Section A6.1.1
Annex Point IIA6.1.1

Acute oral toxicity in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.1/01:** Gxxxx Jxxxx (1989) A comparison of the acute oral toxicity to rats of "Storm", brodifacoum and 1:1 (m/m) combination of "Storm" with brodifacoum. Sxxxx Rxxxx Lxxxx, Sxxxx, Kxxxx, Uxxxx, Report No. SBGR.89.045, May 2, 1989 (unpublished). (BASF-Ref.: FL-460-008)	
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 However, the conduct of the study was consistent in all important aspects to EC method B.1 (92/69/EEC).	
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	97.6%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River U.K. Ltd.	
3.2.4	Sex	Male and female	

Section A6.1.1
Annex Point IIA6.1.1

Acute oral toxicity in rats

3.2.5	Age/weight at study initiation	Not specified
3.2.6	Number of animals per group	5 males and 5 females
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Post-exposure period	21 days
3.3.2	Type	By gavage
3.3.3	Concentration	0.20, 0.25, 0.32, 0.40 and 0.50 mg/kg
3.3.4	Vehicle	Corn oil
3.3.5	Concentration in vehicle	0.05 % m/v test substance in corn oil
3.3.6	Total volume applied	Not stated
3.3.7	Controls	Not applicable
3.4	**Examinations**	Clinical examinations (three times daily for the first three days and once daily thereafter); Body weights (initial (day 1), day 7, 14, and 21); Gross pathology upon necropsy.
3.5	**Method of determination of LD_{50}**	Method based on probit analysis.
3.6	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.1.1- 1 and Table A6.1.1- 2. Overt reactions to the treatment were first apparent on day 4. Among the rats that survived the treatment, two developed a transient pallor of the eyes and/or skin and another three animals showed an unkempt appearance. The great majority of rats surviving oral administration of Flocoumafen did not develop overt changes of appearance or behaviour. The principal signs of reaction to treatment among decedents were pallor of the skin and eyes, abasis/ataxia and an unkempt appearance. Other less common clinical signs included hypothermia and prostration immediately before death, swelling of the jaw or feet, increased lachrymation and bleeding from the ear-marks.
4.2	**Pathology**	Necropsy findings of the decedents were considered to be direct or indirect indications of substantial haemorrhage: pallor of lungs, liver and kidneys was a common finding, darkening of the contents of various levels of the gastro-intestinal tract probably due to the presence of blood was observed and clots were found in or around the meninges, thymus, lungs, urinary bladder, testes, seminal vesicles, locomotor musculature and peri-orbital tissues.

Section A6.1.1
Annex Point IIA6.1.1

Acute oral toxicity in rats

4.3	**Other**	All surviving rats gained weight relative to their day 1 body weights upon study termination.	
4.4	**LD_{50}**	Males: 0.43 mg/kg Females: 0.31 mg/kg Combined: 0.37 mg/kg	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in Fischer 344 rats. Groups of 5 male and 5 female rats received 0.20 to 0.50 mg/kg of Flocoumafen in corn oil orally by gavage. Although not a guideline study, the method used was consistent to method B.1 (92/69/EEC) in all important aspects.	
5.2	**Results and discussion**	Mortalities occurred on day 3 or between days 5 and 8. The great majority of rats surviving oral administration of Flocoumafen did not develop overt changes of appearance or behaviour. The principal signs of reaction to treatment among decedents were pallor of the skin and eyes, abasis/ataxia and an unkempt appearance. A 21-day LD_{50} was calculated using a method based on probit analysis at 0.43 mg/kg for males and 0.31 mg/kg for females. Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, Flocoumafen requires classification with the symbol "T+" and with R28 "very toxic if swallowed" (LD_{50}, oral, rat ≤ 25 mg/kg).	
5.3	**Conclusion**		
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	23 December 2004
Materials and Methods	2.2: In the study report a quality assurance statement was included. The study was performed in compliance with GLP. 3.1.2: specification of test substance: cis:trans isomer ratio = 57:43.
Results and discussion	No comments.
Conclusion	In rats, the oral LD_{50} of flocoumafen was found to be 0.43 mg/kg bw in males and 0.31 mg/kg bw in females.
Reliability	1
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.1- 1: Acute toxicity in male rats.

Dose [mg/kg]	**Number of dead/ number investigated**	**Time of death**	**Observations**
0.20	0/5	–	–
0.25	0/5	–	–
0.32	0/5	–	–
0.40	2/5	Day 3–7	Pale eyes and skin, ataxia, unkempt appearance
0.50	4/5	Day 6–7	Pale eyes and skin, ataxia, unkempt appearance, swollen foot/feet
LD_{50} value	0.43 mg/kg (95 % CI = 0.38–0.52 mg/kg)		

Table A6.1.1- 2: Acute toxicity in female rats

Dose [mg/kg]	**Number of dead/ number of investigated**	**Time of death**	**Observations**
0.20	1/5	Day 7	Pale eyes and skin, ataxia, hypothermia, swollen jaw
0.25	0/5	–	–
0.32	2/5	Day 6–7	Pale eyes and skin, ataxia, unkempt appearance
0.40	5/5	Day 6–8	Pale eyes and skin, unkempt appearance, increased lachrymation, abasia, prostrate able to move
0.50	5/5	Day 5–8	Pale eyes and skin, ataxia, unkempt appearance, abasia
LD_{50} value	0.31 mg/kg (95 % CI = 0.26–0.37 mg/kg)		

Section A6.1.1 Acute oral toxicity in rats

Annex Point IIA6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.1/02:** Pxxxx Jxxxx (1984) Toxicology of rodenticides: the acute and sub-acute oral and acute percutaneous toxicity of WL108366 (technical material) in rats. Sxxxx Rxxxx Lxxxx, Sxxxx, Kxxxxt, Uxxxx, Report No. SBGR.84.124, September 11, 1984 (unpublished). (BASF-Ref.: FL-410-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No However, the conduct of the study was consistent to EC method B.1 (92/69/EEC) in all important aspects.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	ST84/025	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	> 99%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study. The stability of the test substance in the vehicle was assessed using HPLC and judged to be stable for at least seven days.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River U.K. Ltd.	
3.2.4	Sex	Male and female	

Section A6.1.1 Acute oral toxicity in rats

Annex Point IIA6.1.1

3.2.5	Age/weight at study initiation	Age: 10–11 weeks Body weight: 190–230 (males), 120–150 g (females)
3.2.6	Number of animals per group	5 males and 5 females
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Post-exposure period	42 days
3.3.2	Type	Gavage
3.3.3	Concentration	0.06, 0.13, 0.25, 0.50 mg/kg
3.3.4	Vehicle	Corn oil
3.3.5	Concentration in vehicle	0.05 % or 0.1 % m/v test substance in corn oil
3.3.6	Total volume applied	Not stated
3.3.7	Controls	Not applicable
3.4	**Examinations**	Clinical examinations and body weights (initial (day 0), day 7, 14, 21, 35 and 42). Gross pathology upon necropsy.
3.5	**Method of determination of LD_{50}**	Estimation on the basis of mortalities.
3.6	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.1.1- 3 and Table A6.1.1- 4. All animals were free of overt clinical signs for the first few days after dosing. Thereafter lethargy, piloerection, hunched back, pale eyes and skin, blood in urine and around nose, and chromodacryorrhea were observed. Some animals were apparently unable to use their hindlimbs prior to death.
4.2	**Pathology**	Surviving animals did not show any significant treatment-related abnormality upon necropsy at study termination. Necropsy of animals that died during the study revealed internal haemorrhages.
4.3	**Other**	All surviving rats gained weight relative to their day 1 body weights upon study termination.

Section A6.1.1 Acute oral toxicity in rats

Annex Point IIA6.1.1

4.4	**LD_{50}**	The acute oral LD_{50} value of the test material, administered to rats as a solution in corn oil was approximately 0.25 mg/kg for the sexes combined. Based on the obtained mortalities, the following ranges for the LD_{50} value are considered: males: 0.25–0.5 mg/kg females: 0.13–0.25 mg/kg

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in Fischer 344 rats. Groups of 5 male and 5 female rats received 0.06 to 0.50 mg/kg of Flocoumafen in corn oil by gavage. Although not a guideline study, the method used was consistent to EC method B.1 (92/69/EEC) in all important aspects.
5.2	**Results and discussion**	Mortalities occurred after 5 to 7 days. All animals were free of overt clinical signs for the first few days after dosing. Thereafter lethargy, piloerection, hunched back, pale eyes and skin, blood in urine and around nose, and chromodacryorrhea were observed. Surviving animals did not show any significant treatment-related abnormality upon necropsy at study termination. Necropsy of animals that died during the study revealed internal haemorrhages. The acute oral LD_{50} value of the test material, administered as a solution in corn oil, was estimated on the basis of mortalities as approximately 0.25 mg/kg combined for both sexes. Based on the obtained mortalities, the following ranges for the LD_{50} value are considered: males: 0.25–0.5 mg/kg females: 0.13–0.25 mg/kg Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, Flocoumafen requires classification with the symbol "T+" and with R28 "very toxic if swallowed" (LD_{50}, oral, rat ≤ 25 mg/kg).
5.3	**Conclusion**	
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	23 December 2004
Materials and Methods	3.1.2: No information was provided on the cis:trans isomer ratio..
Results and discussion	No comments.
Conclusion	In rats, the oral LD_{50} of flocoumafen was found to be 0.25 – 0.5 mg/kg bw in males and 0.13 – 0.25 mg/kg bw in females.
Reliability	2
Acceptability	Acceptable, provided that the applicant submits information of the cis:trans isomer ratio of the test substance.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.1- 3: Acute toxicity in male rats.

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
0.06[a]	0/5	–	Piloerection, blood around nose
0.13[a]	0/5	–	Lethargy, piloerection
0.25[b]	2/5	Day 6	Lethargy, piloerection, coma, laboured breathing
0.50[b]	5/5	Day 5–7	Chromadacryorrhea, blood in urine, pale eyes and skin, not using hind limbs, coma, blood around nose, hunched back, piloerection, lethargy

a) administered as a 0.05 % solution in corn oil;

b) administered as a 0.1 % solution in corn oil

Table A6.1.1- 4: Acute toxicity in female rats

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
0.06[a]	0/5	–	Piloerection
0.13[a]	0/5	–	–
0.25[b]	4/5	Day 5–7	Ataxia, increased lacrimation, coma, lethargy, chromodacryorrhea, laboured breathing, hunched back, blood around nose, piloerection, hind foot bleeding, not using hind limbs,
0.50[b]	5/5	Day 5–7	Piloerection, lethargy, pale eyes and skin, hunched back, coma, laboured breathing, chromadacryorrhea, blood in urine, blood around nose, ataxia

a) administered as a 0.05 % solution in corn oil;

b) administered as a 0.1 % solution in corn oil

Section A6.1.1 Acute oral toxicity in rabbits

Annex Point IIA6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.1/03:** Sxxxx Rxxxx (1983) The acute oral toxicity of WL108366 in New Zealand White rabbits. Sxxxx Lxxxx, Report, September 28, 1983 (unpublished). (BASF-Ref.: FL-411-008)	
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 The conduct of the study was similar to method B.1 (92/69/EEC).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Yes Post-mortem examination was not performed. The study was restricted to males. However, male and female rabbits were treated in another study (reference A6.1.1/11)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	X
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Not specified	
3.1.3	Purity	Not specified	
3.1.4	Description	Not stated	
3.1.5	Stability	Not stated	
3.2	**Test animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	Bantin & Kingman Ltd., Hull	
3.2.4	Sex	Male	

Section A6.1.1 Acute oral toxicity in rabbits

Annex Point IIA6.1.1

3.2.5	Age/weight at study initiation	Age: not stated Body weight: 1.5–2.0 kg	
3.2.6	Number of animals per group	4 singly caged males	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Post-exposure period	21 days	
3.3.2	Type	By gavage	
3.3.3	Concentration	2.15, 1.0, 0.464, 0.215 (two replicates), 0.10, and 0.0464 mg/kg	X
3.3.4	Vehicle	Polyethylene glycol 200/triethanolamine (9:1) (PEG/TEA)	
3.3.5	Concentration in vehicle	Not specified	
3.3.6	Total volume applied	Each dose was administered in 1.0 ml PEG/TEA per 1.0 kg bodyweight.	
3.3.7	Controls	Not applicable	
3.4	**Examinations**	Clinical examinations (daily)	
3.5	**Method of determination of LD_{50}**	Estimation, not specified	
3.6	**Further remarks**	No necropsy was performed.	

4 RESULTS

4.1	**Clinical signs**	Mortalities are presented in Table A6.1.1- 5. Although mortality in the replicates of the 0.215 mg/kg dose varied between 100 % and 0 %, the derived mean mortality at this dosage level was considered as dose response typical of other known second generation anticoagulants. However, no reason for this unexpected result is given and no control group was run concurrently to the replication, thus it is considered that the results obtained for this dose group should be excluded. Death generally occurred between 5 and 10 days after administration, with exception of two rabbits dying on days 16 and 17. In animals which died, lethargy, hunched posture, blood around the eyes, mouth, ears and anus were observed prior to death. Surviving rabbits of the 0.215 mg/kg dose group exhibited mild symptoms of anticoagulant poisoning. All other surviving animals appeared healthy.	
4.2	**Pathology**	No pathology results were reported.	
4.3	**Other**	Body weights were not reported.	
4.4	**LD_{50}**	The results indicate that the LD_{50} value is in a range of 0.10 to 0.464 mg/kg, excluding the unusual results in rabbits dosed with 0.215 mg/kg.	

Section A6.1.1 Acute oral toxicity in rabbits

Annex Point IIA6.1.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in New Zealand White rabbits. Although not a guideline study, the method used was similar to method B.1 (92/69/EEC), with the exception that necropsy was not performed and only male animals were dosed. However, male and female rabbits were treated in another study (reference A6.1.1/11).	X
5.2	**Results and discussion**	Death generally occurred between 5 and 10 days after administration, with exception of two rabbits dying on days 16 and 17. In animals which died, lethargy, hunched posture, blood around the eyes, mouth, ears and anus were observed prior to death. Surviving rabbits of the 0.215 mg/kg dose group exhibited mild symptoms of anticoagulant poisoning. All other surviving animals appeared healthy. Mortality in the replicates of the 0.215 mg/kg dose varied between 100 % and 0 %. Since no reason for this unexpected result is given and since no control group was tested concurrently to the additional replicate, it is considered that the results obtained for this dose group should be excluded. Thus, the results indicate that the LD_{50} value is in a range of 0.10 to 0.464 mg/kg.	
5.3	**Conclusion**		
5.3.1	Reliability	3	
5.3.2	Deficiencies	Yes A reason for the unexpected results in rabbits dosed with 0.215 mg/kg was not discussed. It was not reported whether a control group was run concurrently to the second 0.215 mg/kg dose group or not.	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 December 2004
Materials and Methods	3.1: No information on the test substance was available, as for instance purity and cis:trans isomer ratio. 3.3.3: the lowest dose was 2.14 instead of 2.15 mg/kg. 5.1 Post-mortem examination was not performed, body weights were not determined, and the study was restricted to only 4 male animals. No details were provided on the test substance (e.g. purity). The reporting of material, methods and results was very limited.
Results and discussion	No comments.
Conclusion	In male rabbits, the results of an acute oral toxicity study indicated that the LD_{50} of flocoumafen is in the range of 0.10 – 0.464 mg/kg bw.
Reliability	3
Acceptability	Acceptable as additional information since there were methodological and reporting deficiencies (see above).
Remarks	It was not clear why the study was included as a key study by the applicant. The study should be considered as supportive data.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.1- 5: Acute toxicity in male rabbits

Dose [mg/kg]	Number of dead/ number investigated	Time of death
2.14	4/4	Day 8–17
1.0	4/4	Day 5–7
0.464	4/4	Day 6–16
0.215[a]	4/4	Day 5–6
0.215[a]	0/4	–
0.10	1/4	Day 9
0.0464	0/4	–
LD_{50}	in a range of 0.10 to 0.464	

[a] replicated dose group, excluded for LD_{50} estimation

Section A6.1.1 Acute oral toxicity in gerbils

Annex Point IIA6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.1/04:** Sxxxx Rxxxx (1983) The acute oral toxicity of WL108366 in Mongolian gerbils. Sxxxx Lxxxx, Report, September 28, 1983 (unpublished). (BASF-Ref.: FL-411-009)	
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 However, the conduct of the study was similar to EC method B.1 (92/69/EEC).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Yes Less than five rodents were used, no necropsy was performed, the study was restricted to males.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	X
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Not specified	
3.1.3	Purity	Not specified	
3.1.4	Description	Not stated	
3.1.5	Stability	Not stated	
3.2	**Test animals**		
3.2.1	Species	Gerbils (*Meriones unguiculatus*)	
3.2.2	Strain	Mongolian gerbil	
3.2.3	Source	Inter-Simian Ltd., Abingdon	
3.2.4	Sex	Male	

Section A6.1.1 Acute oral toxicity in gerbils

Annex Point IIA6.1.1

3.2.5	Age/weight at study initiation	Age: not stated Body weight: 60–80 g	
3.2.6	Number of animals per group	4 males	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Post-exposure period	21 days	
3.3.2	Type	Gavage	
3.3.3	Concentration	2.15, 0.464, 0.215, 0.10, and 0.0464 mg/kg	
3.3.4	Vehicle	Polyethylene glycol 200/triethanolamine (9:1) (PEG/TEA)	
3.3.5	Concentration in vehicle	Not stated	
3.3.6	Total volume applied	Each dose was administered in 2.0 ml PEG/TEA per 1.0 kg bodyweight.	
3.3.7	Controls	None	
3.4	**Examinations**	Clinical examinations (daily)	
3.5	**Method of determination of LD_{50}**	Determined using the tables prepared by Horn (Simplified LD_{50} (or EC_{50}) calculations, Biometrics 12, 311-322)	
3.6	**Further remarks**	No necropsy was performed.	
		4 RESULTS	
4.1	**Clinical signs**	Mortalities are presented in Table A6.1.1- 6. Mortality occurred between day 4 and day 7. Animals which died during the study showed lethargy, hunched posture and the appearance of blood around the orifices. All surviving animals appeared healthy.	
4.2	**Pathology**	No pathology was reported.	
4.3	**Other**	Body weights were not reported.	
4.4	**LD_{50}**	The LD_{50} was found to be 0.18 mg/kg (95% CI = 0.12–0.26 mg/kg).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in Mongolian gerbils. Although not a guideline study, the method used was similar to method B.1 (92/69/EEC), with the exception that less than five rodents were used, no necropsy was performed and toxicity in female gerbils was not investigated.	X

Section A6.1.1 Acute oral toxicity in gerbils

Annex Point IIA6.1.1

5.2	**Results and discussion**	Mortality occurred between day 4 and day 7. Animals which died during the study showed lethargy, hunched posture and the appearance of blood around the orifices. All surviving animals appeared healthy. An acute LD_{50} of 0.18 mg/kg was estimated using the tables prepared by Horn.	
5.3	**Conclusion**		
5.3.1	Reliability	2	X
5.3.2	Deficiencies	Yes No necropsy was performed.	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 December 2004
Materials and Methods	3.1: No information on the test substance was available, as for instance purity and cis:trans isomer ratio. 5.1 Post-mortem examination was noted performed, body weights were not determined, and the study was restricted to only 4 male animals. No details were provided on the test substance (e.g. purity). The reporting of material, methods and results was very limited.
Results and discussion	No comments.
Conclusion	In male gerbils, the results of an acute oral toxicity study indicated that the LD_{50} of flocoumafen is 0.18 mg/kg.
Reliability	3
Acceptability	Acceptable as additional information since there were methodological and reporting deficiencies (see above).
Remarks	It was not clear why the study was included as a key study by the applicant. The study should be considered as supportive data.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.1- 6: Acute toxicity to male gerbils

Dose [mg/kg]	Number of dead/ number investigated	Time of death
2.15	4/4	Day 4–5
0.464	4/4	Day 4–6
0.215	3/4	Day 4–7
0.10	0/4	–
0.0464	0/4	–
LD_{50}	0.18 mg/kg (95% CI = 0.12–0.26 mg/kg)	

Section A6.1.1 Acute oral toxicity

Annex Point IIA6.1.1 Supportive data

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

Reference	Title	System	Results
A6.1.1/05: Sxxxx Rxxxx (1984) Sxxxx Lxxxx, Report, August 2, 1984 (unpublished).	The acute oral toxicity of WL 108366 in C57BL/10 mice	Male and female C57BL/10 mice (5 per sex and group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male mice = 0.79 mg/kg LD_{50}, female mice = 1.47 mg/kg
A6.1.1/06: Sxxxx Rxxxx (1983) Sxxxx Lxxxx, Report, July 12, 1983 (unpublished).	The acute oral toxicity of a series of novel anticoagulants in Wistar rats and C57BL/10 mice	Wistar rats (4 males per group); C57BL/10 mice (5 males per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male rats = 0.46 mg/kg LD_{50}, male mice = 0.79 mg/kg
A6.1.1/07: Sxxxx Rxxxx (1986) Sxxxx Lxxxx, Report, July 10, 1986 (unpublished).	The acute oral toxicity of WL 108366 in female Wistar rats	Female Wistar rats (4 females per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 14 days. LD_{50}, female rats = 0.56 mg/kg
A6.1.1/08: Gxxxx Jxxxx (1987) Axxxx Txxxx Lxxxx, Sxxxx, Uxxxx, Report, March 19, 1987 (unpublished).	Determination of the acute oral LD_{50} of flocoumafen against the ship rat (*Rattus rattus*)	Male and female rats (4 per sex and group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male rats = 1.78 mg/kg LD_{50}, female rats = 1.0 mg/kg
A6.1.1/09: Axxxx (1985) Sxxxx Lxxxx, Report, August 5, 1985 (unpublished).	Summary of protocol for determining acute oral LD_{50} of Flocoumafen ('366) for *Rattus rattus*	Male and female rats (4 per sex and group)	summary of A6.1.1/08 as listed above
A6.1.1/10: Pxxxx Jxxxx (1984) Sxxxx Rxxxx Lxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx, Kxxxx, Uxxxx, Report No: SBGR.84.148 May 1984 (unpublished).	Toxicology of rodenticides: the acute oral toxicity of WL 108366 in mice	Male and female CF1 mice (5 per sex and group)	The test substance was dissolved in corn oil and administered once orally by gavage. The observation period was 42 days. LD_{50}, male mice = 2.9 mg/kg LD_{50}, female mice = 2.0 mg/kg

Section A6.1.1 Acute oral toxicity

Annex Point IIA6.1.1 **Supportive data**

A6.1.1/11: Pxxxx Jxxxx (1986) Sxxxx Rxxxx Lxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx, Kxxxx, Uxxxx, Report No.: SBGR.85.189 January 6, 1986 (unpublished).	Toxicology of rodenticides: the acute oral toxicity of WL 108366 in rabbits and hamsters	Male and female Syrian hamsters (5 per sex and group) male and female New Zealand White rabbits (2 per sex and group)	The test substance was administered enclosed in gelatine capsules to rabbits and by gavage as a 0.5% solution in corn oil to the hamsters. The observation period was 35 days for rabbits and 28 days for hamsters. LD_{50}, rabbits = 0.7 mg/kg LD_{50}, hamster > 50 mg/kg
A6.1.1/12: Sxxxx Rxxxx (1983) Sxxxx Lxxxx, Report No: not stated, July 7, 1983 (unpublished).	The acute oral toxicity of a series of novel anticoagulants in Syrian hamsters	Syrian hamsters (4 males per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male hamsters > 46.40 mg/kg
A6.1.1/13: Sxxxx Rxxxx (1983) Sxxxx Lxxxx, Report No: not stated September 28, 1983 (unpublished).	The acute oral toxicity of WL 108366 in Dunkin-Hartley guinea pigs	Dunkin-Hartley guinea pigs (4 males per group)	The test substance was dissolved in PEG/TEA and administered once orally by gavage. The observation period was 21 days. LD_{50}, male Guinea pigs > 10 mg/kg

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 December 2004
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.1.2 Acute dermal toxicity in rabbits

Annex Point IIA6.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.2/01:** Vxxxx Gxxxx (1988) The acute dermal toxicity of STORM. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.88.080, August 24, 1988 (unpublished). (BASF-Ref.: FL-412-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Testing guidelines for toxicology studies (Japan/MAFF, 1985). Apart from this, the conduct of the study was consistent to EC method B.3 (92/69/EEC) in all important aspects.	
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	96.1%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	The test substance was shown to be stable for 22 months.	
3.2	**Test animals**		
3.2.1	Species	Rabbits	
3.2.2	Strain	New Zealand White	
3.2.3	Source	Sittingbourne Research Centre Breeding unit	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 5 – 7 months Body weight: 2.6 – 3.3 kg (males); 2.5 – 3.8 kg (females)	

Section A6.1.2 Acute dermal toxicity in rabbits

Annex Point IIA6.1.2

3.2.6	Number of animals per group	5 males and 5 females	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Area covered	Not specified	x
3.3.2	Occlusion	Semi-occlusive	
3.3.3	Vehicle	Water	
3.3.4	Concentration in vehicle	0.2, 0.4, 0.8 or 1.6 mg/kg of the test substance wetted with 1 ml of water	
3.3.5	Total volume applied	Not applicable	
3.3.6	Duration of exposure	24 hours	
3.3.7	Removal of test substance	With water	
3.3.8	Observation period	28 days	
3.3.9	Controls	Not applicable	
3.4	**Examinations**	Clinical examinations (three times daily for the first two/three days and once daily thereafter), body weights (initial (day 1), day 7, 14, 21 and 28), gross pathology	
3.5	**Method of determination of LD_{50}**	Method based on probit analysis	
3.6	**Further remarks**	None	

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.1.2- 1 and Table A6.1.2- 2. Deaths occurred mainly in week two and three of the study. Most of the animals which died during the course of the study showed anorexia, lethargy or haemorrhaging prior to death. No signs of toxicity were observed in survivors, except for one female of the high dose group, which lost weight during the second week of the study. This animal recovered during week 3.	
4.2	**Pathology**	Upon necropsy, treatment-related findings were noted at all dose levels and were essentially all haemorrhagic in nature. Negligible effects were recorded in the low dose group and in survivors of the 0.4 mg/kg dose group. More severe effects were noted at 0.8 mg/kg and 1.6 mg/kg.	
4.3	**Other**	All surviving rabbits gained weight relative to their day 1 body weights upon study termination.	
4.4	**LD_{50}**	Males: 0.65 mg/kg Females: 1.14 mg/kg Combined: 0.87 mg/kg	

Section A6.1.2 Acute dermal toxicity in rabbits

Annex Point IIA6.1.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute dermal toxicity of Flocoumafen was tested in New Zealand White rabbits. The method used was consistent to method B.3 (92/69/EEC) in all important aspects.	
5.2	**Results and discussion**	Deaths occurred mainly in week two and three of the study. Most of the animals which died during the course of the study showed anorexia, lethargy or haemorrhaging prior to death. No signs of toxicity were observed in survivors, except for one female of the high dose group, which lost weight during the second week of the study. However, this animal recovered during week 3. The test material administered as wetted powder elicited a LD_{50} of 0.65 mg/kg in males and 1.14 mg/kg in females. Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, Flocoumafen requires classification with the symbol "T+" and with R27 "very toxic in contact with skin" (LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg).	
5.3	**Conclusion**		
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	24 December 2004
Materials and Methods	2.2: In the study report a quality assurance statement was included. The study was performed in compliance with GLP. 3.1.2: specification of test substance: cis:trans isomer ratio = 57:43. 3.3.1 No information was provided on the size of the application area. The size of the application area might influence the height of the LD50 (e.g. due to higher or lower dermal absorption). However, since this study is used for classification and labelling purposes and the test substance is classified as "very toxic in contact with skin" (worst-case classification), the study is considered suitable for evaluation.
Results and discussion	No comments.
Conclusion	In rabbits, the dermal LD_{50} of flocoumafen was found to be 0.65 mg/kg bw in males and 1.14 mg/kg bw in females (no information on application area provided).
Reliability	2, since no information was provided on the application area.
Acceptability	Acceptable for classification and labelling purposes (see material and methods).
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.2- 1: Acute toxicity in male rabbits.

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
0.20	0/5	–	None
0.40	1/5	Day 20	Lethargy, haemorrhage in right conjunctiva and in nails of hind limbs (observed in one animal prior to death)
0.80	3/5	Day 16 – 24	Loss of appetite, lethargy, abasia, pale eyes, bilateral conjunctival oedema, exophthalmia, ataxia, periorbital swelling, conjunctival haemorrhage, occasional bleeding, submandibular swelling and salivation (observed in the 3 animals prior to non-scheduled sacrifice)
1.6	5/5	Day 10 – 28	Loss of appetite, periorbital swelling, occasional conjunctival bleeding, bleeding from nose and anus, lethargy
LD_{50} value	0.65 mg/kg (95 % CI = 0.43 – 1.0 mg/kg)		

Table A6.1.2- 2: Acute toxicity in female rabbits.

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
0.20	0/5	–	None
0.40	0/5	–	None
0.80	1/5	Day 11	Loss of appetite and lethargy (observed in one animal prior to death)
1.6	4/5	Day 8 – 13	Lethargy, loss of appetite
LD_{50} value	1.14 mg/kg (95 % CI = 0.78 – 1.97 mg/kg)		

Section A6.1.2 Acute dermal toxicity of the corn oil manufacturing master mix in rats

Annex Point IIA6.1.2

Official use only

1 REFERENCE

1.1	**Reference**	**A6.1.2/02:** Pxxxx Jxxxx (1988) Toxicology of rodenticides: The percutaneous toxicity of WL108366. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.87.230, September 20, 1988 (unpublished). (BASF-Ref.: FL-412-002) **A6.1.2/03:** Pxxxx Jxxxx (1985) Toxicology of rodenticides: The percutaneous toxicity of WL108366 corn oil manufacturing master mix. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.84.227, January 2, 1985 (unpublished). Remark: References A6.1.2/02 and /03 are duplicate reports issued at different dates, but both referring to the same experiment. Both are included into Document IV-A for the sake of completeness. They are jointly reviewed in the current summary since the bodies of the reports are identical.
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 Method based on Noakes and Sanderson 1969 (A method for determining the dermal toxicity of pesticides; Brit. J. Industry. Med. 26, 59-64). The conduct of the study was similar to EC method B.3 (92/69/EEC).
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes Deviations from the prescribed guideline: the test compound was held in contact with the skin by an aluminium foil instead of a porous gauze dressing. Necropsy was only reported for two animals instead of all animals.

Section A6.1.2 Acute dermal toxicity of the corn oil manufacturing master mix in rats
Annex Point IIA6.1.2

3 MATERIALS AND METHODS

3.1	**Test material**	0.5 % w/v WL108366 in corn oil (corn oil manufacturing master mix)	
3.1.1	Lot/Batch number	25-7-84	
3.1.2	Specification	Not specified	X
3.1.3	Purity	0.48% of a.i.	
3.1.4	Description	Yellow liquid	
3.1.5	Stability	The test compound was judged to be stable for the duration of this study.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River UK Ltd.	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 10 – 11 weeks Body weight: 170 – 220 g (males); 120 – 145 g (females)	
3.2.6	Number of animals per group	5 males and 5 females (50 – 160 mg/kg doses) 10 males and 10 females (125 and 200 mg/kg doses)	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Area covered	Not specified	X
3.3.2	Occlusion	Semi-occlusive (The calculated dose was applied to the shorn skin by syringe and the test material was covered with a piece of aluminium foil which was held in place by adhesive tape)	X
3.3.3	Vehicle	Corn oil (test material was used as supplied)	
3.3.4	Concentration in vehicle	50, 80, 100, 125, 160 and 200 mg/kg of Flocoumafen in corn oil (undiluted test material)	
3.3.5	Total volume applied	Not stated	
3.3.6	Duration of exposure	24 hours	
3.3.7	Removal of test substance	Warm dilute detergent solution	
3.3.8	Observation period	14 days	
3.3.9	Controls	Not applicable	

Section A6.1.2 **Acute dermal toxicity of the corn oil manufacturing master mix in rats**
Annex Point IIA6.1.2

3.4	**Examinations**	Clinical examinations, body weights (initial (day 0), day 7, and 14), gross pathology
3.5	**Method of determination of LD_{50}**	Probit analysis
3.6	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.1.2- 3 and Table A6.1.2- 4. Deaths occurred between days 5 and 10. Signs of anticoagulant poisoning were delayed, but all affected animals died. Clinical signs observed prior to death included bruising and swelling of the limbs, bleeding from the ear marks, pale eyes and skin, lethargy and gait abnormalities. Animals treated with 50 or 80 mg/kg showed no signs of toxicity.
4.2	**Pathology**	One male of the 160 mg/kg group was sacrificed non-scheduled and showed a blood clot on one testis, pale lungs and brain haemorrhage upon necropsy. One female of the 125 mg/kg group was also sacrificed non-scheduled and necropsy revealed pale coloured liver, lungs, spleen and kidneys and subcutaneous haemorrhages in cranial and ventral regions. It was not reported, if further gross pathological examinations were performed.
4.3	**Other**	All surviving rats gained weight relative to their day 0 body weights upon study termination.
4.4	**LD_{50}**	Males: 104 mg/kg (equivalent to 0.56 mg a.i./kg) Females: 80 – 100 mg/kg (equivalent to 0.43 – 0.54 mg a.i./kg) Combined: 100 mg/kg (equivalent to 0.54 mg a.i./kg)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute dermal toxicity of Flocoumafen was tested in Fischer 344 rats. The method used was similar to method B.3 (92/69/EEC). Deviations from the prescribed guideline: the test compound was held in contact with the skin by an aluminium foil instead of a porous gauze dressing. Necropsy was only reported for two animals instead of all animals.

Section A6.1.2 Acute dermal toxicity of the corn oil manufacturing master mix in rats
Annex Point IIA6.1.2

5.2	**Results and discussion**	Mortality occurred between days 5 and 10. Clinical signs observed prior to death included bruising and swelling of the limbs, bleeding from the ear marks, pale eyes and skin, lethargy and gait abnormalities. A LD_{50} value of 0.56 mg a.i./kg for male rats was calculated by probit analysis. The results obtained for female rats indicated a LD_{50} in a range of 0.43 – 0.54 mg a.i./kg. Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, the corn oil manufacturing master mix (0.5 % w/v Flocoumafen in corn oil) requires classification with the symbol "T+" and with R27 "very toxic in contact with skin" (LD_{50}, dermal, rat or rabbit ≤ 50 mg/kg).
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes Gross pathology was only reported for two animals. However, typical signs of anticoagulant poisoning were recorded during the conduct of the study. Clinical signs of toxicity and necropsy reported for two animals showed that the effects were essentially haemorrhagic in nature.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 December 2004
Materials and Methods	3.1.2 No information was provided on the cis:trans isomer ratio of WL108366. 3.3.1 No information was provided on the size of the application area. The size of the application area might influence the height of the LD_{50} (e.g. due to higher or lower dermal absorption). 3.3.2 The test substance was covered by an occlusive dressing (aluminium foil), instead of a semi-occlusive dressing. Occlusive application might result in a higher absorption of the test substance. Since this study is used for classification and labelling purposes and the test substance is classified as “very toxic in contact with skin” (worst-case classification), the study is considered suitable for evaluation.
Results and discussion	No comments.
Conclusion	In rats, the dermal LD_{50} of flocoumafen was found to be 0.56 mg/kg bw in males and 0.43-0.54 mg/kg bw in females (no information on application area provided).
Reliability	2
Acceptability	Acceptable for classification and labelling purposes (see material and methods).
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.2- 3: Acute toxicity in male rats.

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
50	0/5	–	None
80	0/5	–	None
100	3/5	Day 6 – 8	Piloerection, cyanosis, abasia, lethargy, chromodacryorrhoea, bloody ear mark, hunched back, blood around nose, splayed gait, pale eyes and skin, lethargy
125	8/10	Day 5 – 10	Bruised and swollen hind leg, ataxia, splayed gait, pale eyes, slight inflammation on back, cyanosis, lethargy, piloerection, pale skin, unkempt appearance, blood around nose, bleeding from right ear, hunched back
160	5/5	Day 5 – 10	Piloerection, lethargy, pale eyes, pale ears, face swollen, cyanosis, pale skin, respiratory distress, chromodacryorrhoea, hunched back, unkempt appearance, paralysis of the hind legs
200	10/10	Day 5 – 7	Piloerection, chromodacryorrhoea, blood around nose, pale eyes, pale skin, swollen foreleg, lethargy, abasia, bruising around earmarks, slight inflammation on back, swollen face, coma, hunched back
LD_{50} value	104 mg/kg (95% CI = 84 – 118 mg/kg), equivalent to: 0.56 mg a.i./kg (95 % CI = 0.46 – 0.64 mg a.i./kg)		

Table A6.1.2- 4: Acute toxicity in female rats.

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
50	0/5	–	None
80	0/5	–	None
100	5/5	Day 6 – 9	Blood around nose, piloerection, paralysis of the hind legs, chromodacryorrhoea, bloody earmark, cyanosis, splayed gait, abasia, bruised and/or swollen hind leg, pale eyes, pale skin, lethargy, slight inflammation of the back, hunched back
125	8/10	Day 6 – 9	Blood around nose, bloody earmark, pale eyes, pale skin, unkempt appearance, piloerection, lethargy, sore area on chest, slight inflammation and/or small bruised area on back, ataxia, lethargy, bruised and bleeding on chest, hunched back, bruised and swollen leg, paralysis of hind legs
160	5/5	Day 7 – 9	Pale ears, bruising around earmark, swollen foreleg, pale eyes, pale skin, lethargy, hunched back, unkempt appearance, chromodacryorrhoea, bloody earmark, piloerection,
200	10/10	Day 6 – 9	Piloerection, swelling on chest, pale skin, pale eyes, lethargy, piloerection, bloody earmark, bruising around earmark, hunched back, unkempt appearance, bruised and/or swollen leg, blood around nose, chromodacryorrhoea, cyanosis, pale ears
LD_{50} value	80 – 100 mg/kg (LD_{50} not calculable), equivalent to: 0.43 – 0.54 mg a.i./kg		

Section A6.1.2 Acute dermal toxicity of bait concentrate powder

Annex Point IIA6.1.2 **Supportive data**

The following reference is considered to contain additional information concerning acute dermal toxicity of the manufacturing master mix and is thus presented in tabular format as supportive data (non-GLP study):

Reference	Title	System	Results
A6.1.2/04 Pxxxx, Jxxxx (1984), Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Unpublished Report No. SBGR.84.162, August 15, 1984.	Toxicology of rodenticides: The acute percutaneous toxicity of WL108366 bait concentrate.	Male and female Fischer 344 rats (5 per sex and group)	Bait concentrate powder containing 0.5 % w/w of Flocoumafen was dispensed on an aluminium foil and applied occlusive for 24 hours. The observation period was 14 days. The following acute, dermal LD_{50} values were obtained for the bait concentrate powder: LD_{50}, males = 458 mg/kg LD_{50}, females = 367 mg/kg (95% CI = 220 – 443 mg/kg) LD_{50}, combined = 406 mg/kg (95% CI = 329 – 470 mg/kg).

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 December 2004
Conclusion	The presentation of the above study as supportive data is accepted.
Remarks	

	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.1.3 **Annex Point IIA6.1.3**	**Acute inhalation toxicity of the manufacturing master mix in mice**	

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.3/01:** Mxxxx Pxxxx (1989) Storm master mix acute inhalation toxicity study in mice. Ixxxx Rxxxx Ixxxx, Mxxxx, Uxxxx, Report No. 5330, June 28, 1989 (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 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 U.S. EPA Pesticide Assessment Guidelines, Subdivision F, 82-1, and Japanese MHW guidelines for acute toxicity testing (1984).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Not applicable.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Storm master mix contained 0.48 % m/m of Flocoumafen (generated as a particulate dust aerosol).	
3.1.1	Lot/Batch number	ST88/177 Batch No. 003	
3.1.2	Specification	Not specified	X
3.1.3	Purity	0.48 % of a.s.	
3.1.4	Description	Blue milled powder (< 5 µm).	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Test animals**		
3.2.1	Species	Mice	
3.2.2	Strain	CD-1 mice	
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK	
3.2.4	Sex	Male and female	

Section A6.1.3 **Annex Point IIA6.1.3**	**Acute inhalation toxicity of the manufacturing master mix in mice**

3.2.5	Age at study initiation	7 weeks
3.2.6	Weight at study initiation	21–30 g
3.2.7	Number of animals per group	5 males and 5 females
3.2.8	Control animals	Yes (5 males and 5 females)
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Concentrations	Average concentration (range) Group 1: 0 mg/l Group 2: 0.42 mg/l (0.22–0.56), Group 3: 0.97 mg/l (0.90–1.01), Group 4: 0.07 mg/l (0.07–0.08), Group 5: 0.12 mg/l (0.10–0.14)
3.3.2	Particle size	Mass mean diameter of the aerosol particles [µm] (geometric standard deviation [µm]): Group 2: 2.2 µm (2.3) Group 3: 3.0 µm (1.6) Group 4: 2.9 µm (1.6) Group 5: 2.8 µm (1.6)
3.3.3	Type or preparation of particles	The test atmosphere was generated by means of a Wright Dust Feeder using oil-free, dry compressed air.
3.3.4	Type of exposure	Nose-only
3.3.5	Vehicle	None
3.3.6	Concentration in vehicle	Not applicable
3.3.7	Duration of exposure	4 hours
3.3.8	Observation period	14 days
3.3.9	Controls	Air only
3.4	**Examinations**	Clinical examinations Body weights Prothrombin time Kaolin cephalin coagulation time Gross pathology Lung/ body weight ratio
3.4.1	Clinical signs	Continuously for the exposure period and for the first 1–2 hours post dosing, once daily during the 14 day post dose observation period.
3.4.2	Body weights	Initial (day 0), day 2, day 3, day 4, day 7, day 10 and 14.

Section A6.1.3 **Annex Point IIA6.1.3**	**Acute inhalation toxicity of the manufacturing master mix in mice**

3.5	**Method of determination of LC_{50}**	By assessment of the raw data.
3.6	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.1.3- 2 and Table A6.1.3- 3. All animals exposed to 0.42 or 0.97 mg/l of test substance and from which blood sample was obtained showed no coagulation. The majority of animals exhibited classical signs of anticoagulant poisoning, e.g. pale extremities and eyes, green coloured faeces, piloerection, body tremors laboured respiration, hypothermia, hypokinesia. The observed signs were first noted on either day 4 or 5 and became so severe that a number of animals were sacrificed on human ground. These effects were attenuated in the two lower dose groups.
4.2	**Pathology**	Mortalities and observations are presented in Table A6.1.3- 2 and Table A6.1.3- 3. No treatment-related pathological changes were found in low dose animals and survivors of the mid and high dose group upon necropsy. The cause of death in the mid and high dose groups was widespread severe haemorrhaging. Lung / body weight ratios were considered to be within normal limits.
4.3	**Other**	The majority of the animals exposed to 0.42 or 0.97 mg/l test substance lost body weight following exposure. Body weight loss was still evident for several of the surviving animals at the end of the 14 day observation period. Animals exposed to 0.07 or 0.12 mg /l maintained body weight during the study.
4.4	**LC_{50}**	Within the range of 0.12 to 0.42 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute inhalation toxicity of Storm master mix containing 0.48% Flocoumafen was tested in CD-1 mice according to EPA guidelines and Japanese MHW guidelines for acute toxicity testing (1984). Deviations from the prescribed guidelines were not stated.

Section A6.1.3 **Annex Point IIA6.1.3**		**Acute inhalation toxicity of the manufacturing master mix in mice**	
5.2	**Results and discussion**	Animals of the high and mid concentration group exhibited classical signs of anticoagulant poisoning, while these effects were attenuated in the low concentration groups. The test material administered as a predominantly respirable atmosphere of master mix containing 0.48% m/m Flocoumafen resulted in a LC_{50} within the range of 0.12 to 0.42 mg/l, corresponding to 0.0006 – 0.002 mg a.i./l. Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, the master mix already requires classification with the symbol "T+" and with R26 "very toxic by inhalation" (LD_{50}, inhalation, rat, for aerosols or particulates ≤ 0.25 mg/l/4h). Thus, in consequence, by way of read-across, and assuming that the particle size of commercially available Flocoumafen is comparable to that of the master mix, the active ingredient would likewise require classification with "very toxic by inhalation".	
5.3	**Conclusion**		
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	24 December 2004
Materials and Methods	3.1.2: No information was provided on the cis:trans isomer ratio of the test substance.
Results and discussion	No comments.
Conclusion	In mice, the LC_{50} of flocoumafen was found to be in the range of 0.0006 to 0.002 mg a.i./L.
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable, provided that information on the cis:trans isomer ratio of the test substance is submitted by the applicant. Normally, for classification and labelling purposes, an acute inhalation study should be performed in rats instead of mice. As the test substance should be classified as 'very toxic by inhalation", the study is considered suitable for evaluation.
Remarks	In the titles of Table A6.1.3-1 and A6.1.3-2 rats were noted instead of mice.

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

Table A6.1.3- 2: Acute inhalation toxicity in male rats.

