenacoum study with Bob white quail, which was considered invalid due to high mortality in the control has a LC50 of 989. As compared with the flocoumafen 5d LC50 of 62 mg/kg diet (measured conc.), flocoumafen is considered more toxic (factor 16). As the difenacoum study with Bob white quail is double invalid, we consider impropriate ignore this factor. It should be noticed that for derivation of the extrapolation factor the results of high quality flocoumafen studies were used as compared with low quality difenacoum studies. Therefore we have reservations as related to the

	extrapolation factor. For consistency reasons, however, the extrapolation factor of 4.6 will be used in the assessment to calculate the chronic NOEC birds for flocoumafen from the reproduction study with difenacoum. The Netherlands concludes that some hesitation remain whether Difenacoum is representative for evaluating the effect on reproduction for Flocoumafen, but accept with reservations. Finland: 3.3.1, Table A7.5.3.1.3-2: Age range at test start and at time of first dosing should be 7-8 weeks. 3.3.2, Table A7.5.3.1.3-3: Number of animals per dose should be 38-40. 4.2.5, Table A7.5.3.1.3-11: The measured average concentrations of difenacoum were 81.6-103.5% for T3 (0.02 mg/kg) and 78.2-119.9% for T4 (0.1 mg/kg) for the three batches analysed. Table A7.5.3.1.3-14: Validity criteria of < 10% mortality in control animals was not fulfilled. Despite this the test is regarded as acceptable, because the mortality was explained by the aggressive behaviour between pen mates.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.3- 1: Method of administration of the test substance.

Carrier / Vehicle	**Details**
Water	No
Organic carrier	Yes, acetone which was allowed to evaporate
Concentration of the carrier [% v/v]	2%
Other vehicle	None
Function of the carrier / vehicle	Solvent for test substance to facilitate homogeneity

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	*Coturnix coturnix japonica*, strain D1
Source	North West gamebirds, LLC., 228812 E. Game Farm Road, Kennewick, Washington
Age (in weeks), sex and initial body weight (bw)	4 weeks old on arrival at test facility, male and female
Age range within the test	8 weeks old
Breeding population	Reputable and reliable supplier
Amount of food	*Ad libitum* feeding
Age at time of first dosing	8 weeks old
Health condition / medication	Health condition good, no medication
Pre-treatment	Acclimatisation period up to 4 weeks, no adverse observations

Table A7.5.3.1.3- 3: Test system.

Criteria	Details
Test location	Indoors, in cages
Holding pens	Test cages used were galvanised steel, 51 × 50 × 25.5 cm (l × w × h) over a faecal collection pan of absorbent material
Number of animals (male/female)	198 (99/99)
Number of animals per pen [cm²/bird]	2 animals per cage, surface area 2550 cm²
Number of animals per dose	40
Pre-treatment / acclimation	Dry non-medicated Mazuri Exotic Gamebird Starter diet was used. During the last 7 days of acclimatisation the diet was changed to Mazuri Exotic Gamebird breeder diet by adding proportionally more breeder diet and less starter diet. Diet and tap water available *ad libitum*
Diet during test	Mazuri® Exotic Gamebird Breeder was used as the basal feed to prepare all test diets. The test substance was dissolved in HPLC-grade acetone to make a stock solution. For each dietary concentration, an appropriate aliquot of the stock solution was transferred to another container and diluted with additional acetone. The total amount of vehicle added to a batch was set at two percent by weight. The final solution for each dietary level was added to the basal feed in the mixing bowl of a large Hobart mixer. Fresh test diets were prepared at least every two weeks. In the early weeks of the study, fresh batches were sometimes mixed more often to assure adequate supplies. Prepared diets were stored in a walk-in freezer for two weeks, at which time a new batch was mixed No additional supplements were used Treated diets were offered *ad libitum*
Dosage levels (of test substance)	Nominal Dietary Concentrations: 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3), 0.100 (T4) mg/kg diet Treated diets offered *ad libitum* from week 0 to week 20
Replicate/dosage level	Not applicable
Dosing method	Dietary
Dosing volume per application	Diet offered *ad libitum*
Frequency, duration and method of animal monitoring after dosing	The birds were observed daily during the 20 week exposure period. Birds that died on the test were removed, weighed and necropsied. Feed consumption of each pair of birds was measured weekly during the exposure period. At the conclusion of the treatment period, remaining birds were euthanized and necropsied for gross pathological abnormalities. Specific examination was made on the gastro-intestinal tract, liver, kidneys, bile duct, heart, spleen, and reproductive organs. Wet weights of the liver, spleen and testes were measured at the time of necropsy. Other observations were recorded as necessary.
Time and intervals of body weight determination	The body weight of each bird was measured at the initiation of the 14-day acclimation period, on day 0, at the end of week 8, and at the end of week 20

(continued on next page)

Incubation, storing and	All eggs were collected once each day during the 10-week egg laying period.