Dose [mg/l]	Number of dead/ number investigated	Time of death	Observations (clinical signs and gross pathology)
0 (control)	0/5	–	None
0.07	0/5	–	Blue staining around head
0.12	0/5	–	Blue staining around head, yellow gelatinous mass attached to left testicle
0.42	5/5	Day 5 – 7	Blue staining around head, laboured respiration, ataxic, slight blue/ green faeces, moribund, haemorrhage on upper lip, thoracic cavity filled with blood
0.97	3/5	Day 5 – 10	Respiratory depression, laboured respiration, blue staining around head, piloerection, hypothermia, pale extremities, pale eyes, body tremors, subdued, hunched posture, blue/ green faeces, moribund, thoracic cavity filled with blood, kidneys and stomach pale, kidneys pale and large amount of blood present, intestines blue/ black appearance, intestines very pale with occasional red areas, lungs red appearance, lungs very pale, stomach large amounts of red/ black material present and red areas present on stomach wall.
LC_{50} value	within the range of 0.12 – 0.42 mg/l		

Table A6.1.3- 3: Acute inhalation toxicity in female rats

Dose [mg/l]	Number of dead/ number investigated	Time of death	Observations (clinical signs and gross pathology)
0 (control)	0/5	–	None
0.07	0/5	–	Blue staining around head
0.12	0/5	–	Blue staining around head
0.42	4/5	Day 5 – 7	Blue staining around head, laboured respiration, piloerection, hypothermia, pale extremities, pale eyes, vaginal bleeding, head/ body tremors, ataxic, hypokinesia, subdued, hunched, slight blue /green faeces, moribund, thoracic cavity filled with blood, blood stains around vagina, intestines filled with green/ black liquid, intestines blue/ green appearance
0.97	3/5	Day 5 – 7	Respiratory depression, blue staining around head, laboured respiration, piloerection, hypothermia, pale extremities, pale eyes, vaginal bleeding, head/ body tremors, ataxic, hypokinesia, subdued, hunched posture, slight blue /green faeces, moribund, thoracic cavity filled with blood, intestines gas filled, stomach large amount of red/ black material present, kidney, liver and lungs pale
LC_{50} value	within the range of 0.12 to 0.42 mg/l		

Section A6.1.3/02 and /03 Acute inhalation toxicity of the manufacturing master mix in rats

Annex Point IIA6.1.3

Official use only

1 REFERENCE

1.1	**Reference**	**A6.1.3/02:** Bxxxx Dxxxx (1988) Toxicology of a candidate rodenticide: the acute 4 hour inhalation toxicity of technical concentrate containing 0.5% m/m WL108366. Sxxxx Rxxxx Lxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx, Uxxxx, Report No. SBGR.87.229, September 19, 1988 (unpublished). (BASF-Ref.: FL-413-001) **A6.1.3/03:** Bxxxx Dxxxx (1984) Toxicology of a candidate rodenticide: the acute 4 hour inhalation toxicity of manufacturing master mix (bait concentrate) containing 0.5% m/m WL108366. Sxxxx Rxxxx Lxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx, Uxxxx, Report No. SBGR.84.151, September 20, 1984 (unpublished). (BASF-Ref.: FL-460-003) Remark: References A6.1.3/02 and /03 are duplicate reports issued at different dates, but both referring to the same experiment (exp. no. 2925). Both are included into Document IV-A for the sake of completeness. They are jointly reviewed in the current summary since the bodies of the reports are identical.
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 However, the conduct of the study was consistent to EC method B.2 (92/69/EEC) in all important aspects.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes The mass median diameter could not be determined for the lowest concentration atmosphere.

Section A6.1.3/02 and /03 **Acute inhalation toxicity of the manufacturing master mix in rats**

Annex Point IIA6.1.3

3 MATERIALS AND METHODS

3.1	**Test material**	0.5% m/m WL108366 technical/bait concentrate (manufacturing master mix)	
3.1.1	Lot/Batch number	ST84/050	
3.1.2	Specification	Not specified	X
3.1.3	Purity	0.50% of a.s. (0.49% a.s. after atmosphere generation)	
3.1.4	Description	Blue powder	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Albino rats derived from the Cobs Wistar strain	
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 9–10 weeks Body weight: m: 309–484 g; f: 192–263 g	
3.2.6	Number of animals per group	5 males and 5 females	
3.2.7	Control animals	Yes (5 males and 5 females)	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Concentrations	Time weighted average concentrations (range): 0.04 mg/l (0.022–0.059) 0.16 mg/l (0.15–0.16) 1.4 mg/l (1.33–1.44)	
3.3.2	Particle size	MMAD (mass median aerodynamic diameter) $\pm$ GSD (geometric standard deviation): 0.04 mg/l: indeterminable 0.16 mg/l: 3.4 ± 2.1 µm 1.4 mg/l: 4.2 ± 2.3 µm	
3.3.3	Type or preparation of particles	The test atmosphere was generated by means of a Wright Dust Feed generator using oil-free, dry compressed air.	
3.3.4	Type of exposure	Nose-only	
3.3.5	Vehicle	None	
3.3.6	Concentration in vehicle	Not applicable	

Section A6.1.3/02 and /03 **Acute inhalation toxicity of the manufacturing master mix in rats**

Annex Point IIA6.1.3

3.3.7	Duration of exposure	4 hours	
3.3.8	Observation period	13 to 15 days	
3.3.9	Controls	Air only	
3.4	**Examinations**	Clinical examinations (continuously for the first 30 min of exposure and thereafter periodically throughout the exposure period, periodically for 3 hours after exposure and daily thereafter). Prothrombin times (pre-test and upon termination). Body weights (initial (day 0), day 7, and 13/14). Gross pathology upon necropsy.	
3.5	**Method of determination of LC_{50}**	No statistical technique employed. Assessment based on the raw data.	
3.6	**Further remarks**	None	

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.1.3- 4 and Table A6.1.3- 5. The animals exposed to a concentration of 1.4 mg/l of test substance exhibited classical signs of anticoagulant poisoning, e.g. bruised appearance of the feet with bleeding, pale skin, eyes and ears, and green coloured faeces. These effects were attenuated in the two lower dose groups and in the case of the group exposed to a concentration of 0.04 mg/l; only one male exhibited a bruised appearance of its feet. The increased susceptibility of males compared to females was also apparent in the lethargic signs of high dose animals. All males were lethargic in the post-exposure period on the day of exposure, whereas it was not until the fifth day following exposure that three out of five females became lethargic. Similarly three males exhibited a hunched back stance on day 4 but only one female showed the same signs on day 12 following exposure. The first signs of bruising of the feet occurred in the majority of the animals on day 2 and most of the animals exhibiting paleness of the skin were affected on day 4. Few or no symptoms were shown on the day following exposure.	
4.2	**Pathology**	No treatment-related pathological changes were found in low dose animals and survivors of the mid dose group upon necropsy. The cause of death in the mid and high dose groups was widespread severe haemorrhaging. There were similar findings in the surviving female of the high dose group.	
4.3	**Other**	All surviving rats in the low and mid dose group gained weight relative to their day 0 body weights in a similar manner as the control upon study termination, except for one animal in each group. The surviving female in the high dose group lost weight dramatically during the observation period.	X
4.4	**LC_{50}**	Within the range of 0.16 to 1.4 mg/l	

Section A6.1.3/02 and /03 **Acute inhalation toxicity of the manufacturing master mix in rats**

Annex Point IIA6.1.3

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of five male and five female rats were exposed for 4 hours to inhalable dust atmospheres of technical concentrate containing 0.5% Flocoumafen. Although not a guideline study, the method used was consistent to method B.2 (92/69/EEC) in all important aspects. Deviations from the prescribed guideline: The mass median diameter could not be determined for the lowest concentration atmosphere.
5.2	**Results and discussion**	Animals of the high concentration group exhibited classical signs of anticoagulant poisoning, while these effects were attenuated in the intermediate concentration group. The test material administered as a predominantly respirable atmosphere of technical concentrate containing 0.5% m/m Flocoumafen caused a LD_{50} within the range of 0.16 to 1.4 mg/l, corresponding to 0.0008–0.007 mg a.i./l. Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, the master mix requires classification with the symbol "T+" and with R26 "very toxic by inhalation" (LD_{50}, inhalation, rat, for aerosols or particulates ≤ 0.25 mg/l/4h). Thus, in consequence, by way of read-across, and assuming that the particle size of commercially available Flocoumafen is comparable to that of the master mix, the active ingredient would likewise require classification with "very toxic by inhalation".
5.3	**Conclusion**	
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	27 December 2004
Materials and Methods	3.1.2: No information was provided on the cis:trans isomer ratio of the W:108366.
Results and discussion	4.3: No control animals were included in the study, therefore, body weights could not be compared with controls. Animals showed normal body weight gain.
Conclusion	In rats, the LC50 of flocoumafen was found to be in the range of 0.0008 to 0.007 mg a.i./L.
Reliability	2
Acceptability	Acceptable, provided that information is provided on the cis:trans isomer ratio of the W:108366.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.3- 4: Acute inhalation toxicity in male rats

Dose [mg/l]	**Number of dead/ number of investigated**	**Time of death**	**Observations**
0.04	0/5	–	bruised appearance of claws
0.16	2/5	day 6–9	bruised appearance of claws, bleeding from claws, pale skin, pale eyes, pale ears, lethargic- subdued, hunched back, moribund/coma
1.4	5/5	day 4–6	blood around mouth and nasal area, bruised appearance of claws, bleeding from claws, pale skin, pale eyes, pale ears, lethargic- subdued, hind leg parisis, hunched back, eye half closed, eye dark red appearance, green faeces, black faeces, moribund/coma
LC_{50} value	within the range of 0.16 to 1.4 mg/l		

Table A6.1.3- 5: Acute inhalation toxicity in female rats

Dose [mg/kg]	**Number of dead/ number of investigated**	**Time of death**	**Observations**
0.04	0/5	–	no visible signs of intoxication
0.16	1/5	day 8	blood around mouth and nasal area, bleeding from claws, pale skin, pale eyes, pale ears, moribund/coma
1.4	4/5	day 6–7	blood around mouth and nasal area, bruised appearance of claws, bleeding from claws, pale skin, pale eyes, pale ears, lethargic-subdued, hind leg parisis, hunched back, eye dark red appearance, green faeces, black faeces, moribund/coma,
LC_{50} value	within the range of 0.16 to 1.4 mg/l		

Section A6.1.4 Acute dermal irritation in rabbits

Annex Point IIA6.1.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.4/01:** Fxxxx Jxxxx (1983) The acute skin irritation of novel anticoagulants in male rabbits. Sxxxx Lxxxx, Report, July 13, 1983 (unpublished). (BASF-Ref.: FL-415-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No The conduct of the study was similar method B.4 (92/69/EEC).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Yes 1 ml of the 1 % test solution was applied instead of 0.5 ml or 0.5 g of the test substance and the test substance was covered by polyethylene coats instead of a gauze patch. The exposure duration was 24 hours instead of 4 hours. Animals were not observed for signs of oedema and no observations for erythema 60 min and 72 hours after patch removal were reported.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	X
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	Not stated	
3.1.3	Purity	Not stated	
3.1.4	Description	Not stated	
3.1.5	Stability	Not stated	
3.2	**Test animals**		
3.2.1	Species	Rabbits	
3.2.2	Strain	New Zealand White	
3.2.3	Source	Bantin & Kingman Ltd., Hull	

Section A6.1.4 Acute dermal irritation in rabbits

Annex Point IIA6.1.4

3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Age: not stated Body weight: 2.0 – 2.5 kg	
3.2.6	Number of animals per group	3 males	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Preparation of test substance	The compounds were dissolved, without heating, in PEG 200 to give 1.0% solutions.	
3.3.2	Test site and preparation of test site	Four 5 cm square patches were shaved on the dorsal and lateral sides, just forward of the back legs and shoulders, 24 hours before treatment. The two shaved patches just forward of the back legs were lightly abraded with sandpaper, reddening but not breaking the skin.	
3.3.3	Occlusion	Semi-occlusive	
3.3.4	Vehicle	Polyethylene Glycol (PEG 200)	
3.3.5	Concentration in vehicle	1.0% solution	X
3.3.6	Total volume applied	1 ml of the 1% test solution	
3.3.7	Duration of exposure	24 hours	
3.3.8	Removal of test substance	With warm soapy water	
3.3.9	Post-exposure period	48 hours	
3.3.10	Controls	None	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Dermal examination	Yes (erythema only)	
3.4.3	Scoring system	Grade Skin reaction 0 no erythema 1 pink 2 red 3 severe redness 4 beet redness	
3.4.4	Examination time points	24 and 48 hours after patch removal	
3.4.5	Other examinations	Not stated	
3.5	**Further remarks**	None	

Section A6.1.4 Acute dermal irritation in rabbits

Annex Point IIA6.1.4

4 RESULTS

4.1	**Average score**		
4.1.1	Erythema	0	
4.1.2	Oedema	Not investigated	
4.2	**Reversibility**	Not applicable	
4.3	**Other examinations**	The rabbits remained healthy with no abnormal behaviour patterns throughout the experiment.	
4.4	**Overall result**	No erythema reactions were observed in any of the three males. It was concluded that Flocoumafen does not present a primary skin irritancy hazard.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute dermal irritation of Flocoumafen was tested in male New Zealand White rabbits. The method used was similar to method B.4 (92/69/EEC). Deviations from the prescribed guideline as discussed in 5.3.2 below.	
5.2	**Results and discussion**	No erythema reactions were observed in any of the three males. Thus, it can be concluded that Flocoumafen does not present a primary skin irritancy hazard.	
5.3	**Conclusion**		
5.3.1	Reliability	2	X
5.3.2	Deficiencies	Yes Oedema were not investigated. Erythema were not scored 60 min and 72 hours after patch removal. However, this deviation has no effect on the quality and integrity of the study, since no erythema were observed at 24 and 48 hours. The study was performed with a 1% solution in PEG, which was justified at the time by the high toxicity of the test substance, and would appear plausible according to the reported dermal LD_{50} of 0.65 mg/kg in male and 1.14 mg/kg in female rats (reference: IIIA, 6.1.2).	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	27 December 2004
Materials and Methods	3.1 As no information was available on the test substance (e.g. purity and cis:trans isomer ratio), no conclusions can be drawn for flocoumafen. 3.3.5 No clarification is given for the 1% solution in PEG by the authors of the study report. Considering the results of the acute dermal toxicity study, and exposure of the animals for 4 hours, a higher concentration flocoumafen might have been tested. Additional deviations were mentioned by the applicant at 2.3
Results and discussion	No comments.
Conclusion	In a skin irritation study, at 24 and 48 hours after exposure to a 1% solution of flocoumafen (unknown purity and cis:trans isomer ratio), no erythema was noted in male rabbits. The skin irritation study is not considered suitable for conclusions with regard to skin irritating properties of flocoumafen.
Reliability	3, considering the study deviations.
Acceptability	Not suitable for classification and labelling purposes.
Remarks	Considering the results of the acute dermal toxicity studies with flocoumafen, and the new guideline for skin irritation testing, flocoumafen need not to be tested for skin irritation/corrosion.
COMMENTS FROM ...	
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.1.4- 1: Acute dermal irritation in male rabbits.

Score (average animals investigated)	**Time**	**Erythema**	
		abraded	**non-abraded**
Average score (3 animals)	24 h	0	0
	48 h	0	0
Average score	24, 48 h	0	0
Reversibility*		not applicable	
Average time for reversibility		not applicable	

*) c: completely reversible;
nc: not completely reversible;
n: not reversible

Section A6.1.4 Acute eye irritation in rabbits

Annex Point IIA6.1.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.4/02:** Pxxxx Rxxxx Wxxxx (1996) Acute eye irritation/corrosion study with Flocoumafen technical material in the rabbit. Nxxxx Bxxxx, Sxxxx, Txxxx Nxxxx, Report No. 173317, June 14, 1996 (unpublished). (BASF-Ref.: FL-415-003)	
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**	OECD 405 (1987) EC method B.5 (92/69/EEC)	
2.2	**GLP**	Yes Certified laboratory	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	Floc01	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	99.5%	
3.1.4	Description	White to pale buff powder	
3.1.5	Stability	Not specified	X
3.2	**Test animals**		
3.2.1	Species	Rabbits	
3.2.2	Strain	New Zealand White	
3.2.3	Source	Broekman Institute, Someren, The Netherlands	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Age: approx. 8 – 10 weeks Body weight: 1370 – 2331 g	

Section A6.1.4 Acute eye irritation in rabbits

Annex Point IIA6.1.4

3.2.6	Number of animals per group	3 males	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	The test substance was ground to fine powder prior to administration.	
3.3.2	Amount of active substance instilled	22.8 to 22.9 mg of the test substance (a volume of approx. 0.1 ml)	
3.3.3	Exposure period	24 hours (Remnants of the test substance were present in the eyes of all animals on day 1 only. Thus, the eyes were not washed after the 24 hour observation).	
3.3.4	Post-exposure period	48 hours	
3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	Not specified	
3.4.2	Scoring system	According to guidelines	
3.4.3	Examination time points	1, 24, 48 and 72 hours after instillation	
3.4.4	Other investigations	Mortality (twice daily) Toxicity (at least once daily) Body weight (day of treatment prior to instillation)	
3.5	**Further remarks**	In order to prevent anticoagulant poisoning, Vitamin K1 was administered to the animals as the antidote to Flocoumafen technical. The vitamin K1 solution was diluted 1:3 with corn oil and administered by gavage prior to and during the treatment two to three times a day. In addition, each animal received 10 mg of Vitamin K1 by intravenous injection immediately after instillation of the test substance in the eye.	

4 RESULTS

4.1	**Clinical signs**	No mortality or clinical signs of toxicity were observed in any of the animals during the study period.	
4.2	**Average score**		
4.2.1	Cornea	0 (for all three animals)	
4.2.2	Iris	0 (for all three animals)	
4.2.3	Conjunctiva		
	Redness	0.56 (individual mean scores: 0.3, 0.7, 0.7)	X
	Chemosis	0.22 (individual mean scores: 0, 0.7, 0)	X

Section A6.1.4 Acute eye irritation in rabbits

Annex Point IIA6.1.4

4.3	**Reversibility**	Yes Redness, chemosis and discharge of the conjunctiva were observed starting one hour after application and had resolved at 48 to 72 hours after instillation.
4.4	**Other**	There was no evidence of ocular corrosion. No iridic irritation or corneal opacity were observed, and treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals.
4.5	**Overall result**	Based on the results summarised above and in Table A6.1.4- 2 and according to the EEC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC, 27th April 1993), Flocoumafen technical material is non-irritating to the rabbit eye.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute eye irritation potential of Flocoumafen was tested in New Zealand White rabbits according to OECD 405 (1987) and EC method B.5 (92/69/EEC).
5.2	**Results and discussion**	No iridic irritation or corneal opacity were observed during the study. Redness, chemosis and discharge of the conjunctiva were observed starting one hour after application and had resolved at 48 to 72 hours after instillation. Flocoumafen technical material was considered to be non-irritating to the rabbit eye.
5.3	**Conclusion**	
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	27 December 2004
Materials and Methods	3.1.2 No information was provided on the cis:trans isomer ratio of the test substance. 3.1.5 It was stated in the study report that the test substance was stable under storage conditions.
Results and discussion	4.2.3 No overall mean scores should be calculated when a study is performed with three animals.
Conclusion	Flocoumafen does not have to be classified as irritating to eyes.
Reliability	1
Acceptability	Acceptable, provided that information is submitted on the cis:trans isomer ratio of the test substance.
Remarks	None.

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

Table A6.1.4- 2: Acute eye irritation in male rabbits.

	Cornea		Iris	Conjunctiva		
	Opacity	Area		Redness	Chemosis	Discharge
Score (average of animals investigated)	**0 to 4**	**0 to 4**	**0 to 2**	**0 to 3**	**0 to 4**	**0 to 3**
60 min	0	0	0	1.33	0.00	0.33
24 h	0	0	0	1.00	0.33	0.33
48 h	0	0	0	0.67	0.33	0.00
72 h	0	0	0	0.00	0.00	0.00
Average 24, 48, 72 h	0	0	0	0.56	0.22	0.17
Maximum average score (including area affected, maximum: 110)	0		0		3.33	
Reversibility*	n.a.	n.a.	n.a.	c	c	c
Average time for reversion	n.a.	n.a.	n.a.	72 h	72 h	48 h

* c: completely reversible; nc: not completely reversible; n: not reversible

n.a.: not applicable

Calculation of maximum average score according to “Draize Scale for Scoring Ocular Irritation”.

Section A6.1.4 Acute dermal irritation of the manufacturing master mix in rabbits

Annex Point IIA6.1.4

Official use only

1 REFERENCE

Note: In addition to the studies with the active ingredient, a study was also performed investigating the skin and eye irritancy potential of a manufacturing master mix containing 0.5 % Flocoumafen.

1.1	**Reference**	**A6.1.4/03:** Vxxxx Gxxxx (1988) Toxicology of rodenticides: Skin and eye irritancy potential of 0.5% STORM manufacturing master mix. Sxxxx Rxxxx Cxxxx, Kxxxx, Uxxxx, Report No. SBGR. 87.214, February 17, 1988 (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 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 404 (1981)
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	STORM manufacturing master mix 0.5 %
3.1.1	Lot/Batch number	ST87/132
3.1.2	Specification	Not applicable
3.1.3	Purity	0.54 % m/m Flocoumafen
3.1.4	Description	Blue powder
3.1.5	Stability	The test substance was considered stable for the duration of the study.
3.2	**Test animals**	
3.2.1	Species	Rabbits
3.2.2	Strain	New Zealand White
3.2.3	Source	Sittingbourne Research Centre Breeding Unit
3.2.4	Sex	Male and female

Section A6.1.4 Acute dermal irritation of the manufacturing master mix in rabbits

Annex Point IIA6.1.4

3.2.5	Age at study initiation	4 – 9 months	
3.2.6	Weight at study initiation	3686 – 4608 g	
3.2.7	Number of animals per group	3 males and 3 females	
3.2.8	Control animals	None	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Preparation of test substance	The test substance was moistened with water.	
3.3.2	Test site and preparation of test site	Shoulders and hindquarters were closely shorn and a 4 cm^2 test site was selected.	X
3.3.3	Occlusion	Semi-occlusive	
3.3.4	Vehicle	Water	
3.3.5	Concentration in vehicle	500 mg of test substance.	
3.3.6	Total volume applied	500 mg of test substance.	
3.3.7	Duration of exposure	4 hours	
3.3.8	Removal of test substance	With water and gently dried.	
3.3.9	Post-exposure period	7 days	
3.3.10	Controls	None	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Dermal examination	Yes Erythema and oedema	
3.4.3	Scoring system	According to evaluation of skin reaction in OECD 404 (1981).	
3.4.4	Examination time points	4, 24, 48, 72 hours and 7 days after patch removal	
3.4.5	Other examinations	Not stated	
3.5	**Further remarks**	None	

4 RESULTS

4.1	**Average score**	

Section A6.1.4 Acute dermal irritation of the manufacturing master mix in rabbits

Annex Point IIA6.1.4

4.1.1	Erythema	0
4.1.2	Oedema	0
4.2	**Reversibility**	Not applicable
4.3	**Other examinations**	The rabbits remained healthy with no abnormal behaviour patterns throughout the experiment.
4.4	**Overall result**	No erythema or oedema reactions were observed in any of the six animals. It was concluded that STORM manufacturing master mix is non-irritating to the rabbit skin.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute dermal irritation of STORM manufacturing master mix with 0.5% Flocoumafen was tested in male New Zealand White rabbits according to OECD guideline 404. Rabbits were administered a dose of 500 mg moistened test substance per site. No deviations from the methods prescribed by the guideline were reported.
5.2	**Results and discussion**	Administration of the moistened test material caused no erythema or oedema among the test animals. Thus, it can be concluded that STORM manufacturing mix does not present a primary skin irritancy hazard.
5.3	**Conclusion**	
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	27 December 2004
Materials and Methods	3.3.2 The application area was slightly too small (4 cm2 instead of 6 cm2).
Results and discussion	No comments.
Conclusion	STORM Manufacturing Master Mix (containing 0.5% flocoumafen) does not have to be classified as irritating to skin.
Reliability	2
Acceptability	Acceptable.
Remarks	None

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

Section A6.1.4 Acute eye irritation of the manufacturing master mix in rabbits

Annex Point IIA6.1.4

Official use only

1 REFERENCE

1.1	**Reference**	**A6.1.4/03** (see previous 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**	Yes OECD 404 (1981) OECD 405 (1981)
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	STORM manufacturing master mix 0.5%
3.1.1	Lot/Batch number	ST87/132
3.1.2	Specification	Not applicable
3.1.3	Purity	0.54% m/m Flocoumafen
3.1.4	Description	Blue powder
3.1.5	Stability	The test substance was considered stable for the duration of the study.
3.2	**Test animals**	
3.2.1	Species	Rabbits
3.2.2	Strain	New Zealand White
3.2.3	Source	Sittingbourne Research Centre Breeding Unit
3.2.4	Sex	Male and female
3.2.5	Age at study initiation	4 – 9 months
3.2.6	Weight at study initiation	2810 – 4562 g

Section A6.1.4 Acute eye irritation of the manufacturing master mix in rabbits

Annex Point IIA6.1.4

3.2.7	Number of animals per group	(i) Main study group: 3 males and 3 females (ii) Second study group: 1 male and 2 females	
3.2.8	Control animals	None	
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	The test substance was used as delivered.	
3.3.2	Amount of active substance instilled	25 mg of the test substance.	X
3.3.3	Exposure period	(i) 24 hours (ii) 2 minutes, then washed out with 20 ml tap water.	
3.3.4	Post-exposure period	21 days	
3.4	**Examinations**		
3.4.1	Ophthalmoscopic examination	Not specified	
3.4.2	Scoring system	According to guideline.	
3.4.3	Examination time points	1, 4, 24, 48, 72 hours and 7, 14 and 21 days after instillation.	
3.4.4	Other investigations	Conjunctival discharge	
3.5	**Further remarks**	None	

4 RESULTS

4.1	**Clinical signs**	No	
4.2	**Average score**		
4.2.1	Corneal opacity	(i) Main study group: 1.4 (for all six animals) (ii) Second study group: 1.1 (for all three animals)	
4.2.2	Iris	(i) Main study group: 0.4 (for all six animals) (ii) Second study group: 0.5 (for all three animals)	
4.2.3	Conjunctiva		
	Redness	(i) Main study group: 2.6 (for all six animals) (ii) Second study group: 2.5 (for all three animals)	
	Chemosis	(i) Main study group: 1.4 (for all six animals) (ii) Second study group: 1.0 (for all three animals)	

Section A6.1.4 Acute eye irritation of the manufacturing master mix in rabbits

Annex Point IIA6.1.4

4.3	**Reversibility**	Yes (i) Main study group: Redness of the conjunctiva, corneal opacity and corneal area affected were observed starting 1 hour of observation and had resolved at 21 days after instillation. Discharge of the conjunctiva were observed starting 1 hour of observation and had resolved at 14 days after instillation. Chemosis of the conjunctiva and iritic effects were observed starting 1 hour of observation and had resolved at 7 days after instillation. (ii) Second study group: Redness of the conjunctiva, corneal opacity and corneal area affected were observed starting 1 hour of observation and had resolved at 21 days after instillation. Corneal opacity and corneal area affected were observed starting 1 hour of observation and had resolved at 7 days after instillation. Chemosis, discharge of the conjunctiva and iritic effects were observed starting 1 hour of observation and had resolved at 72 hours after instillation.
4.4	**Other**	None
4.5	**Overall result**	Based on these results, STORM manufacturing master mix requires classification with R36 “irritating to eyes” according to the requirements specified by Directive 67/548/EC and subsequent regulations.

5 APPLICANT’S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute eye irritation potential of STORM manufacturing master mix containing 0.5 % Flocoumafen was tested in New Zealand White rabbits according to OECD guideline 405 (1981). Rabbits were administered a dose of 25 mg test substance per eye. No deviations from the methods prescribed by the guideline were reported.
5.2	**Results and discussion**	Average scores of 1.4 for corneal opacity, 0.4 for iridial irritation, 2.6 for conjunctival redness, 1.4 for conjunctival chemosis and 1.4 for conjunctival discharge were calculated for the animals of the main study group based on scorings at 24, 48 and 72 hours.
5.3	**Conclusion**	Thus, according to the requirements specified by Directive 67/548/EC and subsequent regulations, the manufacturing master mix requires classification with R36 “irritating to eyes”.
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	27 December 2004
Materials and Methods	3.3.2 No information was available on the volume of test substance instilled into the eye. Based on the data of the skin irritation study, it can be assumed that 25 mg of test substance, equals a volume of 0.025 ml. According to the guideline, the amount used should have a a volume of 0.1 ml.
Results and discussion	No comments.
Conclusion	STORM Manufacturing Master Mix (containing 0.5% flocoumafen) should be classified as irritating to eyes.
Reliability	2
Acceptability	Acceptable. Although the tested volume was 0.025 ml instead of 0.1 ml, considering the results of the test, the study is considered acceptable.
Remarks	None.

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

Table A6.1.4- 3: Acute eye irritation in rabbits of the main study group.

	Cornea		Iris	Conjunctiva		
	Opacity	Area		Redness	Chemosis	Discharge
Score (average of animals investigated)	**0 to 4**	**0 to 4**	**0 to 2**	**0 to 3**	**0 to 4**	**0 to 3**
60 min	0.5	0.7	0	1.0	0.3	1.0
24 h	1.3	2.0	0.3	2.2	1.8	1.7
48 h	1.5	1.8	0.5	2.8	1.7	1.5
72 h	1.3	1.5	0.3	2.8	0.8	1.2
Average 24, 48, 72 h	1.4	1.8	0.4	2.6	1.4	1.4
Maximum average score (including area affected, maximum: 110)	17.5		2.5		12	
Reversibility*	c	c	c	c	c	c
Average time for reversion	21 days	21 days	7 days	21 days	7 days	14 days

* c: completely reversible

Calculation of maximum average score according to “Draize Scale for Scoring Ocular Irritation”.

Table A6.1.4- 4: Acute eye irritation in rabbits of the second study group.

	Cornea		Iris	Conjunctiva		
	Opacity	Area		Redness	Chemosis	Discharge
Score (average of animals investigated)	**0 to 4**	**0 to 4**	**0 to 2**	**0 to 3**	**0 to 4**	**0 to 3**
60 min	0	0.7	0	1.0	0.3	0.7
24 h	1.3	1.3	0.7	2.7	2.3	1.7
48 h	1.3	1.0	0.7	2.7	0.7	0.7
72 h	0.7	0.3	0	2.0	0	0
Average 24, 48, 72 h	1.1	0.9	0.5	2.5	1.0	1.1
Maximum average score (including area affected, maximum: 110)	13.3		3.3		13.3	
Reversibility*	c	c	c	c	c	c
Average time for reversion	7 days	7 days	72 hours	21 days	72 hours	72 hours

* c: completely reversible

Calculation of maximum average score according to “Draize Scale for Scoring Ocular Irritation”.

Section A6.1.4 Acute eye irritation in rabbits

Annex Point IIA6.1.4 Supportive data

The following reference is considered to contain additional information concerning acute eye irritation in rabbits and is thus presented in tabular format as supportive data: (non-GLP study)			
Reference	Title	System	Results
A6.1.4/04 Axxxx (1983), Sxxxx Lxxxx, Unpublished Report, July 15, 1983. (BASF-Ref.: FL-415-002)	Acute eye irritation of novel anticoagulants in male rabbits.	New Zealand White rabbits (3 males)	Flocoumafen was dissolved in PEG 200 to give a 1.0% solution and instilled into one eye. Examinations were performed 24 and 48 hours after treatment. No effects of the test substance on cornea, iris and conjunctivae were observed.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	27 December 2004
Conclusion	The presentation of the above study as supportive data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.1.5 Skin sensitisation
Annex Point IIA6.1.5 (Guinea pig maximisation test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.1.5/01:** Pxxxx Jxxxx (1986) Toxicology of rodenticides ("Storm"): The skin sensitizing potential of WL108366. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.86.091, June 30, 1986 (unpublished). (BASF-Ref.: FL-416-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No The conduct of the study was similar to method B.6 (96/54/EC).	
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	
2.3	**Deviations**	Yes A test of the experimental technique using a suitable reference substance was not reported. According to the prescribed guideline, the concentration of the test substance used for each induction should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. Seven animals died after the topical induction and the highest achievable test substance concentration used for the topical induction was non-irritating in the pilot study. For the intra-dermal induction the test substance was formulated in a FCA/corn oil mixture instead of a FCA/water or physiological saline mixture.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	As given in Section A2, apart from the purity stated below.	X
3.1.3	Purity	97.6%	
3.1.4	Description	Off-white powder	

Section A6.1.5 Skin sensitisation
Annex Point IIA6.1.5 (Guinea pig maximisation test)

3.1.5	Stability	The test substance was considered to be stable for the duration of this study under the storage conditions employed (dark at ambient temperature). The test substance was shown to be stable in corn oil for up to 7 days. The formulations of the test substance in pure petroleum jelly and also in corn oil: FCA were found to be stable for at least 7.5 hours.	
3.1.6	Preparation of test substance for application	For induction: corn oil (intra-dermal), petroleum jelly (topical) For challenge: petroleum jelly	
3.1.7	Pre-test performed on irritant effects	Yes	
3.2	**Test animals**		
3.2.1	Species	Guinea pigs	
3.2.2	Strain	Not specified	
3.2.3	Source	Porcellus Ltd., UK	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: not stated Body weight: 505 – 598g (males); 501 – 597g (females)	
3.2.6	Number of animals per group	10 males and 10 females	
3.2.7	Control animals	5 males and 5 females	
3.3	**Administration/ Exposure**	Adjuvant	
3.3.1	Induction schedule	(i) day 0; (ii) one week after first induction	
3.3.2	Way of induction	(i) intra-dermal; (ii) topical	
3.3.3	Occlusion	(ii) semi-occlusive (for 48 h)	
3.3.4	Concentrations used for induction	(i) 0.05% test substance in corn oil; (ii) 50% test substance in petroleum jelly	X
3.3.5	Concentration Freund's Complete Adjuvant (FCA)	Test material in 50:50 FCA/solvent (corn oil)	
3.3.6	Challenge schedule	Two weeks after topical induction (for 24 hours)	
3.3.7	Concentrations used for challenge	50% test substance in petroleum jelly	
3.3.8	Re-challenge	No	
3.3.9	Scoring schedule	24h, 48h after challenge	
3.3.10	Removal of the test substance	Not stated	
3.3.11	Positive control substance	None	

Section A6.1.5 Skin sensitisation
Annex Point IIA6.1.5 (Guinea pig maximisation test)

3.4	**Examinations**	
3.4.1	Pilot study	Yes
3.5	**Further remarks**	None

4 RESULTS

4.1	**Results of pilot studies**	Intra-dermal injection of 0.05% test substance in corn oil caused slight redness (edges not defined). Intra-dermal injection of 0.1, 0.5 or 1.0% test substance in corn oil caused irritation scored with 2 (pink/red square with defined edges). Topical applications of 15, 25, or 50% (highest concentration achievable) test substance in petroleum jelly caused no difference from the surrounding skin.
4.2	**Results of test**	
4.2.1	24h after challenge	No positive response.
4.2.2	48h after challenge	No positive response.
4.2.3	Other findings	Seven of the test animals died or were killed for humane reasons between day 8 and 12 (after application of the topical induction patches but before the challenge state). The body weight gain in surviving test animals was noticeably less than in the controls.
4.3	**Overall result**	None of the 13 surviving test animals showed positive responses at 24 or 48 hours after removal of the challenge patches. Thus, the test material was considered to be non-sensitising to the skin of guinea pigs.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The skin sensitizing potential of Flocoumafen was tested using the guinea pig maximisation test. Although not a guideline study, the method used was similar to method B.6 (96/54/EC).
5.2	**Results and discussion**	The test material was classified as non-sensitising to the skin, since none of the surviving animals showed positive responses at 24 or 48 hours after removal of the challenge patches.
5.3	**Conclusion**	
5.3.1	Reliability	2

Section A6.1.5 Skin sensitisation
Annex Point IIA6.1.5 (Guinea pig maximisation test)

5.3.2	Deficiencies	Yes Seven animals died or were killed for humane reasons after the topical induction. Thus, the classification of the test substance could not be based on 20 animals as required by the guideline. However, the concentration of the test substance used for each induction should usually be chosen in such a way that it will be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. However, seven animals died or were killed for humane reasons after the topical induction, and the highest achievable test substance concentration used for the topical induction was therefore one that was non-irritating in the pilot study. For the intra-dermal induction, the test substance was formulated in a FCA/corn oil mixture instead of a FCA/water or physiological saline mixture. Nevertheless, in view of the toxicity of the test substance, the chosen modifications would appear justifiable, and to have no impact on the overall outcome of the test.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	27 December 2004
Materials and Methods	3.1.2: specification of test substance: cis:trans isomer ratio = 57:43. 3.3.4: The highest attainable concentration of 50% used for topical induction was a non-irritating concentration. Therefore, the test area should have been treated with sodium lauryl sulphate. However, since animals died after topical induction, the test substance was systemically available.
Results and discussion	No comments.
Conclusion	In a maximization study, flocoumafen was not sensitizing to the skin of guinea pigs.
Reliability	2
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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

	GPMT		**Observations/Remarks**
	Day	**Application**	
Induction 1	0	Intra-dermal	None stated
Induction 2	6 – 8	Topical	Seven animals died or were killed for humane reasons between day 8-12
Challenge	22 – 23	Topical	Some animals showed responses to treatment immediately after patch removal (slight redness)
Scoring 1	24	–	No positive response
Scoring 2	25	–	No positive response

Table A6.1.5- 2: Result of the skin sensitisation test.

	Number of animals with signs of allergic reactions/ number of animals in group	
	Negative Control	**Test group**
Scored after 24 h	0/10	0/13
Scored after 48 hours	0/10	0/13

Section A6.2 Annex Point IIA6.2 — Metabolism of ^{14}C-Flocoumafen in the rat after oral administration of a single high dose (in vivo test)

Official use only

1 REFERENCE

1.1	**Reference**	**A6.2/01:** Hxxxx Cxxxx (2003): Metabolism of ^{14}C-BAS 322 I in the rat. Bxxxx, Lxxxx, Gxxxx, Report no. 66884, December 18, 2003 (unpublished). (BASF Ref. 2003/1021832)
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

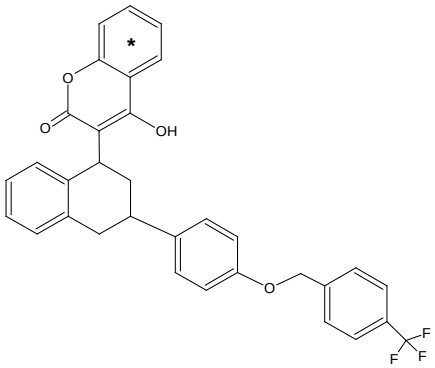
2.1	**Guideline study**	Yes EC method B.36 (87/302/EEC); OECD 417 (1984)
2.2	**GLP**	Yes Certified laboratory
2.3	**Deviations**	Yes Only one dose level instead of two was used. However, in view of the availability of a full set of ADME studies, the current study was conducted only for identification of metabolites. Thus, this deviation does not affect the validity of the study.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2, radio-labelled.
3.1.1	Lot/Batch number	792-1012 (coumarin-U-C^{14}-label) 794-1066 (trifluoromethylphenyl-U-C^{14}-label)
3.1.2	Specification	As given in section 2, apart from purity stated below. Isomeric composition was not specified (i.e. ratio *cis*:*trans* isomers).
3.1.3	Purity	Radiochemical purity: 99.4 % (coumarin-U-C^{14}-label); Chemical purity: 98.2 % Radiochemical purity: 97 % (trifluoromethylphenyl-U-C^{14}-label); Chemical purity: 86 %
3.1.4	Description	Not stated
3.1.5	Stability	Not stated

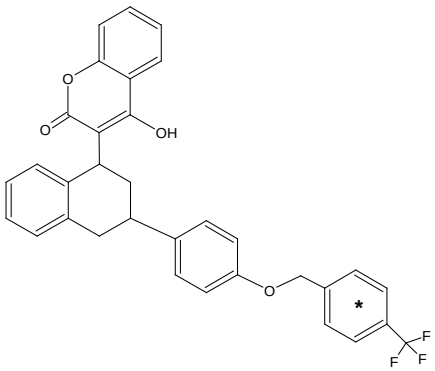
Section A6.2 Annex Point IIA6.2 — Metabolism of ^{14}C-Flocoumafen in the rat after oral administration of a single high dose (in vivo test)

3.1.6	Radiolabelling	coumarin-U-C^{14}-label:



* = position of [U-^{14}C]-label

trifluoromethylphenyl-U-C^{14}-label:



* = position of [U-^{14}C]-label

3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer
3.2.3	Source	Not stated
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Not stated
3.2.6	Number of animals per group	10 males
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Type	Gavage
3.3.2	Concentration of test substance	approximately 14 mg/kg bw (high dose) mean achieved dose: 13.6 mg/kg bw (coumarin-U-C^{14}-label) and 14.3 mg/kg bw (trifluoromethylphenyl-U-C^{14}-label)
3.3.3	Specific activity of test substance	Specific activity: 5.82 MBq/mg (coumarin-U-C^{14}-label) Specific activity: 4.90 MBq/mg (trifluoromethylphenyl-U-C^{14}-label)

Section A6.2 **Metabolism of ^{14}C-Flocoumafen in the rat after oral administration of a single high dose (in vivo test)**
Annex Point IIA6.2

3.3.4	Volume applied	about 2 g	
3.3.5	Vehicle	Corn germ oil	
3.3.6	Post-exposure period	2 days (coumarin-U-C^{14}-label) 3 days (trifluoromethylphenyl-U-C^{14}-label)	
3.3.7	Samples (sampling time)	Urine and faeces (collection for two to three days), Liver (sampling upon necropsy)	
3.3.8	Examinations	Determination of total radioactive residues (TRR) by LSC for liquid samples and by combustion followed by LSC for solid samples. Quantification and identification of metabolites from urine, faeces and liver (acetonitrile extract) was performed by HPLC/UV. Further identification of significant metabolites was conducted by mass spectroscopy.	X

4 RESULTS

4.1	**Toxic effects, clinical signs**	Not stated.	
4.2	**Elimination**	The major part of radioactivity was excreted via faeces amounting to 63.2 % and 71.0 % of the dose for the coumarin-U-C^{14}-label and trifluoromethylphenyl-U-C^{14}-label, respectively. In contrast, 0.6 % and 6.1 % of the dose were detected in urine of animals administered with the coumarin-U-C^{14}-labelled or the trifluoromethylphenyl-U-C^{14}-labelled test item, respectively. The results are summarised in Table A6.2- 1.	
4.3	**Radioactivity in liver**	Radioactive residues detected in pooled liver samples of animals administered with the coumarin-U-C^{14}-labelled or the trifluoromethyl-phenyl-U-C^{14}-labelled test item amounted to 15.2 % and 9.3 % of the dose, respectively.	
4.4	**Recovery of labelled compound**	79.8 % (coumarin-U-C^{14}-label) 87.7 % (trifluoromethylphenyl-U-C^{14}-label)	

Section A6.2 Annex Point IIA6.2 — Metabolism of ^{14}C-Flocoumafen in the rat after oral administration of a single high dose (in vivo test)

4.5	**Identification and quantification of metabolites**	The results of the identification and quantification of metabolites in urine, faeces and liver are summarised in Table A6.2- 2. The proposed metabolic pathway in rats is presented in Figure A6.2- 1. Metabolites detected in urine samples were shown to be more polar than the metabolites detected in faeces, and resulted from the cleavage of the benzyl ether bond of the test compound with further oxidation and conjugation reactions (here with glucuronic acid or amino acid). The methoxy groups in M 322I 03 and M 322I 04 were assumed to be produced as artefact during work-up. The parent compound was not detected in urine samples of both labels. In contrast, the major compounds identified in faeces were the *cis* and *trans* isomers of the parent compound, with the *cis* form at dominant quantities. Further characteristic biotransformation products were the hydroxylated metabolites M 322I 01 and M 322I 02 as well as glucuronic acid conjugates of the parent molecule. Analogous results were obtained in liver samples, with the *cis* and *trans* isomers of the parent compound building the major residue. The *trans* form was the dominant isomer in liver in comparison to faeces with the *cis* isomer at larger quantities. In addition, the hydroxylated metabolites M 322I 01 and M 322I 02 as well as M 322I 08 showing an additional double bond in the non-aromatic ring of the tetralin unit were detected.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of ten male Fischer rats received approximately 14 mg/kg bw of coumarin-U-C^{14}-labelled or trifluoromethyl-phenyl-U-C^{14}-labelled Flocoumafen by gavage. Identification and quantification of metabolites was performed in samples of urine, faeces and liver. The study was conducted according to EC method B.36 (87/302/EEC) and OECD 417 (1984), with exception that only one instead of two dose levels were used based on the results of former studies.	
5.2	**Results and discussion**	The major part of radioactivity was excreted via faeces amounting to 63.2 % and 71.0 % of the dose for the coumarin-U-C^{14}-label and trifluoromethylphenyl-U-C^{14}-label, respectively. Mainly unchanged parent compound was identified in samples of faeces. In contrast, 0.6 % and 6.1 % of the administered dose were detected in urine of animals administered with the coumarin-U-C^{14}-labelled or the trifluoromethylphenyl-U-C^{14}-labelled test item, respectively. Metabolites detected in urine samples showed to be more polar than the metabolites detected in faeces. Radioactive residues detected in pooled liver samples of animals administered with the coumarin-U-C^{14}-labelled or the trifluoromethyl-phenyl-U-C^{14}-labelled test item amounted to 15.2 % and 9.3 % of the dose, respectively. The *cis* and *trans* isomers of the parent compound formed the major residue in liver. The main routes of biotransformation were represented by phase I reactions oxidising all ring systems of the test item. Conjugation of the parent was observed with glucuronic acid. Cleavage of the benzyl ether bridge played a minor role in metabolism of Flocoumafen, but explained the urine metabolites detected at low quantities.	X

Section A6.2 **Annex Point IIA6.2**	**Metabolism of ^{14}C-Flocoumafen in the rat after oral administration of a single high dose (in vivo test)**	
5.3	**Conclusion**	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	10 January 2005
Materials and Methods	(3.1) In a request for information the applicant demonstrated information on the test substance: batch no. 792-1012 with a cis-trans ratio of 56.8:43.2 and batch no. 794-1066 with a cis-trans ratio of 57.9:42.1. (3.3.8) faeces: MeOH extract.
Results and discussion	(5.2) The following additions were made by the RMS: Mainly (56-57% of faeces associated radioactivity) unchanged parent compound was identified in samples of faeces. In total 83-86% of faeces associated radioactivity was identified. No parent was detected in the urine. The *cis* and *trans* isomers of the parent compound formed the major (60-63% of liver associated radioactivity) residue in liver. In total 83-86% of liver associated radioactivity was identified.
Conclusion	Groups of ten male Fischer rats received approximately 14 mg/kg bw of coumarin-U-C^{14}-labelled or trifluoromethyl-phenyl-U-C^{14}-labelled Flocoumafen by gavage. Identification and quantification of metabolites was performed in samples of urine, faeces and liver. The major part of radioactivity was excreted via faeces (63.2 % and 71.0 % of the dose). Mainly (56-57% of faeces associated radioactivity) unchanged parent compound was identified in samples of faeces. In total 83-86% of faeces associated radioactivity was identified. In contrast, 0.6 % and 6.1 % of the administered dose were detected in urine. Metabolites detected in urine samples showed to be more polar than the metabolites detected in faeces. After single oral administration of 14 mg/kg bw of coumarin-U-C^{14}-labelled or trifluoromethyl-phenyl-U-C^{14}-labelled Flocoumafen, total oral absorption amounted to 17% of the administered dose, based on radio label found in urine, liver and cage wash. Radioactive residues detected in pooled liver samples amounted to 9.3-15.2 % of the dose. The *cis* and *trans* isomers of the parent compound formed the major (60-63% of liver associated radioactivity) residue in liver. In total 83-86% of liver associated radioactivity was identified. Based on the metabolite identifications, it is concluded that the main routes of biotransformation were represented by phase I reactions oxidising all ring systems of the test item. Conjugation of the parent was observed with glucuronic acid. Cleavage of the benzyl ether bridge played a minor role in metabolism of Flocoumafen, but explained the urine metabolites detected at low quantities.
Reliability	1
Acceptability	Acceptable.
Remarks	No females were included.

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

Table A6.2- 1: Excretion of radioactivity in % of the administered dose (approx. 14 mg/kg bw).

Matrix	Time interval [h]	% of the administered dose	
		Coumarin-U-C^{14}-label	Trifluoromethylphenyl-U-C^{14}-label
Urine	0–24	0.4	2.6
	24–48	0.2	2.3
	48–72	not determined	1.2
	0–72	0.6	6.1
Faeces	0–24	34.0	25.7
	24–48	29.2	35.2
	48–72	not determined	10.1
	0–72	63.2	71.0
Liver	after sacrifice	15.2	9.3
Cage wash	after sacrifice	0.8	1.3
Total		79.8	87.7

Table A6.2- 2: Composition of radioactivity in urine, faeces and liver after single administration of [^{14}C]-Flocoumafen.

Metabolite identity	Total radioactivity in % of the dose					
	coumarin-U-C^{14}-label			trifluoromethylphenyl-U-C^{14}-label		
	Urine (0–48 h)	Faeces (0–48 h)	Liver	Urine (0–48 h)	Faeces (0–48 h)	Liver
M 322I 01	–	4.52	0.46	–	9.57	0.28
M 322I 02	–	6.71	2.68	–	8.28	1.63
M 322I 03	0.02	–	–	–	–	–
Isomer of M 322I 03	0.02	–	–	–	–	–
M 322I 04	0.04	–	–	–	–	–
M 322I 05	–	1.06	–	–	–	–
M 322I 06	–	2.04	–	–	1.09	–
Isomer of M 322I 06	–	2.06	–	–	1.41	–
M 322I 07	–	0.84	–	–	–	–

M 322I 08	–	–	0.30	–	0.47	0.27
M 322I 09	–	–	–	0.78	–	–
Isomer of M 322I 09	–	–	–	0.96	–	–
M 322I 10	–	–	–	2.41	–	–
M 322I 11	–	–	–	0.86	–	–
cis-BAS 322 I[1]	–	21.41	3.35	–	27.76	1.88
trans-BAS 322 I[1]	–	13.82	5.80	–	12.33	3.96
Total	0.08	52.46	12.59	5.01	60.91	8.02

[1] BAS 322 I is the development code for Flocoumafen

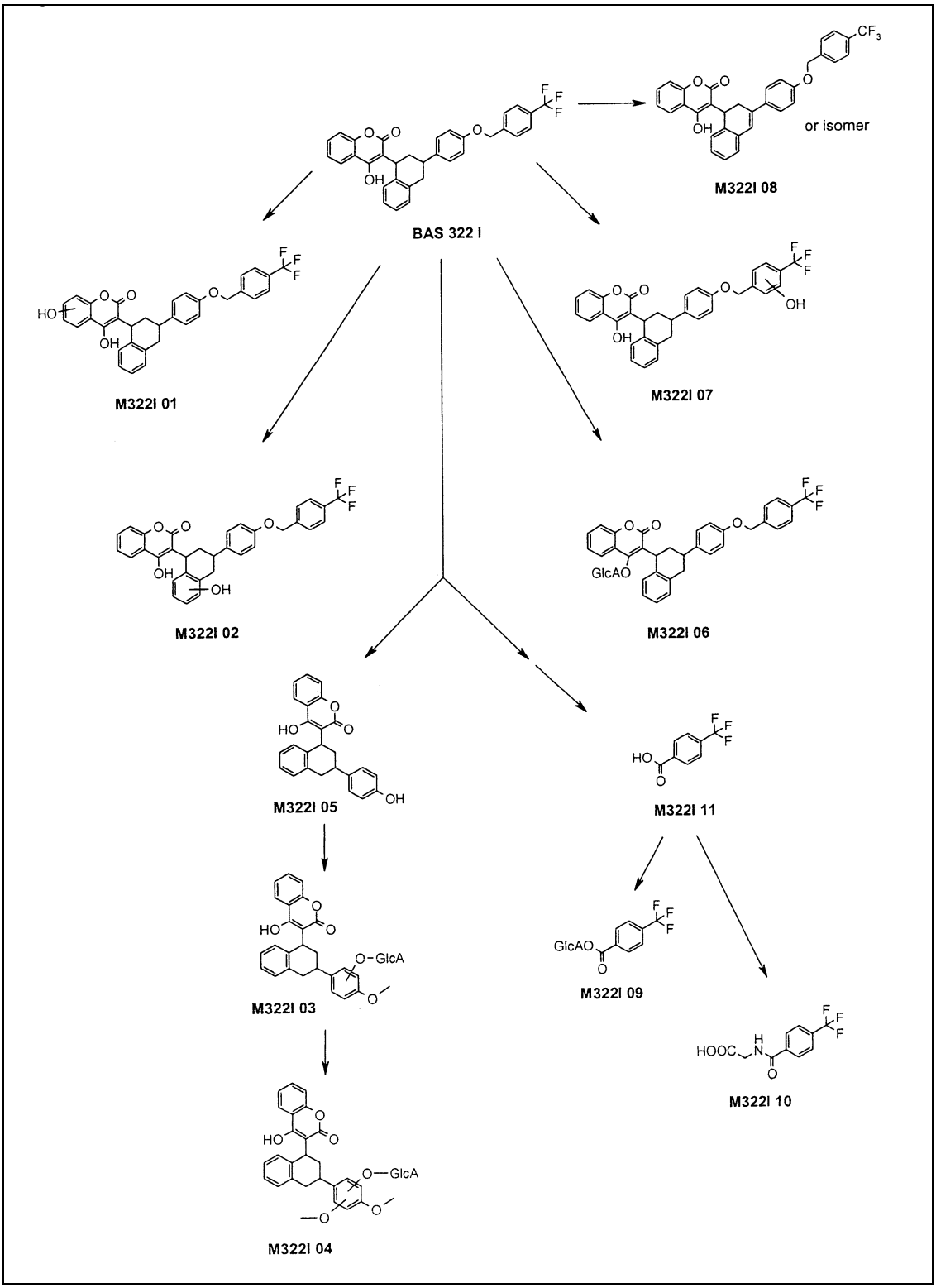


Figure A6.2- 1: Proposed metabolic pathway in the rat.

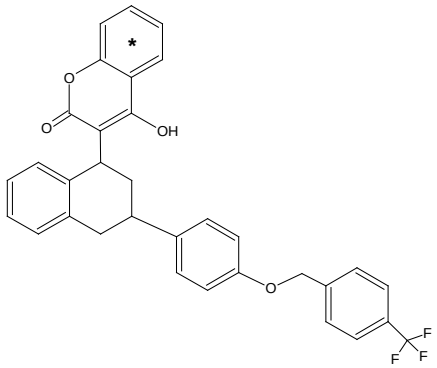
Section A6.2 Percutaneous absorption in the rat (in vivo test)

Annex Point IIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.2/02:** Hxxxx Kxxxx, Wxxxx Pxxxx (1985) Percutaneous absorption, metabolism and elimination of WL108366 in the rat. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.85.218, December 18, 1985 (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 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 The conduct of the study was consistent to EU method B.36 (88/302/EEC) in all important aspects.	X
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2, radio-labelled, in acetone.	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	Radiochemical purity: 97.2%	
3.1.4	Description	Not stated	
3.1.5	Stability	Storage of acetone stock solutions of ^{14}C-Flocoumafen resulted in up to 5% radiochemical degradation in 7 days. Freshly purified material was used.	

Section A6.2 Percutaneous absorption in the rat (in vivo test)

Annex Point IIA6.2

3.1.6	Radiolabelling	coumarin-U-C^{14}-label: 
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age: not stated Body weight: 173–190 g
3.2.6	Number of animals per group	9 males
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Dermal
3.3.1	Preparation of test site	Three days prior to dosing, an area of dorsal fur was removed using electric clippers.
3.3.2	Concentration of test substance	0.17 mg/kg (total dose 30 µg, ca. 3 µg/cm²)
3.3.3	Vehicle	Acetone
3.3.4	Specific activity of test substance	266 µCi/mg
3.3.5	Volume applied	100 µl
3.3.6	Size of test site	At least 10 cm²
3.3.7	Exposure period	7 days, non-occluded (but with the use of neck restraining collars for 6h p.a.)
3.3.8	Sampling time	Urine and faeces daily; blood, organs and tissues upon necropsy
3.3.9	Samples	Urine and faeces (all animals), blood, liver, kidneys, heart, lungs, brain, testes, spleen, skin (with paws and tail attached), gastro-intestinal tract (pylorus to anus) including contents, sub-samples of fat (subcutaneous and peri-renal), muscle (right femoral) and the remaining carcass (for 6 animals). Three animals were subjected to whole body autoradiography.

Section A6.2 Percutaneous absorption in the rat (in vivo test)

Annex Point IIA6.2

3.3.10	Examinations	Determination of total radioactive residues (TRR) by LSC or by combustion followed by LSC. Quantification and identification of metabolites from urine, faeces and liver (acetonitrile extract) was performed by HPLC/UV and two-dimensional TLC.	X
		4 RESULTS	
4.1	**Toxic effects, clinical signs**	No general signs of anticoagulant toxicity were noted. Clinical signs associated with neck restraining collars included enhanced levels of activity, the appearance of peri-nasal and peri-orbital brown staining (chromodacryorrhea). After removal of the restraining collars, the animals rapidly returned to normal behaviour and the brown staining disappeared.	
4.2	**Dermal irritation**	No effects were noted.	
4.3	**Elimination**	Elimination via urine and faeces amounted to 10 % (ca. 1.5 % per day) and 31 % of the administered dose (ca. 4.5 % per day), respectively, within 7 days after treatment. The results are presented in Table A6.2- 4.	
4.4	**Radioactivity in tissues**	Radioactive residues were widely distributed within the sampled tissues and organs, with the major part of radioactivity (i.e. 25 % of dose) located in liver samples. Other significant residues (10-15 fold less) were determined in kidneys, lungs and spleen. In addition, 11.5 % of the administered dose remained at the site of application, while 5 % remained in the carcass. The results are summarised in Table A6.2- 3.	X
4.5	**Recovery of labelled compound**	91.16 ± 2.8 %	
4.6	**Percutaneous absorption**	76.97 % (applying to the sum of radioactivity detected in excreta, removed organs and the remaining carcass)	
4.7	**Identification of metabolites**	Examination of the radioactivity present in the urine extract containing the majority of the radioactivity detected in urine (i.e. 62.5 %), showed the presence of 2 principal polar metabolites (amounting to 70 and 21 % of radioactivity on the plate, TLC) and very little unchanged Flocoumafen (3 % of the radioactivity on the plate, TLC). In contrast, the majority of the radioactivity detected in faecal extracts was present as components coinciding with the *cis* and *trans* isomers of the parent compound (70.3 % of the recovered radioactivity). Residues in liver were identified to be mainly the unchanged parent compound.	X

Section A6.2 Percutaneous absorption in the rat (in vivo test)

Annex Point IIA6.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Absorption, metabolism and elimination of Flocoumafen was tested in male Fischer 344 rats following a single percutaneous administration of 0.17 mg ^{14}C-Flocoumafen/kg. Although not a guideline study, the conduct of the study was consistent in all important aspects to method B.36 (88/302/EEC). It is expressly noted here that a specific guideline for dermal absorption was not available at the time of conduct of the study.
5.2	**Results and discussion**	Extensive penetration of radioactivity was observed, as shown by the amount of radioactivity remaining at the dose site (i.e. 12 % of the applied dose). Elimination of radioactivity via excreta was slow during 7 days following administration, with 10 % of the administered dose eliminated via the urine and 31 % of the dose eliminated via faeces. Radioactive residues were widely distributed within the sampled tissues and organs. The major residue was located in liver (25 % of the dose) and was shown to be predominantly unchanged parent compound. Other significant residues (10-15 fold less) were determined in kidneys, lungs and spleen. Examination of the radioactivity present in extracts of urine or faeces by HPLC and TLC, showed the presence of 2 principal polar metabolites in urine and mainly the unchanged parent compound in faeces. Only a very small amount of unchanged Flocoumafen was detected in urine extracts. Total dermal absorption amounted to 76.97 % of the administered dose (applying to the sum of radioactivity detected in excreta, removed organs and the remaining carcass).
5.3	**Conclusion**	The use of these results in further risk assessment is considered very limited indeed. The choice of vehicle (acetone) and exposure period (7 days, without a post-exposure wash) yield a worst-case result that is not suitable for the assessment of risk of dermal absorption under practically relevant conditions of use (i.e., rodenticide bait handling in pest control operations). Thus, it is proposed to use a 10% default for dermal absorption in view of the high molecular weight (542.6 g/mol) and the high logPow (6.12 at pH=7), and in consideration of principles laid down in the guidance document Sanco/222/2000 rev. 6 (27. November 2002) on dermal absorption.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes (choice of vehicle and exposure period)

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	10 January 2005
Materials and Methods	(2.1) The study does not meet the requirements of OECD 427. (2.2) The study was performed under GLP. (3.21) Specification of test substance: cis:trans isomer ratio = 50:50. (3.3.10) No procedural recoveries of extraction/concentration procedures prior to chromatographic analysis were determined.
Results and discussion	(4.4) Radioactivity was found in the stomach (not defined whether this represent the stomach content or stomach tissue). Considering the fact that after 6 hours the neck collars were removed and the fact that the test substance was applied non-occluded, it cannot be excluded that some amount of oral exposure to the test substance occurred due to licking of the application site. (4.7) more detail added by the RMS: urine: 83.5% of the radioactivity in urine was extractable. The non-extractable activity (16.5%) and the final extract (6%) was not further analysed. Approximately 7.9% of radioactivity in the urine was parent Flocoumafen. Four metabolites (2.7, 4.4, 13.1 and 43.8%) were observed. faeces: 86% of the radioactivity in the faeces was extractable and 60.5% was parent Flocoumafen. Three metabolites, each <12.9% were observed. liver: 88.7% of the radioactivity in the liver was extractable and 81.2% was parent Flocoumafen. One minor metabolite (<5%) was observed.
Conclusion	Dermal absorption, metabolism and elimination of flocoumafen were studied in male rats following a single percutaneous administration of 0.17 mg ^{14}C-Flocoumafen/kg (3µg/cm^2). Animals were exposure non-occlusively for 7 days. Elimination of radioactivity via excreta was slow following administration, with 10.3% of the administered dose via urine and 30.9% of the administered dose via faeces. Radioactive residues were widely distributed within tissues and organs. In liver 25.4% of the administered dose was located, 4.6% of the administered dose was located in GI tract, spleen, heart, brain, testes, kidneys and lungs, and 5.02% was found in the remaining carcass. Total dermal absorption amounted to approximately 76% of the administered dose. However, it should be noted that the exposure period was 7 days, instead of approximately 6-8 hours. As the MOE and the risk index (based on AOEL) are calculated based on a daily dose, the present study is not considered suitable for risk assessment purposes. Furthermore, in the present study flocoumafen was administered in acetone. This vehicle does not represent the normal exposure conditions of flocoumafen, as Storm BB is a cereal based wax-bound bait block. Therefore, as no suitable dermal absorption data are available, an estimate of dermal absorption is made based on the physical and chemical properties of flocoumafen. Based on the molecular weight of 542.6 g/ml and the log Pow of 6.12 (at pH 7), a dermal absorption of 10% should be considered for risk assessment purposes.
Reliability	3
Acceptability	Not acceptable. The study is not considered suitable for risk assessment purposes of Storm BB.
Remarks	None.

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

Table A6.2- 3: Recovery data of ^{14}C-Flocoumafen following percutaneous absorption in rats (mean of 6 animals).

	^{14}C-Flocoumafen	
	Absolute amount	**% of dose**
Compound applied	30 µg, at ca. 3 mg/cm²	100
Compartments with compound detected:		
1. Skin (with substance not removable)	1590 ng/g	11.54 ± 3.5
2. Cage wash	–	2.66 ± 0.8
3. Urine and faeces	–	41.91 ± 4.7
4. Removed organs		
Liver	760 ng/g	25.42 ± 0.8
GI tract, spleen, heart, brain, testes, kidneys and lungs	284.9 ng/g	4.62 ± 0.7
5. Remaining carcass	7.0 ng/g	5.02 ± 1.1
Sum of # 3 – 5: excreta, removed organs, remaining carcass (= absorption)	– [1]	76.97
Sum of all detected labelled compound (#1 – 5) (= recovery)	– [1]	91.16 ± 2.8

1) not stated in the report

Absolute amounts of residues in tissues and organs are expressed as ng Flocoumafen equivalents/g.

Table A6.2- 4: Daily elimination of radioactivity in the urine and faeces following dermal administration of ^{14}C-Flocoumafen (total dose 30 µg, at ca. 3 µg/cm²) to male Fischer 344 rats.

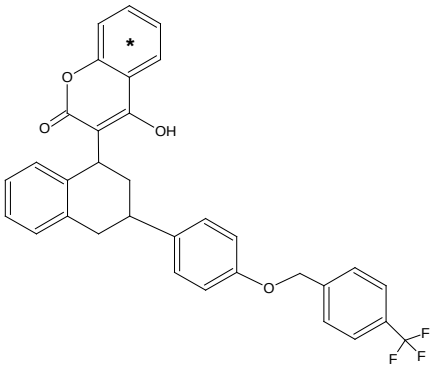
	Radioactivity in % of administered dose (mean of 9 animals)							
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Total**
Urine	0.40 ± 0.22	1.26 ± 0.68	2.12 ± 0.85	2.20 ± 0.88	1.71 ± 0.69	1.47 ± 0.33	1.12 ± 0.21	10.28 ± 3.17
Faeces	1.82 ± 0.80	5.50 ± 1.44	5.49 ± 1.38	5.18 ± 1.09	4.76 ± 1.11	4.25 ± 2.15	3.87 ± 1.01	30.87 ± 2.37

Section A6.2 Annex Point IIA6.2 — Elimination and distribution of ^{14}C-Flocoumafen in the rat after oral administration of a single low dose (in vivo test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.2/03:** Wxxxx Pxxxx, Hxxxx Dxxxx (1985) WL108366: Fate of a single oral dose of [^{14}C]-WL108366 in rats, Part I: Elimination and retention of radioactivity and effect of WL108366 on prothrombin time. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.85.053; March 26, 1985 (unpublished). (BASF-Ref.: FL-440-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No However, the conduct of the study was consistent in all important aspects to EC method B.36 (88/302/EEC).	
2.2	**GLP**	No At the time of the study conduct, GLP were not compulsory. However, the study was conducted in accordance with the principles of GLP	X
2.3	**Deviations**	Yes Only one dose level instead of two was used intentionally. However, a full set of ADME studies (including single low and high dose) are submitted with this dossier.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2, radio-labelled, in acetone.	
3.1.1	Lot/Batch number	Not stated	
3.1.2	Specification	As given in Section A2. (*cis*/*trans* ratio: 61:39)	X
3.1.3	Purity	Radiochemical purity: 97.5%	
3.1.4	Description	Not stated	
3.1.5	Stability	Not stated	

Section A6.2 Annex Point IIA6.2

Elimination and distribution of ^{14}C-Flocoumafen in the rat after oral administration of a single low dose (in vivo test)

3.1.6	Radiolabelling	coumarin-U-C^{14}-label: 
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: not stated Body weight: 204 – 242 g (males); 130 – 148 g (females)
3.2.6	Number of animals per group	(i) 5 males and 5 females (absorption/elimination, retention experiment) (ii) 2 males and 2 females (collection of respired $^{14}CO_2$) (iii) 4 males and 4 females (whole body autoradiography) (iv) 14 males (half-life of ^{14}C-Flocoumafen in the blood)
3.2.7	Control animals	(iv) 5 animals (determination of control plasma prothrombin times)
3.3	**Administration/ Exposure**	Oral
3.3.1	Type	Gavage (single dose)
3.3.2	Concentration of test substance	0.14 mg/kg
3.3.3	Specific activity of test substance	270 µCi/mg
3.3.4	Total volume applied	Not specified
3.3.5	Vehicle	Corn oil
3.3.6	Postexposure period	(i) 7 days (ii) 48 hours (iii) up to 7 days (iv) up to 4 days

Section A6.2 **Annex Point IIA6.2**	**Elimination and distribution of ^{14}C-Flocoumafen in the rat after oral administration of a single low dose (in vivo test)**	
3.3.7 Samples (sampling time)	(i) urine and faeces (daily); blood, liver, kidneys, heart, spleen, lungs, brain, intestines (including stomach and all contents), skin, testes or ovaries, and sub-samples of muscle and abdominal fat (upon necropsy) (ii) collection of respired $^{14}CO_2$ for 48 hours (iii) whole body autoradiography after 2 days (two animals per sex) and after 7 days (two animals per sex) (iv) plasma prothrombin times and concentration of ^{14}C-Flocoumafen in whole blood and plasma (two animals each at 1, 4, 8 and 24 hours, and in groups of three on days 2 and 4)	X
3.3.8 Examinations	Determination of total radioactive residues (TRR) by LSC or by combustion followed by LSC. In addition, liver extracts (acetonitrile) were analysed by HPLC/UV and TLC.	
	4 RESULTS	
4.1 Toxic effects, clinical signs	No clinical signs were reported. Increased prothrombin times occurred after the single oral administration, reaching a maximum at 24 hours. By 48 hours, however, the values had returned to the control range of 10 to 11 seconds. A rapid increase in concentrations of Flocoumafen and/or metabolites was observed for whole blood and plasma with a maximum at 4 hours (0.03 µg/ml in whole blood) followed by a rapid decline. The results are presented in Figure A6.2- 2.	
4.2 Elimination	Seven days after administration, 26 % (males) and 23 % (females) of the radioactivity was excreted via faeces. Elimination via urine accounted for less than 0.5 % of the administered dose (i.e. 0.353 % for males and 0.443 % for females). No $^{14}CO_2$ was detected in the expired air (limit of detection: 0.05 % of dose). The results are presented in Table A6.2- 6.	
4.3 Retention	Radioactive residues were widely distributed within the sampled tissues and organs, with the major part of radioactivity (i.e. 43 and 53 % of dose for males and females, respectively) located in liver samples. In total, 74 % and 79 % of the administered dose were retained in males and females, respectively. This degree of retention was exceptionally high and was assumed to be due to the fact that a low dose of a relatively bio-stable molecule may store selectively in a specific organelle of the liver and thus made unavailable for rapid metabolism. The results are presented in Table A6.2- 5.	
4.4 Recovery of labelled compound	100.36% (males); 102.74% (females)	
4.5 Identification of metabolites in liver	The unchanged parent compound (*cis*/*trans* ratio: 61:39) amounted to 67 % of the residue detected in liver samples.	X

Section A6.2
Annex Point IIA6.2

Elimination and distribution of ^{14}C-Flocoumafen in the rat after oral administration of a single low dose (in vivo test)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Elimination and retention of ^{14}C-Flocoumafen after a single oral dose of 0.14 mg/kg in corn oil was studied in Fischer 344 rats. In addition, the effect of Flocoumafen on prothrombin time was investigated. Although not a guideline study, the conduct of the study was consistent in all important aspects to EC method B.36 (88/302/EEC).	
5.2	**Results and discussion**	Elevated prothrombin times relative to the normal control range of 10–11 seconds were observed at 4 and 8 hours after administration. The maximum prothrombin time was reached after 24 hours (approx. 2-fold of the normal control range) and returned to the control range by 48 hours after administration. Radioactivity in whole blood samples of the rats reached a maximum of 0.025 µg equivalents/ml at 4 hours and rapidly declined thereafter. The major route of elimination was via faeces (23–26% of the administered dose), with urine accounting for less than 0.5% of the administered dose. Upon study termination after 7 days, 74% to 79% of the dose was retained in the animals with approx. 50 % of the retained radioactivity located in the liver. About 70% of the hepatic radioactivity was found to be unchanged Flocoumafen. It was concluded that the concentration of radioactivity in the liver (1.2–1.6 mg equivalents/g) appears to have only a transient effect on the synthesis of blood clotting factors possibly because it was stored in a non-bioavailable form.	
5.3	**Conclusion**		X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	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	11 January 2005
Materials and Methods	(2.2) The study was performed under GLP. (3.1.2) The cis:trans isomer ratio of ^{14}C-labeled Flocoumafen is 51:49 (and not 61:39). (3.3.7) (iv) Only the total radioactivity in whole blood and plasma was determined. The concentration is expressed as Flocoumafen equivalents (µg/mL).
Results and discussion	(4.5) The total amount of radioactivity in the liver after 2 days was not reported. 81% of the liver TRR was extractable of which 83% was parent Flocoumafen (*cis/trans* 61:39). The remaining extractable material consisted of polar metabolites (14%) and non-polar material (3%). No chromatograms (HPLC/TLC) were given in the report.
Conclusion	Elimination and retention of ^{14}C-Flocoumafen after a single oral dose of 0.14 mg/kg in corn oil was studied in Fischer 344 rats. In addition, the effect of Flocoumafen on prothrombin time was investigated. Elevated prothrombin times relative to the normal control range of 10–11 seconds were observed at 4 and 8 hours after administration. The maximum prothrombin time was reached after 24 hours (approx. 2-fold of the normal control range) and returned to the control range by 48 hours after administration. Radioactivity in whole blood samples of the rats reached a maximum of 0.025 µg equivalents/mL at 4 hours and rapidly declined thereafter. The major route of elimination was via faeces (23–26% of the administered dose), with urine accounting for less than 0.5% of the administered dose. Upon study termination after 7 days, 74% to 79% of the dose was retained in the animals with approx. 50 % of the retained radioactivity located in the liver. About 70% of the hepatic radioactivity was found to be unchanged Flocoumafen. After single oral administration of 0.14 mg/kg bw of coumarin-U-C^{14}-labelled Flocoumafen, total oral absorption amounted to 69% in males and 75% in females of the administered dose, based on radio label found in urine, tissues (liver, skin and kidney) and cage wash. It was concluded that the concentration of radioactivity in the liver (1.2–1.6 mg equivalents/g) appears to have only a transient effect on the synthesis of blood clotting factors possibly because it was stored in a non-bioavailable form.
Reliability	2, because of lack of detail in the report: no chromatograms were given.
Acceptability	Acceptable.
Remarks	None.

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

Reliability	
Acceptability	
Remarks	

Table A6.2- 5: Total recovery of radioactivity following oral administration of ^{14}C-Flocoumafen (0.14 mg/kg) to Fischer 344 rats (means of 5 males and 4 females).

	^{14}C-Flocoumafen			
	Absolute amount		**% of dose**	
	Male	**Female**	**Male**	**Female**
Compound applied	0.14 mg/kg		100	
Compartments with compound detected:				
1. Cage wash	–	–	0.014	0.04
2. Blood	0.0011 µg/ml	0.001 µg/ml	–	–
3. Urine	–	–	0.35	0.45
4. Faeces	–	–	26.16	23.07
5. Removed organs				
Liver	1.18 µg/g	1.57 µg/g	43.13	53.37
Skin	0.053 µg/g	0.040 µ/g	10.09	7.30
Intestine	0.060 µg/g	0.055 µg/g	5.15	5.17
Kidney	0.206 µg/g	0.199 µg/g	1.18	1.30
6. Remaining carcass	0.023 µg/g	0.019 µg/g	14.29	12.06
7. Exhaled air	–	–	< 0.05	< 0.05
Sum of all detected labelled compound (#1 – 7) (=recovery)	–	–	100.36	102.74

The residues in all the other tissues examined totalled about 1% of the dose.

Absolute amounts of residues in tissues and organs are expressed as µg Flocoumafen equivalents/g.

Table A6.2- 6: Daily elimination of radioactivity in the urine and faeces following oral administration of ^{14}C-Flocoumafen (0.14 mg/kg) to Fischer 344 rats

	Radioactivity in % of administered dose (means of 5 males and 4 females)							
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Total**
Urine								
Male	0.235	0.054	0.024	0.014	0.011	0.009	0.007	0.353
Female	0.254	0.059	0.051	0.034	0.021	0.014	0.012	0.443
Faeces								
Male	14.7	5.83	2.63	1.23	0.74	0.59	0.44	26.1
Female	12.22	5.31	2.35	1.45	0.86	0.55	0.49	23.0

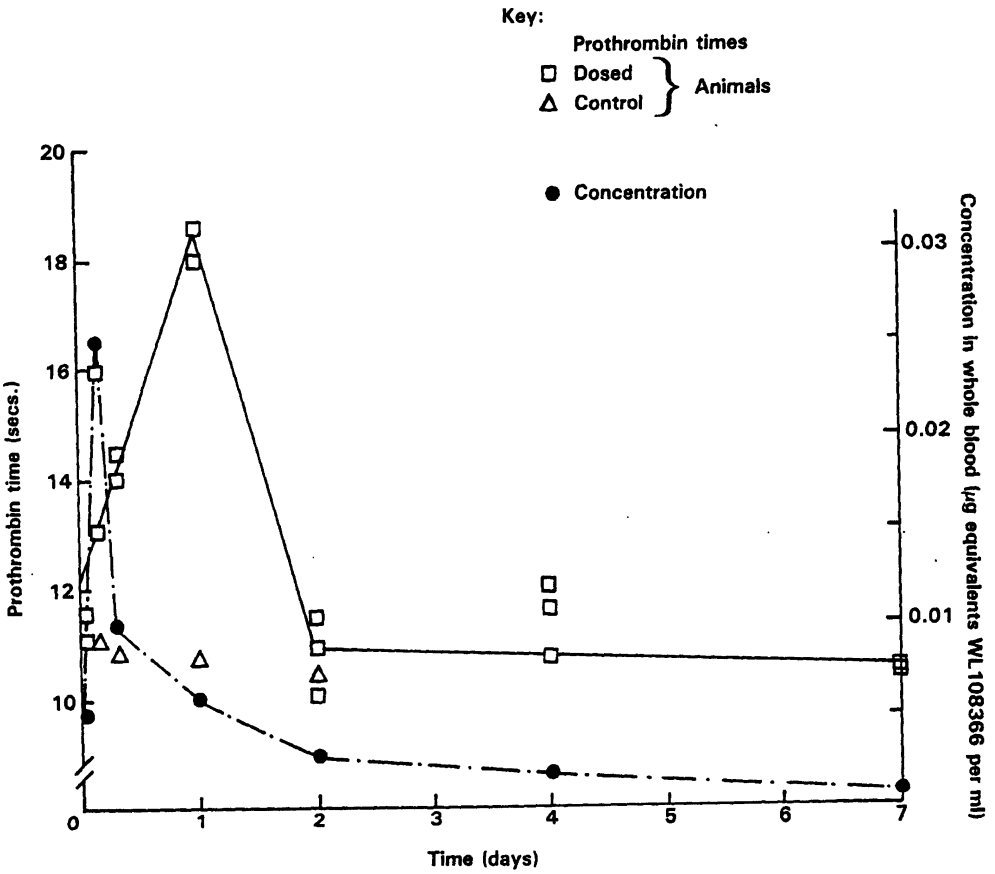


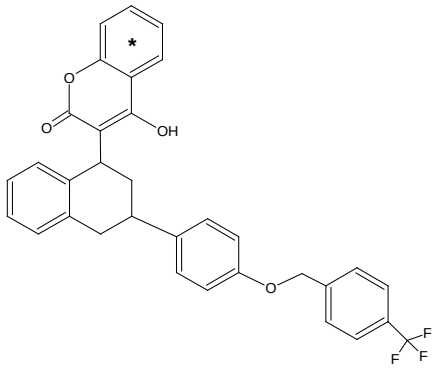
Figure A6.2- 2: Concentration of Flocoumafen and equivalents in whole blood and influence of Flocoumafen on prothrombin time.

Section A6.2 Depletion of ^{14}C-Flocoumafen from selected tissues after oral administration of a single low dose (in vivo test)

Annex Point IIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.2/04:** Wxxxx Pxxxx, Hxxxx Dxxxx (1985) WL108366: Fate of a single oral dose of [^{14}C]WL108366 in rats, Part 2: Rate of depletion of radioactivity from selected tissues. Sxxxx Rxxxx Lxxxx., Sxxxx, Uxxxx, Report No. SBGR.85.177, October 14, 1985 (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**	No However, the conduct of the study was consistent to EC method B.36 (88/302/EEC) in all important aspects.	
2.2	**GLP**	No At the time of the study conduct, GLP were not compulsory. However, the study was conducted in accordance with the principles of GLP	X
2.3	**Deviations**	Yes Only one dose level instead of two was used intentionally. However, a full set of ADME studies (including single low and high dose) are submitted with this dossier.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2, radio-labelled, in acetone.	
3.1.1	Lot/Batch number	Not stated.	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	Radiochemical purity: 97.5%	X
3.1.4	Description	Not stated.	
3.1.5	Stability	Not stated.	X

Section A6.2 Depletion of ^{14}C-Flocoumafen from selected tissues after oral administration of a single low dose (in vivo test)
Annex Point IIA6.2

3.1.6	Radiolabelling	coumarin-U-C^{14}-label: 
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age: not stated Body weight: 214–250 g
3.2.6	Number of animals per group	3
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Type	Gavage (single dose)
3.3.2	Concentration of test substance	0.14 mg/kg
3.3.3	Specific activity of test substance	270 µCi/mg
3.3.4	Total volume applied	Not specified
3.3.5	Vehicle	Corn oil
3.3.6	Post-exposure period	Up to 373 days
3.3.7	Samples (sampling time)	Blood, liver, kidneys, intestines (including stomach and all contents) and also sub-samples of muscle and abdominal fat (groups of three animals sacrificed on days 2, 4, 7, 14, 28, 56, 112, 201, 285 and 373). The animals referred to as being sacrificed on day 7 were three of those from the absorption/elimination study reported in SBGR.85.053 (A6.2/3).
3.3.8	Examinations	Determination of total radioactive residues (TRR) by LSC or by combustion followed by LSC.

Section A6.2 **Annex Point IIA6.2**	**Depletion of ^{14}C-Flocoumafen from selected tissues after oral administration of a single low dose (in vivo test)**	
	4 RESULTS	
4.1 **Toxic effects, clinical signs**	No treatment-related clinical signs were reported. One animal which remained until day 373 showed severe emaciation, weighing 40% less than the mean weight of the other two animals, and was omitted from the mean and S.D. calculations. This animal showed a broken lower front tooth and the corresponding upper tooth was elongated and curving over the lower jaw.	
4.2 **Half-life periods**	Radioactivity was found to be retained for a long period and the depletion of residues from the selected tissues was very slow. Radioactive residues determined in liver samples remained at a plateau value of 1.2 µg/g during the 7 days after administration and depleted slowly thereafter with a calculated half-life of about 222 days. Depletion of radioactivity from kidney, fat and muscle occurred in a bi-phasic manner. Initially, elimination from these tissues was rapid (half-life periods of 4.5 to 9.8 days, α-phase). After 28 days, the depletion slowed considerably resulting in half life periods of 187 to 261 days (β-phase). Very low concentrations were found in blood, which decreased similarly bi-phasic. Radioactive residues were also persistent in intestines with depletion at a similar rate ($t_{½}$ = 222 days) to that for liver and the *β*-phases in the other tissues. The results are summarised in Table A6.2- 8 and Table A6.2- 9.	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 **Materials and methods**	The rate of depletion of ^{14}C-Flocoumafen from selected tissues after a single oral dose of 0.14 mg/kg was tested in Fischer 344 rats. Although not a guideline study, the conduct of the study was consistent in all important aspects to method B.36 (88/302/EEC).	
5.2 **Results and discussion**	Radioactivity was found to be retained for a long period and the depletion of residues from the selected tissues was very slow. Radioactive residues determined in liver samples remained at a plateau value of 1.2 µg/g during the 7 days after administration and depleted slowly thereafter with a calculated half-life of about 222 days. Depletion of radioactivity from kidney, fat and muscle occurred in a bi-phasic manner. Initially, elimination from these tissues was rapid (half-life periods of 4.5 to 9.8 days, *α*-phase). After 28 days, the depletion slowed considerably resulting in half life periods of 187 to 261 days (*β*-phase).	X
5.3 **Conclusion**		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

11 January 2005

Materials and Methods

(2.2) The study was performed under GLP.

(3.1.3) *cis/trans* ratio: 51:49.

(3.1.5) stability during dosing period was demonstrated.

Results and discussion

(4.2) The source code of the computer programme for the calculation of half-lives was given in the report. However, no further description of the method of calculation and of hinge point determination was given. The reported results of the calculations appeared to be not in line with the observations: e.g. the reported half-life for elimination from kidney during the initial phase (day 4 to 14) was 4.5 days whereas the concentration over this period (10 days) did only drop with ~35% . Therefore the calculations were repeated by the RMS. Hinge points were determined using Genstat software and 1st order half-lives were calculated using log-linear regression analysis (where applicable before and after the hinge point). The results are given in the Table below:

Table A6.2- 7 RMS: Half-lives of depletion of radioactivity from tissues after a single oral dose of ^{14}C-Flocoumafen to male rats.

Matrix	Hinge point (days)	before hinge point		after hinge point	
		DT_{50} (days)	r^2	DT_{50} (days)	r^2
kidney	between 14-18 days	18.5	0.96	191	0.95
muscle	between 14-18 days	29.5	1.00	265	0.86
fat	between 28-56 days	28.8	0.81	273	0.96
blood	between 7-14 days	3.1	1.00	341	0.75
intestine	no hinge point	205[1]	0.79	na	na
liver	no hinge point	215[2]	0.97	na	na

[1] all data points were used
[2] calculated for the period following the 7 day lag-phase (7-373 days)
na = not applicable

Conclusion

Radioactivity was found to be retained for a long period in tissues and the depletion of residues from the selected tissues was very slow. Radioactive residues determined in liver samples remained at a plateau value of 1.2 µg/g during the 7 days after administration and depleted slowly thereafter with a calculated half-life of about 215 days. Depletion of radioactivity from kidney, fat and muscle occurred in a bi-phasic manner. Initially, elimination from these tissues was rapid (half-life periods of 18.5 to 29.5 days). After 16-30 days, the depletion slowed considerably resulting in half life periods of 191 to 273 days. Depletion from intestines did not show a biphasic behaviour and occurred with a half-life of 205 days. Depletion of radioactivity from blood occurred in a bi-phasic manner. Initially, elimination from blood was rapid (half-life period of 3.1 days). After 7 days, the depletion slowed considerably resulting in a half-life of 341 days.

Reliability

1

Acceptability

Acceptable.

Remarks

The depletion half-lives were recalculated by the RMS.

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

Table A6.2- 8: Summary of radioactive residues in tissues of male Fischer rats following a single oral dose of ^{14}C- Flocoumafen.

Days after dosing	Mean concentrations expressed as Flocoumafen equivalents [µg/g]					
	Liver	**Kidney**	**Intestine**	**Muscle**	**Fat**	**Whole blood [µg/ml]**
2	1.190 (± 0.148)	0.261 (± 0.008)	0.092 (± 0.008)	0.032 (± 0.002)	0.045 (± 0.004)	0.003 (± 0)
4	1.213 (± 0.022)	0.263 (± 0.009)	0.071 (± 0.008)	0.030 (± 0.003)	0.035 (± 0.004)	0.002 (± 0.0006)
7	1.203 (± 0.116)	0.215 (± 0.033)	0.067 (± 0.011)	0.028 (± 0.003)	0.031 (± 0.002)	0.001 (± 0)
14	1.123 (± 0.044)	0.172 (± 0.019)	0.044 (± 0.007)	0.024 (± 0.006)	0.025 (± 0.001)	0.0009 (± 0.0002)
28	1.008 (± 0.073)	0.141 (± 0.006)	0.046 (± 0.013)	0.020 (± 0.003)	0.022 (± 0.003)	0.0008 (± 0.0002)
56	0.932 (± 0.039)	0.131 (± 0.011)	0.068 (± 0.004)	0.019 (± 0.001)	0.018 (± 0.002)	0.0007 (± 0.0001)
112	0.679 (± 0.050)	0.096 (± 0.001)	0.043 (± 0.007)	0.015 (± 0.001)	0.014 (± 0.001)	0.0006 (± 0.0002)
201	0.647 (± 0.074)	0.089 (± 0.019)	0.038 (± 0.009)	0.015 (± 0.004)	0.013 (± 0.004)	0.0006 (± 0.00006)
285	0.451 (± 0.061)	0.047 (± 0.002)	0.019 (± 0)	0.008 (± 0.0006)	0.009 (± 0.001)	< 0.0004
373	0.344 (± 0.006)	0.043 (± 0.0007)	0.022 (± 0.009)	0.009 (± 0)	0.008 (± 0)	< 0.0004

Values are means ± S.D. of 3 animals except for day 373 (2 animals)

Table A6.2- 9: Half-lives of depletion of radioactivity from tissues after a single oral dose of ^{14}C-Flocoumafen to male rats.

	Liver	**Kidney**	**Intestine**	**Muscle**	**Fat**	**Blood**
Half-life [days] (95% CI)	222 (204 – 242)	4.5 (–6.2 – 7.5)	222 (180 – 286)	9.8[b] (4.9 – [–21.6])	4.9 (3.5 – 6.0)	1.5 (–3.8 – 3.1)
		187[a] (168 – 208)		261[a] (224 – 314)	241[a] (211 – 280)	158[a] (107 – 493)

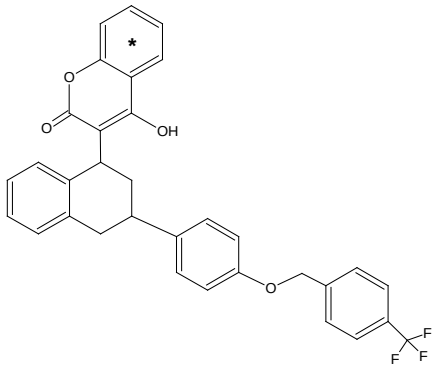
[a]: β-phase; corrected for contribution from the α-phase

[b]: No significant correlation at 95% level (significant at 90%)

Section A6.2 Annex Point IIA6.2 — Meta0.bolism of ^{14}C-Flocoumafen in the rat after single p.o. (including a single high dose) or i.v. application (in vivo test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.2/05:** Wxxxx Pxxxx, Hxxxx Kxxxx (1986) WL108366: Fate of a single oral dose of [^{14}C]-WL108366 in rats, Part III: Metabolism. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx; Unpublished Report No. SBGR.85.294; July 16, 1986. (BASF-Ref.: FL-440-003)	
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 However, the conduct of the study was consistent to EU method B.36 (88/302/EEC) in all important aspects.	
2.2	**GLP**	No At the time of the study conduct, GLP were not compulsory. However, the study was conducted in accordance with the principles of GLP	X
2.3	**Deviations**	Yes Clinical signs of toxicity and relevant clinical features were not reported.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2, radio-labelled, in acetone or acetonitrile.	
3.1.1	Lot/Batch number	Not stated in the report.	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	Radiochemical purity: (i) 97.5% (ii) 98.9% (iii) 95.9% (iv) 95.2, 94.9%	
3.1.4	Description	Not stated in the report.	
3.1.5	Stability	^{14}C-Floocumafen stock solutions were found to deteriorate on storage (-20°C in the dark) with time. Repurification was required where radiochemical purities of < 90% were determined.	