Table A7.5.3.1.3- 3: Test system.

hatching	The eggs were placed in an egg cooler (Model ESC-3-110 and ESC-6-110. Kuhl Corporation, Flemington, NJ.) after collection. The egg cooler trays rotate automatically each hour. Temperature and relative humidity of the egg cooler was monitored daily with a digital hygrometer/thermometer. All intact eggs (except eggs used to determine eggshell thickness) were set weekly to an incubator (Model 1, Petersime Incubator Company, Gettysburg, OH). The hatchlings were housed in box type poultry brooders (90 cm long x 80 cm wide x 25 cm high). The floor surface area of the brooders was 7200 cm2. Two brooder compartments were used for each group each week.
Test period after egg-laying	From week 11 to week 20
Turning of eggs	Yes up to incubation day 15, then placed in a non-turning compartment of the incubator
Collection period for eggs	From week 11 to week 20

Table A7.5.3.1.3- 4: Test conditions (housing).

Criteria	Details
Test temperature	During the acclimatisation period the mean minimum and maximum daily temperature were 20 and 23ºC, respectively. During the 20 week treatment period the mean minimum and maximum daily temperature were 20 and 23ºC, respectively.
Shielding of the animals	Not stated in the report but the animals were obviously shielded against excessive noise, activity or other disturbance as the study was undertaken as a competent laboratory
Ventilation	10–15 room changes per hour
Relative humidity	During the acclimatisation period the mean minimum and maximum relative humidity were 42 and 68%, respectively During the 20 week treatment period the mean minimum and maximum relative humidity were 54 and 73%, respectively
Photoperiod and lighting	Lighting was provided by full spectrum fluorescent bulbs, which were illuminated 7 hours per day during the first eight weeks of the exposure period. At the beginning of the 9th week of the exposure period, the light cycle was increased in increments of two hours per day over 5 days until a light cycle of 17 hours light per day was attained. The average light intensity was 72.5 lux, and was measured at the front of each rack at each level.
Storing, incubation and hatching conditions for eggs	All eggs were collected once each day during the 10-week egg laying period. All intact eggs (except eggs used to determine eggshell thickness) were set weekly to an incubator. On day 15 of incubation, eggs were transferred to the non-turning hatcher compartment that maintained separation of eggs from each parental pen. The hatchlings were removed from the hatcher over a 24-hour period beginning on day 17. Hatchlings were observed for 14 consecutive days after the 24-hour hatch period. The hatchlings were housed in box type poultry brooders. Two brooder compartments were used for each group each week. The hatchlings were divided evenly among the two brooders each of the two hatch days, with odd hatchlings (if any) going in one brooder 1 of 2 the first day and brooder 2 of 2 the second day. The most hatchlings occupying a single brooder at any time in this study was 45.
Environmental conditions for young birds	In the hatchling room the mean minimum and maximum temperature were 34 and 37ºC, respectively. The mean minimum and maximum relative humidity were 40 and 49%, respectively. Lighting was provided by incandescent lighting on a 12 hour cycle.

Table A7.5.3.1.3- 5: Effect data: adverse effects observed in adult birds during treatment period.

Group	Nominal Concentration (mg a.i./kg diet)	Birds Euthanized [a]	Birds Died [b]	Week(s) Found Dead	Initial Number of Birds	Dead (%)	Group Observations
VC	0	9	5	5,7,9,14	40	12.5	Feather loss (head,back), pecking (head), abrasion (head,ear,eye,foot), found dead, and sacrificed.
T1	0.001	4	2	10,16	38	5.3	Feather loss (head,back,neck), pecking (head), abrasion (head), hemmorhage (beak). Subdermal Hematoma (head), growth on foot.
T2	0.005	6	1	20	40	2.5	Feather loss (head, eye,neck, back), pecking (head), abscess (beak), abrasion (head,foot), sacrificed, subdermal hematoma (head), ataxic, growth on beak, injured (right leg).
T3	0.020	5	1	7	40	2.5	Feather loss (head,neck), pecking (head), abscess (head), abrasion(foot), hypo-reactivity, abrasion healing, feathers growing, found dead, sacrificed, wing drop, injured (wing), growth on beak, subdermal hematoma (head).
T4	0.100	7	1	16	40	2.5	Feather loss (head, back), pecking (head) abrasion (head,foot), sacrificed, ataxic, fluffed feathers, found dead, growth on beak.

a Single birds were euthanized if their pen-mate had died. Both members of a pair were euthanized if the pair was incompatible, described as repeated or routine agonistic behavior which was resulting in severe injury to one or both members of the pair. Both members of some pairs were also euthanized if they met the criterion for excessivly low egg production in week 13 of the test. The criterion was egg production in week 13 which was less than or equal to two standard deviations below the mean egg production of the VC group in week 13.

b Includes only those birds that were found dead during the 20 week adult observation period. Not included are any birds euthanized during the test.

Table A7.5.3.1.3- 6: Values of reproductive performance: summary of egg data.

Group	Nominal Concentration (mg a.i./kg diet)	Egg Data							
		Eggs Laid	Mean Eggs/Hen/ Week	Mean Hatchlings/ Hen/Week	Mean Shell Thickness (mm)	Cracked Eggs (%)[a]	Viable Embryos (%)[b]	Live Embryos (%)[c]	Hatch (%)[d]
VC	0	889	6.5	4.0	0.215	9.8	94.4	95.3	83.7
T1	0.016	985	6.2	3.5	0.204	13.3	95.0	96.1	81.8
T2	0.075	977	5.7	3.0	0.216	9.9	87.5	95.2	77.2
T3	0.317	1056	6.2	4.0	0.215	7.7	95.5	96.2	81.4
T4	1.593	1056	5.8	3.3	0.213	10.2	90.5	97.5	83.0

a Percent Cracked Eggs = (cracked eggs/eggs candled) * 100.

b Percent Viable embryos = (viable embryos/eggs set) * 100.

c Percent Live Embryos = (live embryos/viable embryos) * 100.

d Percent Hatch = (hatchlings/viable embryos) * 100.