Section A6.2 Annex Point IIA6.2 — Meta0.bolism of ^{14}C-Flocoumafen in the rat after single p.o. (including a single high dose) or i.v. application (in vivo test)

3.1.6	Radiolabelling	coumarin-U-C^{14}-label: 
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344 (oral or intravenous dosing) Wistar (intraperitoneal dosing)
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK
3.2.4	Sex	Male or male and female
3.2.5	Age/weight at study initiation	Age: not stated Body weight: (i) 204–242 g (males); 130–148 g (females) (ii) 181–220 g (males) (iii) 201–214 g (males) (iv) 323–360 g (males)
3.2.6	Number of animals per group	(i) 11 males and 5 females (low oral dose) (ii) 9 males (high oral dose) (iii) 5 males (intravenous dose) (iv) 4 males (intraperitoneal dose, bile duct cannulated)
3.2.7	Control animals	Not stated
3.3	**Administration/ Exposure**	Oral, intravenous or intraperitoneal
3.3.1	Type	(i) gavage (single dose) (ii) gavage (single dose) (iii) caudal tail vein injection (single dose) (iv) intraperitoneal (single dose)
3.3.2	Concentration of test substance	(i) 0.14 mg/kg (low dose, oral) (ii) 14 mg/kg (high dose, oral) (iii) 0.13 mg/kg (intravenous dose) (iv) 0.07 – 0.11 mg/kg (intraperitoneal dose)
3.3.3	Specific activity of test substance	(i) 270 µCi/mg (ii) 15 µCi/mg (iii) 266 µCi/mg (iv) 266 µCi/mg
3.3.4	Total volume applied	1.0 ml/kg (low and high oral dose) 0.5 ml/kg (i.v. and i.p. dose)

Section A6.2 **Annex Point IIA6.2**		**Meta0.bolism of ^{14}C-Flocoumafen in the rat after single p.o. (including a single high dose) or i.v. application (in vivo test)**	
3.3.5	Vehicle	Corn oil for dosing via the oral route polyethylene glycol 200 (PEG 200, BDH) for dosing via i.v. or i.p.	
3.3.6	Postexposure period	(i) up to 285 days (ii) 2 days (iii) 7 days (iv) 8 hours	
3.3.7	Samples (sampling time)	(i) determination of total radioactivity and metabolites in faeces (combined 1st day samples), subcellular localisation of radioactive hepatic residue (sacrifice 2 days or 285 days after dosing) (ii) determination of total radioactivity and metabolites in faeces (combined 1st and 2nd day samples), enzymic deconjugation tests in faecal extract (day 2), livers (upon necropsy) (iii) urine and faeces (daily), metabolites in faeces (day 1 and day 5), liver and various tissues (upon necropsy) (iv) bile (before dosing and continuously afterwards), liver (upon necropsy)	X
3.3.8	Examinations	Determination of total radioactive residues (TRR) by LSC or by combustion followed by LSC. Quantification and identification of metabolites from urine, faeces and liver was performed by HPLC/UV and TLC. Further identification of significant metabolites was conducted by mass spectroscopy.	
3.3.9	Further remarks	In addition, *in vitro* metabolism studies were performed using subcellular fractionated livers from male Fischer 344 rats and male Syrian hamsters. Test microsomes (viable), boiled (non-viable) mircosomes or controls where microsomal protein was omitted were incubated with the test substance for 15 min and the obtained extracts were subjected to radioanalysis, HPLC analysis and TLC analysis.	
		4 RESULTS	
4.1	**Toxic effects, clinical signs**	Monitoring of clinical signs was not reported.	X
4.2	**Elimination and analysis of excreta**	In high oral dose rats, 75% of the administered radioactivity was recovered from faeces 48 hours after dosing, while only 1% was excreted via urine. The major product in faeces was the unchanged parent compound (53 %). In addition, unidentified degradation products were observed. In intravenously dosed rats, 6.29% of the administered dose was recovered in faeces within 7 days, whereas only 0.8% was detected in urine. The results of intravenously dosed rats are presented in Table A6.2- 11. In rats fitted with biliary fistulae for 8 hours, 1.37% of the administered dose was eliminated via the bile while 64.2% and 0.51% of the dose were disposed in liver and blood, respectively.	X

Section A6.2 Annex Point IIA6.2 — Meta0.bolism of ^{14}C-Flocoumafen in the rat after single p.o. (including a single high dose) or i.v. application (in vivo test)

4.3	**Retention and metabolism**	64.2% of the administered dose was present in the livers of bile duct cannulated rats after 8 hours. In rats administered a single oral, low dose, 43.7% and 16.4% of the administered dose were detected in livers on day 2 and day 285, respectively. Livers of rats of the high dose group contained 11.3% of the administered dose on day 2 of the i.v. dosed group 37.5% of the administered dose on day 7. The major compound found in liver extracts was unchanged Flocoumafen. Liver extracts of low dose rats sacrificed on day 2 showed a different *cis:trans* ratio (61:39) than the dose solution (51:49). The results of the analysis of radioactivity extracted from rat liver observed in all tests are presented in Table A6.2- 10. 49.2% of liver associated radioactivity was extracted in washed microsomes of low dose animals sacrificed 2 days after dosing. The radioactivity was equally distributed between smooth (26.51%) and rough (22.23%) membranes. In animals sacrificed on day 285, a small change in sub-microsomal localisation of radioactivity was observed (smooth : 4.22%, rough: 9.45%). Analysis of radioactivity in bile extracts provided evidence for the presence of largely polar radioactive products. No unchanged Flocoumafen was identified in the bile. Radioactive residues were widely distributed within the sampled tissues and organs of intravenous dosed rats, with the major part of radioactivity (i.e. 37.5 % of dose) located in liver samples. The results are presented in Table A6.2- 12.	X
4.4	**Recovery of labelled compound**	52.88 to 95.75% (intravenous administration). Low recoveries obtained for two animals may be explained by poor injection technique, necessitating re-injection at another site in the tail.	
4.5	***In vitro* metabolism**	Hamster as well as rat microsomes catalysed the conversion of ^{14}C-Flocoumafen to at least three minor components. Boiled microsomes of both species failed to convert Flocoumafen.	X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The metabolic fate of ^{14}C-Flocoumafen after a single low (0.14 mg/kg) or a single high oral dose (14 mg/kg) in corn oil was studied in Fischer 344 rats. Further tests were performed with male rats dosed intravenously and bile duct cannulated rats dosed intra-peritoneally. In addition, *in vitro* metabolism studies were carried out using sub-cellular fractionated livers from male Fischer 344 rats and male Syrian hamsters. Although not a guideline study, the conduct of the study was consistent in all important aspects to method B.36 (88/302/EEC), except that clinical signs of toxicity and relevant clinical features were not reported.	

Section A6.2 Meta0.bolism of ^{14}C-Flocoumafen in the rat after single p.o. (including a single high dose) or i.v. application (in vivo test)

Annex Point IIA6.2

5.2	**Results and discussion**	Elimination of radioactive Flocoumafen via faeces was markedly less in rats dosed intravenously (6.3% of the administered dose within 7 days) than in rats dosed orally (26.1% of the administered dose within 7 days; reference A6.2/3), denoting incomplete absorption when administered via the oral route (up to 30% unabsorbed). Extraction of faeces collected from high dose animals indicated that the major product was the unchanged parent compound. Results from bile duct cannulated rats showed that only minor amounts of the test substance are eliminated via the bile (1.37% of the i.p. administered dose after 8 hours). In all animals, appreciable cellular accumulation occurred in the liver, predominantly in bile duct cannulated rats (64.2% of the administered dose, after 8 hours), low oral dosed rats (43.7% of the dose, on day 2) and in intravenously dosed rats (37.5% of the dose, on day 7). The major compound found in liver extracts was unchanged Flocoumafen, which was associated sub-cellularly with the microsomal membranes. The authors considered that the findings support the existence of a high affinity, low capacity binding site in rat liver which prevents, or severely reduces, biotransformation and efficient removal of the compound. Supplementary *in vitro* studies showed no apparent inability of rat microsomes to metabolise Flocoumafen as compared with those from a more tolerant rodent species (hamster).	
5.3	**Conclusion**		X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	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	12 January 2005
Materials and Methods	(2.2) The study was performed under GLP. (3.3.7) (i) determination of total radioactivity and metabolites in faeces (combined 1st day samples) *and liver (day 2)*, subcellular localisation of radioactive hepatic residue (sacrifice 2 days or 285 days after dosing).
Results and discussion	(4.1) The high dose (14 mg/kg bw) was reported to be lethal. It was stated that samples were taken before toxic effects were noted. (4.2) This section was rewritten by the RMS to include more detail: In high oral dose rats, 75% of the administered radioactivity was recovered from faeces 48 hours after dosing, while only 1% was excreted via urine. Approximately 90% of faeces associated radioactivity was extractable and contained (TLC) 41% parent Flocoumafen, 42% unidentified polar components and 17% unidentified non-polar components. In low dose rats, the amount of radioactivity recovered from faeces and urine was not reported. Approximately 90% of faeces associated radioactivity was extractable and contained (HPLC) 53% parent Flocoumafen, unidentified polar and non-polar components (not quantified). TLC analysis indicated only 10% parent Flocoumafen. In intravenously dosed rats, 6.29% of the administered dose was recovered in faeces within 7 days, whereas only 0.8% was detected in urine. Approximately 76-81% of faeces associated radioactivity was extractable and contained (TLC, day 2) 37% parent Flocoumafen, unidentified polar components (29-39%) and unidentified non-polar components (rest). The results of intravenously dosed rats are presented in Table A6.2- 11. In rats fitted with biliary fistulae for 8 hours, 1.37% of the administered dose was eliminated via the bile. (4.3) Additions by RMS are inserted in italics. …..*The liver* of the i.v. dosed group *contained* 37.5% of the administered dose on day 7. The major compound found in liver extracts was unchanged Flocoumafen *(81-97% of the liver extract and 81-96% of the liver associated radioactivity was extractable).* Liver …… 49.2% of liver associated radioactivity was *associated with* washed microsomes …. (4.5) The total extent of conversion of the parent was 13% (hamster) and 8.1% (rat).
Conclusion	The metabolic fate of ^{14}C-Flocoumafen after a single low (0.14 mg/kg) or a single high oral dose (14 mg/kg) in corn oil was studied in Fischer 344 rats. Further tests were performed with male rats dosed intravenously and bile duct cannulated rats dosed intra-peritoneally. Flocoumafen was a major component of faeces (37-53%) and liver (81-97%) associated radioactivity. Other components were characterised as polar and non-polar components, but not identified. Bile did not contain Flocoumafen.
Reliability	3 (the metabolism pathway of Flocoumafen in rat cannot be determined from this study)
Acceptability	Not acceptable for the establishment of metabolism pathway of Flocoumafen in rat. Absorption, excretion and distribution results confirm the results of other

	studies.
Remarks	The reports lacks detail on important aspects of the study: no TLC chromatograms are presented, only one HLPC chromatogram is given, no integration results are given, several measurements were not reported (e.g. combustion results after extraction). Due to lack of identification of degradation products, the metabolic pathway of Flocoumafen in rat cannot be determined from this study (which was the primary purpose of this study). [The metabolism pathway was adequately determined in study A6.2/01].
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.2- 10: Extraction of radioactivity from rat liver tissues and analysis.

Treatment	**% dose present in liver (± SD)**	**% recovery of radioactivity**	**Extract**	
			% unchanged Flocoumafen [a]	**Isomer ratio *cis*:*trans***
Low dose, oral, day 2	43.7 ± 4.4	81.2	82.7 (29.3)	61:39
Low dose, oral, day 285	16.4 ± 1.5	–	–	–
High dose, oral, day 2	11.3	–	–	–
Intravenous dose, day 7	37.5 ± 8.0	95.6	80.7 (28.9)	55:45
Bile duct cannulated rats, 8 hours	64.2 ± 7.4	91.5	96.9 (56.9)	–

a) Figures in parentheses show % of dose.

Table A6.2- 11: Daily elimination of radioactivity in urine and faeces following intravenous administration of ^{14}C-Flocoumafen (0.13 mg/kg) to male Fischer 344 rats.

	Radioactivity in % of administered dose							
	0–24 h	**24–48 h**	**48–72 h**	**72–96 h**	**96–120 h**	**120–144 h**	**114–168 h ***	**Total**
Urine	0.41 (± 0.11)	0.10 (± 0.06)	0.08 (± 0.04)	0.07 (± 0.04)	0.05 (± 0.02)	0.04 (± 0.03)	0.05 (± 0.03)	0.8 (± 0.33)
Faeces	0.97 (± 0.66)	1.84 (± 0.88)	0.94 (± 0.35)	1.03 (± 0.69)	0.58 (± 0.19)	0.56 (± 0.17)	0.37 (± 0.14)	6.29 (± 2.29)

* including collection vessel washings

Table A6.2- 12: Total recovery of radioactivity from male Fischer rats following intravenous administration of ^{14}C-Flocoumafen (0.13 mg/kg)

Animal no.	Radioactivity as % of administered dose									
	Excreta		Liver	Skin	Tail	Intestine	Kidney	Lungs	Remaining carcass	Total
	Urine	Faeces								
1	0.50	3.70	29.0	ND	3.27	ND	ND	ND	16.40*	52.88
2	0.71	6.51	44.2	6.50	1.99	3.58	1.13	0.47	16.74	81.83
3	0.70	6.71	38.8	7.89	2.55	3.12	1.23	0.49	16.57	78.04
4	0.87	4.81	29.5	2.55	9.55	2.50	0.67	0.34	8.30	59.08
5	1.23	9.73	45.8	ND	8.17	ND	ND	ND	30.83*	95.75
Mean	0.80	6.29	37.5	5.65	5.11	3.07	1.01	0.43		
SD	0.33	2.29	7.9	2.8	3.5	0.54	0.30	0.08		

* data obtained by solubilisation of carcass after removal of tail and liver only

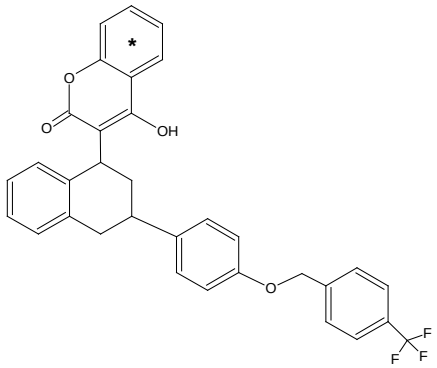
ND = not determined

For animals no. 2, 3 and 4, the residue in all the other tissues examined (i.e. heart, brain and testes) totalled about 0.6% of dose)

Section A6.2 Annex Point IIA6.2 — Metabolism of ^{14}C-Flocoumafen in the rat after repeated oral administration at a low and a high dose (in vivo test)

			Official use only
		1 REFERENCE	
1.1	**References**	**A6.2/06:** Hxxxx Kxxxx, Wxxxx Pxxxx (1986) Elimination, metabolism and disposition of ^{14}C-WL108366 in the Fischer 344 rat following repeated oral administration. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No.: SBGR.86.084; September 24, 1986 (unpublished). **A6.2/07:** Huckle KR, Hutson DH, Warburton PA (1988) Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. Xenobiotica 18 (12): 1465-1479. **Remark:** References A6.2/06 and /07 are based on the same data and report essentially identical results. Thus, they are jointly reviewed in the current 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**	No However, the conduct of the study was consistent in all important aspects to method B.36 (88/302/EEC).	
2.2	**GLP**	No At the time of the study conduct, GLP were not compulsory. However, the study was conducted in accordance with the principles of GLP	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2, radio-labelled, in acetone or acetonitrile.	
3.1.1	Lot/Batch number	S0765	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	Radiochemical purity: 97.8%	
3.1.4	Description	Not stated in the report.	
3.1.5	Stability	^{14}C-Flocoumafen is radiochemically unstable on storage and was therefore repurified regularly.	

Section A6.2 Annex Point IIA6.2 — Metabolism of ^{14}C-Flocoumafen in the rat after repeated oral administration at a low and a high dose (in vivo test)

3.1.6	Radiolabelling	coumarin-U-C^{14}-label: 	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River UK Ltd., Manston, Kent, UK	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Age: 6 to 8 weeks Body weight: 214 – 268 g	
3.2.6	Number of animals per group	36 (low dose) divided into subgroups of 3 animals 27 (high dose) divided into subgroups of 3 animals	
3.2.7	Control animals	12 (vehicle only at 7 day intervals) divided into subgroups of 3 animals	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Type	Gavage (repeated dose)	
3.3.2	Concentration of test substance	0.1 mg/kg b.w. (high dose) 0.02 mg/kg b.w. (low dose)	
3.3.3	Specific activity of test substance	266 µCi/mg	
3.3.4	Total volume applied	1 ml/kg	
3.3.5	Vehicle	Corn oil	
3.3.6	Exposure period	Up to 14 weeks (control and low dose group) Up to 10 weeks (high dose group)	
3.3.7	Frequency of administration	Weekly	
3.3.8	Sacrifice	(i) Low dose: groups of 3 animals at 1, 2, 4, 6, 8, 10, and 12 weeks and the remaining 15 animals at 14 weeks. (ii) High dose: groups of 3 animals at 1, 2, and 4 weeks, two animals surviving to term at 10 weeks were maintained for further 222 days (recovery group). (iii) Control: groups of 3 animals at 1, 6 and six animals at 14 weeks.	X

Section A6.2 **Annex Point IIA6.2**		**Metabolism of ^{14}C-Flocoumafen in the rat after repeated oral administration at a low and a high dose (in vivo test)**	
3.3.9	Samples (sampling time)	(i) Clinical signs (daily), body weight (weekly), urine and faeces from one animal of each subgroup (daily for 3 days post dosing), tissue analysis in 3 animals (upon study termination), prothrombin extension times, haematology and clinical chemistry, ^{14}C-Flocoumafen residues in blood, liver, kidneys, muscle and fat (upon sacrifice), analysis of samples of liver and faeces at 8 and 14 weeks for metabolite profiles. (ii) Clinical signs (daily), body weight (weekly), urine and faeces from one animal of each subgroup (daily for 3 days post dosing), prothrombin extension times, haematology and clinical chemistry, ^{14}C-Flocoumafen residues in blood, liver, kidneys, muscle and fat (upon sacrifice), analysis of samples of liver and faeces at 6 and 10 weeks for metabolite profiles. (iii) Clinical signs (daily), body weight (weekly), prothrombin extension times, haematology and clinical chemistry, ^{14}C-Flocoumafen residues in blood, liver, kidneys, muscle and fat (upon sacrifice).	X
3.3.10	Examinations	Determination of total radioactive residues (TRR) by LSC or by combustion followed by LSC. Further Quantification and identification of metabolites was performed by HPLC/UV .	X
		4 RESULTS	
4.1	**Toxic effects, clinical signs**	No clinical signs and no mortalities were reported for control and low dose group animals. Body weights recorded in the low dose group were similar to the control group. In the high dose group, two animals died during the study and eleven animals were sacrificed non-scheduled. Clinical signs, including perinasal and perorbital (chromodacryorrhea) dark staining, limb paralysis, piloerection, pale skin and eyes, hunched back and lassitude usually appeared 1 to 3 days prior to death or humane sacrifice. No statistically significant differences were detected between the high dose animals and control rats when growth curves were compared. The onset of clinical signs and resultant death of animals in the high dose group was, however, associated with an approximate 10% reduction in body weight. A net body weight increase was seen throughout the recovery period of the two high dose animals.	
4.2	**Elimination**	Urine: 0.8% and 0.7% of the administered dose over 3 days post dosing for the low dose and high dose group, respectively; 80 and 65% of total radioactivity eliminated was recorded within 24 hours after dosing. Faeces: 28% and 42% of the administered dose over 3 days post dosing for the low dose and high dose group, respectively; 45 and 40% of total radioactivity eliminated was recorded within 24 hours after dosing.	X

Section A6.2 Annex Point IIA6.2 — Metabolism of ^{14}C-Flocoumafen in the rat after repeated oral administration at a low and a high dose (in vivo test)

4.3	**Retention**	For both treatments, the highest tissue concentration of radioactivity was found in liver, followed by kidney > skin > muscle > fat > blood. Progressive increases in tissue radioactivity were seen for all tissues throughout the treatment period. Residues detected in high dose animals which died or were sacrificed non-scheduled were approx. 1- to 7-fold higher than in animals sacrificed scheduled after the 6th dose, with exception of residues in skin which were approx. 2-fold lower. Radioactive residue concentrations in liver, kidney, skin and muscle of the recovery animals were 2- to 2.5-fold lower than those seen in animals exhibiting clinical signs. The results are presented in Table A6.2- 13 and Table A6.2- 14.	X
4.4	**Metabolism**	Mainly unchanged Flocoumafen was detected in faecal samples of both dosage groups. However, the proportion present in extracts decreased with the cumulative dose received. There was also an apparent change in the isomer ratio of *cis* and *trans* products, showing a decrease in the amount of *trans* products. A concurrent increase in the percentage of polar components was observed. The results are presented in Table A6.2- 15. The major component detected in liver extracts of both dose groups was unchanged Flocoumafen. In addition, an increase in the percentage of a minor more polar product was noted with the cumulative dose received (metabolite B). Although not established as the same product(s) as those seen in the faecal extracts, it showed very similar retention characteristics. In contrast to metabolite profiles seen in rat faecal extracts, the isomer ratio of hepatic Flocoumafen remained similar throughout the course of the study. The results are presented in Table A6.2- 16.	X
4.5	**Haematology and clinical biochemistry**	Haematology and clinical biochemical measurements were essentially normal for animals in the low dose treatment group. Some minor changes were seen in the high dose animals 6 weeks after commencement of the study. These included a decrease in the mean platelet volume (also seen in low dose animals), a depressed plasma Ca^{2+} ion concentration and increased aminotransferase (AST, ALT) levels. In addition, total plasma protein was increased at week 1 for both treatment groups.	

Section A6.2
Annex Point IIA6.2

Metabolism of ^{14}C-Flocoumafen in the rat after repeated oral administration at a low and a high dose (in vivo test)

4.6	**Gross necropsy**	Weekly doses of 0.02 mg Flocoumafen/kg did not induce any haemorrhagic lesions, hepatic or renal histological changes. The absence of treatment-related effects was also noted in rats treated with 0.1 mg Flocoumafen/kg for up to 42 days. Animals of the high dose group which died during the study or were sacrificed non-scheduled exhibited treatment-related multifocal haemorrhagic lesions. These haemorrhages were primarily localised in tissues which were sites of vigorous muscular activity during life. The major organs of most of these animals demonstrated diffuse pallor. In addition, treatment-related histological changes in livers of these animals were observed. The early histological change in liver was a marked reduction in cytoplasmic vacuolation of glycogenic type. The cumulative effects of Flocoumafen in liver produced severe parenchymal atrophy and in some animals progressed to diffuse centrilobular coagulative necrosis. However, no consistent degree of severity of hepatotoxic changes was found for all high dose animals. No treatment-related histological changes were identified in the kidney, femoral muscle and perirenal fat of any treated animal.
4.7	**Recovery of labelled compound**	Not specified.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Male Fischer 344 rats were treated with multiple oral doses of ^{14}C-Flocoumafen at a rate of 0.1 mg/kg (high dose) or 0.02 mg/kg (low dose) at weekly intervals. The elimination, metabolism and disposition of radioactive Flocoumafen was determined in pre-selected animals and the pathological, haematological and clinical biochemical condition of animals was determined at strategic times throughout the study. Although not a guideline study, the conduct of the study was consistent in all important aspects to EC method B.36 (88/302/EEC).

Section A6.2 Metabolism of ^{14}C-Flocoumafen in the rat after repeated oral administration at a low and a high dose (in vivo test)
Annex Point IIA6.2

5.2	**Results and discussion**	No mortality and no signs of toxicity were observed in the control group and the low dose group, while in the high dose group two animals died during the study and eleven animals were sacrificed non-scheduled. Gross necropsy of these animals revealed extensive haemorrhagic lesions and treatment-related histological changes in livers. In all animals, appreciable cellular accumulation of radioactivity occurred in the liver. The major component detected in liver extracts of both dose groups was unchanged Flocoumafen. At the low dose level, hepatic residues increased proportionately with the dose received, whereas at the upper dose level, hepatic residues reached a plateau 4 weeks after commencement of the study. At 6 weeks after commencement of the study, a decrease in the mean platelet volume was observed in high and low dose animals and high dose animals showed a depressed plasma Ca^{2+} ion concentration and increased aminotransferase (AST, ALT) levels. The major route of elimination of radioactivity was via faeces, principally as the unchanged parent compound. Two other more polar metabolites of unknown identity were determined in rat faecal extracts, at both of the dose levels investigated. The quantitative significance of these metabolites appeared to increase with cumulative dose. There was an apparent change in isomer ratio of *cis* and *trans* Flocoumafen present in rat faecal extracts, showing a decrease in the amount of *trans* Flocoumafen.	X
5.3	**Conclusion**		X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	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	18 January 2005
Materials and Methods	(2.2) The study was performed under GLP. (3.1.2) cis: trans isomer ratio = 54:46. (3.3.8) (ii) High dose: groups of three animals at 1,2, 4 *and 6* weeks, …. (3.3.9) Additions by RMS are inserted in italics. (i) …… and clinical chemistry, *total ^{14}C* residues in blood, liver, kidneys, muscle and fat (upon sacrifice), analysis of samples of liver and faeces at *1, 2,* 8 and 14 weeks for metabolite profiles. (ii) …. and clinical chemistry, *total ^{14}C* residues in blood, liver, kidneys, muscle and fat (upon sacrifice), analysis of samples of liver and faeces at *1, 2,* 6 and 10 weeks for metabolite profiles. (iii) Results for ^{14}C analysis in control animals is not reported. It was not clear to the RMS whether this was performed (as stated by the notifier). (3.3.10) Analysis was performed by HPLC-UV and HPLC-RAD.
Results and discussion	(4.2) Urine: 0.8% and 0.7% *per week [mean of the weekly administered dose]* over 3 days post ….. Faeces: 28% and 42% *per week [mean of the administered dose]* over 3 days post ….. (4.3) Additions by RMS are inserted in italics. For both treatments, the highest tissue concentration of radioactivity was found in liver *(up to 34-51% of the cumulative dose)*, followed by kidney > skin > muscle > fat > blood. (4.4) Additions by RMS are inserted in italics. Mainly *(44-74% of faeces associated radioactivity)* unchanged Flocoumafen was detected in faecal samples of both dosage groups. However …… ….. .A concurrent increase in the percentage of polar components *(at least three components)* was observed *(maximum up to 28-44% of faeces associated radioactivity). One less polar metabolite was observed (maximum 2.7-4.2% of faeces associated radioactivity).* The major component detected in liver extracts of both dose groups was unchanged Flocoumafen *(76-88% of liver associated radioactivity).* In addition, an increase in the percentage of a minor more polar product was noted with the cumulative dose received (metabolite B, *maximum 10-11% of of liver associated radioactivity).* Although not established as the same product(s) as those seen in the faecal extracts, it showed very similar retention characteristics. *Metabolite B consisted of two peaks. One other polar metabolite (maximum 2-3%) and one less polar metabolite (maximum 1%) were observed*. In …… (5.2) Additions by RMS are inserted in italics. In all animals, appreciable cellular accumulation of radioactivity occurred in the liver *(up to 34-51% of the cumulative dose).* The major …. ……. .Two other more polar metabolites *and one les polar metabolite* of unknown identity were determined in rat faecal extracts, at both of the dose levels investigated.
Conclusion	Male Fischer 344 rats were treated with multiple oral doses of ^{14}C-Flocoumafen at a rate of 0.1 mg/kg (high dose) or 0.02 mg/kg (low dose) at weekly intervals for

	10 and 14 weeks, respectively. No mortality and no signs of toxicity were observed in the control group and the low dose group, while in the high dose group two animals died during the study and eleven animals were sacrificed non-scheduled. The major route of elimination of radioactivity was via faeces (28-42% of the weekly dose per week), principally as the unchanged parent compound (44-74% of faeces associated radioactivity). Two other more polar metabolite fractions and one less polar metabolite of unknown identity were determined. Elimination via the urine was low (0.7-0.8%/week). In all animals, appreciable cellular accumulation of radioactivity occurred in the liver (up to 34-51% of the cumulative dose). The major component detected in liver extracts of both dose groups was unchanged Flocoumafen (76-88% of liver associated radioactivity). At the low dose level, hepatic residues increased proportionately with the dose received, whereas at the upper dose level, hepatic residues reached a plateau 4 weeks after commencement of the study. The order of tissue concentrations was liver > kidney > skin > muscle > fat > blood. Progressive increases in tissue radioactivity were seen for all tissues throughout the treatment period. Radioactive residue concentrations in liver, kidney, skin and muscle of the recovery animals decreased (222 days after the last dose) 2- to 2.5-fold.
Reliability	1
Acceptability	Acceptable.
Remarks	Due to lack of identification of degradation products, the metabolic pathway of Flocoumafen in rat cannot be determined from this study. [The metabolism pathway was adequately determined in study A6.2/01].

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

Table A6.2- 13: Tissue concentrations of radioactivity (low dose).

Dose number	Flocoumafen equivalents [ng / g tissue] (means of 3 animals)					
	Liver	**Kidney**	**Skin**	**Muscle**	**Fat**	**Blood**
1	109	8.5	1.0	0.8	0.9	<0.2
2	444	40	5.1	4.0	3.2	0.2
4	1251	138	28	16	12	1.2
6	1435	161	38	18	14	1.3
8	1621	209	55	24	19	1.3
10	1754	259	69	30	24	2.0
12	2015	264	76	33	27	2.1
14	2110	308	103	43	29	3.2

Table A6.2- 14: Tissue concentrations of radioactivity (high dose).

Dose number	Flocoumafen equivalents [ng / g tissue]					
	Liver	**Kidney**	**Skin**	**Muscle**	**Fat**	**Blood**
Scheduled necropsy (means of 3 animals):						
1	1023	91	14	9.1	9.0	0.7
2	2137	278	67	34	29	2.5
4	2683	422	189	76	44	7.4
6	2687	469	246	92	48	11.7
Non-scheduled necropsy (means of 13 animals):						
	4180	598	155	128	118	79
Recovery animals (means of 2 animals):						
	1742	231	61	57	25	1.3

Table A6.2- 15: Extraction of faeces and metabolite profile.

Treatment and dose number	Extraction efficiency (%)	Components expressed as % of total faecal residue[(a)]				Flocoumafen % isomer composition (*cis*:*trans*)
		Metabolite A	Metabolite B	Flocoumafen	Metabolite C	
Low dose:						
1	93.0	5.3	4.6	73.7	2.9	55:45
2	91.9	9.3	6.1	67.8	4.2	54:46
8	91.5	14.0	13.9	58.5	1.3	61:39
14	90.2	14.8	23.4	47.7	1.3	60:40
High dose:						
1	91.0	11.5	7.1	64.1	2.7	55:45
2	90.0	11.7	13.0	58.3	2.1	47:53
6	90.0	15.8	28.6	43.6	0.3	66:34
10	90.2	15.9	27.3	44.2	0.8	60:40

(a) data are corrected for extraction efficiency

Metabolites A and B: unidentified polar material, Metabolite C: unidentified material

Table A6.2- 16: Extraction of liver and metabolite profile.

Treatment and dose number	Extraction efficiency (%)	Components expressed as % of total liver residue[(a)]				Flocoumafen % isomer composition (*cis*:*trans*)
		Metabolite A	Metabolite B	Flocoumafen	Metabolite C	
Low dose:						
1	95.6	1.7	3.9	88.1	1.0	54:46
2	96.7	1.7	4.6	88.2	1.2	51:49
8	94.2	1.4	8.6	82.6	0.8	59:41
14	95.5	2.5	10.9	80.8	0.6	59:41
High dose:						
1	89.3	1.3	4.6	81.6	1.1	55:45
2	92.5	1.0	8.0	81.9	1.1	56:44
6	88.9	1.3	10.3	76.0	0.8	57:43
10	93.9	1.5	9.1	82.0	0.7	53:47

[(a)] data are corrected for extraction efficiency

Section A6.2 Metabolism studies in mammals

Annex Point IIA6.2 Supportive data

The following references are considered to contain additional information concerning metabolism in rats and are thus presented in tabular format as supportive data: (all studies were non-GLP studies)

Reference	**Title**	**System**	**Results**
A6.2/08: Hxxxx Dxxxx, Bxxxx Rxxxx, Cxxxx Dxxxx, Bxxxx Cxxxx (1991) Hxxxx Rxxxx Cxxxx, Hxxxx, UK, Report no.: HRC/LPA 158/891590, July 26, 1991 (unpublished).	Determination of the residues and the half-life of the rodenticides Brodifacoum, Bromadiolone and Flocoumafen in the livers of rats during 200 days after single oral doses of each at a dose level of 0.2 mg/kg	Male Sprague-Dawley rats	Test compounds (0.2 mg/kg) dissolved in PEG 300 were administered to male rats orally by gavage and residues in liver were determined for up to 200 days after dosing. In contrast to reference A.6.2/04, a biphasic depletion of residues in liver was observed. During the initial 28 days the half-life of elimination was approx. 6 days for Flocoumafen. Estimates of the terminal half-life from the slope of the fitted bi-exponential curve gave a half-life of 159 days.
A6.2/09: Huckle KR, Morrison, BJ, Warburton PA (1989) Xenobiotica 19 (1), 63-74	The percutaneous fate of the rodenticide flocoumafen in the rat: role of non-biliary intestinal excretion	a) Male Fischer 344 rats (dermal or i.v. administration) b) Male Wistar rats (bile duct cannulation, i.p. administration)	The results reported for the dermal exposure are identical to reference A6.2/02. The results reported for the i.v. and i.p. administration were already summarised in reference A6.2/5.
A6.2/10: Sxxxx Rxxxx (1984) Sxxxx Lxxxx, Unnumbered Report, December 10, 1984 (unpublished).	An experimental note on: The release of body residues of WL108366 with phenylbutazone	Four male New Zealand White rabbits	Phenylbutazone was administered to rabbits from previous body residence studies with Flocoumafen, whose prothrombin times had returned to within the normal range, or near to the normal range. Sequential prothrombin time determinations indicated the potential of phenylbutazone to release bound Flocoumafen from the body. The results of the study also suggested that elimination of Flocoumafen had occurred as a result of phenylbutazone administration.
A6.2/11: Mxxxx Bxxxx (1987) Sxxxx Rxxxx Lxxxx, Sxxxx Rxxxx Cxxxx, Uxxxx, Report no. SBRN.87.035, February 1987 (unpublished).	The effect of phenobarbitone and Warfarin administration on hepatic ^{14}C-WL108366 residues in Fischer 344 rats	Male Fischer 344 rats (5 animals per group)	Seven days after receiving a single oral dose of ^{14}C-Flocoumafen (0.10 mg/kg) male Fischer rats received either a single oral dose of Warfarin (0.12 mg/kg) or single daily, oral doses of phenobarbitone (50 mg/kg) for 5 days. Fourteen days after the administration of Flocoumafen no significant differences could be detected in the concentration of radioactivity determined in the livers of either the Warfarin- or phenobarbitone-treated animals when compared to the respective control animals. Both substances caused a 2-fold increase in the amount of urinary elimination of radioactivity, which had a negligible effect on the body burden of Flocoumafen.

Section A6.2 Metabolism studies in mammals

Annex Point IIA6.2 Supportive data

A6.2/12: Huckle KR, Hutson DH, Logan CJ, Morrison BJ, Warburton PA (1989) Pestic. Sci. 25, 297-312 (published)	The fate of the rodenticide Flocoumafen in the rat: retention and elimination of a single oral dose	Fischer 344 rats	This publication is based on the following references: A6.2/03, A6.2/04 and A6.2/11.
A6.2/13: Veenstra GE, Owen DE, Huckle KR (1991) Arch. Toxicol., Suppl. 14, 160-165 (published).	Metabolic and toxicological studies on the anticoagulant rodenticide, Flocoumafen	Male and female Beagle dogs (4 per sex)	Dogs received one to two single doses of 0.5 mg Flocoumafen/kg in corn oil, followed by 7 days of therapy with vitamin K (2 or 5 mg/kg). This therapeutic regime was effective and suitable for dogs intoxicated with Flocoumafen, whereas no difference was observed in antidotal response using either 2 or 5 mg/kg vitamin K per day. Severe intoxication, as observed in gross haemorrhaging was successfully treated by whole blood transfusion. Flocoumafen was retained in the liver at concentrations similar to those in other species.
A6.2/14: Hxxxx Kxxxx, Wxxxx Pxxxx, Lxxxx Cxxxx, Hxxxx Dxxxx (1988) Sxxxx Rxxxx Lxxxx Sxxxx Rxxxx Cxxxx, Sxxxx, Uxxxx, summary of report no. SBB/54/88 (unpublished).	The fate of the anticoagulant rodenticide Flocoumafen in the rat and in quail	Male Fischer 344 rats; male quail	Flocoumafen is extremely toxic to rats, it undergoes very limited biotransformation and is eliminated very slowly. By contrast, it is less toxic to quail, being extensively metabolised and also rapidly eliminated. However, persistent hepatic Flocoumafen residues were seen in both species.

Evaluation by Competent Authorities	
Use separate ”evaluation boxes” to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	27 January 2005
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.3.1 Short-term repeated dose feeding study in rats

Annex Point IIA6.3

Official use only

1 REFERENCE

1.1	**Reference**	**A6.3.1/01:** Pxxxx Jxxxx (1984) WL108366: a 28 day feeding study in rats. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report no. SBGR.84.235, September 1984 (unpublished). (BASF-Ref.: FL-420-003)
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 However, the conduct of the study was consistent to EU method B.7 (96/54/EC) in all important aspects.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes No motor activity assessment and no functional observations were performed. No determination of glucose levels in plasma or serum was included. Adrenals, epididymides and thymus were not weighed upon necropsy.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	ST84/071	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	> 99%	
3.1.4	Description	White crystalline powder.	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River U.K. Ltd.	

Section A6.3.1 Short-term repeated dose feeding study in rats

Annex Point IIA6.3

3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 6 to 8 weeks Body weight: not stated
3.2.6	Number of animals per group	8 males and 8 females
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	Daily
3.3.3	Post-exposure period	None
3.3.4	Type	In food
3.3.5	Concentration	0, 0.01, 0.05, 0.1 and 0.2 ppm
3.3.6	Vehicle	Acetone
3.3.7	Concentration in vehicle	0.1 to 2.0 mg of test solution diluted to 25 ml with acetone.
3.3.8	Total volume applied	Not stated
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.2	Clinical signs	Yes (once or twice daily)
3.4.3	Mortality	Yes (once or twice daily)
3.4.4	Body weight	Yes (before treatment, weekly throughout the study and upon sacrifice)
3.4.5	Food consumption	Yes (twice weekly)
3.4.6	Water consumption	No
3.4.7	Ophthalmoscopic examination	No
3.4.8	Haematology	Yes Number of animals: all animals Time points: end of study Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, prothrombin time, thromboplastin time, mean corpuscular and mean platelet volumes.

Section A6.3.1 Short-term repeated dose feeding study in rats

Annex Point IIA6.3

3.4.9	Clinical chemistry	Yes Number of animals: all animals Time points: end of study Parameters: sodium, potassium, calcium, chloride, inorganic phosphate, triglyceride, total cholesterol, blood urea nitrogen, total bilirubin, creatinine, total protein, lactate dehydrogenase, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transpeptidase.
3.4.10	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: liver, kidneys, testes, spleen, brain, and heart
3.5.2	Gross and histopathology	Yes Full necropsies were performed on rats of all dose groups and tissues were examined histologically from animals of the high dose group and the control group. Histopathology was performed on following tissues of all control and high dose animals: adrenals, aorta, brain, bone marrow, cervix, epididymes, eyes, head, heart, intestines, kidneys, lachrymal glands, larynx, liver, lungs, lymph nodes, mammary gland, muscle, nerves, ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicles, spinal cord, spleen, stomach, testes, thyroids, thymus, tongue, urinary bladder, uterus and any non-haemorrhagic macroscopic lesion observed upon necropsy.
3.5.3	Other examinations	No other examinations were reported.
3.5.4	Statistics	Two-way analysis of variance, covariance analysis, Dunnett's test, Williams' test,
3.6	**Further remarks**	Vitamin K_3 in form of a menadione derivative was added to the experimental diets at 3 ppm. This was reported to be a standard practice at the laboratory to counteract the loss of vitamin K in the diet during processing. It was considered that the quantity of vitamin K_3 added to the experimental diets would be insufficient to counteract any haemorrhagic effects caused by the test substance.

4 RESULTS

4.1	**Observations**	
4.1.1	Clinical signs	No overt clinical signs were observed.
4.1.2	Mortality	All animals survived to scheduled necropsy.
4.2	**Body weight gain**	No effects on mean or individual bodyweights were observed.
4.3	**Food consumption and compound intake**	Female rats dosed with 0.10 ppm of Flocoumafen showed a slight decrease in food consumption in week 4, resulting in a slight decrease in body weights. Since no effects on food consumption were observed in the high dose group, this decrease was considered to have no biological significance.

Section A6.3.1 Short-term repeated dose feeding study in rats

Annex Point IIA6.3

4.4	**Ophthalmoscopic examination**	No ophthalmoscopic examination was performed.	
4.5	**Blood analysis**		
4.5.1	Haematology	No statistically significant differences between control animals and any dosed males were observed for the haematology parameters determined. Increases in the prothrombin and activated partial thromboplastin times were recorded in females of the high dose group.	X
4.5.2	Clinical chemistry	A statistical significant decrease in total protein and calcium concentration and an increase in chloride ion concentration was observed in high dose males. In female rats significant decreases in total protein were observed at 0.10 and 0.20 ppm. In females of the 0.10 ppm dose group, significant decreases in alkaline phosphatase activity and cholesterol concentration was noted when compared to the controls.	
4.5.3	Urinalysis	Urinalysis was not performed.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No effects on organ weights (adjusted for terminal body weights) were observed.	X
4.6.2	Gross and histopathology	No treatment-related macroscopic changes or haemorrhagic lesions were identified upon necropsy. Treatment-related minor histological changes were observed only in the livers of four male rats fed 0.2 ppm. In periportal parenchymal cells there was reduced cytoplasmic vacuolation of glycogenic type.	
4.7	**Other**	None	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of 8 male and 8 female Fischer 344 rats received diets containing 0, 0.01, 0.05, 0.1 or 0.2 ppm of Flocoumafen for 28 days. Although not a guideline study, the method used was consistent to method B.7 (96/54/EC) in all important aspects. Deviating from the prescribed guideline, no motor activity assessment and no functional observations were performed. Further, no determination of glucose levels in plasma or serum was included. Adrenals, epididymes and thymus were not weighted upon necropsy.

Section A6.3.1 Short-term repeated dose feeding study in rats

Annex Point IIA6.3

5.2	**Results and discussion**	No overt clinical signs of toxicity, no mortalities and no-treatment related effects on body weight or food consumption were observed during the study. A statistically significant reduction of liver weight in males of the 0.2 ppm groups was not considered to be of toxicological relevance in view of the absence of this finding after adjustment to terminal body weight. High dose (0.2 ppm) females showed a slight but significant increase in mean prothrombin and activated partial thromboplastin times (not observe din males). Decreased levels of plasma protein, alkaline phosphatase and cholesterol were determined in females dosed with 0.1 or 0.2 ppm of Flocoumafen. Males did not show any changes in the clotting function or any other haematology or clin.-biochem. parameters, except for reduced plasma protein at 0.2 ppm. No treatment-related macroscopic changes or haemorrhagic lesions were identified upon necropsy. Histopathology revealed reduced cytoplasmic vacuolation of glycogenic type in the periportal parenchymal cells in livers of high dose males.
5.3	**Conclusion**	
5.3.1	LO(A)EL	0.1 ppm (females), 0.2 ppm (males)
5.3.2	NO(A)EL	0.05 ppm (females), 0.1 ppm (males)
5.3.3	Other	It was concluded that feeding rats diets containing up to 0.2 ppm Flocoumafen for 28 days did not give rise to any haematological, biochemical or pathological findings indicative of any toxic effect other than that of an indirect anticoagulant.
5.3.4	Reliability	1
5.3.5	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	27 December 2004
Materials and Methods	3.1.2: specification of test substance: cis:trans isomer ratio = 55:45. The study was performed in accordance with OECD 407 and EC B.7, with exception of the following items: ophthalmoscopy and urinalysis were not performed, no motor activity assessments and no functional observations were performed, no determination of glucose levels in plasma or serum was included, adrenal, epididymes and thymus were not weighed upon necropsy. These deviations to the guideline are not considered to have affected the study outcome, considering the mode of action of flocoumafen. Test substance intake was not calculated in the study report. Therefore, a factor 20 was used for calculation of intakes in mg/kg bw/day, resulting in intakes of 0.0005, 0.0025, 0.005 and 0.01 mg/kg bw/day. 3.6 Vitamin K3 was administered to the diet, to counteract the loss of vitamin K in the diet during processing, according to the authors of the study report. However, it should be noted that flocoumafen, just like other coumarin derivatives, acts as a vitamin K antagonist. Furthermore, patients suffering from flocoumafen poisoning are treated with vitamin K. The applicant submitted additional information to support the statement that „the quantity of vitamin K_3 added to the experimental diets would be insufficient to counteract any haemorrhagic effects caused by the test substance“. In the rat study, vitamine K3 was supplemented at a concentration of 3 ppm in the diet. Considering the apparent loss of vitamin K1 below the specified level and the tenfold lower activity of the used vitamin K3 in relation to K1, it may be safely be assumed that supplementation at this level did in fact not interfere with the effects of the test substance Flocoumafen to any relevant extent. Instead, this merely reflects practical experience in preparing an optimised laboratory diet.
Results and discussion	4.5.1: A slight non-statistically significant increase in activated partial thromboplastin time was noted in females at 0.1 mg/kg food. (111% of controls). 4.6.1: A slight statistically significant decrease in absolute liver weight was noted in males at 0.2 mg/kg food (93% of controls).