Table A7.5.3.1.3- 7: Values of reproductive performance: summary of hatchling data.

Group	Nominal Cumulative Dose (mg/kg bw)	Hatchling Data					
		Hatchlings	Normal Hatchlings (%)	14-day Survivors/ Normal Hatchlings (%)	14-day Survivors/Eggs Laid (%)	Mean 14-day Survivors/Hen	Mean 14-day Hatchling Body Weight (g)
VC	0	567	96.1	80.8	51.9	33.7	68
T1	0.016	563	95.7	84.7	48.7	30.2	65
T2	0.075	526	93.2	83.5	45.4	25.9	68
T3	0.317	674	94.8	80.7	52.2	32.4	59
T4	1.593	622	90.5	79.9	47.9	27.8	65

Table A7.5.3.1.3- 8: Adult body weight data.

Group	Nominal Concentration (mg a.i./kg diet)	Mean Body Weight (g) Week 0[a,b]	Week 8[c]	Week 20[d]
		Male		
VC	0	211	236	273
T1	0.001	214	251	290
T2	0.005	214	251	284
T3	0.020	206	238	275
T4	0.100	202	234	266
		Female		
VC	0	225	278	304
T1	0.001	224	284	312
T2	0.005	229	279	315
T3	0.020	227	280	302
T4	0.100	231	291	318

[a] Differences in initial body weights (male and female) among groups were not significant when analyzed using ANOVA (F = 2.45, calculated F = 0.228).

b Differences in initial male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.774). Differences in initial female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.335).

c Differences in initial male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.976). Differences in initial female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.628).

d Differences in initial male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.852). Differences in initial female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.924).

Table A7.5.3.1.3- 9: Body weight data of hatchlings.

Mean Body Weight of *Coturnix* Hatchlings During the Reproduction Test With

Group	Nominal Concentration (mg a.i./kg diet)	Mean 14-day Body Weight (g)[a]
VC	0.000	68
T1	0.001	65
T2	0.005	68
T3	0.020	59
T4	0.100	65

[a] Differences in mean day-14 body weight among groups were significant when analyzed using ANOVA (F = 2.53, calculated F = 8.420).

Table A7.5.3.1.3- 10: Food consumption of adult Japanese quails.

Mean Feed Consumption of Adult *Coturnix*
During the Avian Reproduction Test With Difenacoum

Group	Nominal Concentration (mg a.i./kg diet)	Feed Consumption (grams/bird/day)																			
		Week																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean[a]
VC	0	28	29	26	26	28	26	26	28	30	31	35	36	35	36	36	37	36	37	34	35	32
T1	0.001	28	30	27	28	31	29	29	31	32	34	35	37	37	38	38	38	37	39	36	37	34
T2	0.005	28	30	26	27	30	27	28	29	29	31	33	36	34	36	34	37	35	37	35	36	32
T3	0.020	28	30	27	27	31	27	27	29	30	31	34	36	36	37	37	38	36	36	36	38	33
T4	0.100	26	30	27	27	29	27	28	30	32	34	34	37	36	38	36	38	37	37	37	39	33

[a] Difference in mean feed consumption among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.182).

Table A7.5.3.1.3- 11: Results of the analytical verification of the test substance in diet.

		Mean Measured Concentration (mg a.i./kg diet)				
Batch #	Date Prepared	VC (0.000 mg a.i./kg diet)	T1 (0.001 mg a.i./kg diet)	T2 (0.005 mg a.i./kg diet)	T3 (0.020 mg a.i./kg diet)	T4 (0.100 mg a.i./kg diet)
1	May 9, 2005	-	-	-	0.0177	0.1017
2	June 7, 2005	-	-	-	0.0225	0.1054
11	September 9, 2005	-	-	-	0.0198	0.0845
Mean Measured Concentration		-	-	-	0.0200	0.0972
Standard Deviation		-	-	-	0.0024	0.0112
Percent of Nominal		-	-	-	100.0	97.2

Table A7.5.3.1.3- 12: Results of terminal necropsies of adult birds.

Gross Necropsy Results of Adult *Coturnix* During the Avian Reproduction Test With Difenacoum
(The number in each column represents the number of birds that displayed the listed findings.)