Conclusion	28-day exposure of rats to diets containing 0, 0.01, 0.05, 0.1 or 0.2 mg/kg food (equivalent to 0.0005, 0.0025, 0.005 and 0.01 mg/kg bw/day) resulted in increased mean prothrombin and activated partial thromboplastin times in females at 0.2 mg/kg food. At 0.1 mg/kg food, a slight non-statistically significant increase in activated partial thromboplastin time was noted in females. Decreased levels of plasma protein, alkaline phosphatase and cholesterol were noted in females at 0.1 and 0.2 mg/kg food. A statistically significant decrease in calcium and a statistically significant increase in chloride were noted in males at 0.2 mg/kg food. At histopathology, a slight reduction of cytoplasmatic vacuolation of glycogenic type in the periportal parenchymal cells in livers of males at 0.2 mg/kg food was noted. Based on the decreased levels of plasma protein, alkaline phosphatase and cholesterol and increased activated thromboplastin times in females at 0.1 mg/kg food, the NOAEL was established at 0.05 mg/kg food (equivalent to 0.0025 mg/kg bw/day).
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable.
Remarks	None.

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

Table A6.3.1- 1: Results of the 28-day feeding study in rats.

Parameter	**Control**		**0.01 ppm**		**0.05 ppm**		**0.10 ppm**		**0.20 ppm**		**Dose-response +/–**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals examined	8	8	8	8	8	8	8	8	8	8		
Mortality	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	–	–
Clinical signs[a]											–	–
Body weight week 4	250.6g	160.3g	-0.8%	+0.9%	-0.9%	-1.2%	-1.1%	-3.3%	-1.5%	+1.2%	–	–
Food consumption week 4	134.8g	94.0g	-2.3%	-3.7%	-4.2%	-2.2%	-1.5%	-10.1%	-4.6%	+2.0%	–	–
Clinical chemistry												
Protein								↓	↓	↓	–	–
AP								↓			–	–
Cholesterol								↓↓			–	–
Total calcium									↓		–	–
Chloride									↑		–	–
Haematology												
Prothrombin time										↑↑	–	–
Thromboplastin[1]										↑↑	–	–
Terminal body weight [g]											–	–
<u>*Liver*</u>												
Microscopic pathology												
Cytoplasmic vacuolation									↓[a]		–	–

↑, ↓ = significantly increased or decreased compared to controls, p≤0.05, using Williams' test
↑↑, ↓↓ = significantly increased or decreased compared to controls, p≤0.01, using Williams' test

Thromboplastin[1] = activated partial thromboplastin time, AP = alkaline phophatase

[a] Periportal parenchymal cells showed reduced cytoplasmic vacuolation of glycogenic type.

Section A6.3.1 Short-term repeated dose feeding study in rats

Annex Point IIA6.3 **Supportive data**

The following references are considered to contain additional information concerning short-term repeated dose toxicity and are thus presented in tabular format as supportive data: (all studies were non-GLP studies)

Reference	Title	System	Results
A6.3.1/02: Pxxxx Jxxxx (1984) Sxxxx Rxxxx Lxxxx, Sxxxx Rxxxx Cxxxx, Sxxxx, Kxxxx, Uxxxx, Report no: SBGR.84.279, November, 1984 (unpublished).	Toxicology of rodenticides WL108366: a five day range finding feeding study in rats.	Male and female Fischer 344 rats (5 per group and sex)	The study was conducted as a range finding study to reference A6.3.1/1. Groups of five male and five female rats were fed diets containing 0, 0.2, 0.4 or 0.8 ppm Flocoumafen for five days and were maintained for a recovery period of ten days. Four females and four males in the high dose group died during the study. No toxicological significant signs were observed in animals fed diets containing 0.4 ppm or less.
A6.3.1/03: Sxxxx Rxxxx (1983) Sxxxx Lxxxx, Report, October 12, 1983 (unpublished).	The sub-acute oral toxicity of WL108366 in Wistar rats.	Male Wistar rats	Groups of 4 male rats were administered orally by gavage with 0.215, 0.10, 0.0464 and 0.0215 mg Flocoumafen/kg in PEG/TEA daily for five days. Following dosing the animals were maintained for a 21 day observation period. In the two highest dose groups, all animals died during the study. Thus, the sub-acute oral LD_{50} was determined to be: LD_{50} = 0.34 mg/kg b.w.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	27 December 2004
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	None. In study A6.3.1/03, observations for deaths and clinical signs were made. No further observations were included in the study. Therefore, the study is only considered suitable for establishment of a subacute LD_{50}.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.3.2 Short-term repeated dose toxicity (dermal)

Annex Point IIA6.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	The conduct of a percutaneous 28-day toxicity study in the rat is not considered to be required since route-to-route extrapolation is not considered to be restricted in any way.	
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	27 December 2004
Evaluation of applicant's justification	A repeated dose dermal toxicity study is required, where significant dermal exposure to the active substance is to be expected and when route-to-route extrapolation is not possible. Considering the exposure assessments, dermal exposure to flocoumafen is to be expected. However, there are no contra-indications for route-to-route extrapolation. From the results of the acute oral and dermal toxicity studies with flocoumafen, it can be concluded that there are no route-specific effects to be expected after repeated exposure to flocoumafen. Furthermore, there is no evidence of enterohepatic circulation or a first-pass effect. Therefore, it is concluded that for the dermal exposure route, oral toxicity data can be used for risk characterisation of flocoumafen and Storm BB.
Conclusion	Non-submission of data is accepted.
Remarks	None.

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

Section A6.3.3 Short-term repeated dose toxicity (inhalation)

Annex Point IIA6.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	(1) The vapour pressure of Flocoumafen (IIA, 3.2) was determined experimentally under GLP to a value below 1 x 10^{-5} hPA (20°C). Therefore, Flocoumafen is not a volatile substance. (2) In consideration of the intended use as a rodenticide in a ready-to-use wax block bait, inhalation is considered to be a negligible route of exposure. In conclusion, the performance of a 28-day inhalation toxicity study in the rat is consequently not considered to be required predominantly for 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	27 December 2004
Evaluation of applicant's justification	A repeated dose inhalation toxicity study is required for volatile substances (vapour pressure > 1 x 10^{-2} Pa) and in special cases e.g. for aerosols and dusts. Since flocoumafen has a vapour pressure of < 1 × 10^{-3} Pa and the formulation under consideration (Storm BB) is a wax bound block bait, it is concluded that for the inhalation exposure route, oral toxicity data can be used for risk characterisation of flocoumafen and Storm BB.
Conclusion	Non-submission of data is accepted.
Remarks	None.

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

Remarks	

Section A6.4.1 Subchronic oral toxicity test in a non-rodent species

Annex Point IIA6.4

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:

Performance of a sub-chronic toxicity study with Flocoumafen in the second animal species (e. g. the dog) is not considered to be required for lack of secondary exposure and due to the toxicity profile. A detailed justification, with explicit reference to the "refined waiving concept" in the TNsG on data requirements, is given as follows:

Secondary exposure:

In view of the intended uses as outlined in Sections A5 and B5, secondary exposure (i. e., persons not involved in production and application) is considered to be extremely unlikely. Any contact with the active substance is a priori minimal in view of the nature and composition of the product in the form of ready-to-use rodenticide baits with an a.s. content of 0.005%. The deployment of bait in tamper-resistant bait stations ensures protection of children, pets and livestock from contact with bait. Any accidental direct consumption of bait by humans is effectively prevented by the inclusion of a bittering agent (Section B2). Any secondary exposure to Flocoumafen would be acute. In support of this viewpoint, chronic secondary exposure is denoted as "not relevant" in the TNsG on human exposure. This applies in the same way to sub-chronic exposure. Furthermore, indirect exposure via the environment is considered to be of minor importance since the release of rodenticides to the environment is limited. Accordingly, it is justified to generally consider secondary exposure as negligible.

Toxicological profile:

a) The 90-d sub-chronic study on rats (A6.4.1/01) is considered to be performed in agreement with the most recent EU method (B.26, 2001/59/EC) in all important aspects and to be fully appropriate for risk assessment. The NOAEL was established at 0.005 mg/kg bw/d. The expected primary exposure levels are $\leq 3.6 \times 10^{-5}$ mg/kg bw/d (worst-case). Thus, taking into consideration a satisfactory MOE (in this case > 100), substance-related adverse effects for human exposure are not expected.

b) The mechanism of toxicity of Flocoumafen is well known; it is equivalent to the mode of action in target organisms. The sole toxic effect is inhibition of vitamin K recycling, subsequent interruption of the formation of blood clotting factors, and consequently sustained haemorrhages (A5.4/01, 02). At the expected exposure levels including a satisfactory MOE, as specified under (a) above, it is justified that the toxicological effects in the target rodent species are not relevant to humans regarding the expected exposure levels.

c) The acute toxicity of Flocoumafen in the (susceptible) target species rat is either similar or even lower than for other mammalian species, corresponding to its intended use as a rodenticide. This is also commonly the case for other rodenticides (EHC 175, Anticoagulant Rodenticides, WHO; 1995). In view of the particular sensitivity of rats, sub-chronic testing in a second, non-rodent species is not

Section A6.4.1 Subchronic oral toxicity test in a non-rodent species

Annex Point IIA6.4

expected to yield endpoints that reflect any particularly enhanced sensitivity. Acute oral toxicity studies demonstrated that Flocoumafen is similarly highly toxic by the oral route to rats, mice and dogs: The lowest LD_{50} obtained for rats was approximately 0.25 mg/kg b.w. (reference A6.1.1/2). The lowest LD_{50} obtained for mice was 0.79 mg/kg b.w. (males) and 1.47 mg/kg b.w. (females) (reference A6.1.1/5). On the basis of the results of an acute oral toxicity study with Beagle dogs, it was concluded that the maximum non-lethal single dose of Flocoumafen is in a range of 0.075 to 0.25 mg/kg b.w. since dogs dosed with 0.25 mg/kg were killed for humane reasons (reference A6.13/1). Similar clinical signs of toxicity and haematological effects were noted in all three species following acute and short-term exposure (references A6.13/1, A6.13/2, B7.8.7.2/13). A short-term repeated dose feeding study in rats (reference A6.3.1/1) and a sub-chronic oral toxicity test in rats (reference A6.4.1/1) showed that feeding rats a diet containing up to 0.2 ppm for 28 days or 0.10 ppm for 90 days did not give rise to any haematological, biochemical or pathological findings indicative of any toxic effect other than that of an indirect anticoagulant. Similarly, in the palatability study in dogs, comparable toxic endpoints were observed following weekly exposure to Flocoumafen in the diet for up to 6 weeks (reference A6.13/2).

d) The conduct of a sub-chronic toxicity study with Flocoumafen in the dog is not considered to be required since the product is intended for use solely as a rodenticide, a non-food use. Thus, repeat dietary exposure would not be anticipated and any dermal exposure would likely be limited. In addition, a sub-chronic dietary toxicity study of 3-6 month duration with Flocoumafen in the dog would not provide any new relevant information to the already existing toxicological database for this compound. Thus, the conduct of a sub-chronic dietary toxicity study in dogs is also not justified from an ethical point of view.

In conclusion, it is argued for that based on available toxicological data and the well-established mechanism of toxicity, as well as the predicted negligible human secondary exposure levels, sub-chronic exposure of humans to Flocoumafen is generally insignificant. Therefore, non-performance of a sub-chronic toxicity study in a second animal species is considered to be fully 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	29 December 2004
Evaluation of applicant's justification	According to the waiving concept for rodenticides, a subchronic toxicity study in the second animal species can be waived if the level of secondary exposure to the rodenticidal active substance is negligible. In case of flocoumafen, secondary exposure can occur due to handling of dead rodents. However, secondary exposure for the general public will be occur at a low level, in a low frequency and can be considered acute or accidental. According to the waiving concept for rodenticides, a subchronic toxicity study in the second animal species can be waived if the repeated dose studies in the first species are without indication of substance-related adverse effects for primary exposure, and, if the mechanism of toxicity is known and it is justified that the toxicological effects in the target rodent species are not relevant to humans regarding the expected exposure levels. In case of flocoumafen, repeated dose studies in the first species (rat) were with indication of substance-related adverse effects for primary exposure. Furthermore, effects are not considered species-specific. No clear human medical data on flocoumafen are available, however, from studies with different species it is to be expected that the anticoagulant action of flocoumafen in rats and mice will also apply for humans. Based on the guidance given in the waiving document for rodenticides, a subchronic toxicity study in the second animal species cannot be waived. However, one should consider the already available toxicological information with non-rodent species.

Evaluation of applicant's justification (continued)

The mode of action of flocoumafen is well understood. Flocoumafen acts as an anticoagulant in several species, including non-rodent species. In cats and pigs, higher LD_{50}'s were established when compared to rats (10 and 60 mg/kg in cats and pigs, respectively, versus an LD_{50} of 0.24-0.5 mg/kg in male rats and 0.13-0.31 mg/kg in female rats). In dogs, an LD_{50} of 0.075 – 0.25 mg/kg was established (A.6.13/1). Male and female dogs treated with 0.25 or 1.00 mg/kg b.w. of Flocoumafen were sacrificed non-scheduled for humane reasons between day 4 and day 9 after dose administration. Dogs given 0.025 and 0.075 showed no treatment-related signs of toxicity. Macroscopic post mortem examination of dogs sacrificed non-scheduled showed widespread haemorrhaging throughout the body which was found to be especially marked in the thoracic cavity for three of the four animals sacrificed non-scheduled and was associated with a general pale appearance of all tissues. No treatment-related abnormalities were recorded upon necropsy of dogs administered with 0.025 or 0.075 mg/kg b.w.
Based on the absence of effects in the 0.075 mg/kg bw, and the fact that the dogs given 0.25 were sacrificed for humane reasons, it can be concluded that the oral LD_{50} for dogs will be comparable to the oral LD_{50} in rats.
In a study to investigate the effectiveness of vitamin K1 therapy as an antidote to single exposure intoxication by flocoumafen (A.6.13./06), one male and one female dog received a single dose of 0.5 mg/kg. The female dog showed in-appetence, pale gums and subdued behaviour 5 days after dosing. Post mortem examinations after sacrifice for humane reasons showed free blood in the abdominal cavity and massive haemorrhages. The male showed no clinical signs of intoxication after the single dose despite showing elevated clotting times. Thus, a second dose of 0.5 mg Flocoumafen/kg b.w. was administered. On day 6, in-appetence, pale gums and laboured breathing was observed. Gross pathology revealed free blood in the thoracic cavity and massive blood clots. Clotting times were elevated on day 5 after the first (both dogs) and the second dose (male dog only).

In a palatability study (A.6.13/14), dogs received 50 ppm in the diet once a week for up to 3 or 6 weeks. All dogs were killed in extremis or died during the study. Animals showed increased PT and APTT values and comparable clinical signs of toxicity as seen in rats. Furthermore, haemorrhages were seen at post-mortem examination.

The palatability study does not indicate an enhanced or different sensitivity of dogs to flocoumafen than rats.

Conclusion	Based on the studies with non-rodent species described above a higher sensitivity of non-rodent species when compared to rats is not to be expected. Therefore, additional semichronic toxicity study in a second non-rodent species is not considered necessary. The justification for non-submission of a semichronic toxicity study in a second non-rodent species is considered acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.4.1 Subchronic oral toxicity test in rats

Annex Point IIA6.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.4.1/01:** Cxxxx Dxxxx, Exxxx Dxxxx (1989) WL108366: A 90 day feeding study in rats. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.88.147, June 7, 1989 (unpublished). (BASF-Ref.: FL-425-002)	
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**	No However, the conduct of the study was consistent to EU method B.26 (2001/59/EC) in all important aspects.	
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	
2.3	**Deviations**	Yes No motor activity assessment and no functional observations were performed. Uterus, ovaries, epididymes and thymus were not weighed upon study termination. Histopathology was not reported for parathyroid, oesophagus and skin.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	96.1%	
3.1.4	Description	Off-white powder	

Section A6.4.1 Subchronic oral toxicity test in rats

Annex Point IIA6.4

3.1.5	Stability	The test substance was considered to be stable for the duration of the study. On the basis of a stability test, the test substance was considered to be stable for at least one month in laboratory animal diet when stored at approximately –4°C. Diets containing 0.05 to 0.6 ppm of the test substance were analysed by high performance liquid chromatography with fluorescence detection. The results showed that the uniformity of distribution of the test substance in the diet was satisfactory and that the desired concentrations had been achieved.
3.2	**Test animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	Charles River U.K. Ltd., Manston, Kent
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 6 to 8 weeks Body weight: within ± 20% of the overall mean body weight
3.2.6	Number of animals per group	10 males and 10 females
3.2.7	Control animals	20 males and 20 females
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	Not specified
3.3.3	Post-exposure period	None
3.3.4	Type	In food
3.3.5	Concentration	0, 0.01, 0.02, 0.05, 0.1, 0.25 and 0.6 ppm
3.3.6	Vehicle	Acetone
3.3.7	Concentration in vehicle	Correct quantities of the stock solutions (test substance in acetone) were diluted to 25 ml with acetone.
3.3.8	Total volume applied	Not stated
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
	Clinical signs	Yes (once or twice daily)
	Mortality	Yes (once or twice daily)
3.4.2	Body weight	Yes (before treatment, weekly throughout the study and upon sacrifice)

Section A6.4.1 Subchronic oral toxicity test in rats

Annex Point IIA6.4

3.4.3	Food consumption	Yes (weekly)	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	Yes Prior to commencement of exposure in all control rats and rats dosed with 0.25 or 0.60 ppm; prior to necropsy at 12 weeks in control animals and rats dosed with 0.10 ppm (highest surviving dose group)	
3.4.6	Haematology	Yes Number of animals: each surviving animal Time points: end of study Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, prothrombin time, partial thromboplastin time, mean corpuscular and mean platelet volumes, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, platelet distribution.	
3.4.7	Clinical chemistry	Yes Number of animals: each surviving animal Time points: end of study Parameters: sodium, potassium, calcium, chloride ion, glucose, inorganic phosphate, triglyceride, cholesterol, urea nitrogen, bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transpeptidase.	
3.4.8	Urinalysis	Yes Number of animals: each surviving animal Time points: collection during the last week of the study Parameters: urine volume and appearance, osmolality, glucose concentration	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes Organs: liver, kidneys, testes, spleen, brain, pituitary, salivary glands, prostate and adrenal glands and heart	X
3.5.2	Gross and histopathology	Yes Gross necropsies were performed on rats of all dose groups. All organs and tissues taken at necropsy from rats in the control, 0.1, 0.25 and 0.60 ppm groups and lung, liver and kidney from the 0.01, 0.02 and 0.05 ppm groups were subjected to histopathology. Histopathology was performed on following tissues of all control animals and animals dosed at 0.1, 0.25 and 0.60 ppm: adrenals, aorta, brain, bone, bronchi, epididymes, eyes, heart, intestines, kidneys, lachrimal glands, larynx, liver, lungs, lymph nodes, mammary gland, muscle, nerves, ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicles, spinal cord, spleen, stomach, testes, thyroids, thymus, tongue, urinary bladder, uterus, vagina and any non-haemorrhagic macroscopic lesion observed upon necropsy.	
3.5.3	Other examinations	No other examinations were reported.	

Section A6.4.1 Subchronic oral toxicity test in rats

Annex Point IIA6.4

3.5.4	Statistics	Two-way analysis of variance, analysis of covariance, Dunnett's test, Williams' test, Wilcoxon two-sample rank sum test.
3.6	**Further remarks**	Vitamin K_3 in form of a menadione derivative was added to the experimental diets at 3 ppm. This was reported to be a standard practice at the laboratory to counteract the loss of vitamin K in the diet during processing. It was considered that the quantity of vitamin K_3 added to the experimental diets would be insufficient to counteract any haemorrhagic effects caused by the test substance.

4 RESULTS

4.1	**Observations**	
4.1.1	Clinical signs	Generally, rats in the 0.25 and 0.60 ppm dose groups showed pale eyes and skin, dark or swollen areas on the body, blood around the nose and eyes and blood in the urine prior to death or non-scheduled sacrifice. Animals surviving to the end of the 90-day period did not show any of these typical "anticoagulant" signs.
4.1.2	Mortality	All animals fed 0.60 ppm of Flocoumafen died or were sacrificed non-scheduled between day 10 and 14. All animals fed 0.25 ppm died or were sacrificed non-scheduled between day 26 and 30 (females) or day 38 to 51 (males).
4.2	**Body weight gain**	No toxicologically significant effects were observed.
4.3	**Food consumption and compound intake**	Slightly higher food consumption was observed for 0.10 and 0.25 ppm males and 0.10 ppm females reaching statistical significance on three occasions. This effect was not observed in the high dose group.
4.4	**Ophthalmoscopic examination**	Dietary exposure of rats to 0.10 ppm of Flocoumafen for 12 weeks resulted in no treatment-related ophthalmic changes.
4.5	**Blood analysis**	
4.5.1	Haematology	Male and female rats dosed with 0.10 ppm of Flocoumafen showed markedly significant increases in their coagulation times in both the prothrombin and activated partial thromboplastin assays. In addition, female rats of this dose group had increased platelet counts and platelet count values. Male rats at 0.05 and 0.10 ppm dose levels showed decreases in percent monocytes. However, this finding was considered to be toxicologically insignificant by the authors, since no atypical cells were observed on examination of the blood films. Some other statistically significant effects were also observed, which were however considered incidental. Statistically significant results are summarised in Table A6.4.1- 1. For further details please refer to the original report. No statistical analysis of the data obtained for the 0.60 and 0.25 ppm dose groups was performed, since animals died or were sacrificed non-scheduled. All animals sacrificed non-scheduled showed extremely prolonged prothrombin times. Although red blood cells seemed normal, there were less of them, consistent with loss through haemorrhage.

Section A6.4.1 Subchronic oral toxicity test in rats

Annex Point IIA6.4

4.5.2	Clinical chemistry	Statistically significant increased cholesterol levels were noted in both sexes at 0.10 ppm, and in males only at 0.05 ppm. However, this finding in males is not a toxicologically relevant adverse effect, since (i) there was no clear dose-response relationship (for example, the values for the 0.01 ppm group were higher than for the 0.02 ppm group), (ii) the individual values for the 0.05 ppm group are all exactly within the same range as the control group. Further, males in the 0.10 ppm dose group showed reduced plasma chloride and increased albumin concentrations. However, the change in albumin concentrations was observed only after a high result from a control animal had been excluded on statistical grounds. Statistically significant results are summarised in Table A6.4.1- 1. For further detail please refer to the original report. No statistical analysis of the data obtained for the 0.60 and 0.25 ppm dose groups was performed, since animals died or were sacrificed non-scheduled. Animals sacrificed non-scheduled showed increased concentrations of blood urea and triglyceride, while other clinical chemical parameters showed large inter-individual variation, probably depending on the severity of haemorrhagic lesion.	X
4.5.3	Urinalysis	Females dosed with 0.10 ppm showed a slight but significant increase in urinary glucose concentration. It was concluded that this effect may have been caused by the anti-coagulant properties of Flocoumafen, although a concomitant increase in urinary erythrocytes/haemoglobin was not observed. No further toxicological significant effects were observed except perhaps for a 24% increase in urinary volume in males treated with 0.10 ppm of Flocoumafen. Statistically significant results are summarised in Table A6.4.1- 1. For further detail please refer to the original report.	
4.6	**Sacrifice and pathology**		X
4.6.1	Organ weights	Males dosed with 0.1 ppm of the test substance showed higher mean heart weights compared to the controls. No other biologically significant differences were observed.	
4.6.2	Gross and histopathology	The cause of death for animals of the 0.25 or 0.60 ppm groups was haemorrhage in each case. The site of haemorrhage varied greatly, but was reported to be frequently widespread. No other treatment-related macroscopic lesions were observed. Animals of the 0.10 ppm dose group showed a higher incidence of lymph node haemorrhage of a severity not uncommonly seen in controls. No other treatment-related microscopic findings were noted.	
4.7	**Other**	None	

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.4.1 Subchronic oral toxicity test in rats

Annex Point IIA6.4

5.1	**Materials and methods**	Groups of 10 male and 10 female Fischer 344 rats received diets containing 0.01, 0.02, 0.05, 0.10, 0.25 or 0.60 ppm of Flocoumafen for up to 90 days. Additionally, a control group with 20 males and 20 females was included. Although not a guideline study, the method used was consistent to EC method B.26 (2001/59/EC) in all important aspects. Deviations from current guideline as stated under 2.3 above.
5.2	**Results and discussion**	All animals of the 0.25 and 0.60 ppm groups died or were sacrificed non-scheduled due to severe signs of typical "anticoagulant" toxicity. Gross pathology showed that the only cause of death were haemorrhages. In addition, prothrombin times of rats dosed with 0.60 or 0.25 ppm were increased to such an extent that they could not be measured and blood urea and triglyceride concentrations were also elevated. Animals in the remaining dose groups showed no treatment-related clinical signs or macroscopic findings. Animals of the 0.10 ppm dose group showed a higher incidence of lymph node haemorrhage of a severity not uncommonly seen in controls. Furthermore, prothrombin and activated partial thromboplastin times were markedly increased in this dose group. Females fed 0.10 ppm also had elevated platelet counts. Whereas statistically significant increased cholesterol levels were noted in both sexes at 0.10 ppm, the slight elevation lost statistical significance in females of the 0.05 ppm group, and was without a clear does-relationship in males. Females dosed with 0.10 ppm showed a slight increase in glucose and males of this dose group showed an increased urinary volume. Male heart weights were increased after treatment with 0.10 ppm.
5.3	**Conclusion**	
5.3.1	LO(A)EL	0.1 ppm
5.3.2	NO(A)EL	0.05 ppm, corresponding to 0.005 mg/kg b.w./day applying a factor of 0.1 for the conversion from "ppm in feed" to "mg/kg bw/d" (GDCh, BUA Grundsatzpapiere, August 1992)
5.3.3	Other	It was concluded that feeding rats at a level of 0.10 ppm Flocoumafen for 90 days did not give rise to any clinical, haematological, biochemical or pathological findings indicative of any toxic effects other than those of anticoagulation. At 0.05 ppm in feed, no toxicologically relevant adverse findings could be observed. Therefore, the no-adverse-effect-level can be established at this dietary level, which in turn can be converted into a NOAEL for risk assessment by applying a conversion factor of 0.1 (GDCh, BUA Grundsatzpapiere, August 1992) from "ppm in feed" to "mg/kg bw/d", yielding a NOAEL of 0.005 mg/kg b.w./d.
5.3.4	Reliability	1
5.3.5	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	4 December 2006
Materials and Methods	3.1.2: specification of test substance: cis:trans isomer ratio = 57:43. 3.4.8 In addition, colour changes and deposit were investigated at urinalysis. 3.5.1. In addition, ovary weights were recorded. 3.6 Vitamin K3 was administered to the diet, to counteract the loss of vitamin K in the diet during processing, according to the authors of the study report. However, it should be noted that flocoumafen, just like other coumarin derivatives, acts as a vitamin K antagonist. Treatment with vitamin K1 is recommended where, based on diagnostic results, this appears to be necessary (severe intoxication only). In this 90-day study the quantity of vitamin K3 added to the experimental diets would be insufficient to counteract any haemorrhagic effects caused by the test substance (tenfold lower activity of the used vitamin K3 in relation to K1) .
Results and discussion	4.5.2 Cholesterol levels were increased at 0.05 (109% and 106% of control values in males and females, respectively) and at 0.1 mg/kg food (111% and 114% of control values in males and females, respectively). The increased cholesterol levels at 0,05 and 0.1 mg/kg food were not toxicologically relevant, since observed changes were within the same range as the historical control values and the absence of a dose-response up to 0.1 mg/kg food. 4.6 Additional effects were included in Table A.6.4.1-1, when compared to the effects described in paragraph 4 and 5, as for instance the changes in weights of kidneys and salivary glands and changes in body weight and food consumption. Changes in body weight and food consumption were slight and in absence of a dose-response. Absolute and relative kidney weights were increased 103-104% when compared to controls, absolute and relative weights of salivary glands were decreased 96-98% when compared to controls but there was no microscopic correlate. These changes in organ weights were therefore not considered adverse. Furthermore, it should be noted that macroscopic findings were not included in in Table A.6.4.1-1. In animals given 0.25 and 0.60 mg/kg food, heamorrhages were noted in a wide range of organs. With regard to histopathology, the applicant included only the heamorrhages in lymphnodes in males in Table A.6.4.1-1. In females the following incidence was noted for heamorrhage in lymphnodes for the 0.01, 0.02, 0.05, 0.10, 0.25 and 0.60 mg/kg food groups, respectively: 8/20, -, 1/1, -, 3/10, 8/10 and 4/10 (- = not examined). Considering the incidence in control females, the observation in males might be within the control range. However, the incidence of haemorrhage in lymphnodes in males was statistically significantly increased at 0.02 mg/kg food and above. Historical control data showed that the observed haemorrhages in lymphnodes at 0,1, 0,25 and 0,6 mg/kg food are due to flocoumafen.

Conclusion	In a 90-day oral toxicity study, rats were given diets containing 0, 0.01, 0.02, 0.05, 0.1, 0.25 or 0.6 mg/kg food (equivalent to 0.0005, 0.001, 0.0025, 0.005, 0.0125 and 0.03 mg/kg bw/day; based on conversion factor of 20 (JMPR)). All animals given 0.25 and 0.6 mg/kg food died during the study. Animals found dead or sacrificed during the study showed typical anticoagulant signs are pale eyes and skin, dark or swollen areas on the body, blood around nose and eyes and blood in the urine. Increased mean prothrombin and activated thromboplastin times were noted in males and females at 0.1 mg/kg food. In females an increased platelet count and plateletcrit were noted at 0.1 mg/kg food. Decreases in monocytes were noted in males at 0.05 and 0.1 mg/kg food (61 and 59% of control values, respectively), however, these changes were not accompanied by further haematological changes. Cholesterol levels were increased at 0.05 (109% and 106% of control values in males and females, respectively) and at 0.1 mg/kg food (111% and 114% of control values in males and females, respectively). The increased cholesterol levels at 0,05 and 0.1 mg/kg food were not toxicologically relevant, since observed changes were within the same range as the historical control values and the absence of a dose-response up to 0.1 mg/kg food. Slightly increased albumin values (2.1%) and slightly decreased chloride values (2%) were noted in males at 0.1 mg/kg food. At urinalysis, increased urine volume was noted in males at 0.1 mg/kg food and increased glucose was noted in females at 0.1 mg/kg food. Absolute and relative heart weights were increased in males at 0.1 mg/kg food (108% of control), in the absence of histopathological correlates. At histopathological examination, increased incidences of haemorrhage were noted in several organs in the two highest dose groups, mainly in males (e.g. testes, prostate, epididymides, urinary bladder). Centrilobular degeneration was noted at 0.25 and 0.60 mg/kg food in males and females and multifocal necrosis in liver of males at 0.6 mg/kg food. Haematopoiesis was noted in spleen of males at 0.25 and 0.6 mg/kg food and of females at 0.1 and 0.25 mg/kg food. A statistically significant increase in haemorrhages in lymphnodes was noted in males at 0.02, 0.05, 0.10, 0.25 and 0.60 mg/kg food. Historical control data showed that the observed haemorrhages in lymphnodes at 0,1, 0,25 and 0,6 mg/kg food are due to flocoumafen. Based on the haemorrhages in lymphnodes, a NOAEL of 0.05 mg/kg food is established (equivalent to 0.0025 mg/kg bw/day)..
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable.
Remarks	No further remarks.

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

Table A6.4.1- 1: Results of the 90-day feeding study in rats.

Parameter	**Control**		**0.01 ppm**		**0.02 ppm**		**0.05 ppm**		**0.10 ppm**		**0.25 ppm**		**0.60 ppm**		**Dose-response +/–**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals examined	20	20	10	10	10	10	10	10	10	10	10	10	10	10		
Mortality [a]	0/20	0/20	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10	+	+
Clinical signs [b]																
Pale eyes/ skin											+	+	+	+	+	+
Dark or swollen areas											+	+	+	+	+	+
Blood around the eyes/ nose											+	+	+	+	+	+
Blood in urine											+	+	+	+	+	+
Body weight											@	@	@	@		
Week 1										↑					–	–
Week 7					↓										–	–
Week 10							↓								–	–
Food consumption											@	@	@	@		
Week 1										↑↑	↑				–	–
Week 4				↓											–	–
Week 8										↑					–	–
Clinical chemistry											@	@	@	@		
Chloride									↓						–	–
Cholesterol							↑	NS↑	↑↑	↑↑					+	+
Albumin									↑						–	–
Haematology											@	@	@	@		
Prothrombin[1]									↑↑	↑↑					+	+
Thromboplastin[2]									↑↑	↑↑					+	+
MCV					↑										–	–
MCH				↓	↑										–	–
Monocytes							↓		↓						–	–
Leucocytes								↑							–	–
Platelet count										↑↑					–	–
RBC width										↑					–	–
Plateletcrit										↑↑					–	–
Urinalysis											@	@	@	@		
Leucocytes[3]			↓		↓										–	–
Ketones			↓↓	↓											–	–
Blood[4]			↓↓				↓↓		↓						–	–
Volume									↑↑						–	–
Vacteria						↓									–	–
Urobilinogen						↓									–	–
Glucose										↑					–	–

(Table continued on next page)

Table A6.4.1–1 (continued): Results of the 90-day feeding study in rats.

Parameter	Control		0.01 ppm		0.02 ppm		0.05 ppm		0.10 ppm		0.25 ppm		0.60 ppm		Dose-response +/–	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Terminal body weight [g]											@	@	@	@	–	–
Heart																
organ weight[c]									↑↑						–	–
Kidneys																
organ weight[c]								↑		↑					–	–
Salivary glands																
organ weight[c]									↓						–	–
Lymph nodes																
Microscopic pathology [d,e]	(19)		(2)		(3)		(1)		(10)		(10)		(10)			
haemorrhage	0		1		2↑		1↑		3↑		7↑↑		4↑↑		+	
mesenteric haemorrhagic	0		0		1		0		0		0		0		–	
mesenteric haemorrhage	2		0		0		0		0				0		–	
mesenteric[5]	1		0		0		0		0		0		0		–	
no microscopic evidence of macroscopic finding	0		1		0		0		0		0		0		–	
mast-cell hyperplasia	1		0		0		0		0		0		0		–	

↑ or ↓ = significantly greater or less than control mean ($p<0.05$)

↑↑ or ↓↓ = significantly greater or less than control mean ($p<0.01$)
NS↑ means an increase not statistically significant at the 95% confidence level

[a] dead or sacrificed non-scheduled
[b] +, – increased or decreased in comparison to the controls
[c] adjusted for terminal body weight
[d] numerals in brackets indicate the number of tissues examined histologically
[e] incidence of microscopic findings (numeric)
[1] prothrombin time
[2] activated partial thromboplastin time
[3] urinary leucocytes
[4] males of the control group had abnormally high level of blood in urine
[5] mesenteric no microscopic evidence of macroscopic finding
MCV = mean corpuscular volume
MCH = Mean corpuscular haemoglobin
RBC width = Red cell distribution width
[@] all animals died during the study

Section A6.4.1 Subchronic oral toxicity tests in rats

Annex Point IIA6.4 **Supportive data**

The following reference is considered to contain additional information concerning subchronic repeated dose toxicity and is thus presented in tabular format as supportive data: (non-GLP study)			
Reference	Title	System	Results
A6.4.1/02: Fxxxx Jxxxx, Pxxxx Nxxxx (1985), Sxxxx Lxxxx, Report dated January 28, 1985 (unpublished).	An evaluation of the long term sub-acute oral toxicity of WL108366 in Wistar rats.	Male Wistar rats (12 per group)	Groups of male rats received oral doses of 0, 0.0125, 0.0625 or 0.125 mg/kg by gavage once a week for up to 12 weeks. Prothrombin times were determined in rats sacrificed at 3, 6, 9 or 12 weeks. It was considered that a significant amount of the weekly administered dose was eliminated. Thus, showing that the long term sub-acute oral toxicity of Flocoumafen is less than additive.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	29 December 2004
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	Rats administered doses of 0.0625 and 0.125 mg/kg weekly, died during the study, between days 56-71 and 32-36, respectively. These rats exhibited symptoms typical of anticoagulant poisoning: lethargy, hunched posture and bleeding from the mouth and anus. Autopsies confirmed that these animals had died of anticoagulant poisoning. Animals given 0.0125 mg/kg for 12 weeks, showed no increase in prothrombin times. Rats given 0.0625 mg/kg showed slightly elevated prothrombin times at week 9. One animal administered 0.125 mg/kg showed elevated prothrombin times at week 6, the other animals died before week 6. Body weight gain of surviving animals was similar to those of the control groups. Lower body weights were noted for animals that died during the study.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.4.2 Subchronic dermal toxicity test

Annex Point IIA6.4

	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:	The conduct of a percutaneous 90-day toxicity study in the rat is not considered to be required since route-to-route extrapolation is not restricted in any way.	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	27 December 2004
Evaluation of applicant's justification	A repeated dose dermal toxicity study is required, where significant dermal exposure to the active substance is to be expected and when route-to-route extrapolation is not possible. Considering the exposure assessments, dermal exposure to flocoumafen is to be expected. However, there are no contra-indications for route-to-route extrapolation. From the results of the acute oral and dermal toxicity studies with flocoumafen, it can be concluded that there are no route-specific effects to be expected after repeated exposure to flocoumafen. Furthermore, there is no evidence of enterohepatic circulation or a first-pass effect. Therefore, it is concluded that for the dermal exposure route, oral toxicity data can be used for risk characterisation of flocoumafen and Storm BB.
Conclusion	Non-submission of data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.4.3 Subchronic inhalation toxicity test

Annex Point IIA6.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	(1) The vapour pressure of Flocoumafen (IIA, 3.2) was determined experimentally under GLP to a value below 1 x 10^{-5} hPA (20°C). Therefore, Flocoumafen is not a volatile substance. (2) In consideration of the intended use as a rodenticide in a ready-to-use wax block bait, inhalation is considered to be a negligible route of exposure. In conclusion, the performance of a 90-day inhalation toxicity study in the rat is consequently not considered to be required predominantly for 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	29 December 2004
Evaluation of applicant's justification	A repeated dose inhalation toxicity study is required for volatile substances (vapour pressure > 1 x 10^{-2} Pa) and in special cases e.g. for aerosols and dusts. Since flocoumafen has a vapour pressure of < 1 × 10^{-3} Pa and the formulation under consideration (Storm BB) is a wax bound block bait, it is concluded that for the inhalation exposure route, oral toxicity data can be used for risk characterisation of flocoumafen and Storm BB.
Conclusion	Non-submission of data is accepted.
Remarks	None.

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

Section A6.5 Chronic toxicity

Annex Point IIA6.5

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The non-submission of a chronic toxicity study is considered to be justified according to the "refined waiving concept" in the TNsG on data requirements, based on exposure pattern and toxicological profile. In the following, the fulfilment of the waiving criteria for chronic toxicity as outlined in the TNsG is demonstrated. *1. Secondary exposure*: In view of the intended uses as set forth in Sections A5 and B5, the extent of secondary exposure (i. e., persons not involved in production and application) is considered to be negligible: The deployment of bait in tamper-resistant bait stations or directly into rodent burrows ensures protection of pets, livestock and children from contact with bait. Any accidental direct consumption of bait by humans is effectively prevented by the inclusion of a bittering agent (Section B2). Any secondary exposure to Flocoumafen would be acute. In support of this viewpoint, chronic secondary exposure is denoted as "not relevant" in the TNsG on human exposure. Furthermore, indirect exposure via the environment is considered to be of minor importance since the release of rodenticides to the environment is limited. Overall, it appears justified to generally consider secondary exposure as negligible. *2. Primary exposure*: Primary exposure during production of the active substance (reference 6.12.3/01) has been shown to be negligible, as judged on the basis of biological monitoring (prothrombin time) of active substance manufacturing workers. Further, primary exposure during formulation of ready-to-use baits (references 6.12.1/01 and 6.12.1/02) has similarly been shown also not to elicit any changes in prothrombin times, interpreted as a sign of negligible exposure. Concerning exposure of pest control workers during application of baits, exposure may be interpreted as intrinsically low in view of the low active substance content (0.005%) of bait materials, and the fact that these are ready-to-use baits which require only a minimum of direct handling. This is clearly indicated by a dedicated PCO monitoring study (reference B6.6/02). However, we explicitly note that the default levels of lower concern with regard to long-term toxicity testing are defined as "up to once per month or a 3-month period" by the waiving concept in the TNsG. This exposure frequency is completely unrealistic for professional pest control operators (PCO). These defaults in the opinion of the applicant are clearly overruled by estimates obtained from the human exposure study conducted on behalf of the industry companies participating in the CEFIC rodenticide working group. Based on these data, a detailed exposure assessment was performed (see Doc. II-B), providing evidence that primary exposure is of very low concern, in conjunction with the toxicological profile below.	

Section A6.5 Chronic toxicity

Annex Point IIA6.5

4. Toxicological profile:

a) The 90-d sub-chronic study on rats (A6.4.1/01) is considered to have been performed in agreement with the most recent EU method (B.26, 2001/59/EC) in all important aspects and to be fully appropriate for risk assessment. The NOAEL was established at 0.005 mg/kg bw/d. The expected primary exposure levels are $\leq 3.6 \times 10^{-5}$ mg/kg bw/d (worst-case) even on Tier 1 level (unprotected worker). Thus, taking into consideration a satisfactory MOE (in this case > 100), substance-related adverse effects for humans are not expected. However, since pest control operators wear protective gloves and clothing by default, as explained in detail in the product section of this dossier, realistic worst estimates of exposure (Tier 2) are amount to a maximum of 3.6×10^{-6} mg/kg bw/d. The MOE is thus > 1000, therefore fulfilling the criterion of lower concern as specified in the TNsG on data requirements, chapter 1.

b) The mechanism of toxicity of Flocoumafen is well established, and can be considered equivalent for humans and the target species. The sole toxic effect is the inhibition of vitamin K recycling, subsequent interruption of the formation of blood clotting factors, and consequently sustained haemorrhages (A5.4/01, 02). At the expected exposure levels including a satisfactory MOE, as specified under (a) above, it is justified that the toxicological effects in the target rodent species are not relevant to humans regarding the expected exposure levels.

c) Rats receiving Flocoumafen in the diet for 28 days (reference A6.3.1/1) or 90 days (reference A6.4.1/1) showed no treatment-related macroscopic lesions apart from haemorrhages upon necropsy. Short-term studies with Beagle dogs, where vitamin K was administered after signs of intoxication by Flocoumafen occurred (referred to as antidotal administration), have not shown any other toxic effects at doses that would have otherwise been lethal (A6.13/6 and A6.13/11), supporting that anticoagulation is the sole pharmacological action of Flocoumafen.

4. Toxicokinetic profile:

Flocoumafen and its metabolites are eliminated predominantly via faeces (while bile and urine are of minor importance, and exhalation is negligible), and depletion from kidney, fat and muscle follows a bi-phasic pattern:

- the initially depletion from these tissues is rapid with a $t_{½}$ of approximately 4.5–9.8 days, thus effectively clearing the bulk of absorbed a.s. rapidly

- after 28 days, depletion slows down, after which however a large portion of the dose has already been eliminated

- the depletion profile of the generally low concentrations in blood is similarly biphasic.

5. Technical feasibility:

A unique feature of rodenticides is that this test species used in long-

Section A6.5 Chronic toxicity

Annex Point IIA6.5

term toxicity and carcinogenicity studies is also the target species. A comparison of LD_{50} values for other mammals shows that the range of tolerance is generally within one order of magnitude, and all values are very low in absolute terms. However, using other species than rats or mice is not feasible for technical reasons such as duration of study. The following acute oral LD_{50} values were reported for several species (IPCS 1995, ref. A5.4/03):

Species	LD_{50} (mg/kg)	(Reference)
rat	m: 0.43; f: 0.31	(A6.1.1/01)
gerbil	m: 0.18	(A6.1.1/04)
mice	m: 0.79; f: 1.47	(A6.1.1/05)
hamster	m/f: > 50	(A6.1.1/11)
guinea pig	m: > 10	(A6.1.1/13)
dog	m/f: 0.075 – 0.25	(A6.13/01)
cat	m/f: >10	(A6.13/02)
pig	m/f: approx. 60	(A6.13/03)

In addition, the administration of a maximum tolerated dose which is used in order to demonstrate the validity of long-term carcinogenicity/toxicity studies, is difficult since Flocoumafen accumulates in the liver, the site of Vitamin K regeneration. The half-life of depletion of ^{14}C-Flocoumafen from rat livers was reported at 222 days (A6.2/03). Further, the route of administration of the test substance for long-term studies would be a problem: It is not feasible to prepare accurately homogenous rodent test diets at the very low concentrations needed for the maximum tolerated dose and lower dose levels. Oral administration by gavage, apart from being unfeasible for a life-time study, is barred because handling for gavage can be expected to lead to haemorrhages, and administration via injection or inhalation (limited volatility) are obviously also not feasible. Finally, administration via drinking water is not feasible because of the extremely limited water solubility of the test substance.