Observations		Nominal Concentrations (mg a.i./kg diet)				
		VC (0)	T1 (0.001)	T2 (0.005)	T3 (0.020)	T4 (0.100)
Fate:	Found dead	5	2	1	1	1
	Sacrificed	35	36	39	39	39
Total necropsies:		40	38	40	40	40
Feather loss:		20	18	19	17	18
Emaciated:		2	1	2	0	1
Breast muscle atrophy:		2	1	2	0	1
Ventriculus contents:	No feed/grit	2	0	0	0	0
	1/2 full	2	4	2	2	2
	Full	36	34	38	38	38
Enlarged: [a]	Liver	n/a	n/a	n/a	n/a	n/a
	Kidneys	0	2	0	0	0
	Spleen	n/a	n/a	n/a	n/a	n/a
	Bile duct	0	0	0	0	0
Discolored:	Liver	6	3	6	1	5
	Heart	0	0	0	0	0
	Kidneys	0	0	1	0	0
	Spleen	0	1	0	0	0
	Bile duct	0	0	0	0	0
Lesions/Abrasions:	Skin	7	5	3	5	4
Lesions/Growths:	Mouth	0	0	0	0	0
	Esophagus/Crop	1	0	0	0	0
	Proventriculus	0	0	0	0	1
	Ventriculus	0	0	0	0	1
	Intestines	0	0	0	0	0
	Heart	0	0	0	0	0
	Liver	0	0	0	0	0
	Bile Duct	0	0	0	0	0
	Spleen	0	0	0	0	0
	Kidneys	0	0	0	0	0
	Uro-Genital	0	0	0	0	0
Reproductive organs:	Mature follicles	18	19	19	20	20
	Egg in oviduct	14	15	15	17	16
	Immature Testes	0	0	1	0	0

[a] Classification as "enlarged" is subjective for kidneys and bile duct. Livers, spleens, and male testes were weighed and the results presented in Table 21 and in Appendix C3.

Table A7.5.3.1.3- 13: Organ weights of adult birds upon terminal necropsy.

Mean Organ Weights of Adult *Coturnix*
at the End of the Twenty Week Exposure Period

Group	Nominal Concentration (mg a.i./kg diet)	Organ Body Weight (g)			
		Liver[a]	Spleen[b]	Right Testes[c]	Left Testes[d]
Male					
VC	0.000	6.0	0.11	3.9	3.7
T1	0.001	7.5	0.16	3.6	3.4
T2	0.005	7.1	0.16	3.2	3.2
T3	0.020	6.7	0.14	3.7	3.4
T4	0.100	6.4	0.18	3.4	3.2
Female					
VC	0	9.9	0.20	---	---
T1	0.001	10.9	0.27	---	---
T2	0.005	11.6	0.21	---	---
T3	0.020	9.3	0.22	---	---
T4	0.100	10.8	0.18	---	---

[a] Differences in liver weights among males in the groups were not significant when analyzed using ANOVA Male (F = 2.53, calculated F = 2.299). Differences in liver weights among females in the groups were significant when analyzed using ANOVA (F = 2.53 , calculated F = 4.693). Although the ANOVA declared significant differences, pair-wise comparisons of each treatment group with the VC group did not find significant differences (Bonferroni's t-test).

[b] Differences in spleen weights among groups were not significant when analyzed using ANOVA Male (F = 2.53, calculated F = 1.609), Female(F = 2.53, calculated F = 0.765).

[c] Differences in right testes weights among groups were not significant when analyzed using ANOVA Male (F = 2.53, calculated F = 1.797).

[d] Differences in left testes weights among groups were not significant when analyzed using ANOVA Male (F = 2.53, calculated F = 0.931).

Table A7.5.3.1.3- 14: 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 ≥ 14, 12 and 24 for mallard duck, bobwhite quail and Japanese quail	X	
Average eggshell thickness for the control group ≥ 0.34, 0.19 and 0.19 mm for mallard duck, bobwhite quail and Japanese quail	X	
Concentration of the test substance in the diet ≥ 80 % of the nominal concentration throughout the test period	X	

*) Mortality of the control animals was 12.5%, the highest of any of the groups.

Section A7.5.4.1 Acute toxicity to honeybees and other beneficial arthropods

Annex Point IIIA 8.3.1

	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:	The intended use of Flocoumafen as a rodenticide is not expected to result in any relevant exposure of bees or other terrestrial arthropods to the active substance. The wax block formulation is considered to be unattractive to bees. Moreover, when the recommendations of good baiting practice are followed, the bait will be largely unavailable to beneficial insects. Thus, for lack of any relevant exposure, testing for effects on honey bees is not considered to be required. Nevertheless, it is noted that a toxicity study testing insecticidal effects of Flocoumafen against *Musca domestica* and *Blatella germanica* was conducted (A7.5.6/01). As a result, the substance did not cause any toxic effects to these insects.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The waiver is accepted, considering also that this type of test is not required for rodenticides.
Conclusion	The waiver is accepted.
Remarks	No further remarks.

	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.5.1 Bioconcentration, terrestrial

Annex Point IIA 7.5

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.5.1/01:** Sxxxx Txxxx (2003) Estimation of the terrestrial bioconcentration factor (BCF) of Flocoumafen. Exxxx Cxxxx Gxxxx, Hxxxx, Gxxxx, Report no. BAS-20031107-01, November 07, 2003 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
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**	As given in Section A2.
3.1.1	Lot/Batch number	Not applicable
3.1.2	Specification	Not applicable
3.1.3	Purity	Not applicable
3.1.4	Further relevant properties	Not applicable
3.1.5	Method of analysis	Not applicable
3.2	**Reference substance**	Not applicable
3.2.1	Method of analysis for reference substance	Not applicable
3.3	**Testing procedure**	
3.3.1	Test system/ performance	Not applicable

Section A7.5.5.1 Bioconcentration, terrestrial

Annex Point IIA 7.5

3.3.2	Estimation of bioconcentration	On the basis of log P_{ow}, as specified in the TGD on risk assessment. Experimentally determined log P_{ow} values are reported by reference A3.9/01. log P_{ow} (pH 7) = 6.12 log P_{ow} (pH 9) = 5.11

4 RESULTS

4.1	**Experimental data**	
4.1.1	Mortality/ behaviour	Not applicable
4.1.2	Lipid content	Not applicable
4.1.3	Concentrations of test material during test	Not applicable
4.1.4	Bioconcentration factor (BCF)	Not applicable
4.1.5	Uptake and depuration rate constants	Not applicable
4.1.6	Depuration time	Not applicable
4.1.7	Metabolites	Not applicable
4.1.8	Other observations	Not applicable
4.2	**Estimation of bioconcentration**	pH 7: BCF = 15820 pH 9: BCF = 1547

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Estimation of the terrestrial bioconcentration factor ($BCF_{earthworm}$) based on the partition coefficient P_{ow}, as specified by the TGD on risk assessment.
5.2	**Results and discussion**	Based on experimentally determined partition coefficients (log P_{ow} = 6.12 for pH 7 and 5.11 for pH 9), bioconcentration factors were estimated at $BCF_{earthworm}$ = 15820 (pH 7) $BCF_{earthworm}$ = 1547 (pH 9).
5.3	**Conclusion**	Since the estimation was performed using an officially recommended model, based on measured values determined under GLP by fully valid experimental procedures, this calculation is considered valid without restrictions.
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	9 December 2004
Materials and Methods	No comments.
Results and discussion	No comments.
Conclusion	No comments.
Reliability	No comments.
Acceptability	Acceptable.
Remarks	No comments.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.6 Effects on other terrestrial non-target organisms: Toxicity to insects
Annex Point IIIA 13.3.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.6/01:** Sxxxx Rxxxx (1983) The insecticidal effects of novel anticoagulants against *Musca domestica* and *Blatella germanica*. Sxxxx Lxxxx, Report, July 13, 1983 (unpublished). (BASF-Ref.: FL-531-001) Remark: Several anticoagulants were tested; reference is made only to Flocoumafen in this summary.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
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**	No
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	Not stated
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	Not stated; substance was “considered to be pure”.
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to affect the test performance.
3.1.5	Method of analysis	Not applicable
3.2	**Administration of the test substance**	Topical application
3.3	**Reference substance**	No reference substance examined.
3.3.1	Method of analysis for reference substance	Not applicable

Section A7.5.6 Effects on other terrestrial non-target organisms: Toxicity to insects
Annex Point IIIA 13.3.4

3.4	**Testing procedure**	
3.4.1	Test organisms	*Musca domestica*, 3 day old females, own laboratory culture; *Blatella germanica*, 3 – 4 weeks old males, own laboratory culture.
3.4.2	Test system	See Table A7.5.6- 1.
3.4.3	Test conditions	Test conditions are provided in Table A7.5.6- 2.
3.4.4	Duration of the test	96 h (*Musca domestica*) 48 h (*Blatella germanica*)
3.4.5	Test parameter	Apparent mortality (immobility of individuals)
3.4.6	Timing of observation	After 24 h and 48 h; in *M. domestica* additionally after 96 h.
3.4.7	Statistics	No statistical procedures employed.

4 RESULTS

4.1	**Effect data**	There was no evidence for an insecticidal effect. The apparent mortality data are presented in Table A7.5.6- 3.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Potential insecticidal effects of Flocoumafen were investigated in a screening test using *Musca domestica* and *Blatella germanica*. The study was designed as a limit test: A single dose level was applied topically in form of a 4 % solution in acetone. The study did not follow agreed guidelines.
5.2	**Results and discussion**	Flocoumafen is well soluble and sufficiently stable in acetone, and non-volatile. Thus, the physico-chemical properties (also see Section A3) are not considered to have affected the test results. Treatment-related mortality is not different from control mortality in both species. Hence, there is no evidence of an insecticidal effect of Flocoumafen.
5.3	**Conclusion**	The study did not follow agreed guidelines. Nevertheless, the employed method seems appropriate for a rough assessment of insecticidal potential. This type of study is not a mandatory or additional data requirement for PT 14. Thus the study at hand is considered to be of limited relevance. Nevertheless, it may provide some additional information for risk assessment. There is no evidence of an insecticidal effect of Flocoumafen.
5.3.1	Reliability	3

Section A7.5.6 **Annex Point IIIA 13.3.4**	**Effects on other terrestrial non-target organisms: Toxicity to insects**	
5.3.2 Deficiencies	Yes Although no apparent methodological deficiencies occurred, the report is very brief, which restricts the comprehensibility of the study.	X

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	9 December 2004
Materials and Methods	No comments.
Results and discussion	No comments.
Conclusion	No comments.
Reliability	No comments.
Acceptability	Acceptable as additional information since there were methodological and reporting deficiencies (see below).
Remarks	The study was not performed according to an accepted guideline. The major methodological deficiency is the lack of evidence that exposure was adequate (no positive control and/or treatment solutions not analysed). Major reporting deficiencies were: purity test substance and expiry date not reported; weight of the flies not reported; insufficient information on preparation of treatment solutions ("4% in acetone").

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.6- 1: Test system.