In conclusion, it is considered that based on negligible secondary exposure and very low primary exposure of humans, the anticipated lack of substance-related adverse effects in consideration of data from sub-chronic studies and a satisfactory MOE, and the well-established mechanism of toxicity of anticoagulants, the performance of a chronic toxicity study with Flocoumafen is 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	30 December 2004
Evaluation of applicant's justification	The waiving principles for chronic toxicity studies include the aspects exposure and toxicity profile. Exposure: Chronic toxicity studies need not to be submitted if the level of secondary exposure is negligible and if the frequency or duration of primary exposure is below the level of lower concern with regard to long-term toxicity testing, e.g. up to once per month or a 3 month period. Secondary exposure might include exposure to flocoumafen through contamination of food or feed or by handling of dead rodents. Secondary exposure through contamination of food and feed is expected to be negligible and/or accidental, and is therefore considered to be acute. At the removal or disposal stage, the tasks of both the non-professional and professional user include collection of uneaten bait and emptying packages and dead animals. Secondary exposure through handling of dead animals is considered to be chronic of nature for professional users. Primary exposure will be limited to trained or non-trained professionals. For professional users the use of STORM BB is not fully protected. STORM BB is delivered in plastic snap-lid buckets or bait stations. The tasks of the professional user includes the placing of bait in rodent burrows or securing was block in bait stations. For both trained professional (vermin control companies, local authorities), and non-trained professional users (farmers) chronic occupational primary exposure cannot be excluded. Toxicity profile: According to the refined waiving concept for rodenticides, chronic toxicity studies need not to be submitted if the sub-chronic repeated dose studies are without indication of substance-related adverse effects for human exposure and if the mechanism of toxicity is known and it is justified that the toxicological effects in the target rodent species are not relevant to humans regarding the expected exposure levels. Flocoumafen has shown in subacute and semichronic toxicity studies to induce adverse anticoagulant effects. Considering the differences between the NOAELs of the available subacute and semichronic toxicity studies with flocoumafen, an effect of exposure duration cannot be excluded. The mode of action as seen in rodent species (inhibition of vitamin K recycling) is expected to be equivalent in humans.

Conclusion	Considering the guidance given for waiving of chronic toxicity studies, and the exposure to flocoumafen and toxicity profile of flocoumafen, a chronic toxicity study should be submitted. However, Storm BB is intended to act against rodents, including rats and mice, the preferred species for chronic toxicity testing. Therefore, there might some technical problems in performing a chronic toxicity study with flocoumafen without causing unnecessary harm in laboratory animals, e.g. very low doses should be given. Extremely low doses should be considered, taking into account the results of the semichronic toxicity study with flocoumafen in combination with the half-life time of 215 days of flocoumafen in liver. These low doses might be difficult to achieve homogenously in diets. In addition, test substance administration by gavage is not considered suitable for a chronic toxicity study and administration via drinking water is not considered feasible because of the extremely limited water solubility of flocoumafen. Based on the expected exposure pattern, for trained and non-trained professional users chronic primary and secondary exposure cannot be excluded. However, performance of a chronic toxicity study with rodents might be difficult due to technical problems (extremely low doses necessary) and might induce unnecessary harm to laboratory animals.
Remarks	Considering the differences between the NOAELs of the available subacute and semichronic toxicity studies with flocoumafen, and considering the fact that flocoumafen accumulates, it is concluded that an additional factor for exposure duration should be considered at chronic risk assessment.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.6.1 *In-vitro* gene mutation study in bacteria

Annex Point IIA6.6

Official use only

1 REFERENCE

1.1	**Reference**	**A6.6.1/01:** Bxxxx Txxxx, Cxxxx Mxxxx, Wxxxx Dxxxx (1984) Genotoxicity studies with WL108366 (candidate rodenticide). Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.84.160, August 6, 1984 (unpublished). (BASF-Ref.: FL-435-001)
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.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No However, the conduct of the study was consistent to EU method B.13/14 (2000/32/EC) in all important aspects.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed. However, the study was audited by a quality assurance unit.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	ST84/051	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	> 99%	
3.1.4	Description	Off-white crystalline solid	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Study type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	*S. typhimurium:* TA 1535, TA 1537, TA 98, TA 100, TA 1538 *E. coli:* WP2 uvr A pKM 101	
3.2.2	Deficiencies / Proficiencies	Amino-acid requiring strains, histidine for *S. typhimurium* and tryptophane for *E. coli*.	

Section A6.6.1 *In-vitro* gene mutation study in bacteria

Annex Point IIA6.6

3.2.3	Metabolic activation system	S9 mix Rat liver S9 fraction, not further specified.	X
3.2.4	Positive control	TA1538, TA98, TA100: benzo(a)pyrene (with metabolic activation), TA1537: neutral red, TA1535 and *E.coli*: potassium dichromate or sodium azide.	
3.3	**Application of test substance**		
3.3.1	Concentrations	31.25, 62.5, 125, 250, 500, 1000 or 2000 µg/plate (both in the presence and in the absence of rat liver S9 fraction).	
3.3.2	Way of application	20 µl volumes of solutions of Flocoumafen dissolved in dimethyl sulphoxide (DMSO) were added to top agar mix and plated. The cultures were incubated at 37°C for 48-72 hours.	
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**	Number of revertant colonies.	

4 RESULTS

4.1	**Genotoxicity**		
4.1.1	Without metabolic activation	No The results are presented in Table A6.6.1- 1.	X
4.1.2	With metabolic activation	No The results are presented in Table A6.6.1- 1.	
4.2	**Cytotoxicity**	No	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The mutagenic potential of Flocoumafen was tested in the bacterial reverse mutation test using the plate incorporation assay, and the method used was consistent to method B.13/14 (2000/32/EC) in all important aspects. No relevant deviations from the prescribed test guideline were reported.
5.2	**Results and discussion**	Concentrations of up to 2000 µg Flocoumafen/plate were tested in the plate incorporation assay. No increase in the reverse gene mutation rate was found in any of the tested strains either in presence or absence of rat liver S9 fraction.
5.3	**Conclusion**	Flocoumafen was found to be not genotoxic under the conditions of the test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	13 May 2005
Materials and Methods	3.1.2: No information was provided on the cis:trans isomer ratio of the test substance. However, the actual manufacturing process was not changed over the years since Flocoumafen was introduced into the market. Thus an evaluation of the typical variation of the cis/trans-ratio in commercial batches is considered to be feasible to generally demonstrate adequate consistency of the isomeric composition. The cis:trans ratio was very likely within the range specified by the notifier. 3.2.3: Rats pre-treated with Aroclor 1254
Results and discussion	4.1.1: No positive results for positive control without metabolic activation for TA1538, TA98, TA100
Conclusion	The test substance was not mutagenic in a *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay
Reliability	1
Acceptability	Acceptable.
Remarks	The report included also the *Saccharomyces cerevisiae* mutation assay. These results (negative) were not evaluated as this assay was not considered relevant.

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

Table A6.6.1- 1*:* Relative reverse mutation rates in *E. coli* or *S. typhimurium* after treatment with Flocoumafen (two plate-incorporation assays per concentration).

Concentration [µg per plate]	Relative reverse mutation rates[1]												Comments
	– S9						+ S9						
	WP_2	TA1535	TA1537	TA1538	TA98	TA100	WP_2	TA1535	TA1537	TA1538	TA98	TA100	
Control (mean number of revertant colonies)	60.3±5.0 58.7±6.8	7.3±2.1 12.0±3.5	8.3±0.6 8.0±1.0	15.0±4.6 12.0±1.7	12.3±0.6 14.0±2.6	79.3±8.5 69.0±7.2	61.0±6.2 70.7±2.3	9.3±1.5 11.7±2.5	14.0±2.6 12.3±3.1	18.7±3.2 18.0±3.6	12.3±3.2 18.7±4.0	87.7±8.0 79.7±9.7	
31.25	0.9 1.0	1.5 0.9	1.1 0.8	1.0 0.9	0.9 0.8	1.1 1.2	1.1 0.9	0.9 1.1	0.8 0.9	1.2 1.0	1.9 1.1	0.9 1.2	No evidence of cytotoxicity was observed, on microscopical examination of the background lawn, at any amount tested.
62.5	1.0 1.1	1.2 0.9	1.0 1.1	0.9 0.9	0.9 0.8	1.1 1.2	1.1 1.1	0.9 0.8	1.0 0.9	1.4 1.2	1.6 0.9	0.9 1.0	
125	1.1 1.1	1.4 0.8	1.0 0.9	0.8 1.0	1.0 0.7	0.9 1.1	1.3 1.1	0.7 0.8	0.8 0.9	1.1 1.3	1.6 0.9	1.1 1.0	
250	1.1 1.1	1.1 0.9	0.9 0.9	0.7 0.9	0.9 0.8	1.2 1.3	1.0 1.3	1.0 1.0	0.8 0.7	1.4 1.3	2.0 0.8	1.0 1.0	
500	0.8 1.2	0.9 0.7	0.9 1.0	0.7 0.6	0.8 1.0	1.0 1.1	1.2 1.1	1.2 0.9	1.0 0.9	1.1 1.2	1.3 0.9	1.0 1.1	
1000	0.9 1.3	0.8 0.6	1.1 0.8	0.7 0.9	0.8 0.8	1.2 1.1	1.1 1.2	0.9 0.9	1.0 0.6	1.1 0.9	1.5 0.8	1.1 1.1	
2000	1.0 1.0	1.1 0.8	0.7 0.8	0.8 0.9	1.0 0.7	1.1 1.1	1.2 1.1	1.0 0.9	1.1 0.9	1.1 1.0	1.2 1.0	1.1 1.3	
Sodium azide (5 µg)	– –	121.4* 87.0*	– –	– –	– –	– –	– –	47.9* 70.9*	– –	– –	– –	– –	
Benzo(a)pyrene (20 µg)	– –	– –	– –	1.0 1.0	1.0 1.0	1.2 1.3	– –	– –	– –	9.5* 4.9*	23.2* 18.3*	4.6* 5.2*	
Potassium dichromate (20 mg)	7.6* 7.9*	– –	– –	– –	– –	– –	5.4* 5.7*	– –	– –	– –	– –	– –	
Neutral red (20 mg)	– –	– –	1.4 1.2	– –	– –	– –	– –	– –	22.6* 19.6*	– –	– –	– –	

[1] results are expressed as ratio: Mean number of revertant colonies per treated plate / Mean number of revertant colonies per control plate
- not tested
* Reproducible values of 2.5 × control value or greater were considered to indicate mutagenic response.

Section A6.6.2 *In-vitro* cytogenicity study in mammalian cells

Annex Point IIA6.6

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.6.1/01 (Cross-reference):** Bxxxx Txxxx, Cxxxx Mxxxx, Wxxxx Dxxxx (1984) Genotoxicity studies with WL108366 (candidate rodenticide). Sxxxx Rxxxx Lxxxx, Sxxxx, Kxxxx, Uxxxx, Report No. SBGR.84.160, August 6, 1984 (unpublished). (BASF-Ref.: FL-435-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No However, the conduct of the study was similar to method B.10 (2000/32/EC).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed. However, the study was audited by a quality assurance unit.	
2.3	**Deviations**	Yes The cells were not treated in the presence of a metabolic activation system. The assay procedure is not described in detail, instead only reference to an in-house SOP is made.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	ST84/051	
3.1.2	Specification	As given in Section A2.	X
3.1.3	Purity	> 99%	
3.1.4	Description	Off-white crystalline solid.	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Study type**	*In vitro* mammalian chromosome aberration test	
3.2.1	Organism/cell type	Rat liver (RL_4) cells	

Section A6.6.2 *In-vitro* cytogenicity study in mammalian cells

Annex Point IIA6.6

3.2.2	Deficiencies/ Proficiencies	Not stated in the report.		
3.2.3	Metabolic activation system	None		
3.2.4	Positive control	7,12- Dimethylbenzanthracene (DMBA)		
3.3	**Application of test substance**			X
3.3.1	Concentrations	1st experiment:	37.5, 17.5 or 8.75 µg/ml (not reported since insufficient cells at metaphase for analysis were obtained)	
		2nd experiment:	25, 20, 10 or 5 µg/ml	
		3rd experiment:	20, 10 or 5 µg/ml	
3.3.2	Way of application	The test compound was formulated in dimethyl sulphoxide (DMSO), but came out of suspension when a solution of 200 mg/ml was diluted in medium.		
3.3.3	Pre-incubation time	None		
3.3.4	Other modifications	Not stated in the report.		
3.4	**Examinations**	Evaluation of chromosome or chromatid aberrations.		
3.4.1	Number of cells evaluated	300 for test concentrations of 5 to 20 µg/ml and the negative control; 135 to 226 for the positive control and the highest tested concentration.		

4 RESULTS

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	No The results are summarised in Table A6.6.2- 1 and Table A6.6.2- 2.
4.1.2	With metabolic activation	Not applicable
4.2	**Cytotoxicity**	Yes An inhibition of growth of 50 % was judged to lie within the range of 10 to 50 µg Flocoumafen/ml. A concentration of 30 µg Flocoumafen/ml reduced the cloning efficiency of RL_4 cells to 67.4 % when compared to the solvent control. A test substance concentration of 40 µg/ml caused a reduction to 35 %.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The *in-vitro* genotoxicity of Flocoumafen was tested in rat liver cells. Although not a guideline study, the method used was similar to method B.10 (2000/32/EC).

Section A6.6.2 *In-vitro* cytogenicity study in mammalian cells

Annex Point IIA6.6

5.2	**Results and discussion**	No consistent dose-related increase in any single type of aberration was observed and the amount of damage observed at each concentration was generally comparable with the untreated cultures. Therefore, it was concluded that the damage observed in cultures exposed to Flocoumafen did not represent a compound-related effect.
5.3	**Conclusion**	Under the conditions of the test, Flocoumafen was considered not genotoxic. However, cells were not exposed to the test substance in the presence of a metabolic activation system. Thus, the extent of testing in this study may be questioned, and the result is valid with this restriction only.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes The cells were not treated in the presence of a metabolic activation system.

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 May 2005
Materials and Methods	3.1.2: No information was provided on the cis:trans isomer ratio of the test substance. However, the actual manufacturing process was not changed over the years since Flocoumafen was introduced into the market. Thus an evaluation of the typical variation of the cis/trans-ratio in commercial batches is considered to be feasible to generally demonstrate adequate consistency of the isomeric composition. The cis:trans ratio was very likely within the range specified by the notifier.
Results and discussion	Although, in this *in vitro* cytogenetic assay the rat liver cells were not exposed to the test substance in the presence of a metabolic activation system, the RMS doubts this should be considered as a restriction since, the rat liver cell itself has the compatibility of metabolic activation. However, no cells were exposed to an indirect acting mutagen (control group), requiring metabolic activation which indicates that the metabolic activation functioned properly.
Conclusion	In an *in vitro* cytogenetic assay flocoumafen was not genotoxic with the restriction that no control chemical was tested to indicate the metabolic activation of the rat liver (RL4) cells. Data on cis:trans ratio and exposure duration need to be provided.
Reliability	2
Acceptability	Acceptable with restrictions, see results and discussion
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.2- 1: Metaphase chromosome analysis of RL_4 cells after exposure to Flocoumafen or 7,12-Dimethylbenzanthracene (DMBA), 2nd experiment.

	Control	**5 µg/ml**	**10 µg/ml**	**20 µg/ml**	**25 µg/ml**	**DMBA**
(No. of cultures)	**(3)**	**(3)**	**(3)**	**(3)**	**(2)**	**(2)**
No. of cells analysed	**300**	**300**	**300**	**300**	**226**	**135**
Cytotoxicity	An inhibition of growth of 50 % was judged to lie within the range of 10 to 50 µg Flocoumafen/ml					
	No. of cells showing:					
Chromatid aberrations						
Gaps	8	4	5	2	1	23
Breaks	3	0	1	0	0	12
Single fragments	0	0	0	0	1	5
Exchange figures	0	0	1	0	0	19
Chromosome aberrations						
Acentric fragments	0	0	1	0	0	4
Dicentrics	0	0	1	0	0	0
Translocation	0	0	0	0	0	0
Rings	0	0	0	0	0	0
Mitotic index[1]	100 %	–	–	60 %	40 %	–
Severe damage[2]	0	0	0	0	0	3
Polyploidy	10	6	12	3	3	1
Endo reduplication	0	0	0	1	0	0

–) Not stated in the report.

1) Mitotic index relative to the control cultures.

2) Damage so severe that differentiation of aberrations was not possible.

Table A6.6.2- 2: Metaphase chromosome analysis of RL_4 cells after exposure to Flocoumafen or 7,12-Dimethylbenzanthracene (DMBA), 3rd experiment.

	Control	**5 µg/ml**	**10 µg/ml**	**20 µg/ml**	**DMBA**
(No. of cultures)	**(3)**	**(3)**	**(3)**	**(3)**	**(2)**
No. of cells analysed	**300**	**300**	**300**	**300**	**200**
Cytotoxicity	An inhibition of growth of 50 % was judged to lie within the range of 10 to 50 µg Flocoumafen/ml.				
	No. of cells showing:				
Chromatid aberrations					
Gaps	5	6	6	3	17
Breaks	1	0	1	1	9
Single fragments	0	0	1	0	9
Exchange figures	0	1	0	0	32
Chromosome aberrations					
Acentric fragments	0	0	2	1	4
Dicentrics	0	0	0	0	0
Translocation	0	0	0	0	0
Rings	0	0	0	0	0
Mitotic index[1]	100 %	–	–	63 %	–
Severe damage[2]	0	0	0	0	0
Polyploidy	5	7	8	7	8
Endo reduplication	1	0	0	0	0

–) Not stated in the report.

1) Mitotic index relative to the control cultures.

2) Damage so severe that differentiation of aberrations was not possible.

Section A6.6.3 *In-vitro* gene mutation study in mammalian cells

Annex Point IIA6.6

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.6.3/01:** Cxxxx Mxxxx, Wxxxx Dxxxx (1986) In vitro mutagenicity studies with WL108366 (rodenticide) using cultured Chinese hamster V79 cells. Sxxxx Rxxxx Lxxxx, Sxxxx, Kxxxx, Uxxxx, Report No. SBGR.86.014, May 7, 1986 (unpublished). (BASF-Ref.: FL-435-003)	
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 However, the conduct of the study was consistent with method B.17 (2000/32/EC) in all important aspects.	
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	ST85/126	
3.1.2	Specification	As given in Section A2, apart from purity stated below.	X
3.1.3	Purity	96.6%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Study type**	*In vitro* mammalian cell gene mutation test.	
3.2.1	Organism/cell type	Chinese hamster lung fibroblasts (V79).	
3.2.2	Deficiencies/ Proficiencies	Hypoxanthine-guanine phosphoribosyl transferase (HPRT) proficient cells.	

Section A6.6.3 *In-vitro* gene mutation study in mammalian cells

Annex Point IIA6.6

3.2.3	Metabolic activation system	S9 mix The S9 mix was used at 10% and comprised a buffered solution of 10% Aroclor-induced rat-liver homogenate (S9 fraction), glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate.
3.2.4	Positive control	7,12- Dimethylbenz [a] anthracene (DMBA), ethyl methanesulphonate (EMS)
3.3	**Application of test substance**	
3.3.1	Concentrations	0, 5, 50, 100, 150 µg/ml
3.3.2	Way of application	The test compound was formulated as a solution in dimethyl sulphoxide (DMSO).
3.3.3	Pre-incubation time	Not applicable
3.3.4	Other modifications	Not stated in the report.
3.4	**Examinations**	The effect of the test substance on the frequency of mutation was tested by measuring resistance to the base analogue 6-thioguanine.
3.4.1	Number of cells evaluated	Not applicable

4 RESULTS

4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	No
4.1.2	With metabolic activation	No
4.2	**Cytotoxicity**	Yes The test substance was markedly cytotoxic at concentrations of 150 and 200 µg/ml.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The *in-vitro* genotoxicity of Flocoumafen was tested in Chinese hamster lung fibroblasts (V79). Although not a guideline study, the method used was consistent in all important aspects to method B.17 (2000/32/EC).
5.2	**Results and discussion**	Flocoumafen did not cause any dose-related or reproducible increases in the mutant frequencies in Chinese hamster lung fibroblasts (V79) with or without metabolic activation. Statistically significant increases in gene mutation were observed for both positive control substances when compared to the negative controls ($p \leq 0.01$).
5.3	**Conclusion**	It was concluded that the test compound was **non mutagenic** under the conditions of this test.

Section A6.6.3 *In-vitro* gene mutation study in mammalian cells

Annex Point IIA6.6

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	24 May 2005
Materials and Methods	3.1.2: specification of test substance: cis:trans isomer ratio = 57.6:42.4.
Results and discussion	No comments
Conclusion	Flocoumafen is non mutagenic in an *in vitro* gene mutation study (HPRT) with Chinese hamster lung fibroblasts.
Reliability	1
Acceptability	Acceptable
Remarks	None

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

Table A6.6.3- 1: The effect of Flocoumafen or ethyl methane sulphonate (EMS) on forward mutation rates to 6-thioguanine resistance in V79 cells in absence of metabolic activation on day 7.

Concentration [mg/ml]	**– S9**				**Comments**
	Experiment 1		**Experiment 3**		
	Number of mutant colonies per 10^5 cells	Mean plating efficiency %	Number of mutant colonies per 10^5 cells	Mean plating efficiency %	
0	0.728	58	1.500	76	
5	0.827	57	1.554	73	
50	0.563	61	1.869	70	
100	1.270	67	0.995	75	
150	0.530	63	0.666	61	cytotoxic[1]
EMS (400)	77.889	59	43.528	70	

[1] Markedly cytotoxic in the cytotoxicity assay (plating efficiency: 18% of control)

Table A6.6.3- 2: The effect of Flocoumafen or ethyl methane sulphonate (EMS) on forward mutation rates to 6-thioguanine resistance in V79 cells in presence of metabolic activation on day 7.

Concentration [mg/ml]	**+ S9**				**Comments**
	Experiment 2		**Experiment 4**		
	Number of mutant colonies per 10^5 cells	Mean plating efficiency %	Number of mutant colonies per 10^5 cells	Mean plating efficiency %	
0	0.434	59	1.682	68	
5	0.653	57	0.072	77	
50	0.824	55	1.169	76	
100	0.819	60	1.227	82	
150	0.905	52	1.910	68	cytotoxic[1]
DMBA (10)	19.977	66	40.224	71	

[1] Markedly cytotoxic in the cytotoxicity assay (plating efficiency: 33% of control).

Section A6.6.3 *In-vitro* gene mutation study in mammalian cells

Annex Point IIA6.6 Supportive data

The following reference is considered to contain additional information concerning in vitro genotoxicity and is thus presented in tabular format as supportive data (non-GLP study):			
Reference	Title	System	Results
A6.6.3/02: Mxxxx Axxxx, Wxxxx Dxxxx (1986) Sxxxx Rxxxx Lxxxx, Sxxxx, Kxxxx, Uxxxx, Report No. SBGR.85.289, January 14, 1986 (unpublished). (BASF-Ref.: FL-435-002)	Genotoxicity studies with WL108366 (rodenticide): in vitro cell transformation studies	Mouse embryo fibroblasts (C3H10T ½)	It was concluded that Flocoumafen did not induce in vitro cell transformation in C3H10T ½ fibroblasts, either in the presence or in the absence of metabolic activation under the experimental conditions of the study.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 May 2005
Conclusion	The presentation of the above study as supportive data is accepted.
Remarks	None.

	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.6.4 *In vivo* mammalian bone marrow chromosome aberration test

Annex Point IIA6.6

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.6.4/01:** Axxxx Jxxxx, Pxxxx Rxxxx, Mxxxx Kxxxx (1986) Genotoxicity studies with WL 108366 (rodenticide): in vivo chromosome studies with rat bone marrow cells. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 85/8610, January 3, 1986 (unpublished). (BASF-Ref.: FL-435-004)	
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 475 (1984); EEC Directive 79/831 Annex V (1982) Part B.	
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	X
3.1.1	Lot/Batch number	ST85/126	
3.1.2	Specification	Not stated.	
3.1.3	Purity	Not stated.	
3.1.4	Description	Off-white powder	
3.1.5	Stability	Not stated.	
3.1.6	Maximum tolerable dose	A dosage of 1000 mg/kg b.w. was considered to be the maximum tolerable dose over 48 to 72 hours, based on a preliminary toxicity test.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Specific Pathogen Free CD rats of Sprague-Dawley origin.	
3.2.3	Source	Charles River U.K. Ltd., Margate, UK	
3.2.4	Sex	Male and female	

Section A6.6.4 *In vivo* mammalian bone marrow chromosome aberration test
Annex Point IIA6.6

3.2.5	Age/weight at study initiation	Age: Not stated. Body weight: Not stated.
3.2.6	Number of animals per group	15 males and 15 females.
3.2.7	Control animals	Yes 15 males and 15 females (vehicle control), 5 males and 5 females (positive control)
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	One
3.3.2	Interval between applications	Not applicable
3.3.3	Post-exposure period	6, 24 or 48 hours
3.3.4	Type	By gavage
3.3.5	Concentration	0.25 or 1000 mg/kg b.w.
3.3.6	Vehicle	Corn oil
3.3.7	Concentration in vehicle	Not stated.
3.3.8	Total volume applied	10 ml/ kg b.w.
3.3.9	Controls	Corn oil (vehicle control) Cyclophosphamide (positive control, administered intra-peritoneal)
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Bone marrow
		Number of animals: 5 per sex at each of 3 time points
		Number of cells: 50
		Time points: 6, 24, 48 hours after treatment
		Type of cells: Bone marrow cells
		Parameters: Numbers and types of structural aberrations
3.5	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	No animals died during the course of the study. Clinical signs commonly observed in animals treated with the test substance were slight to moderate diarrhoea, pilo-erection and hunched posture. In addition, lethargy, decreased respiratory rate, ptosis, pallor of extremities, gasping, cyanosis or thin appearance were observed in animals sacrificed 48 hours after treatment.

Section A6.6.4 ***In vivo* mammalian bone marrow chromosome aberration test**
Annex Point IIA6.6

4.2	**Haematology/ Tissue examination**	Not stated in the report.
4.3	**Genotoxicity**	No
4.4	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of Flocoumafen on the incidence of chromosomal damage was tested in rats receiving 0.25 or 1000 mg/kg b.w. The test method was based on OECD 475 (1984) and EEC Directive 79/831 Annex V (1982) Part B. to be No relevant deviations from the prescribed guidelines were reported.
5.2	**Results and discussion**	Treatment with Flocoumafen at 0.25 or 1000 mg/kg b.w. did not cause any increase in the proportion of cells showing chromosomal damage at any of the three sampling times at either dose level. Statistically significant increases in the proportion of cells showing chromosomal damage were observed in positive control animals.
5.3	**Conclusion**	Flocoumafen was **not genotoxic** under the conditions of the test.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes The study was conducted according to guidelines recommended at that time. However, the following deviations from the actual required guideline, method B.11 (2000/32/EC), were reported: no mitotic index determined as a measure of cytotoxicity was reported. 50 cells instead of at least 100 cells were analysed for each animal. Increases in polyploidy or endoreduplication were not reported.

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	24 May 2005
Materials and Methods	3.1. In a request for information the applicant demonstrated information on the test substance : purity of 96.6% and a cis-trans ratio of 57.6:42.4.
Results and discussion	No comments
Conclusion	WL 108366 (with unknown purity and isomer ratio) did not induce chromosome aberrations in an *in vivo* chromosome aberration test with rat bone marrow cells.
Reliability	2
Acceptability	Acceptable.,
Remarks	2.2 The study was performed under GLP conditions. A quality assurance statement was included in the study report.

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

Table A6.6.4- 1*:* Metaphase chromosome analysis of bone marrow cells from rats receiving Flocoumafen, corn oil (vehicle control) or Cyclophosphamide (positive control)

	Positive control	**Vehicle control**			**0.25 mg/kg b.w.**			**1000 mg/kg b.w.**		
Sampling time [h]	24	6	24	48	6	24	48	6	24	48
No. of cells evaluated (per animal)	50	50	50	50	50	50	50	50	50	50
Toxicity (clinical signs)										
ptosis						+	+		+	+
lethargy, decreased respiratory rate, pallor of extremities, gasping, cyanosis, thin appearance							+			+
	No. of aberrations per culture									
Chromatic aberrations										
gaps	8	0	0	0	0	1	0	0	0	0
breaks	79	0	0	0	0	0	0	0	0	0
interchanges	14	0	0	0	0	0	0	0	0	0
complex rearrangement	6	0	0	0	0	0	0	0	0	0
single minute	18	0	0	0	0	0	0	0	0	0
Isochromatid aberrations										
gaps	1	0	0	0	0	0	0	0	0	0
breaks	3	0	0	0	0	0	0	0	0	0
interchanges	0	0	0	0	0	0	0	0	0	0
acentric fragment	28	0	0	0	0	0	0	0	0	0
Cells with more than 10 aberrations	14	0	0	0	0	0	0	0	0	0
Mitotic index	Not stated in the report.									
Polyploidy	Not stated in the report.									
Endo reduplication	Not stated in the report.									
Incidence of aberrant cells (%)										
excluding gaps	15.0***	0	0	0	0	0	0	0	0	0
including gaps	15.6***	0	0	0	0	0.2	0	0	0	0

***) $p < 0.001$ (Wilcoxon's sum of ranks test)
+) increased when compared to the vehicle control

Section A6.6.5 Second *in vivo* genotoxicity study

Annex Point IIA6.6

	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:	Performance of a second *in vivo* genotoxicity study is not considered to be required, for the following reasons: (1) There was no evidence of any mutagenic potential of Flocoumafen in the lower tiers of the genotoxicity screening, i.e.: - A6.6.1 (in a bacterial system), - A6.6.2 (in an *in-vitro* cytogenicity test), - A6.6.3 (in an *in-vitro* gene mutation assay). (2) Further, Flocoumafen also failed to elicit any genotoxic response in an *in-vivo* chromosomal aberration test in rat bone marrow cells. Accordingly, further *in vivo* genotoxicity testing is 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	30 December 2004
Evaluation of applicant's justification	An in vivo genotoxicity study (bone marrow assay for chromosomal damage or a micronucleus test) is required when active substance has shown to be positive in the in vitro gene mutation study in bacteria, the in vitro cytogenicity study in mammalian cells or in the in vitro gene mutation assay in mammalian cells. Flocoumafen has shown to be negative in these in vitro studies (A6.6.1, A.6.6.2 and A.6.6.3). Furthermore, flocoumafen has shown to be negative in an in vivo bone marrow chromosome aberration study in rats.
Conclusion	Based on the available genotoxicity data on flocoumafen, the performance of a second in vivo genotoxicity study is not considered necessary.
Remarks	None.

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

Remarks	

Section A6.6.6 Investigation of germ cell effects

Annex Point IIA6.6

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The assessment of germ cell effects is not considered to be required, for the following reasons: (1) There was no evidence of any mutagenic potential of Flocoumafen in the lower tiers of the genotoxicity screening, i.e.: - A6.6.1 (in a bacterial system), - A6.6.2 (in an in-vitro cytogenicity test), - A6.6.3 (in an in-vitro gene mutation assay). (2) Further, Flocoumafen also failed to elicit any genotoxic response in an in-vivo chromosomal aberration test in rat bone marrow cells. Accordingly, further *in vivo* genotoxicity testing in germ cells is 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	30 December 2004
Evaluation of applicant's justification	Investigation of germ cell effects is required when active substance has shown to be positive in the in vivo gene mutation study. Flocoumafen has shown to be negative in an in vivo bone marrow chromosome aberration study in rats.
Conclusion	Based on the available genotoxicity data on flocoumafen, the performance of a dominant lethal test or in vivo mammalian germ cell cytogenetics assay is not considered necessary.
Remarks	None.

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

Section A6.6.7 Further genotoxicity tests

Annex Point IIA6.6

	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:	Performance of a second *in vivo* genotoxicity study is not considered to be required, for the following reasons: (1) There was no evidence of any mutagenic potential of Flocoumafen in the lower tiers of the genotoxicity screening, i.e.: - A6.6.1 (in a bacterial system), - A6.6.2 (in an in-vitro cytogenicity test), - A6.6.3 (in an in-vitro gene mutation assay). (2) Further, Flocoumafen also failed to elicit any genotoxic response in an in-vivo chromosomal aberration teat in rat bone marrow cells. According to chapter 2 of the TNsG on common core data requirements, this data requirement for further genotoxicity testing normally refers only to the case where metabolites of concern are formed in mammals. However, in the case of Flocoumafen, it has been demonstrated that the molecule is metabolised to only a minimal degree and then largely only by conjugation, whereas the bulk of ingested material is either excreted unchanged, or remains in the body similarly unchanged. Therefore, any such further *in vivo* genotoxicity testing is 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	30 December 2004
Evaluation of applicant's justification	Further genotoxicity testing is required if metabolites of concern are formed in ammals. Flocoumafen is metabolised in the rat liver at a very low rate. The main routes of biotransformation were represented by phase-I reactions oxidising all ring systems of the test item. Conjugation of the parent with glucuronic acid was observed. Based on the available mammalian toxicokinetic and environmental data on flocoumafen, there are no indications for metabolistes of concern.
Conclusion	Based on the available data on flocoumafen, further genotoxicity testing is not considered necessary.
Remarks	None.

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

Section A6.7 Carcinogenicity

Annex Point IIA6.7

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The non-submission of a carcinogenicity study is considered justified according to the "refined waiving concept" in the TNsG on data requirements, based on exposure pattern and toxicological profile. In the following, the fulfilment of the waiving criteria for chronic toxicity as set forth in the TNsG is demonstrated. *Secondary exposure*: In view of the intended uses as outlined in Sections A5 and B5, secondary exposure (i. e., of persons not involved in production and application) is considered to be extremely unlikely. The deployment of bait in tamper-resistant bait stations ensures protection of pets, livestock and children from contact with bait. Any accidental direct consumption of bait by humans is effectively prevented by inclusion of a bittering agent (Section B2). Any secondary exposure to Flocoumafen would be acute. In support of this viewpoint, chronic secondary exposure is denoted as "not relevant" in the TNsG on human exposure. Furthermore, indirect exposure via the environment is considered to be of minor importance since the release of rodenticides to the environment is limited. Accordingly, it is justified to generally consider secondary exposure as negligible. *Primary exposure*: The default levels of lower concern with regard to long-term toxicity testing are defined as "up to once per month or a 3-month period" by the waiving concept in the TNsG. This exposure frequency is completely unrealistic for professional pest control operators (PCO). Therefore, the above defaults are anticipated to be overwritten by estimates obtained from an experimental human exposure study conducted on behalf of the industry companies participating in the CEFIC rodenticide working group. Based on these data, a detailed exposure assessment was performed (see Doc. II-B), providing evidence that primary exposure is of very low concern, in conjunction with the toxicological profile below. *Toxicological profile*: As an overall conclusion based on the available genotoxicity tests (A6.6.1, A6.6.2, A6.6.3, A6.6.4) which are considered to be performed in agreement with currently valid EU methods, no evidence for genotoxic potential was identified. Further, there is an absence of any other effects that may lead to non-genotoxic carcinogenesis: rats receiving Flocoumafen in the diet for 28 days (reference A6.3.1/1) or 90 days (reference A6.4.1/1) showed no treatment-related macroscopic lesions apart from haemorrhages upon necropsy. Short-term studies with Beagle dogs, where vitamin K was administered after signs of intoxication by Flocoumafen occurred (referred to as antidotal administration), did not show any other toxic effects at doses that would have otherwise been lethal (A6.13/6 and A6.13/11), supporting that anticoagulation is the sole	

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pharmacological action of Flocoumafen.

The molecule Flocoumafen does not entail any structural alerts for carcinogenicity. Instead, in analogy to Warfarin which is in use as a human anticoagulant drug and for which long-term use has demonstrated an absence of any carcinogenic potential, it may be assumed that at the very low dose levels required for eliciting an anticoagulant response, carcinogenic effects are not anticipated.

The 90-d sub-chronic study on rats (A6.4.1/01) is considered to have been performed in agreement with the most recent EU method (B.26, 2001/59/EC) in all important aspects and to be fully appropriate for risk assessment. The NOAEL was established at 0.0014 mg/kg bw/d. The expected primary exposure levels are $\leq 3.6 \times 10^{-5}$ mg/kg b.w. /d (worst case). Thus, taking into consideration a satisfactory MOE (in this case > 100), substance-related adverse effects for human exposure are not expected.

A waiver is further supported by the practical difficulties of performing such a study, the ratio of the predicted dose in rodent studies and the actual dose of the anticoagulant Warfarin administered to humans, and the low risk of exposure in manufacturing and use. The practical difficulties of long-term administration of anticoagulants are such that an attempt at a study would be certain to fail. Thus, the conduct of a chronic toxicity/ cancerogenicity study is also not justified from an ethic point of view.

Technical feasibility: A unique feature of rodenticides is that this test species used in long-term toxicity and carcinogenicity studies is also the target species. A comparison of LD_{50} values for other mammals shows that the range of tolerance is generally within one order of magnitude, and all values are very low in absolute terms. However, using other species than rats or mice is not feasible for technical reasons such as duration of study. The following acute oral LD_{50} values were reported for several species (EHC 175, Anticoagulant Rodenticides, WHO; 1995):

Species	LD_{50} (mg/kg)	(Reference)
rat	m: 0.43; f: 0.31	(A6.1.1/1)
gerbil	m: 0.18	(A6.1.1/4)
mice	m: 0.79; f: 1.47	(A6.1.1/5)
hamster	m/f: > 50	(A6.1.1/11)
guinea pig	m: > 10	(A6.1.1/13)
dog	m/f: 0.075 – 0.25	(A6.13/1)
cat	m/f: >10	(A6.13/2)
pig	m/f: approx. 60	(A6.13/3)

In addition, the administration of a maximum tolerated dose which is used in order to demonstrate the validity of long-term carcinogenicity/toxicity studies, is difficult since Flocoumafen accumulates in the liver, the site of Vitamin K regeneration. The half-life of depletion of ^{14}C-Flocoumafen from rat livers was reported at 222 days (A6.2/3). Further, the route of administration of the test substance for long-term studies would be a problem: It is not feasible to prepare accurately homogenous rodent test diets at the very low concentrations needed for the maximum tolerated dose and lower dose levels. Oral administration by gavage, apart from being unfeasible for a life-time study, is barred because handling for gavage

Section A6.7 Carcinogenicity

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	can be expected to lead to haemorrhages, and administration via injection or inhalation (limited volatility) are obviously also not feasible. Finally, administration via drinking water is not feasible because of the extremely limited water solubility of the test substance. In conclusion, it is considered that based on negligible secondary exposure and very low primary exposure of humans, the anticipated lack of substance-related non-genotoxic and genotoxic effects in relevant experimental studies, the anticipated satisfactory MOE, and the absence of structural alerts for carcinogenicity, the performance of a chronic toxicity study with Flocoumafen is 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	3 January 2005
Evaluation of applicant's justification	The waiving principles for carcinogenicity studies include the aspects exposure and toxicity profile. Exposure: Carcinogenicity studies need not to be submitted if the level of secondary exposure is negligible and if the frequency or duration of exposure is below the level of lower concern with regard to long-term toxicity testing, e.g. up to once per months or a 3 month period. Primary and secondary exposure cannot be excluded. For professional users the use of STORM BB is not fully protected (there will be exposure and gloves could be wear). STORM BB is delivered in plastic snap-lid buckets or bait stations. The tasks of the professional user includes the placing of bait in rodent burrows or securing wax block in bait stations. At the removal or disposal stage, the tasks of both the non-professional and professional user include collection of uneaten bait and emptying packages and dead animals. Secondary exposure as handling dead rodents by adults and mouthing of poison bait by infants is also not negligible. Toxicity profile: Carcinogenicity studies need not to be submitted if the test substance has no genotoxic potential, if possible mechanisms of toxic effects observed in subchronic or chronic toxicity studies are without any indications of non-genotoxic carcinogenicity and there are no structural alerts for carcinogenicity and if the subchronic studies are without indication of substance-related adverse effects relevant to humans regarding the expected exposure level.

Conclusion	Flocoumafen did not induce point mutations in S. typhimurium tester strains TA 98, TA 100, TA 1537, TA 1535, TA 1538 and the E. coli strain WP2uvrA, both with and without metabolic activation. Flocoumafen was negative in a gene mutation test with Chinese hamster lung fibroblasts with and without metabolic activation. In a chromosome aberration test with rat liver cells, flocoumafen was negative in the absence of metabolic activation. Flocoumafen did not induce a increase in chromosomal aberration in an in vivo bone marrow chromosome aberration test in rats. Based on the results of the available genotoxicity studies, flocoumafen is considered to be non-genotoxic. Flocoumafen has shown in subacute and semichronic toxicity studies to induce adverse anticoagulant effects. Moreover, flocoumafen has shown to have a half-life time of 215 days in liver. The mode of action as seen in rodent species (inhibition of vitamin K recycling) is expected to be equivalent in humans. Considering the guidance given for waiving of carcinogenicity studies, and the exposure to flocoumafen and toxicity profile of flocoumafen, a carcinogenicity study should be submitted. However, STORM BB is intended to act against rodents, including rats and mice, the preferred species for chronic toxicity testing. Therefore, there might some technical problems in performing a chronic toxicity study with flocoumafen without causing unnecessary harm in laboratory animals, e.g. very low doses should be given. Extremely low doses should be considering, taking into account the results of the semichronic toxicity study with flocoumafen in combination with the half-life time of 222 days of flocoumafen in liver. These low doses might difficult to achieve homogenously in diets. In addition, test substance administration by gavage is not considered suitable for a chronic toxicity study and administration via drinking water is not considered feasible because of the extremely limited water solubility of flocoumafen. Based on the expected exposure pattern, for skilled and non-skilled professional users chronic primary and secondary exposure cannot be excluded. However, performance of a carcinogenicity study with rodents might be difficult due to technical problems (extremely low doses necessary) and might induce unnecessary harm to laboratory animals. Therefore, the performance of carcinogenicity studies with Flocoumafen are not required.
Remarks	None.

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

Section A6.8.1 Teratogenicity test in the rabbit

Annex Point IIA6.8.1

			Official use only
		1 REFERENCE	
1.1	**References**	**A6.8.1/01:** Jxxxx Pxxxx, Jxxxx Kxxxx, Mxxxx Rxxxx (1989) The effect of WL 108366 on pregnancy of the rabbit. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 144/881513, January 13, 1989 (unpublished). (BASF-Ref.: FL-432-002 **A6.8.1/02:** Bxxxx Mxxxx (1988) A study of the effect of WL108366 on the pregnancy of the rabbit – Hxxxx Rxxxx Cxxxx experiment numbers SLL/141/R (preliminary study) and SLL/144/R (main study). Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report, July 20, 1988 (unpublished). Remark: Reference A6.8.1/02 is the analytical report with respect to dose verification of the test diet applied in the reference A6.8.1/01 and is therefore included into the current 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**	No However, the conduct of the study was consistent with EC method B.31 (88/302/EEC) in all important aspects.	X
2.2	**GLP**	No At the time of the study conduct, GLP was not compulsory. However, the study was conducted in accordance with the principles of GLP.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	As given in Section A2, apart from purity stated below.	
3.1.3	Purity	97.6%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	Formulations of Flocoumafen in corn oil were stable for at least 10 days.	

Section A6.8.1 Teratogenicity test in the rabbit

Annex Point IIA6.8.1

3.2	**Test animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	Interfauna UK Ltd., Huntingdon, UK	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: 14–24 weeks. Body weight: 3.0–3.9 g.	X
3.2.6	Number of animals per group	16 females	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Not stated in the report.	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Day 6–18 post mating	
3.3.2	Post-exposure period	11 days	
3.3.3	Type	By gavage	
3.3.4	Concentration	0.001, 0.002, 0.004 mg/kg b.w./day	
3.3.5	Vehicle	Corn oil	
3.3.6	Concentration in vehicle	Not stated in the report.	
3.3.7	Total volume applied	0.1 ml/kg	
3.3.8	Controls	Vehicle (corn oil)	
3.4	**Examinations**		
3.4.1	Body weight	Yes (on day 1, 6, 8, 10, 14, 19, 23 and 29 of gestation)	
3.4.2	Food consumption	Yes (from ‘weigh day’ to ‘weigh day’)	
3.4.3	Clinical signs	Yes (daily)	
3.4.4	Examination of uterine content	Number of corpora lutea, pre- and post-implantation loss, embryonic death	
3.4.5	Examination of foetuses	General: Litter size, no. of dead foetuses, foetal weight, sex ratio, foetal abnormalities. Skeleton: Yes Soft tissue: Yes	
3.5	**Further remarks**	None	

Section A6.8.1 Teratogenicity test in the rabbit

Annex Point IIA6.8.1

4 RESULTS

4.1	**Maternal toxic effects**	No animals died during the study and no treatment-related effects on food consumption or body weight were noted. Treatment with 0.004 mg/kg b.w./day was associated with three instances of abortion, the presence of blood on the tray paper on days 19 to 29 in 6 out of 11 dams with live young and a slight increase in the incidence of animals showing fur loss in the post dosing period. No adverse effects on dams were observed in the 0.002 and 0.001 mg/kg b.w./day groups.	
4.2	**Teratogenic/ embryotoxic effects**	The incidences and distribution of malformations, visceral or skeletal anomalies observed were considered not to be treatment related. Slight intergroup differences from controls were not statistically significant ($p > 0.05$). Slight, not treatment-related intergroup differences were observed for the incidence of foetuses with extra (13) ribs and those with variant sternebrae.	X
4.3	**Other effects**	None	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of Flocoumafen on the pregnancy and embryonic or foetal development of the rabbit was investigated at 0 (control), 0.001, 0.002 or 0.004 mg/kg b.w./day, administered in corn oil from day 6 to day 18 post mating. The conduct of the study was consistent to EC method B.31 (88/302/EEC) in all important aspects.	
5.2	**Results and discussion**	Treatment with 0.004 mg/kg b.w./day was associated with three instances of abortion, the presence of blood on the tray paper on days 19 to 29 in 6 out of 11 dams with live young and a slight increase in the incidence of animals showing fur loss in the post dosing period. No adverse effects on resulting litters were observed. Animals dosed with 0.002 or 0.001 mg/kg b.w./day showed no signs of treatment-related maternal or embryotoxic effects. Based on the results of this study, Flocoumafen was considered not to be teratogenic when orally administered to New Zealand White rabbits at levels up to and including 0.004 mg/kg b.w./day.	
5.3	**Conclusion**		X
5.3.1	LO(A)EL maternal toxic effects	0.004 mg/kg b.w./day	
5.3.2	NO(A)EL maternal toxic effects	0.002 mg/kg b.w./day	
5.3.3	LO(A)EL embryotoxic/ teratogenic effects	> 0.004 mg/kg b.w./day	

Section A6.8.1 Teratogenicity test in the rabbit

Annex Point IIA6.8.1

5.3.4	NO(A)EL embryotoxic/ teratogenic effects	>0.004 mg/kg b.w./day
5.3.5	Reliability	2
5.3.6	Deficiencies	Yes The study report exhibits some reporting deficiencies (see results tables).