Criteria	Details
Musca domestica	
Holding of test animals	Plastic cup with gauze cover
Sample size	40 (4 replicates of 10 individuals)
Observation period	96 h
Tested concentration(s)	2 mg/g b.w.
Application of test substance	4 % solution in acetone 1 µl drop to the dorsal thorax of the CO_2-anaesthesised animal
Food	10 % sucrose solution on a cotton wool pad
Control group	Yes control animals treated with 1 µl pure acetone
Blatella germanica	
Holding of test animals	Glass ring
Sample size	30 (3 replicates of 10 individuals)
Observation period	48 h
Tested concentration(s)	0.08 mg/insect
Application of test substance	4 % solution in acetone 2 µl drop between the rear coxae of the CO_2-anaesthesised animal
Food	No
Control group	Yes control animals treated with 2 µl pure acetone

Table A7.5.6- 2: Test conditions; these conditions were identical for *Musca domestica* and *Blatella germanica*.

Criteria	Details
Test temperature	25 ± 2.5 °C
Relative humidity	No humidity control
Photoperiod and lighting	Continuous photoperiod; type of lighting not further specified

Table A7.5.6- 3: Apparent mortality in insects topically treated with Flocoumafen.

	% immobilised individuals		
	24 h	**48 h**	**96 h**
Musca domestica			
Treatment: 2 mg/g	2.5	7.5	7.5
Control	5.0	5.0	10.0
Blatella germanica			
Treatment: 0.08 mg/ind.	6.7	20.0	—
Control	5.0	10.0	—

Section A7.5.7.1.1 Ecotoxicology – effects to mammals: acute oral toxicity

Annex Point IIIA 13.3.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A7.5.7.1.1/01:** Lxxxx Mxxxx, Lxxxx Jxxxx (1986) LD_{50} trials with the anticoagulant Flocoumafen. Dxxxx Pxxxx Ixxxx Lxxxx, Lxxxx, Dxxxx, Report No. B.651, July 1986 (unpublished). (BASF-Ref.: FL-901-010)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
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**	No Although not a guideline study, the method employed is similar to EC method B.1 (acute oral toxicity).	
2.2	**GLP**	No GLP was not compulsory at the time the study was conducted.	
2.3	**Deviations**	Yes Number of animals per dose level, group composition with respect to sex, reporting of pathological findings (see 3.2.6, 3.4, 4.2).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	B.2149	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	Not stated	X
3.2	**Test animals**	The study was carried out in several European and one African wild rodent species, as given below.	
3.2.1	Species	*Microtus agrestis* *Microtus arvalis* *Clethrionomys glareolus* *Apodemus flavicollis* *Apodemus sylvaticus* *Mastomys natalensis* *Arvicola terrestris*	
3.2.2	Strain	All species: Wild type	

Section A7.5.7.1.1 Ecotoxicology – effects to mammals: acute oral toxicity

Annex Point IIIA 13.3.4

3.2.3	Source	Own laboratory breeding	
3.2.4	Sex	Male and female	
3.2.5	Age/ weight at study initiation	Age: not stated Body weight: species-specific, see results section (Tables A7.5.7.1.1-1 to 7).	
3.2.6	Number of animals per group	4 (2 males, 2 females)	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Post-exposure period	21 d	
3.3.2	Type of exposure	By gavage	
3.3.3	Dosing	0.10, 0.316, 1.0, 3.16, 10.0, and 31.6 mg/kg b.w. The 31.6 mg/kg dose was only applied to *Mastomys natalensis* and *Arvicola terrestris*.	
3.3.4	Vehicle	Corn oil	
3.3.5	Concentration in vehicle	As appropriate to ensure application of a volume of 10 mg/kg b.w.	X
3.3.6	Total volume applied	10 mg/kg b.w.	X
3.3.7	Controls	Pure vehicle	
3.4	**Examinations**	Inspection for mortality: daily; Body mass: prior to dosing and after death.	
3.5	**Method of determination of LD_{50}**	*M. arvalis, A. terrestris*: Litchfield & Wilcoxon (1949). All other species: Horn (1956).	X
3.6	**Further remarks**		
		4 RESULTS	
4.1	**Mortality**	Mortality data for the various species are presented in Table A7.5.7.1.1-1 to 7.	X
4.2	**Clinical signs**	Several incidents of “weakness” prior to death; see Table A7.5.7.1.1-1 to 7.	
4.3	**Pathology**	No pathological findings reported.	

Section A7.5.7.1.1 Ecotoxicology – effects to mammals: acute oral toxicity

Annex Point IIIA 13.3.4

4.4	**Other**	Some mortalities were apparently not treatment-related: In *Microtus agrestis*, *M. arvalis*, and *Arvicola terrestris*, some control animals died. No obvious cause of death was noted in the *Microtus* species. Death of control animals was attributed to digestive problems with corn oil. One control individual of *A. terrestris* was ensnarled in the wire net with its incisors. The LD_{50} values given below are estimated from data excluding these unintentional deaths.		X
4.5	**LD_{50} (95% CI)**	*Microtus agrestis* *Microtus arvalis* *Clethrionomys glareolus* *Apodemus flavicollis* *Apodemus sylvaticus* *Mastomys natalensis* *Arvicola terrestris*	0.18 mg/kg (0.07 – 0.46) 0.13 mg/kg (0.06 – 0.32) 0.24 mg/kg (0.13 – 0.42) 4.22 mg/kg (2.37 – 7.50) > 10 mg/kg 1.33 mg/kg (0.75 – 2.37) 0.22 mg/kg (0.06 – 0.72)	X
4.6	**NOEL**	*Microtus agrestis* *Microtus arvalis* *Clethrionomys glareolus* *Apodemus flavicollis* *Apodemus sylvaticus* *Mastomys natalensis* *Arvicola terrestris*	< 0.1 mg/kg < 0.1 mg/kg 0.1 mg/kg 1.0 mg/kg 0.316 mg/kg 0.316 mg/kg < 0.1 mg/kg	X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute oral toxicity of Flocoumafen to various wild rodent species was tested in an approach similar to standard test guidelines, e.g. EC method B.1. Relevant deviations from this guideline were that only four individuals per dose level were tested and that sexes were mixed for each dose level.	
5.2	**Results and discussion**	The physico-chemical properties of Flocoumafen (Section A3) are not considered to affect the results of oral toxicity tests. When the unintentional deaths of control animals in *Microtus* and *Arvicola* are ignored, the resulting LD_{50} values appear plausible. There is a distinct dose-response relationship in all tested species. The LD_{50} values vary considerably and reflect the varying susceptibility to Flocoumafen across species. Notwithstanding this, Flocoumafen is generally highly toxic.	X
5.3	**Conclusion**		X
5.3.1	Reliability	2	

Section A7.5.7.1.1 Ecotoxicology – effects to mammals: acute oral toxicity

Annex Point IIIA 13.3.4

5.3.2	Deficiencies	Yes The deviations from the guideline discussed above can be considered as minor methodological deficiencies. The same applies to the lack of reporting of pathological findings. Nevertheless, the study appears to be conducted according to agreed scientific principles. The results are consistent and plausible. The impact of the deficiencies on the results is considered negligible. Therefore, the study is considered valid with restrictions. The study is deemed acceptable for risk assessment of wild non-target small mammals.	X

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	14 December 2004
Materials and Methods	(3.1.3) Purity and cis-trans ratio were not reported. Data on purity are essential. (3.3.5 & 3.3.6) The dose volume for the larger species *M. natalensis* and *A. terrestris* was 5 mL/kg bw. (3.5) The method by Horn (1956) was used for all species.
Results and discussion	(4.1) Table A.7.5.7.1.1-1; body weight 30.5 should read 31.4. Table A.7.5.7.1.1-3; body weight 56/6 should read 56.6. Table A.7.5.7.1.1-4; LD50 not valid, see below. Table A.7.5.7.1.1-5; LD50 not valid, see below. (4.4 & 4.5 & 4.6 & 5.2 & 5.3.2) Due to mortality in the control (3/4 for *M. agrestis* and 1/4 for *M. arvalis*), possibly due to digestive problems with the vehicle corn oil, reliable LD50 and NOEL values for these species cannot be estimated.
Conclusion	(5.3) The conclusion should be stated (see below). Acute LD50 and NOEL values (mg/kg) in 5 wild rodent species: *Clethrionomys glareolus* 0.24 and 0.1 *Apodemus flavicollis* 4.22 and 1.0 *Apodemus sylvaticus* > 10 and 0.316 *Mastomys natalensis* 1.33 and 0.316 *Arvicola terrestris* 0.22 and <0.1
Reliability	3 (Purity not reported; only 2 animals/sex/dose; no reporting of clinical and pathological findings).
Acceptability	Not acceptable (acceptable with reliability 2 if purity is reported and found to be acceptable).
Remarks	No comments.

	COMMENTS FROM ...
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Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.7.1.1-1: Table for acute toxicity in *Apodemus flavicollis*.

Dose (mg/kg)	Deaths/tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	0/4	—	33.5/32.8/28.6/28.4	
0.1	0/4	—	33.4/31.4/25.4/28.2	
0.316	0/4	—	30.5/31.0/24.8/24.6	
1.0	0/4	—	52.5/42.2/27.6/23.0	
3.16	1/4	6 d	29.0/27.7/27.8/34.0	
10.0	4/4	4 – 6 d	27.8/30.8/24.0/28.1	
31.6	—	—	—	—

LD_{50} = 4.22 mg/kg (95 % CI = 2.37 – 7.50)

Table A7.5.7.1.1-2: Table for acute toxicity in *Apodemus sylvaticus*.

Dose (mg/kg)	Deaths/ tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	0/4	—	32.1/26.4/15.4/14.0	
0.1	0/4	—	23.2/25.4/15.4/14.5	
0.316	0/4	—	23.6/18.8/16.7/18.2	
1.0	1/4	6 d	27.4/27.8/19.4/15.8	“weak” 1 d prior to death
3.16	1/4	8 d	25.8/34.1/17.9/17.7	“weak” 1 d prior to death
10.0	1/4	8 d	25.4/31.5/18.6/11.8	“weak” 1 d prior to death
31.6	—	—	—	—

LD_{50} > 10 mg/kg

Table A7.5.7.1.1-3: Table for acute toxicity in *Mastomys natalensis*.