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	5 January 2005
Materials and Methods	2.1 The study does not fulfil the requirements of the most recent OECD guideline for teratogenicity testing (OECD 414, January 2001) e.g. number of animals used. However, the study meets the requirements of OECD guideline 414 of May 1981. Considering the mode of action of the test substance and the results of the present study, the study is considered acceptable. 2.2. A Quality assurance statement and GLP compliance statement are included in the study report.
Results and discussion	3.1.2 Based on additional information the cis:trans isomer ratio of the test substance is 57:43 with a purity of 97.6% 3.2.5 kg instead of g. 4.2 (see also table A.6.8.1-2) A slight decrease in foetal body weight was noted at the high dose group (93% of control value). A the change in body weight was only slight, and probably due to the slightly higher litter size at this dose group (live young: 6.9, 6.6, 7.2 and 7.6 for the respective dose groups), this finding is not considered toxicologically relevant. 4.2 (see also table A.6.8.1-2) There was a dose-related increase in the percentage of male foetuses, which was not the result of increased foeto-mortality for females. The percentage males in the control group is considered to be quite low (44.6%) which determines to a large extent the observed dose-relationship in the data. Therefore, the increased percentage of male foetuses in the highest dose group was considered to be incidental and of no toxicological relevance. 4.2 (see also table A.6.8.1-3) A slight general increase in the percentage pups with external malformations and gross/visceral anomalies was noted in the high dose group. However, the incidence of these changes in control animals was rather low, there was no increase in one or more specific variation and there was no clear dose-response. Therefore, the slight increase in external malformations and gross/visceral anomalies was not considered toxicological relevant.
Conclusion	In a teratogenicity study, rabbits were dosed with 0, 0.001, 0.002 or 0.004 mg/kg bw/day by gavage, from day 6 to day 18 post mating. Maternal animals at 0.004 mg/kg bw/day showed abortions (3 of 14 animals), and the presence of blood on the tray paper on days 19 to 29 in 6 out of 11 dams with live young, and a slight increased incidence of fur loss in the post dosing period. The NOAEL for maternal effects is established at 0.002 mg/kg bw/day. Since no toxicologically relevant developmental effects were observed and no teratogenic effects were reported, the NOAEL for developmental and teratogenic effects was set at > 0.004 mg/kg bw/day.
Reliability	2
Acceptability	Acceptable.
Remarks	None.

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

Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.8.1- 1: Maternal effects

Parameter	**Control**	**0.001 mg/kg/d**	**0.002 mg/kg/d**	**0.004 mg/kg/d**	**Dose response +/–**
Number of dams examined	16	16	16	16	
Clinical findings (day 19–29) blood on the cage paper (no. dams/ no. of dams with live young)	0/14	0/16	0/13	6/11	–
Mortality of dams [%]	0	0	0	0	
Abortions	0	0	0	3	–
Body weight gain at day 29, [g]	608	559	482	650	–
Food consumption [g/rabbit/day], day 23–28	152	152	144	162	–
Pregnancy rate [%]	87.5	100	81.3	87.5	–
Necropsy findings in dams dead before end of test	n.a.	n.a.	n.a.	n.a.	n.a.

n.a.: not applicable

Table A6.8.1- 2: Litter data of dams with live young at day 29 (Caesarean section data)

Parameter	**Control**	**0.001 mg/kg/d**	**0.002 mg/kg/d**	**0.004 mg/kg/d**	**Dose response +/–**
Corpora lutea (total/number of dams)	152/14	174/16	144/13	119/11	–
Implantations (total/number of dams)	122/14	127/16	113/13	96/11	–
Resorptions (total/number of dams)	n.d.	n.d.	n.d.	n.d.	n.a.
Number of live young (total/number of dams)	96/14	105/16	93/13	84/11	–
Pre-implantation loss (%)	20.1	24.5	20.9	19.0	–
Post-implantation loss (%)	20.1	14.7	18.7	10.6	–
Total number of litters	n.d.	n.d.	n.d.	n.d.	n.a.
Foetuses/ litter	n.d.	n.d.	n.d.	n.d.	n.a.
Live foetuses/ litter	n.d.	n.d.	n.d.	n.d.	n.a.
Mean % males per group	44.6	47.3	52.1	58.4	–
Mean litter weight [g]	303.1	283.9	297.8	317.7	–
Mean foetus weight [g]	46.0	44.1	44.1	42.6	–
Mean placenta weight [g]	n.d.	n.d.	n.d.	n.d.	n.a.
Mean crown-rump length (mm)	n.d.	n.d.	n.d.	n.d.	n.a.
Fetal sex ratio (% males)	44.6	47.3	52.1	58.4	–

n.d.: not determined
n.a.: not applicable

Table A6.8.1- 3: Examination of the foetuses

Parameter	**Control**	**0.001 mg/kg/d**	**0.002 mg/kg/d**	**0.004 mg/kg/d**	**Dose response +/–**
External malformations (%)	2.2	4.8	4.8	5.8	–
External anomalies (%)		Not stated in the report.			
Skeletal malformations (%)		Not stated in the report.			
Skeletal anomalies (%)	13.0	18.8	10.3	16.0	–
Skeletal variants (%)					
12 ribs	34.1	41.8	29.8	36.9	–
13 ribs	65.9	58.2	70.2	63.1	–
Normal sternebrae	74.6	82.4	72.7	73.8	–
Variant sternebrae	25.4	17.6	27.3	26.2	–
Visceral malformations (%)					
Gross/ visceral anomalies (%)	3.3	4.2	3.8	9.2	–
Variants visceral (%)		Not stated in the report.			

Section A6.8.1 Embryotoxicity test in the rat

Annex Point IIA6.8.1

			Official use only
		1 REFERENCE	
1.1	**References**	**A6.8.1/03:** Exxxx Dxxxx (1987) WL108366: A CKA embryotoxicity study in rats. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No. SBGR.86.232, March 23, 1987 (unpublished). (BASF-Ref.: FL-432-001)	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No An embryotoxicity study was performed according to an in-house test method (Sxxxx CKA test, Chernoff, Kavlock Assay), which is based on the method of Chernoff and Kavlock (1981). Teratogenicity was not investigated.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	As given in Section A2, apart from purity stated below.	X
3.1.3	Purity	97.6%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	Charles River U.K. Ltd., Manston, UK	
3.2.4	Sex	Female	

Section A6.8.1 Embryotoxicity test in the rat

Annex Point IIA6.8.1

3.2.5	Age/weight at study initiation	Age: not stated in the report. Body weight: not stated in the report.	
3.2.6	Number of animals per group	18 females	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Not specified in the report. (One male : four females)	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Day 8-17 post mating	X
3.3.2	Postexposure period	Up to the fifth day *post partum*	X
3.3.3	Type	By gavage	
3.3.4	Concentration	0.01, 0.04 mg/kg b.w./day	X
3.3.5	Vehicle	Corn oil	
3.3.6	Concentration in vehicle	Not stated in the report.	
3.3.7	Total volume applied	2 ml/kg b.w.	
3.3.8	Controls	Vehicle (corn oil)	
3.4	**Examinations**		
3.4.1	Body weight	Yes (on day 1, 21 and upon study termination)	X
3.4.2	Food consumption	No	X
3.4.3	Clinical signs	Yes (daily)	
3.4.4	Examination of uterine content	Females failing to produce litter by day 25 were sacrificed and the uterine content was assayed for implantation sides.	X
3.4.5	Examination upon delivery	Day of birth, number of live pups, total weight of the live litter, number of still-born pups and external abnormalities of each pup	X
3.4.6	Examination on the 5th day *post partum*	General: number of surviving pups, total litter weight, maternal body weight Skeleton: No Soft tissue: No	X
3.5	**Further remarks**	Dams delivered naturally.	

Section A6.8.1 **Embryotoxicity test in the rat**
Annex Point IIA6.8.1

4 RESULTS

4.1	**Maternal toxic effects**	Anticoagulant poisoning was observed in high dose females. Of the 18 mated females administered with 0.04 mg/kg/day, three were found to be not pregnant. They died or were killed on humane grounds. Internal haemorrhage was noted upon gross necropsy. In addition, five of the 15 pregnant females in this group died or were sacrificed non-scheduled. Gross necropsy indicated that slow bleeding from within the uterus was the cause of anaemia observed in these animals. All embryonic and placental tissues appeared normal and no abortions or resorptions were reported. Bleeding from the vulva after 8 or 9 days of dosing was observed in seven of the ten surviving females. However, all ten females produced litters and no difference to the control group was noted. Maternal parameters obtained in the 0.01 mg/kg/day group were comparable to the control group.
4.2	**Teratogenic/ embryotoxic effects**	No differences when compared to the controls were observed for mean number of pups per litter, mean weight of pups and % pup survival in both treatment groups. Females of the high dose group, which died during pregnancy had normal foetuses in the uterus. No skeletal or visceral examinations of the foetuses were performed.
4.3	**Other effects**	No other effects were reported.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The potential of Flocoumafen to induce embryotoxic effects in the rat was investigated at 0 (control), 0.01 or 0.04 mg/kg b.w./day in corn oil, administered from day 8 to day 17 post mating. The study was performed according to an in-house test method (Sxxxx CKA test, Chernoff, Kavlock Assay), which is based on the method of Chernoff and Kavlock (1981). The conduct of the study deviated from EC method B.31 (88/302/EEC) in several aspects: Foetuses were not delivered by hysterectomy and not examined for visceral or skeletal abnormalities. Less than 20 pregnant rats were investigated at each dose level and less than three dose levels were tested. Female rats were dosed from day 8 to 17 post mating instead of day 6 to 15. No food consumption was measured and body weights of the dams were determined in less than weekly intervals. The number of *corpora lutea* and the sex of the foetuses were not reported.

Section A6.8.1 Embryotoxicity test in the rat

Annex Point IIA6.8.1

5.2	**Results and discussion**	Three non-pregnant and five pregnant rats of the high dose group died or were sacrificed non-scheduled. Gross necropsy revealed internal haemorrhages, typical of this class of rodenticide. The pregnant animals which died had normal foetuses in the uterus. No abortions were observed. The ten high dose females littering had normal pups with normal growth and survival. No abnormalities of females and litters in the low dose group and the control group were observed. It was concluded that there is no evidence of embryotoxicity due to Flocoumafen and no indication that pregnancy increases the susceptibility of rats to Flocoumafen.	
5.3	**Conclusion**		X
5.3.1	LO(A)EL maternal toxic effects	0.04 mg/kg b.w./day	
5.3.2	NO(A)EL maternal toxic effects	0.01 mg/kg b.w./day	
5.3.3	LO(A)EL embryotoxic	> 0.04 mg/kg b.w./day	
5.3.4	NO(A)EL embryotoxic	> 0.04 mg/kg b.w./day	
5.3.5	Reliability	2	
5.3.6	Deficiencies	Yes No skeletal or visceral examinations of the foetuses were performed.	

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	5 January 2005
Materials and Methods	3.1.2: specification of test substance: cis:trans isomer ratio = 55.6:42.0. The study was not performed in accordance with EC guideline B.31 and OECD guideline 414. The following methodological deficiencies were noticed. 3.3.1 Females were exposure from day 8 to 17 post mating, instead of day 6-15 post mating. 3.3.2 Females were exposed up to the fifth day post partum and animals delivered naturally, instead of exposure until 1 day prior to the scheduled caesarean section. 3.3.4 Only two dose levels were investigated instead of three. 3.4.1 Body weights were not determined in three-day intervals. 3.4.2 Food consumption was not determined in three-day intervals. 3.4.4 Since animal delivered naturally, the number of corpea lutea could not be reported. In addition, pre- and post implantation loss was not calculated. 3.4.5 The pups were not weighed individually and the sex of the foetuses was not reported. 3.4.6 Pups were not examined for skeletal and soft tissue alterations.
Results and discussion	4.1/5.1 Clinical observation of maternal animals of the high dose group included pale eyes, lethargy and haemorrhage from vulva.
Conclusion	In a teratogenicity study, pregnant rats were given doses of 0, 0.01 or 0.04 mg/kg bw, from day 8 to 17 post mating. At the high dose level, females showed clinical signs of toxicity (pale eyes, lethargy and haemorrhage from vulva), indicative of coagulant poisoning. At necropsy, animals showed internal haemorrhage. Maternal animals at 0.01 mg/kg bw showed no signs of toxicity. No effects on number of live pups, litter weight and surviving pups were noted. No external abnormalities were observed. As animals delivered naturally, the number of corpea lutea could not be reported and pre- and post implantation loss was not calculated. Pups were not examined for skeletal and soft tissue alterations. Under the circumstances of the study, flocoumafen did not induce developmental effects in rats at dose levels up to 0.04 mg/kg bw. However, considering the limited study design, a NOAEL for developmental and teratogenic effects is not established. The NOAEL for maternal effects is established at 0.01 mg/kg bw/d.
Reliability	3, The study is not considered suitable for the establishment of NOAELs for developmental and teratogenic effects.
Acceptability	Acceptable as supplementary information. The study does not meet the majority of requirements of a teratogenicity study.
Remarks	In general: the reporting of the results was very limited.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks	

Table A6.8.1- 4: Summary of results for maternal and foetal toxicity.

Parameter	**Control**	**0.01 mg/kg/d**	**0.04 mg/kg/d**	**Dose response +/–**
Number of dams examined	18	18	18	
Clinical findings during application of the substance		None reported		
Mortality of pregnant dams (%)	0	0	33*	+
Pregnancy rate (%)	88.9	94.4	83.3	–
No. of litters at birth	16	17	10	–
No. of litters on day 5	16	17	10	–
Gestation period	22	23	23	–
Pups/litter alive at birth	9.3 ± 1.96	8.1 ± 2.25	8.1 ± 2.96	–
Mean pup weights at birth [g]	4.7 ± 0.22	4.8 ± 0.23	4.8 ± 0.40	–
Pups per litter on day 5 *post partum*	8.9 ± 1.95	8.0 ± 2.29	7.8 ± 2.66	–
Mean pup weights on day 5 *post partum* [g]	6.9 ± 0.65	7.7 ± 0.72	7.1 ± 0.67	–
% pup weight gain, birth – day 5	47.9 ± 12.47	59.2 ± 11.10	47.7 ± 11.77	–
% pup survival	95.9 ± 6.93	99.2 ± 3.46	97.3 ± 5.84	–
% dam weight gain, day 21	44.5 ± 5.66	42.5 ± 7.60	41.7 ± 6.04	–
% dam weight gain, study termination	17.1 ± 2.99	17.0 ± 3.21	16.3 ± 2.98	–
Necropsy findings in dams dead before end of test	n.a.	n.a.	internal haemorrhages	+

* 5 of the 15 pregnant females died or were sacrificed before gestation was complete.
n.a. not applicable

Section A6.8.1 Teratogenicity study in the rat

Annex Point IIA6.8.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.8.1/04:** Jxxxx Pxxxx (1989) A study of the effect of WL 108366 on the pregnancy of the rat with rearing of F1 offspring (Japanese experiment 2). Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 143/881544, February 13, 1989 (unpublished). (BASF-Ref.: FL-430-001) **A6.8.1/05:** Bxxxx Mxxxx (1988) A study of the effect of WL108366 on the pregnancy of the rat – Hxxxx Rxxxx Cxxxx experiment number SLL/143/R. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report, July 20, 1988 (unpublished). Remark: Reference A6.8.1/05 is the analytical report with respect to dose verification of the test diet applied in the reference A6.8.1/04 and is therefore included into the current 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**	No The conduct of the teratogenicity part of the study (parental females sacrificed on day 20 of pregnancy) was consistent to method B.31 (88/302/EEC) in all important aspects with exception that the rats were dosed from day 7–17 post mating. The additional part of the study investigating development of the offspring does not correspond to current guidelines.	
2.2	**GLP**	No The study was conducted in compliance with Good Laboratory Practice Regulations but prior to implementation of GLP.	X
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	5003ST86/053	
3.1.2	Specification	As given in Section A2, apart from purity stated below.	X

Section A6.8.1 Teratogenicity study in the rat

Annex Point IIA6.8.1

3.1.3	Purity	97.6%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	Formulations of Flocoumafen in corn oil were stable for 14 days.	
3.2	**Test animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Crl: CD® (SD) BR VAF/Plus strain	
3.2.3	Source	Charles River Ltd., Portage, USA	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: 8–10 weeks. Body weight: 141–207g (batch A and B), 164–223g (batch C)	
3.2.6	Number of animals per group	(A) 20 females (B) 20 females (C) 15 females (allocated for day 20 sacrifice only)	
3.2.7	Mating	Refer to Table A6.8.1- 7.	
3.2.8	Duration of mating	Not stated for the parental generation. The offspring was mated on a one male to one female basis for 20 days (F1 generation).	
3.2.9	Deviations from standard protocol	Not applicable	
3.2.10	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	See table below	
3.3.2	Duration of exposure before mating	Not applicable	
3.3.3	Duration of exposure in general (P, F1, F2, males, females)	P generation: day 7–17 post mating No further administration of the test substance was performed.	X
3.3.4	Type	By gavage	
3.3.5	Concentration	0.01, 0.02, 0.04 mg/kg bw/day	
3.3.6	Vehicle	Corn oil	
3.3.7	Concentration in vehicle	0.001–0.004 % w/v	
3.3.8	Total volume applied	1 ml/kg	
3.3.9	Controls	Vehicle (corn oil)	
3.4	**Examinations**		X
3.4.1	Clinical signs	Yes (daily)	

Section A6.8.1 Teratogenicity study in the rat

Annex Point IIA6.8.1

3.4.2	Body weight	Yes (initial, day 3, 7, 8, 10, 12, 14, 16, 18 and 20 of pregnancy; dams allowed to litter were weighed on day 0, 7, 14 and 21 post partum)	
3.4.3	Food consumption	Yes (from 'weigh day to weigh day' between day 2 and 20 of pregnancy and from day 1 port partum up to day 20 post partum)	
3.4.4	Litter data for animals sacrificed on day 20 of pregnancy	Number of *corpora lutea*, pre- and post- implantation loss, number and sex of live young, number and distribution of embryofoetal deaths, individual foetal weight, external foetal abnormalities, visceral and skeletal foetal abnormalities	
3.4.5	Oestrus cycle	Not stated in the report.	
3.4.6	Sperm parameters	Not determined during the study.	
3.4.7	Offspring	Number and sex of pups Stillbirths Live births Presence of gross anomalies Weight gain Physical or behavioural abnormalities	
3.4.8	Organ weights P and F1	Not determined during the study.	
3.4.9	Histopathology P and F1	Uterus	
3.4.10	Histopathology F1 not selected for mating, F2	Not stated in the report.	
3.5	**Further remarks**	In addition, pre-weaning development was assessed by the mean day post coitum for achieving surface and air righting reflexes and startle response. Post weaning behavioural tests were also included in the study (accelerating rotarod, actimat, passive avoidance).	**X**

Section A6.8.1 Teratogenicity study in the rat

Annex Point IIA6.8.1

4 RESULTS

4.1 Effects

4.1.1	Parent animals (P)		
	Parental animals	Twelve of 55 mated females dosed with 0.04 mg/kg bw/day died during the study. Ten animals died or were sacrificed non-scheduled prior to day 20, one died post day 20 and one was sacrificed in poor condition following the loss of its litter post partum. All animals which died during the study were non-pregnant, with the exception of the total litter loss post partum. High dose females which were sacrificed non-scheduled showed pale extremities, shallow respiration, loss of body tone, coldness and collapse. Autopsy findings of dead and sacrificed animals included some combination of free blood in the brain/ thoracic cavity, enlarged/ haemorrhagic thymus, haemorrhagic subcutis/ skeletal muscle and enlarged cervical lymph nodes. Similar findings were reported for one animal showing total resorption and one further non-pregnant animal surviving to termination. No treatment-related effects on food consumption, body weight gain or on gross necropsy findings were observed in dams with live young at day 20 or rearing young to weaning.	X
	Litter data of females sacrificed on day 20 of gestation	No treatment-related effects were observed for litter size, pre- and post implantation loss, litter and mean foetal weight and sex ratio. Incidences of visceral or skeletal anomalies were not statistically significant ($p > 0.05$). No significant skeletal variants were noted when compared to the control group. The results are summarised in Table A6.8.1- 5 and Table A6.8.1- 6.	
	Litter (F1), up to weaning at day 21	No treatment-related effects on litter size, pup mortality, litter weight, mean pup weight or sex ratios were observed. In addition, no treatment-related effect on pre-weaning development, which was assessed by the mean day post coitum for achieving surface and air righting reflexes and startle response, was noted. No treatment-related effects were reported upon gross necropsy. The results are summarised in Table A6.8.1- 8.	
4.1.2	F1 animals		
	Parental animals	The mean age of occurrence of both the vaginal opening and balano-preputial cleavage was comparable in all groups. Minor intergroup differences in food intake over the two weeks prior to mating were unrelated to dosage. Weekly bodyweight gains did not reflect any treatment-related trend. Only one male pup of the 0.04 mg/kg/day group showed atypical performances on both the actimat and passive avoidance tests. There was no indication that treatment of parent females (P generation) with Flocoumafen affected the performance of their offspring on the rotarod, actimat or passive avoidance tests. No mortalities occurred among parental F1 animals. The incidence and nature of signs and macroscopic findings upon necropsy of the F1 parents did not suggest any relationship to treatment of their parent females.	

Section A6.8.1 Teratogenicity study in the rat

Annex Point IIA6.8.1

	Litter (F2), up to sacrifice on day 21 post partum	One instance of total litter loss occurred in the females of the 0.02 mg/kg/day group. This incidence was low and thus considered not treatment-related. In addition, no treatment-related effect on pre-birth loss was observed. The only statistically significant difference was the lower litter weight in the 0.02 mg/kg/day group, associated with the lower litter size at this dosage. Sex ratios did not reflect any derivation-related trends or any selective mortality of either sex. No treatment-related anomalies were reported upon gross necropsy of F2 offspring sacrificed at weaning.
4.2	**Other**	No other significant effects were reported.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of Flocoumafen on the pregnancy and development of the offspring were investigated in the rat. Dosages of 0 (control), 0.01, 0.02 or 0.04 mg/kg/day dissolved in corn oil were administered orally by gavage from day 7 to day 17 of gestation. At least 20 females per group were sacrificed on day 20 of gestation and the uterine content as well as the foetuses were examined. Ten females per group littered to rear their offspring to weaning. From these litters, selected F1 offspring were retained and their performance in specific behavioural tests was assessed. At 12 weeks of age they were paired and females were allowed to litter and rear their offspring (F2) to weaning. The conduct of the teratogenicity part of the study (parental females sacrificed on day 20 of pregnancy) was similar to method B.31 (88/302/EEC) with exception that the rats were dosed from day 7–17 post mating. The additional part of the study investigating development of the offspring is not guideline-conform.
5.2	**Results and discussion**	Litter data obtained from females sacrificed on day 20 of gestation showed no treatment-related effects. No significant visceral or skeletal anomalies and no significant skeletal variants were noted when compared to the control group. Thus, Flocoumafen can be considered not teratogenic when orally administered to rats up to and including 0.04 mg/kg bw/day. Further, no treatment-related effects on F1 litter parameters were observed. Examination of parental F1 animals and their litter (F2) did not suggest any relationship to treatment of the parent P females. Based on these results, it was concluded that oral doses of up to and including 0.04 mg Flocoumafen/kg bw/day did not adversely affect embryofoetal development as assessed in dams at day 20 of pregnancy, ability of dams to rear young to weaning or maturation of the selected F1 generation.
5.3	**Conclusion**	
5.3.1	LO(A)EL	
	Parent males	Not applicable
	Parent females	0.04 mg/kg bw/day
	F1 animals	> 0.04 mg/kg/day

Section A6.8.1 Teratogenicity study in the rat

Annex Point IIA6.8.1

5.3.2	NO(A)EL		
	Parent males	Not applicable	
	Parent females	0.02 mg/kg bw/day	
	F1 animals	> 0.04 mg/kg/day	X
5.3.3	Reliability	2	
5.3.4	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 January 2005
Materials and Methods	2.2. A Quality assurance statement and GLP compliance statement are included in the study report. 3.1.2 Based on additional information the cis:trans isomer ratio of the test substance is 57:43 with a purity of 97.6% 3.3.3 Test substance was administered to the P generation on days 7–17 post mating instead of day 6-15 post mating. This deviation from the guideline is considered acceptable. 3.4 In addition to the mentioned parameters, the pregnancy rate, duration of gestation period, copulation index, fertility index, gestation index, birth index and weaning index were determined. 3.5 Ten females littered to rear their offspring. F1 offspring were retained and there performance in specific behavioural tests was assessed. In addition to the developmental tests, the pupil reflex was assessed. When the offspring were approximately 84 days of age they were mated on a one male to one female basis for 20 days. During the mating period all females were weighed daily. Pregnancy rate and the percentage of surviving pared females that became pregnant were determined and mating performance was assessed.
Results and discussion	4.1.1 Parental animals: In addition to the reported effects: during the lactation period, body weight gain of treated groups was slightly lower than of control, but attained no statistical significance. No treatment-related signs of toxicity were noted at doses of 0.01 and 0.02 mg/kg bw/day. Litter data of females sacrificed on day 20 of gestation: in addition to the reported effects: a slightly higher percentage males was noted in treated groups, but attained no statistical significance. Litter (F1) up to weaning: in addition to the reported effects: a slight non-statistically significant decrease in mean pup weight (95% of control) was noted in high dose group at day 21 post-partum. The ratio of males to females was lower at birth at both 0.02 and 0.04 mg/kg bw/d. by day 21 these differences had attained statistical significance but were considered to be due to unusually high values in control groups. F1 animals: In addition to the reported effects: no effect was noted on mating performance and duration of gestation.
Conclusion	In a teratogenicity study, rats were given doses of 0, 0.01, 0.02 and 0.04 mg/kg bw/day from day 7 to 17 post mating. At day 20 of gestation 20 females/group were sacrificed and their foetuses were preserved for visceral and skeletal examination. 10 females/group littered to rear their offspring to weaning. From these litters selected F1 offspring were selected and examined in specific behavioural tests and excess pups were sacrificed and examined for abnormalities. F1 offspring were mated at 12 weeks and females were allowed to litter. F2 pups and F1 adults were sacrificed and examined for abnormalities. At 0.04 mg/kg bw/day P females showed mortality, anticoagulant signs and/or haemorrhages at necropsy. Based on these observations the NOAEL for maternal toxicity was established at 0.02 mg/kg bw/day. No toxicological relevant effects were observed on F1 and F2 litter. Pre-weaning development was similar in offspring from all groups. Therefore, the NOAEL for developmental toxicity was established at > 0.04 mg/kg bw/day. Since no teratogenic effects were reported, the NOAEL for teratogenic effects was set at > 0.04 mg/kg bw/day.
Reliability	2, see acceptability

Acceptability	The study was not performed in accordance with any specific guideline, but the study shares many characteristics with OECD guideline 414. The study is considered acceptable for evaluation.
Remarks	None
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.8.1- 5: Litter data for females sacrificed on day 20 of gestation (P generation)

Parameter	**Control**	**0.01 mg/kg/d**	**0.02 mg/kg/d**	**0.04 mg/kg/d**
Corpora lutea (total/number of dams)	381/28	348/26	429/33	422/30
Implantations (total/number of dams)	340/28	316/26	394/33	380/30
Number of live young (total/number of dams)	325/28	298/26	375/33	357/30
Pre-implantation loss (%)	12.7	9.5	8.1	9.4
Post-implantation loss (%)	4.5	5.3	4.8	6.0
Mean % males per litter	48.0	57.9	54.0	54.0
Mean litter weight [g]	38.46	37.38	37.19	38.58
Mean foetus weight [g]	3.32	3.26	3.23	3.25

Table A6.8.1- 6: Examination of the foetuses of females sacrificed on day 20 of gestation (P generation)

Parameter	**Control**	**0.01 mg/kg/d**	**0.02 mg/kg/d**	**0.04 mg/kg/d**
No. of foetuses examined external	325	298	375	357
External malformations (%)	0.8	2.0	0.7	0.5
No. of foetuses examined skeletal	162	147	187	180
Skeletal anomalies (%)	22.6	27.5	27.2	18.3
Skeletal variants (%)				
13 ribs	100	99.4	98.9	98.2
14 ribs	0.0	0.6	1.1	1.8
Normal sternebrae	23.8	18.7	25.7	19.6
Variant sternebrae	76.2	81.3	74.3	80.4
No. of foetuses examined visceral	160	145	185	175
Visceral anomalies (%)	6.3	9.4	5.6	11.0

Table A6.8.1- 7: Animal assignment for mating (excluding animals sacrificed on day 20 of pregnancy)

		Number of animals			
		Control	**0.01 mg/kg/d**	**0.02 mg/kg/d**	**0.04 mg/kg/d**
Parents (P)	male	Not stated in the report.			
	female	10	10	10	10
F1	male	10	10	10	10
	female	10	10	10	10

Table A6.8.1- 8: Summary of results for all parental females and reared litter

Parameter		Genera-tion	Control male	Control female	0.01 mg/kg/d male	0.01 mg/kg/d female	0.02 mg/kg/d male	0.02 mg/kg/d female	0.04 mg/kg/d male	0.04 mg/kg/d female
Mortality		P	n.a.	0/10	n.a.	0/10	n.a.	0/10	n.a.	1/10
(no. of animals dead/total no. of animals)		F1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Food consumption										
week 12 (g/rat/week)	% of control	F1	202g	148g	102%	107%	105%	106%	95%	99%
Body weight gain										
day 20 of gestation	% of control	P	n.a.	118.8g	n.a.	97%	n.a.	100%	n.a.	103%
day 21 post partum	% of control	P	n.a.	40.5g	n.a.	75%	n.a.	78%	n.a.	77%
day 20 of gestation	% of control	F1	n.a.	138.3g	n.a.	82%	n.a.	89%	n.a.	91%
day 21 post partum	% of control	F1	n.a.	19.8g	n.a.	57%	n.a.	67%	n.a.	81%
Mating index	(%)	F1	n.a.	100	n.a.	100	n.a.	100	n.a.	100
Fertility index	(%)	F1	n.a.	100	n.a.	100	n.a.	90	n.a.	100
Number of implantation sites	Mean	P	n.a.	13.3	n.a.	11.1	n.a.	11.4	n.a.	13.0
Duration of pregnancy	Mean (days)	P	n.a.	22.0	n.a.	22.0	n.a.	22.0	n.a.	22.0
		F1	n.a.	21.8	n.a.	21.7	n.a.	22.0	n.a.	21.7
Birth index	(%)	P	n.a.	90.9	n.a.	90.5	n.a.	90.5	n.a.	92.1
	(%)	F1	n.a.	93.1	n.a.	91.5	n.a.	90.7	n.a.	95.9
Weaning index[1]	(%)	F1	n.a.	97.0	n.a.	99.2	n.a.	98.2	n.a.	98.9
	(%)	F2	n.a.	97.4	n.a.	97.7	n.a.	99.3	n.a.	97.9
Gestation index	(%)	P	n.a.	100	n.a.	100	n.a.	100	n.a.	100
	(%)	F1	n.a.	100	n.a.	100	n.a.	100	n.a.	100
Litter size	Mean	F1	n.a.	12.2	n.a.	10.0	n.a.	10.4	n.a.	12.3
		F2	n.a.	14.1	n.a.	11.3	n.a.	14.6	n.a.	13.3
Litter weight	Mean	F1	n.a.	73.5	n.a.	64.2	n.a.	66.9	n.a.	75.9
		F2	n.a.	82.1	n.a.	65.6	n.a.	88.6	n.a.	74.9
Pub weight	Mean	F1	n.a.	6.1	n.a.	6.5	n.a.	6.5	n.a.	6.3
		F2	n.a.	5.9	n.a.	6.0	n.a.	6.2	n.a.	5.8
Sex ratio	% males	F1	n.a.	55.5	n.a.	54.1	n.a.	41.0	n.a.	46.0
		F2	n.a.	47.4	n.a.	55.4	n.a.	54.6	n.a.	47.1

n.a.: not applicable
[1]: (no. of live weanlings/no. of live young at birth) * 100

Section A6.8.2 Two generation reproduction study

Annex Point IIA6.8.2

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

Other existing data [ ] **Technically not feasible [X]** **Scientifically unjustified [X]**

Limited exposure [X] **Other justification [ ]**

Detailed justification:

In the following, the non-submission of a two-generation reproduction study is justified according to the "refined waiving concept" in the TNsG on data requirements, based on exposure pattern and toxicological profile, and supported by anticipated practical difficulties in performing such a study.

Secondary exposure:

In view of the intended uses as outlined in Sections A5 and B5, secondary exposure (i. e., persons not involved in production and application) is considered to be extremely unlikely. The deployment of bait according to the label instructions ensures protection of pets, livestock and children from contact with bait. Any accidental direct consumption of bait by humans is effectively prevented by inclusion of a bittering agent (Section B2). Any secondary exposure to Flocoumafen would be acute. In support of this viewpoint, chronic secondary exposure is denoted as "not relevant" in the TNsG on human exposure. Furthermore, indirect exposure via the environment is considered to be of minor importance since the release of rodenticides to the environment is limited. Residues in food or feedstuff can be excluded in view of the anticipated use patterns: Any contact of the biocidal product with food or feedstuff is not foreseen. Thus, any relevant exposure of e.g. children via this path can be ruled out. Accordingly, it is justified to generally consider secondary exposure as negligible.

Primary exposure:

Primary exposure resulting from application of bait is demonstrated to be negligible according to the results of an experimental human exposure study (refs. B6.6/02, 04) and subsequent exposure assessment Exposure to Flocoumafen from direct contact would in any case be expected to be low in view of the product characteristics (ready-to-use rodenticide bait), and mixing and loading is excluded for the same reason.

Toxicological profile:

a) In teratogenicity studies in rabbits and rats (A6.8.1) performed in satisfactory agreement with current testing guidelines, developmental effects were not observed up to dose levels producing maternal toxicity (0.004 mg/kg/d in rabbits, 0.04 mg/kg/d in rats). In addition to examining teratogenic effects, the study in rats further investigated possible effects of maternal (P) Flocoumafen treatment on the reproductive performance of their offspring (F1). Examination of parental F1 animals and their litter (F2) did not reveal any adverse effects related to the treatment of the P generation females. Accordingly, this study provides additional, basic experimental evidence for the lack of reproductive toxicity of Flocoumafen

b) In a 90-d sub-chronic study on rats (A6.4.1/01), performed in agreement with the most recent EU method (B.26, 2001/59/EC) in all important aspects and considered fully adequate for risk assessment, potential effects on reproductive organs were monitored

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Section A6.8.2 Two generation reproduction study

Annex Point IIA6.8.2

by assessing organ weights (testes and prostate), as well as gross and histopathology (prostate, seminal vesicles, testes, ovaries, uterus and vagina). Based on these examinations, there was no evidence for any adverse effects to the reproductive organs at the doses tested.

c) Short-term studies with Beagle dogs aimed at evaluation of vitamin K_1 as an antidote following intoxication by Flocoumafen did not reveal any other toxic effects than symptoms of anticoagulant poisoning. Vitamin K1 therapy successfully counteracted the otherwise lethal anticoagulant intoxication (A6.13/06 and A6.13/11). The absence of any other toxic effects support the statement that vitamin K inhibition is the sole pharmacological action of Flocoumafen.

d) Non-performance of a two-generation reproduction study is further supported by the anticipated experimental difficulties: During the course of a multi-generation study, each adult generation is exposed to the test diets for at least 113 days. Flocoumafen accumulates in the liver and depletes from body tissues with a half-life up to 222 days (A6.2/3). Several events in the reproductive cycle are associated with incidental or inevitable haemorrhage (mating, ovulation, change over in placental nutrition, parturition). Due to accumulation and the long half-life, Flocoumafen levels are likely to disturb blood coagulation to a degree that ultimately fatal haemorrhages from the aforementioned events are likely to occur.

e) By analogy, long term experience with the hydroxycoumarin derivative Warfarin as an anticoagulant which is widely used in anti-coagulation therapy in humans showed no association with adverse effects on fertility in humans (WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides, WHO Geneva 1995).

In conclusion, primary and secondary human exposure to Flocoumafen can be considered insignificant. Based on the toxicological profile it was demonstrated that Flocoumafen not only failed to elicit any developmental or reproductive effects in several teratogenicity studies, but also was without any effect on weight, morphology and histopathology of reproductive organs. Thus, the performance of a two-generation reproduction study with Flocoumafen is not considered to be required.

Undertaking of intended data submission **[ ]**

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

EVALUATION BY RAPPORTEUR MEMBER STATE (*)

Date

4 December 2006

Evaluation of applicant's justification

The waiving principles for two-generation reproduction studies include the aspects exposure and toxicity profile.
Two-generation reproduction studies need not to be submitted if the level of primary and secondary exposure is negligible.
Primary and secondary exposure cannot be excluded. For professional users the use of STORM BB is not fully protected (there will be exposure and gloves could be wear). STORM BB is delivered in plastic snap-lid buckets or bait stations. The tasks of the professional user includes the placing of bait in rodent burrows or securing wax block in bait stations. At the removal or disposal stage, the tasks of both the non-professional and professional user include collection of uneaten bait and emptying packages and dead animals. Secondary exposure as handling dead rodents by adults and mouthing of poison bait by infants is also not negligible.

Toxicity profile:
A two-generation reproduction study need not to be submitted if
a) no developmental or reproductive effects are observed in the teratogenicity studies and
b) if the subchronic and chronic studies have shown no adverse effects on the reproductive organs (macroscopic investigation, organ weight analysis and histology) and endocrine functions and
c) if the absence of effects on reproductive organs is not only investigated at a morphological level but also on their functionality and additional data on sperm quality and/or oestrus cycle to confirm no effect on functionality of the reproductive organs are sufficient.

Evaluation of applicant's justification	No developmental effects were observed in teratogenicity studies with rats and rabbits. In a subchronic toxicity study in rats, at histopathological examination, increased incidences of haemorrhage were noted in several organs 0.0125 and 0.03 mg/kg bw/day, including testes, prostate and epididymides. In the available data on flocoumafen no data were available on sperm quality and/or oestrus cycle after exposure to flocoumafen. At the supportive data for reproduction toxicity, the applicant provided a reference from open literature (Sangha et al., 1992). In this study one group received oral doses (17 females per dose group, gavage) of 0.08, 0.11 or 0.14 mg/kg bw. After one week ovaries were weighted and investigated histopathologically. A second group received 0 and 0.14 mg/kg (13 animals per group, gavage); after one week levels of total lipids, total cholesterol, phospholipids, free fatty acids, glycolipids and triglycerides were determined in ovary in half of the animals. The other half of the animals was paired and the breeding time and litter size were recorded. In the first group, ovarian cyclicity was disturbed in all the treated rats, in the two highest dose groups most of them remained in di-oestrous stage. Decreased ovary weights, artretic follicles and degenerating corpora lutea with pyknotic granules were noted at 0.14 mg/kg bw. In the second group increased levels of total lipids, triglycerides and cholesterol and decreased levels of phospholipids, free fatty acids and glycolipids were noted. Of the paired animals, control animals bred after 30 days and gave seven or eight pups per litter. Treated animals bred after 60 days and gave two to four pups per litter. 45 days after parturition, the second breeding was normal with a equivalent litter size (five to eight pups) for both groups. In this study with female rats with flocoumafen, effects on ovary and fertility were noted after a single dose of flocoumafen. However, this study did not fulfil requirements of guideline studies, and reporting of methods and results was very limited. Furthermore, the study indicated that effects on ovary and fertility occurred after single oral dosing with flocoumafen with doses close to LD50 values possibly causing internal bleeding.
Evaluation of applicant's justification	. One should exclude the occurrence of reproductive and developmental effects at the NOAEL used for risk assessment purposes. The applicant noted that there might be some experimental difficulties in performing a multi-generation study. However, a 90-day oral toxicity study has been performed with flocoumafen. A two-generation reproduction study is of equivalent length. When a two-generation reproduction study is performed at the four lowest dose levels used in the 90-day oral toxicity study in rats (0, 0.01, 0.02, 0.05 and 0.1 mg/kg food), one can investigate possible reproductive effects of flocoumafen. However, there might be a risk that animals bleed to death during labour. Furthermore, it is questionable whether off-spring will survive treatment before reproduction.
Conclusion	The toxic effects observed in the male and female reproductive tracts caused by flocoumafen could be related to the anticoagulation and neither specific nor restricted to the reproductive system-i.e., they are non-specific accompaniments to severe generalised toxicity. Based on the fact that the fertility effects are possibly non-specific accompaniments to severe generalised toxicity and on read-across from warfarin as discussed at the 14th and 15th of November 2006 the Classification&Labelling group for biocides and pesticides made a provisional decision not to classify all anticoagulant rodenticides and especially flocoumafen with R62. Therefore, a new multi-generation study is not necessary.
Remarks	None.
	COMMENTS FROM ...

Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.8.2 Two generation reproduction study

Annex Point IIA6.8.2 **Supportive data**

The following reference is considered to contain additional information concerning toxicity to fertility and is thus presented in abbreviated tabular format as supportive data: (non-GLP study)

Reference	Title	Method	Results
A6.8.2/01: Sangha GK, Bilaspuri GS, Guraya SS (1992), Pest. Biochem. Physiol. 44, 15-20 (published).	Effects of oral administration of rodenticide Flocoumafen on the rat ovary.	Female Wistar rats, 3–6 months of age, 150–180 g; *Group A:* single dosing (gavage) with Flocoumafen at 0.08, 0.11 and 0.14 mg/kg b.w. (n = 17 per dose level); after 1 week investigation of ovarian weights and histomorphology; *Group B:* single dosing (gavage) at 0.08, 0.11 and 0.14 mg/kg b.w. (n = 13); after 1 week investigation of ovarian levels of total lipids, total cholesterol, phospholipids, free fatty acids, glycolipids and triglycerides of half of the rats; the other half was paired and breeding time and litter size recorded.	*Group A:* The ovarian cycle was disturbed in all treated rats, rate of atresia was related to the dose, increased incidence of degenerated *corpora lutea* at higher doses; *Group B:* Several of the biochemical parameters were out of normal; the treated rats used for mating showed a delayed onset of breeding and reduced litter size in their first reproductive event immediately after dosing; at the second reproductive event (45 days after first parturition), all these parameters returned to normal. The authors concluded that Flocoumafen caused transient infertility. Conclusion: Upon evaluation, the study was found to be compromised by several severe deficiencies: Basic descriptors of toxicity that are commonly required were not reported; lack of reporting of any general organ macroscopic findings; the dosages employed (up to 0.14 mg/kg b.w.) are relatively close to the LD_{50} (range from 0.13–0.56 mg/kg bw, see section A6.1.1) and can therefore be expected to have elicited severe toxic effects; this will thus invariably be reflected in changes of organ weight, lipid content etc.; it is therefore concluded that the study does not fulfil any criteria required for investigations in the context of assessing reproductive toxicity; the study is therefore considered as invalid. Reliability: **4**

Section A6.9 Neurotoxicity study

Annex Point IIIA6.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, this kind of study needs to be performed for substances of similar or related structures to those capable of inducing delayed neurotoxicity. Further, if anti-cholinesterase activity is detected, a test for response to reactivating agent may be required. A neurotoxicity study with Flocoumafen is not required due to the chemical structure of the compound, since neuro-toxicological effects (as for example in organophosphates) would not be expected. In addition, no adverse effects other than anticoagulation have been reported from the available set of basic toxicology studies.	
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	4 March 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substance and biocidal products, a neurotoxicity study is required if there are any indications that the active substance may have neurotoxic properties. From the results of the available toxicity studies with flocoumafen there are no indications for neurotoxicity. Furthermore, flocoumafen is not similar or related to substances capable of inducing (delayed) neurotoxicity.
Conclusion	The justification for non-submission of a neurotoxicity study is considered acceptable.
Remarks	None.