Dose (mg/kg)	Deaths/ tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	0/4		70.8/64.7/37.7/48.8	
0.1	0/4		65.0/52.4/37.2/83.4	
0.316	0/4		62.0/56/6/42.0/35.0	
1.0	1/4	7 d	62.0/78.2/45.0/42.0	
3.16	4/4	3 – 13 d	94.4/99.4/38.1/52.2	
10.0	4/4	6 d	63.6/75.0/46.6/40.0	
31.6	4/4	5 d	64.0/64.4/52.4/39.4	

LD_{50} = 1.33 mg/kg (95 % CI = 0.75 – 2.37)

Table A7.5.7.1.1-4: Table for acute toxicity in *Microtus agrestis*.

Dose (mg/kg)	Deaths/ tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	3/4	2 – 3 d	45.8/48.1/24.5/33.0	
0.1	2/4	3 d	26.6/52.3/24.8/38.4	
0.316	2/4	4 – 7 d	25.6/17.2/34.2/34.1	
1.0	4/4	3 – 5 d	24.4/25.0/15.2/15.1	
3.16	4/4	3 – 6 d	28.2/42.9/17.0/17.6	
10.0	4/4	1 – 4 d	44.6/40.7/21.5/19.1	
31.6	—	—	—	—

LD_{50} = 0.18 mg/kg (95 % CI = 0.07 – 0.46)

Table A7.5.7.1.1-5: Table for acute toxicity in *Microtus arvalis*.

Dose (mg/kg)	Deaths/ tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	1/4	2 d	17.7/15.4/19.1/13.6	
0.1	2/4	3 – 5 d	21.7/25.4/16.9/13.0	
0.316	3/4	2 – 7 d	18.8/20.6/13.5/18.0	
1.0	4/4	2 – 4 d	24.6/25.9/23.7/19.6	
3.16	4/4	2 – 4 d	30.2/21.5/14.3/18.0	
10.0	4/4	3 – 8 d	25.8/27.0/18.6/16.6	
31.6	—	—	—	—

LD_{50} = 0.13 mg/kg (95 % CI = 0.06 – 0.32)

Table A7.5.7.1.1-6: Table for acute toxicity in *Clethrionomys glareolus*.

Dose (mg/kg)	Deaths/ tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	0/4	—	23.9/20.1/15.3/14.1	
0.1	0/4	—	23.5/23.9/18.2/14.4	
0.316	3/4	3 – 6 d	22.0/13.7/17.0/13.2	
1.0	4/4	3 – 6 d	23.1/12.8/14.4/16.0	
3.16	4/4	2 – 6 d	22.4/25.2/19.8/17.8	
10.0	4/4	4 – 6 d	23.6/19.5/19.6/14.2	
31.6	—	—	—	—

LD_{50} = 0.24 mg/kg (95 % CI = 0.13 – 0.42)

Table A7.5.7.1.1-7: Table for acute toxicity in *Arvicola terrestris*.

Dose (mg/kg)	Deaths/ tested individuals	Time of death (range)	Body weights (g)	Observations
0.0	1/4	3 d	274.7/258.0/251.3/262.1	accidental death
0.1	1/4	3 d	204.3/233.6/157.0/82.2	
0.316	3/4	5 – 6 d	264.4/220.2/82.4/136.6	
1.0	3/4	4 – 11 d	202.0/244.6/170.6/254.4	
3.16	4/4	6 – 12 d	227.7/246.6/188.5/256.8	
10.0	4/4	8 – 18 d	223.8/258.4/211.2/256.9	
31.6	4/4	3 – 8 d	235.2/211.6/236.0/251.8	

LD_{50} = 0.22 mg/kg (95 % CI = 0.06 – 0.72)

Section A7.5.7.1.2 Short term toxicity on mammals

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	It is not likely that mammals other than rodents show a higher toxicity towards Flocoumafen than rats. Actually, toxicity of Flocoumafen to various wild rodent species was demonstrated to be comparable or lower than to rats (ref. A7.5.7.1.1/01). Therefore the mammalian short term toxicity is covered in sufficient detail by Section A6.3.1. Furthermore, for product type 14 (rodenticide) a study on mammalian short term toxicity is not a mandatory additional data requirement. Thus, the conduct of further mammalian toxicity studies is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The waiver is accepted, considering also that a 28-day repeated oral dose study in rat was performed (see document IIA, point 3.5).
Conclusion	The waiver is accepted.
Remarks	No further remarks.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.5.7.1.3 Ecotoxicology – effects to mammals: effects on reproduction

Annex Point IIIA 3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	It is not likely that mammals other than rodents show a higher toxicity towards Flocoumafen than rats, therefore the reproduction on mammals is covered in sufficient detail by Section A6.8.1. Furthermore, for product type 14 (rodenticide) a study on reproduction on mammals is not a mandatory additional data requirement. Thus, the conduct of further mammalian toxicity studies is not considered to be required.	
Undertaking of intended 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 (*)
Date	1 July 2005.
Evaluation of applicant's justification	The waiver is accepted, considering also that a 90-day repeated oral dose study in rat was performed (see document IIA, point 3.5). A two-generation study in rat is not available, but the performance of this study was considered to be undesirable for reasons of animal welfare (see document IIA, point 3.8.2).
Conclusion	The waiver is accepted.
Remarks	No further remarks.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	