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

Section A6.10 Mechanistic study

Annex Point IIIA6.7

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The conduct of mechanistic studies with Flocoumafen to clarify effects reported in toxicity studies, is not considered to be required, since no adverse effects other than anticoagulation effects have previously been reported in any of the available basic toxicology studies.	
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	4 March 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substance and biocidal products, studies of the mechanism of toxicity are considered necessary when there are indications that the active substance may have e.g. a non-genotoxic mechanism for carcinogenicity, species specific effects, adverse effects on reproduction, immunotoxicity or hormone related effects. From the results of the toxicity studies with flocoumafen, there are no indications that studies on the mechanism of toxicity are considered necessary for flocoumafen.
Conclusion	The justification for non-submission of mechanistic studies is considered acceptable.
Remarks	None.

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

Section A6.11 Study on other routes of administration (parenteral routes)

Annex Point IIIA (–)

	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, studies on other routes of administration are required only in exceptional cases, for example when (i) studies on parenteral routes may supplement the information received from toxicokinetic studies and give valuable information e.g. in cases when the gastrointestinal absorption of the chemical in question is poor, or when (ii) acute toxicity studies on intraperitoneal, intravenous subcutaneous and intramuscular routes have been conducted, these should also be submitted. Since the available data from conventional routes of administration and the extensive toxicokinetic data base are considered to provide information adequate for risk assessment, the conduct of studies on other routes of administration 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	4 March 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substance and biocidal products, studies on other routes of administration (parenteral routes) are considered necessary when e.g. the gastrointestinal absorption of the active substance is poor. If available acute toxicity studies with intraperitoneal, intravenous, subcutaneous and intramuscular dose administration should be submitted. As approximately 70% of the orally administered dose of flocoumafen was absorbed, studies on other routes of administration (parenteral routes) are not considered necessary. Intravenous dosing was included in the ADME studies.
Conclusion	The justification for non-submission of studies on other routes of administration is considered acceptable.
Remarks	None.

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

Section A6.12.1 Medical surveillance data on manufacturing plant personnel

Annex Point IIA6.9.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.12.1/01:** Txxxx Cxxxx, vxxxx Sxxxx Nxxxx (1987) Biomedical monitoring of plant workers engaged in Storm® master mix repacking and formulation and in small pack filling of Storm® loose grain bait, Cairo, Egypt, June 1986. Sxxxx Ixxxx Pxxxx Mxxxx Bxxxx, Txxxx Hxxxx, Txxxx Nxxxx; Report no.: HSE 85.006 (unpublished). (BASF-Ref.: FL-445-003)	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.1	**Substance**	(i) Storm® manufacturing master mix, a free flowing greenish-blue powder containing 0.5% Flocoumafen (ii) Storm® loose grain bait, containing 0.005% Flocoumafen	
3.2	**Persons exposed**		
3.2.1	Sex	Not stated.	
3.2.2	Age/ weight	Not stated.	
3.2.3	Known diseases	No diseases were reported. Blood samples were taken of all recruited workers to asses their liver function, the blood coagulation and vitamin K status prior to the study.	
3.2.4	Number of persons	Group I: five Group II: about 20 plant workers	
3.2.5	Other information	Group I: workers engaged in master mix repacking, formulation and occasionally small pack filling for 7 days Group II: workers employed in the small pack filling of ready-to-use grain bait for 6 days Group III: comprising workers who were involved in other jobs who were not employed in the formulation plant	X
3.3	**Exposure**	Oral, inhalation or dermal	
3.3.1	Reason of exposure	Occupational	
3.3.2	Frequency of exposure	Multiple	
3.3.3	Overall time period of exposure	Group I: seven days Group II: six days	
3.3.4	Duration of single exposure	Not stated.	

Section A6.12.1 Medical surveillance data on manufacturing plant personnel
Annex Point IIA6.9.1

3.3.5	Exposure concentration/ dose	Not available	
3.3.6	Other information	Plant location: Middle East company plant facilities 45 km S.E. of Cairo	
3.4	**Examinations**	Exposure assessment by observation from beginning to end of the start-up period; Observation for symptoms and signs of intoxication during the operations; Measurement of the prothrombin time, as well as prothrombin (factor II) and PIVKA II (Protein Induced by Vitamin K Absence or Antagonists) concentrations in the blood whenever possible on a daily base during repacking, formulation or small pack filling operations;	X
		Determination of the concentration of Flocoumafen in the blood.	X
3.5	**Treatment**	Not applicable, since no signs of intoxication were observed during the study.	
3.6	**Remarks**	None	

4 RESULTS

4.1	**Clinical signs**	No clinical signs or symptoms of intoxication were observed during the period in any subject.	
4.2	**Results of examinations**	All workers involved with master mix repacking and dosing of pre-weighted master mix into the mixing vessel (group I) showed blue stains, indicating that dermal exposure had occurred. Although personal protective equipment was provided, the face around the oronasal mask, lower arm above the gloves, hand and sometimes the chest were reported to be the predominant area's of skin contact. No visible contamination of the skin was recorded for worker of group II. Concentrations of Flocoumafen in blood were below the limit of detection of 10 ng/ml with exception of two blood samples of one plant worker belonging to group I, in which traces of Flocoumafen were tentatively detected, i.e. there was some evidence from the chromatograms that both isomers were present at a level less than 10 ng/ml. All determined prothrombin times or prothrombin ratios were reported to be within the expected normal intra-individual variation. Small decreases in prothrombin concentration were observed in one worker of group I and in one worker of group II. However, no trends could be detected in their prothrombin/ prothrombin + PIVKA II ratios. The results are summarised in Table A6.12.1- 1 and Table A6.12.1- 2.	
4.3	**Effectivity of medical treatment**	Not applicable	
4.4	**Outcome**	No biological or adverse health effects were detected in any plant worker, apart from small decreases in prothrombin concentration in two subjects. It was not clear if this finding was associated with absorption of Flocoumafen into the body since concentrations of Flocoumafen in blood were below the limit of detection.	
4.5	**Other**	No other significant observations were recorded.	

Section A6.12.1 Medical surveillance data on manufacturing plant personnel
Annex Point IIA6.9.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A biomedical monitoring study was conducted during the start-up period of a Flocoumafen rodenticide formulation plant. Plant workers engaged in master mix repacking, formulation and occasionally small pack filling (group I) or only employed in the small pack filling of ready-to-use grain bait (group II) were examined for exposure by observation, effects on the blood coagulation system and the concentration of Flocoumafen in the blood.	
5.2	**Results and discussion**	No clinical signs or symptoms of intoxication were observed during the period in any subject. All workers of group I showed blue stains, indicating that dermal exposure had occurred. However, concentrations of Flocoumafen in blood were below the limit of detection of 10 ng/ml. In two blood samples of one plant worker belonging to group I traces of Flocoumafen were tentatively detected. One subject in group I and one subject in group II showed a small decrease in prothrombin concentrations, probably resulting from a marginal decrease in availability of vitamin K in the liver. However, no PIVKA II could be detected in the blood of any subject, using the indirect PIVKA II assay, indicating that PIVKA II concentrations, if present, were below 10%.	
5.3	**Conclusion**	It was concluded that, apart from small decreases in prothrombin concentration in two subjects, all or not related with absorption of Flocoumafen into the body, no biological or adverse health effects were detected in any plant worker.	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	02 May 2005
Materials and Methods	3.4 Exposure assessment: Biological monitoring was not accompanied by actual exposure measurements. Therefore, the results of the study are of limited value for the overall evaluation of flocoumafen. 3.4 Determination of flocoumafen in blood: Blood samples were stored at 4°C for 4-7 months before analysis took place. No information was provided on the stability of flocoumafen in blood samples during the storage period. Furthermore, no information was available on the validation of the method of analysis. Therefore, the results of the measurements of flocoumafen in blood are of limited value.
Results and discussion	5.3: due to the limitations described at materials and methods above, the results of the present study are of limited value for the overall evaluation of flocoumafen.
Conclusion	Although the study has its limitations, negative effects on the workers were not observed..
Reliability	3, considering the study deviations.
Acceptability	Acceptable with its limitations
Remarks	None.

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

Table A6.12.1- 1: Prothrombin time (mean and S.E.M.) of groups examined before and at the end of the start-up period.

Group	Prothrombin time		Prothrombin ratio*	
	Mean clotting time (sec)			
	before	end	before	end
I) Master mix (n=5)	29.4 ± 1.7	28.4 ± 1.5	0.93 ± 0.08	0.92 ± 0.05
II) Small pack filling (n=6)	33.8 ± 2.3	32.5 ± 2.2	1.15 ± 0.09	1.02 ± 0.07
All pre-employment (n = 38)	29.9 ± 0.64	na	1.01 ± 0.03	na

na: not applicable
*: clotting time plasma sample / clotting time control plasma

Table A6.12.1- 2: Prothrombin and prothrombin + PIVKA II concentrations (mean and S.E.M.) of groups examined before and at the end of the start-up period

Group	Concentration (%)				Ratio prothrombinase/TSV	
	prothrombin[1]		prothrombin + PIVKA II[2]			
	before	end	before	end	before	end
I) Master mix (n=5)	97.2 ± 2.6	92.8 ± 4.5	99.0 ± 3.2	92.0 ± 4.6	0.98 ± 0.02	1.01 ± 0.00
II) Small pack filling (n=6)	95.3 ± 5.1	86.7 ± 4.1	96.1 ± 5.1	89.0 ± 4.0	0.99 ± 0.01	0.97 ± 0.01
All pre-employment (n = 38)	95.5 ± 2.1	na	95.6 ± 2.1	na	1.00 ± 0.01	na

na: not applicable
[1]: Prothrombin was determined after activation with prothrombinase.
[2]: Prothrombin + PIVKA II was determined after activation with Taipan snake venom.

Section A6.12.1 Medical surveillance data on manufacturing plant personnel

Annex Point IIA6.9.1

Official use only

1 REFERENCE

1.1	**Reference**	**A6.12.1/02:** Txxxx Cxxxx, vxxxx Sxxxx Nxxxx (1985) Biomedical monitoring of personnel in Sorex Ltd. (Widness, U.K.) involved in a formulation run with the rodenticide WL 108366. Sxxxx Ixxxx Pxxxx Mxxxx Bxxxx, Txxxx Hxxxx, Txxxx Nxxxx, Report No. HSE 85.006, October 1985 (unpublished). (BASF-Ref.: FL-445-002)

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	(i) manufacturing master mix containing 0.5% Flocoumafen technical (ii) ready-to-use grain bait, containing 0.005% Flocoumafen technical
3.2	**Persons exposed**	
3.2.1	Sex	Group A: male Group B: male Group C: male Group D: female
3.2.2	Age/ weight	Not stated in the report.
3.2.3	Known diseases	No diseases were reported.
3.2.4	Number of persons	Group A: four Group B: two Group C: two Group D: four
3.2.5	Other information	Group A: subjects of the study team with no previous exposure to anticoagulants Group B: staff members of Sorex Ltd. with occasional exposure to various rodenticides such as Brodifacoum and Warfarin Group C: process operators employed for four and six years in rodenticide formulation Group D: packers, three had been employed for twenty two years with possible exposure to anticoagulants and other chemicals
3.3	**Exposure**	Oral, inhalation or dermal
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Multiple

Section A6.12.1 Medical surveillance data on manufacturing plant personnel
Annex Point IIA6.9.1

3.3.3	Overall time period of exposure	3 days	
3.3.4	Duration of single exposure	Not stated in the report.	
3.3.5	Exposure concentration/ dose	Not available	X
3.3.6	Other information	None	
3.4	**Examinations**	Determination of blood coagulation factors using the classical prothrombin time test (PTT), a modified prothrombin time technique ('Normotest') and the measurement of individual clotting factor II in pre-exposure, first and second exposure blood samples.	
3.5	**Treatment**	Not applicable, since no signs of intoxication were observed during the study.	
3.6	**Remarks**	It was reported by the Sorex plant physician that two individuals in group C had previously prolonged prothrombin times, due to over-exposure to other coumarin derivatives, and that after oral administration of vitamin K, prothrombin times returned to normal and remained normal.	
		4 RESULTS	
4.1	**Clinical signs**	No symptoms of intoxication were observed during the period in any subject.	
4.2	**Results of examinations**	No effects which could be ascribed to absorption of Flocoumafen were observed. Coagulation times determined by the PTT and the 'Normotest' were shorter than the average value of a reference population (100%) in most individuals. However, the factor II levels of all subjects were within the normal range of 80 – 120%. Results obtained with the PTT test showed no difference between the pre-exposure and the two exposure samples. Coagulation times measured with the Normotest were shorter for the first exposure sample and longer for the second exposure sample. Factor II levels were reduced in both exposure samples when compared to the pre-exposure samples. The major changes were observed in group C. However, differences with the other groups were small and individual values were within the normal range. The results are summarised in Table A6.12.1- 3.	
4.3	**Effectivity of medical treatment**	Not applicable	
4.4	**Outcome**	No changes associated with absorption of Flocoumafen were observed.	
4.5	**Other**	No other significant observations were recorded.	

Section A6.12.1 Medical surveillance data on manufacturing plant personnel
Annex Point IIA6.9.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A biomedical monitoring study was conducted during a demonstration formulation run with the rodenticide Flocoumafen. Plant workers engaged in the formulation of ready-to-use grain baits were examined for effects on the blood coagulating system using three different tests (classical prothrombin time test, a modified prothrombin time test and the measurement of individual clotting factor II).	
5.2	**Results and discussion**	No symptoms of intoxication were observed during the study period in any person involved in this study. No effects which could be ascribed to absorption of Flocoumafen were observed. Coagulation times as measured using the PTT test and the Normotest were generally determined to be shorter than those to be expected in a healthy control population. It was considered that these shorter coagulation times probably do not reflect activation of clotting factors *in vivo*, but result from the use of glass tubes in Sorex Ltd. during blood sampling instead of the generally recommended plastic or siliconised tubes.	
5.3	**Conclusion**	The results of the determination of blood coagulation factors using three different tests showed no changes associated with absorption of Flocoumafen.	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	2 May 2005
Materials and Methods	3.2.5: No information was provided on the tasks of the workers of group A, B, C and D. Furthermore, no information was provided on personal protection equipment worn by the workers during the tasks in the plant. Therefore, the results of the study are of limited value for the overall evaluation of flocoumafen. 3.3.5: Biological monitoring was not accompanied by actual exposure measurements. Therefore, the results of the study are of limited value for the overall evaluation of flocoumafen.
Results and discussion	No comments.
Conclusion	5.3: Considering the remarks made at material and methods, and considering the remark made by the applicant on the use of glass tubes for blood sampling, the results of the present study are of limited value for the overall evaluation of flocoumafen. Although the study has its limitations, no symptoms of intoxication were detected.
Reliability	3, considering the study deviations.
Acceptability	Acceptable with its limitations.
Remarks	None.

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

Table A6.12.1- 3: Coagulation times assessed with the PTT and the Normotest and activities of factor II in personnel of Sorex Ltd. (group B – D) and in persons of the study team (group A).

Group	**PTT test**			**Normotest**			**Factor II assay**		
	Mean clotting time (sec.)			**Mean % of normal[1]**			**Mean % of normal[1]**		
	sampling time			sampling time			sampling time		
	1	2	3	1	2	3	1	2	3
A (n = 4)	11.0	10.2	10.4	118	131	121	105	104	104
B (n = 2)	10.7	10.8	10.5	112	122	111	102	106	101
C (n = 2)	10.1	10.3	10.8	117	109	98	104	96	91
D (n = 4)	10.4	10.8	10.4	129	134	123	107	104	106
Normal range	10 – 14 sec			70 – 130%			80 – 120%		

[1] Results are expressed as a percentage of the mean normal value (100%), which was assigned to a plasma pool from an unexposed control group.
Sampling time: 1: pre-exposure; 2: first exposure; 3: second exposure

Section A6.12.2 **Direct observation of acute, unintentional, paediatric superwarfarin ingestions**
Annex Point IIA6.9.2

Official use only

1 REFERENCE

1.1	**Reference**	**A6.12.2/01:** Ingels M et al. (2002) A prospective study of acute, unintentional, pediatric superwarfarin ingestions managed without decontamination. Annals of Emergency Medicine 40 (1), July 2002, p 73-78 (published).

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Superwarfarin rodenticides, no further specification was available.
3.2	**Method of data collection**	Patients with acute unintentional superwarfarin ingestions, which were reported to poison control centres during 16 month (January 1999 to April 2000) were studied. Forty-eight- to 96-hour prothrombin time or normalised ratio (INR) blood tests were recommended, and telephone contact was attempted at least 3 days after ingestion. The exact location of where calls included in the study originated was not recorded.
3.3	**Test persons**	
3.3.1	Selection criteria	All children younger than 6 years of age with single- substance, acute, unintentional superwarfarin rodenticide ingestions, which were reported within 96 hours of the time of ingestion were enrolled in the study.
3.3.2	Exclusion criteria	Children (1) having received induced emesis, activated charcoal or prophylactic vitamin K, (2) history of a pre-existing bleeding disorder, (3) ingested amount known with certainty to have been 1 pellet or less and (4) ingested amount greater than one box.
3.3.3	Known diseases	Not stated
3.3.4	Number of persons	A total of 545 patients: 82 (15 %) patients were lost to follow-up. 222 (40.7 %) patients with telephone follow-up only 62 (11.4 %) patients with laboratory follow-up only 179 (32.8 %) patients with laboratory and telephone follow-up
3.4	**Exposure**	Oral
3.4.1	Reason of exposure	Accidental
3.4.2	Frequency of exposure	Single
3.4.3	Exposure concentration	The amount of poison ingested was unknown in most of the cases.

Section A6.12.2
Annex Point IIA6.9.2

Direct observation of acute, unintentional, paediatric superwarfarin ingestions

3.5 Examinations

Forty-eight- to 96-hour prothrombin time or international normalised ratio (INR) blood tests were recommended. A prolonged INR was considered ≥ 1.5. If an INR was not available, the prothrombin time ratio (patient to control) was used and was also considered prolonged if ≥ 1.5. Patients with clinical or laboratory evidence of coagulopathy were followed up to ensure complete resolution of the abnormality.

3.6 Data analysis

For data analysis, patients were grouped according to the type of follow-up (i.e. telephone and laboratory, telephone only, laboratory only, lost to follow-up, excluded) and according to the amount ingested (i.e. unknown, unknown with product residue present in the patient's mouth, a few pellets, up to a box).

4 RESULTS

4.1 Outcome

None of the patients had clinically important coagulopathy. Only 2 patients showed a prolonged INR (1.5 and 1.8), both without symptoms of intoxication.

One was a 2-year-old girl who had ingested an unknown amount of superwarfarin rat poison, with residue present in her mouth. Laboratory examinations showed a prothrombin time of 13.8 seconds, an INR of 1.5 and an activated partial thromboplastin time (PTT) of 65 seconds at 48 hours. The child received no therapy and remained asymptomatic. A follow-up examination on day 5 revealed a prothrombin time of 11.9 seconds, an INR of 1.1 and a PTT of 39 seconds.

The second child was a 1-year-old boy who had ingested an unknown amount of superwarfarin, with no residue present. Coagulations studies performed at 48 hours showed a prothrombin time of 15.9 seconds and an INR of 1.8. The child received no therapy and remained asymptomatic. Follow-up examinations on day 10 indicated a prothrombin time of 10.6 seconds and an INR of 0.9.

In addition, incidents of nosebleed (2 patients), small amount of blood crusted in the nose (1 child) and blood-streaked stools that were thought to be caused by anal fissure (1 child) were reported. However, no other symptoms and no prolongation of blood parameters (if available) were observed for these children.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A total of 545 patients younger than 6 years of age who were reported to poison control centres with acute unintentional superwarfarin ingestions were studied, excluding patients who received gastrointestinal decontamination or prophylactic vitamin K. Forty-eight- to 96-hour prothrombin time or normalised ratio (INR) blood tests were recommended, and telephone contact was attempted at least 3 days after ingestion.

5.2 Results and discussion

No clinically important coagulopathy appeared, even though none of the patients received gastrointestinal decontamination or prophylactic vitamin K. Only 2 patients showed a prolonged INR (1.5 and 1.8) at 48 hours, which returned to the normal level in follow-up examinations. Both children remained without symptoms of intoxication.

Section A6.12.2 **Annex Point IIA6.9.2**	**Direct observation of acute, unintentional, paediatric superwarfarin ingestions**	
5.3 Conclusion	The authors concluded that children with acute unintentional superwarfarin ingestions of less than 1 box may be managed without gastric decontamination or prophylactic vitamin K. Laboratory testing for coagulophathy was recommended for cases involving clinically evident bleeding abnormalities only.	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	2 May 2005
Materials and Methods	No comments.
Results and discussion	5.3: It is not clear what is meant by ingestion of one box. One should further specify the amount of superwarfarin ingestion for which no gastric decontamination of prophylactic vitamin K is considered necessary.
Conclusion	Laboratory testing for coagulophathy was recommended for cases involving clinically evident bleeding abnormalities only.
Reliability	2, since no detailed report of the study was available.
Acceptability	Acceptable as supplementary information.
Remarks	None.

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

Section A6.12.2 Supportive data

Annex Point IIA6.9.2

The following reference is considered to contain additional information concerning the direct observation of acute, unintentional, pediatric superwarfarin ingestions and is thus presented in tabular format as supportive data:

Reference	Title	System/methods	Results
A6.12.2/02 Smolinske SC et al. (1989) PEDIATRICS 84 (3), September 1989, p 490-494 (published)	Superwarfarin poisoning in children: a prospective study.	110 patients younger than 12 years of age reported to a poison control centre with accidental superwarfarin ingestions; including patients who received dilution or gastrointestinal decontamination. No child received vitamin K. At least one prothrombin time ≥ 24 hours was recommended, and telephone follow-up was attempted.	Prothrombin times obtained 48 hours after ingestion were more likely to be prolonged than values obtained at 24 hours. The occurrence of an abnormal prothrombin time could not be predicted based on the history of amount ingested or on the presence of the characteristic green-blue product dye in or around the child's mouth. Acute toxicity was evidenced by transient abdominal pain, vomiting, and haeme positive stools in 2 patients. The authors recommended the determination of prothrombin time values 24- and 48 hours after a child has ingested a superwarfarin.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2 May 2005
Conclusion	The presentation of the above study as supportive data is accepted.
Remarks	None.

	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.12.3/1 Health records

Annex Point IIA6.9.3

Official use only

1 REFERENCE

1.1	**Reference**	**A6.12.3/01:** Lxxxx Qxxxx Lxxxx (2003) Results of Sorex CoaguCheck routine prothrombin times. Sxxxx Pxxxx Dxxxx Lxxxx, Uxxxx; October 21, 2003. (unpublished).	

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Not specified	
3.2	**Persons exposed**		
3.2.1	Number of persons	17	
3.2.2	Other information	Staff, not specified	
3.3	**Exposure**	Not specified	
3.3.1	Reason of exposure	Occupational	
3.3.2	Frequency of exposure	Not specified	
3.3.3	Overall time period of exposure	Not specified	
3.3.4	Duration of single exposure	Not specified	
3.3.5	Exposure concentration/ dose	Not specified	
3.4	**Examinations**	Routine prothrombin time tests on October 21, 2003.	X

4 RESULTS

4.1	**Results of examinations**	The prothrombin times determined in 17 staff members were in a range of 10.9 to 13.2 seconds.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The results of a recent, routine prothrombin time check at Sxxxx Pxxxx Dxxxx Lxxxx. are reported.	

Section A6.12.3/1 Health records

Annex Point IIA6.9.3

5.2	**Results and discussion**	The prothrombin times determined in 17 staff members were in a range of 10.9 to 13.2 seconds.	
5.3	**Conclusion**	The results were all within the normal range (i.e. 10–14 seconds) reported for the prothrombin time test (refer to reference A6.12.1/02).	

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 May 2005
Materials and Methods	3.4: No details were provided on the method of prothrombin time tests. It should be noted that PTT is only suitable for measuring effects on vitamin K dependent coagulation factors, possibly leading to acute health effects. PTT is not the most suitable test for determination of early effects which do not lead to adverse health effects. For the latter, the determination of the concentration of PIVKA II in blood is considered to be a more suitable method.
Results and discussion	No comments.
Conclusion	At a health check, the prothrombin times determined in 17 staff members were in a normal range.
Reliability	2, since no details were available on the method of blood sampling, sample storage and method of prothrombine time tests.
Acceptability	Acceptable as supplementary information.
Remarks	None.

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

Section A6.12.4 Epidemiological studies on the general population

Annex Point IIA6.9.4

Official use only

1 REFERENCE

1.1	**Reference**	**A6.12.2/01 (Cross-reference):** Ingels M et al. (2002) A prospective study of acute, unintentional, pediatric superwarfarin ingestions managed without decontamination. Annals of Emergency Medicine 40 (1), July 2002, p 73-78 (published).

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Superwarfarin rodenticides, no further specification was available.
3.2	**Method of data collection**	Patients with acute unintentional superwarfarin ingestions, which were reported to poison control centres during 16 month (January 1999 to April 2000) were studied. Forty-eight- to 96-hour prothrombin time or normalised ratio (INR) blood tests were recommended, and telephone contact was attempted at least 3 days after ingestion. The exact locations of where calls included in the study originated from, were not recorded.
3.3	**Test persons**	
3.3.1	Selection criteria	All children younger than 6 years of age with single- substance, acute, unintentional superwarfarin rodenticide ingestions, which were reported within 96 hours of the time of ingestion were enrolled in the study.
3.3.2	Exclusion criteria	Children (1) having received induced emesis, activated charcoal or prophylactic vitamin K, (2) history of a pre-existing bleeding disorder, (3) ingested amount known with certainty to have been 1 pellet or less and (4) ingested amount greater than one box.
3.3.3	Known diseases	Not stated
3.3.4	Number of persons	A total of 545 patients: 82 (15 %) patients were lost to follow-up. 222 (40.7 %) patients with telephone follow-up only 62 (11.4 %) patients with laboratory follow-up only 179 (32.8 %) patients with laboratory and telephone follow-up
3.4	**Exposure**	Oral
3.4.1	Reason of exposure	Accidental
3.4.2	Frequency of exposure	Single
3.4.3	Exposure concentration	The amount of poison ingested was unknown in most of the cases.

Section A6.12.4 Epidemiological studies on the general population

Annex Point IIA6.9.4

3.5	**Examinations**	Forty-eight- to 96-hour prothrombin time or normalised ratio (INR) blood tests were recommended. A prolonged INR was considered ≥ 1.5. If an INR was not available, the prothrombin time ratio (patient to control) was used and was also considered prolonged if ≥ 1.5. Patients with clinical or laboratory evidence of coagulopathy were followed up to ensure complete resolution of the abnormality.
3.6	**Data analysis**	For data analysis, patients were grouped according to the type of follow-up (i.e. telephone and laboratory, telephone only, laboratory only, lost to follow-up, excluded) and according to the amount ingested (i.e. unknown, unknown with product residue present in the patient's mouth, a few pellets, up to a box).

4 RESULTS

4.1	**Outcome**	None of the patients had clinically important coagulopathy. Only 2 patients showed a prolonged INR (1.5 and 1.8), both without symptoms of intoxication. One was a 2-year-old girl who had ingested an unknown amount of superwarfarin rat poison, with residue present in her mouth. Laboratory examinations showed a prothrombin time of 13.8 seconds, an INR of 1.5 and an activated partial thromboplastin time (PTT) of 65 seconds at 48 hours. The child received no therapy and remained asymptomatic. A follow-up examination on day 5 revealed a prothrombin time of 11.9 seconds, an INR of 1.1 and a PTT of 39 seconds. The second child was a 1-year-old boy who had ingested an unknown amount of superwarfarin, with no residue present. Coagulations studies performed at 48 hours showed a prothrombin time of 15.9 seconds and an INR of 1.8. The child received no therapy and remained asymptomatic. Follow-up examinations on day 10 indicated a prothrombin time of 10.6 seconds and an INR of 0.9. In addition, incidents of nosebleed (2 patients), small amount of blood crusted in the nose (1 child) and blood-streaked stools that were thought to be caused by anal fissure (1 child) were reported. However, no other symptoms and no prolongation of blood parameters (if available) were observed for these children.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A total of 545 patients younger than 6 years of age who were reported to poison control centres with acute unintentional superwarfarin ingestions were studied, excluding patients who received gastrointestinal decontamination or prophylactic vitamin K. Forty-eight- to 96-hour prothrombin time or normalised ratio (INR) blood tests were recommended, and telephone contact was attempted at least 3 days after ingestion.
5.2	**Results and discussion**	No clinically important coagulopathy appeared, even though none of the patients received gastrointestinal decontamination or prophylactic vitamin K. Only 2 patients showed a prolonged INR (1.5 and 1.8) at 48 hours, which returned to the normal level in follow-up examinations. Both children remained without symptoms of intoxication.

Section A6.12.4 Epidemiological studies on the general population

Annex Point IIA6.9.4

5.3	**Conclusion**	The authors concluded that children with acute unintentional superwarfarin ingestions of less than 1 box may be managed without gastric decontamination of prophylactic vitamin K. Laboratory testing for coagulophathy was recommended for cases involving clinically evident bleeding abnormalities only.

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 May 2005
Materials and Methods	No comments.
Results and discussion	5.3: It is not clear what is meant by ingestion of one box. One should further specify the amount of superwarfarin ingestion for which no gastric decontamination of prophylactic vitamin K is considered necessary.
Conclusion	Laboratory testing for coagulophathy was recommended for cases involving clinically evident bleeding abnormalities only.
Reliability	2, since no detailed report of the study was available.
Acceptability	Acceptable as supplementary information.
Remarks	None.

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

Section A6.12.4 Supportive data

Annex Point IIA6.9.4

The following reference is considered to contain additional information concerning the direct observation of acute, unintentional, pediatric superwarfarin ingestions and is thus presented in tabular format as supportive data:

Reference	Title	System/methods	Results
Cross-reference A6.12.2/02 Smolinske SC et al. (1989) PEDIATRICS 84 (3), September 1989, p 490-494 (published)	Superwarfarin poisoning in children: a prospective study.	110 patients younger than 12 years of age reported to a poison control centre with accidental superwarfarin ingestions; including patients who received dilution or gastrointestinal decontamination. No child received vitamin K. At least one prothrombin time ≥ 24 hours was recommended, and telephone follow-up was attempted.	Prothrombin times obtained 48 hours after ingestion were more likely to be prolonged than values obtained at 24 hours. The occurrence of an abnormal prothrombin time could not be predicted based on the history of amount ingested or on the presence of the characteristic green-blue product dye in or around the child's mouth. Acute toxicity was evidenced by transient abdominal pain, vomiting, and haeme positive stools in 2 patients. The authors recommended the determination of prothrombin time values 24- and 48 hours after a child has ingested a superwarfarin.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2 May 2005
Conclusion	The presentation of the above study as supportive data is accepted.
Remarks	None.

	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.12.5 Diagnosis of poisoning including specific signs of poisoning and clinical tests
Annex Point IIA6.9.5

Official use only

1 REFERENCE

1.1 Reference

A6.12.5/01:

Anonymous (1984) Shell rodenticide WL 108366 – Advice to physicians, medical specialists and poison information centres. Shell, Document No. 74.5279, November 1984 (published).

2 GUIDELINES AND QUALITY ASSURANCE
(NOT APPLICABLE)

3 MATERIALS AND METHODS
(NOT APPLICABLE)

4 RESULTS
(NOT APPLICABLE)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Test substance

Flocoumafen is sold ready-to-use as either a pellet bait or a wax block bait both containing 0.005% Flocoumafen. A 0.01% Flocoumafen bait concentrate available at the time of writing the reference is not anymore marketed. Flocoumafen in a more concentrated form is only to be found in the formulation plants that produce the baits and bait concentrate and in laboratories and testing stations that might have very small (gram) quantities for use either as analytical standards or in efficacy experiments.

5.2 Type of exposure

Ingestion, inhalation or dermal absorption of Flocoumafen can lead to severe intoxication when handling the concentrated products found within the formulation plants, laboratories and testing stations. However, for the ready-to-use baits containing 0.005% Flocoumafen, ingestion of, or contact with a large quantity would be required before a toxic effect is expected.

Section A6.12.5 Diagnosis of poisoning including specific signs of poisoning and clinical tests
Annex Point IIA6.9.5

5.3	**Mode of action**	Flocoumafen acts as competitive antagonist of vitamin K and may cause vitamin K_1 deficiency with an attendant deficiency of vitamin K-dependent clotting factors. The development of excessive hypoprothrombinaemia or the occurrence of bleeding is related to the individual turnover of these proteins, their half lives being 60 hours (prothrombin), 6 hours (factor VII), 24 hours (factor IX) and 40 hours (factor 10), rather than the dose level of the anticoagulant. The latency time is expected to be 36 – 72 hours. In view of the long biological half life, Flocoumafen is considered to exert a prolonged anticoagulant effect.
5.4	**Signs and symptoms of intoxication**	Signs and symptoms may vary from some small manifestations of bleeding, such as bruising easily, bleeding from the nose or gum, swelling and tenderness of joints, haematomas and haematuria. In severe cases, massive bleeding from internal organs may result in circulatory shock which may prove fatal. Since formulated Flocoumafen is always associated with water-soluble dye, exposition to Flocoumafen may be indicated by stain at the exposure site.
5.5	**Diagnosis of intoxication**	Initial screening for an increased tendency to bleed includes the measurement of bleeding time, platelet count, partial thromboplastin time and prothrombin time.
5.6	**Treatment**	If ingestion was recent then gastric lavage should be considered. Treatment in mild cases of poisoning: 1. measurement of prothrombin time and haemoglobin level in venous blood 2. administration of 10 mg vitamin K_1 (phytomenadione) orally four times per day 3. daily check of response to therapy If prothrombin time has stabilised within normal limits for three days the daily oral dose may be reduced to 10 mg vitamin K_1 twice a day. Continued treatment may be required for a period of at least 60 days. Treatment of severe cases of poisoning: 1. measurement of prothrombin time and haemoglobin level in venous blood 2. immediately administration of 20 mg vitamin K_1 (phytomenadione) by slow intravenous injection 3. check of response to therapy after 3 hours: if there has been no reduction of prothrombin time another 20 mg of vitamin K_1 should be given intravenously if prothrombin time starts to decrease, oral administration of 10 mg vitamin K_1 four times per day should be given 4. daily check of prothrombin time If prothrombin time has stabilised within normal limits for three days the daily oral dose may be reduced to 10 mg vitamin K_1 twice a day. Continued treatment may be required for a period of at least 60 days. In cases of severe bleeding, apart from vitamin K_1 medication and treatment of the hypovolaemic shock syndrome, the treatment may include blood component therapy.

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 May 2005
Materials and Methods	No comments.
Results and discussion	5.5: Considering the results of A6.12.5/02, one should consider to add the measurement of PIKVA II in blood to the diagnosis of intoxication for detecting early effects of flocoumafen on the coagulation system.
Conclusion	See 5.4, 5.5 and 5.6.
Reliability	0, since report is an advice, not a study report.
Acceptability	Acceptable.
Remarks	None.

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

Section A6.12.5 **Diagnosis of poisoning including specific signs of poisoning and clinical tests**
Annex Point IIA6.9.5

Official use only

1 REFERENCE

1.1 Reference

A6.12.5/02:

Vxxxx Sxxxx Nxxxx (1987) The selection of laboratory tests for the detection of effects on vitamin K dependent coagulation factors by the rodenticide Flocoumafen. Sxxxx Ixxxx Pxxxx Mxxxx Bxxxx, Txxxx Hxxxx, Txxxx Nxxxx, Report No. HSE 87-002, January 1987 (unpublished).

(BASF-Ref.: FL-452-013)

2 GUIDELINES AND QUALITY ASSURANCE

(NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1 Examinations

3.1.1 Coagulation assays

The Quick's prothrombin time test, the 'Thrombotest' and the 'Normotest' were compared using dilutions in 0.9% NaCl of a pooled plasma from 5 healthy persons. The Quick test and the 'Normotest' were further evaluated by determining the clotting times of serial dilutions form a citrated plasma pool taken from 13 healthy subjects.

In addition, the analytical variation of the 'Normotest' was determined by measuring 20 replicates of the same plasma and by measuring commercial plasma from one batch during 5 days. Inter-individual variation of the 'Normotest' was determined using 50 healthy individuals, while intra-individual variation was determined during five days, using two healthy subjects.

3.1.2 Prothrombin assays

In six human plasma samples, prothrombin was determined after activation with either prothrombinase, Taipan snake venom or Echis carinatus venom using optimal experimental conditions. The Taipan snake venom was found to be more suitable for testing than Echis carinatus venom and was used for the following assays. The biological variation of prothrombin determinations was investigated as described for the coagulation assays. The analytical variation was determined by measuring 8 replicates of the same plasma and by measuring plasma of one person during 7 different runs.

Prothrombin and prothrombin + PIVKA II concentrations were determined in samples from 14 patients treated with the oral anticoagulant sintrom [R].

3.2 Remarks

None

Section A6.12.5 **Diagnosis of poisoning including specific signs of poisoning and clinical tests**
Annex Point IIA6.9.5

4 RESULTS

4.1 Coagulation assays

All three coagulation assays were more suitable for measuring changes in the activity of coagulation factors below 50% than in the 100 – 50% normal range. Comparing the three assays, the 'Normotest' was the most sensitive test for measuring changes in the activity of coagulation factors in the normal range.

Testing of the analytical variation of the 'Normotest' showed a mean plasma activity of 96.4% and a coefficient of variation (C.V.) of 5.7% for the 'within-run' precision, while the mean plasma activity for the 'between-run' precision was 84.4%, C.V. 3.9%. The mean activity for inter-individual variation was 84.0% and the C.V. 14%. The C.V.'s for intra-individual variation determined during five days using two healthy subjects were found to be 7.9 and 13.6%, respectively. Thus, for the detection of a biologically significant effect on the coagulation system the difference in coagulation time between a person's pre-exposure value and any subsequent measurement should be substantial.

4.2 Prothrombin assays

The mean PIVKA II concentration determined by subtraction of the prothrombin concentration from the sum of the concentration of prothrombin and PIVKA II was 37% (range: 26 – 51%) in patients administered with sintrom ^{R}. The mean prothrombin concentration was 11% (range: 5 – 24%) in patients compared to 90.5% (range: 69 – 112%) in healthy subjects. The mean prothrombin/ prothrombin + PIVKA II ratio was determined in the same 14 patients to be 0.23 (range: 0.12 – 0.44) compared to 0.99 (0.92 – 1.05) in healthy subjects.

4.3 Other

No other significant observations were reported.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Several laboratory tests for the detection of effects on vitamin K dependent coagulation factors caused by Flocoumafen were evaluated. Three coagulation assays - the Quick's prothrombin time test, the 'Thrombotest' and the 'Normotest' - were compared. In addition, spectrophotometric prothrombin assays were performed.

Prothrombin was determined, by first converting it into thrombin by its physiological activator prothrombinase (factors Xa and Va, Ca^{2+} ions and phospholipids), and subsequent reaction of thrombin with a chromogenic substate. In addition, Taipan snake venom or Echis carinatus venom were used to generate thrombin activity from prothrombin as well as from PIVKA II in blood.

Section A6.12.5 Diagnosis of poisoning including specific signs of poisoning and clinical tests
Annex Point IIA6.9.5

5.2	**Results and discussion**	The results of the coagulation assays showed that the 'Normotest' was the most sensitive method to detect changes in the activity of coagulation factors in the normal range of 100 – 50%. However, this test was considered to be more suitable for measuring significant deviations from normal levels of vitamin K dependent coagulation factors than for measuring a gradual decline within normal levels, e.g. after occupational exposure to Flocoumafen. It was considered that at normal prothrombin concentrations the spectrophotometric assay for determining the ratio prothrombin/ prothrombin + PIVKA II may detect PIVKA II at concentrations ≥ 10%.
5.3	**Conclusion**	The following two tests were recommended for monitoring effects on the coagulation system produced by exposure to Flocoumafen: 1. The 'Normotest' was found to be suitable for detecting effects possibly leading to acute health effects, and 2. for detection of early effects of exposure to Flocoumafen the determination of PIVKA II (the decarboxylated precursor of prothrombin) was recommended by measureing either by subtraction or by calculating the ratio prothrombin/ prothrombin + PIVKA II was suggested.

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 May 2005
Materials and Methods	No comments.
Results and discussion	No comments.
Conclusion	No comments.
Reliability	2 , since at the study was not performed under GLP conditions.
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.12.5 Supportive data

Annex Point IIA6.9.5

The following references are considered to contain additional information concerning the detection of minimal intoxication with vitamin K or unintentional pediatric superwarfarin exposure and are thus presented in tabular format as supportive data (non-GLP studies):

Reference	Title	System/methods	Results
A6.12.5/03 Hxxxx Hxxxx, Dxxxx Pxxxx (1988) Sxxxx Axxxx Rxxxx Axxxx, Report, January 15, 1988 (unpublished). (BASF-Ref.: FL-452-014)	Report on the development of a test to detect minimal intoxication with vitamin K antagonists.	Human serum collected from a healthy volunteer or from a patient under deep stable anticoagulant treatment	Since the first biochemical sign of vitamin K deficiency and intoxication by vitamin K antagonists intake is the occurrence of uncarboxylated forms of clotting proteins in the plasma, the authors developed a test that indicates the presence of such proteins. This test was based upon the action of staphylocoagulase on carboxylated prothrombin in serum.
A6.12.5/04 Mullins ME et al. (2000) PEDIATRICS 105 (2), p402-404, February 2000	Unintentional pediatric superwarfarin exposures: Do we really need a prothrombin time?	Review of poison center charts: 398 consecutive pediatric SWR exposures (January 1993 to December 1994 during which follow-up prothrombin measurements were recommended at 24 and 48 hours); and for 144 consecutive pediatric SWR exposures (January 1996 to December 1997 during which a single PT measurement at 48 hours was recommended)	Of 542 children in 4 years of data collection, follow-up prothrombin times and/or international normalised ratios measurements did not detect any significant coagulation abnormalities. No child developed bleeding complications. No child required or received antidotal treatment with vitamin K. Thus, the authors concluded that medical attention is needed only in the unlikely occurrence of unusual bleeding or bruising. Only intentional ingestions, ingestions involving suspected child abuse or neglect, and ingestions by children with abnormal neurological or psychological development warrant close follow-up and laboratory monitoring.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2 May 2005
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	None.

	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.12.6 Sensitisation/ allergenicity observations

Annex Point IIA6.9.6

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	According to chapter 2 of the TNsG on common core data requirements, information on the sensitisation/allergenicity of workers and others exposed must be provided and included, and where relevant, any incidence of hypersensitivity. It is further expressly mentioned, that any such evidence that a substance can induce specific respiratory hypersensitivity will usually be based on human experience data. The clinical history data including both medical and occupational history, and reports from appropriate lung functions tests related to exposure to the substance should be submitted, if available. However, the high oral, dermal and inhalation toxicity associated with "pure" Flocoumafen implicitly requires the extent of exposure of humans to be at an absolute minimum. Thus, for basic exposure considerations, any relevant potential for development of allergenicity or sensitisation can be practically ruled out	
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 May 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substances and biocidal products, data on sensitisation/allergenicity should be submitted if available, e.g. data on health effects of workers or epidemiological data. Within the available human data on within chapter A6.12, no indications for sensitisation or allergenicity are available. Furthermore, there are no indications for sensitizing properties of flocoumafen and Storm BB, in available animal experimental data.
Conclusion	Non-submission of data is accepted.
Remarks	None.

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

Section A6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

Annex Point IIA6.9.7

Official use only

1 REFERENCE

1.1 Reference

A6.12.7/01:

Anonymous (1987) Storm rodenticide – Advice to physicians, medical specialists and poison information centres. Shell International Chemical Company Ltd., London, July 1987 (published).

(BASF-Ref.: FL-190-003)

2 GUIDELINES AND QUALITY ASSURANCE

(NOT APPLICABLE)

3 MATERIALS AND METHODS

(NOT APPLICABLE)

4 RESULTS

(NOT APPLICABLE)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Test substance

Flocoumafen is sold ready-to-use as either a molasses-bound pellet bait or a wax block bait both containing 0.005% Flocoumafen. Flocoumafen in a more concentrated form is only to be found in the formulation plants that produce the baits and bait concentrate and in laboratories and testing stations that might have very small (gram) quantities for use either as analytical standards or in efficacy experiments.

5.2 Type of exposure

Ingestion, inhalation or dermal absorption of Flocoumafen can lead to severe intoxication when handling the concentrated products found within the formulation plants, laboratories and testing stations. However, for the ready-to-use baits containing 0.005% Flocoumafen, ingestion of, or contact with a large quantity would be required before a toxic effect is expected.

Section A6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment
Annex Point IIA6.9.7

5.3	**Mode of action**	Similar to 4-hydroxy-coumarin derivatives, Flocoumafen rodenticide is an indirect acting anticoagulant. It inhibits the metabolism of vitamin K1 and thus depletes vitamin K1 dependent clotting factors in plasma. (Factors II, VII, IX and X, respectively). Differing from the widely used coumarin drugs, Flocoumafen has along biological half life in the liver. In the absence of vitamin K1, biologically inactive precursors of the clotting factors appear in the circulating plasma in humans. (PIVKA = Protein Induced by Vitamin K1 Absence/Antagonist)
5.4	**Signs and symptoms of intoxication**	The latency time after complete blocking of the synthesis of clotting factors is expected to be 36-72 hours, at which time an increased bleeding tendency occurs such as bruising, nose or gum bleeds, haematuria and haematomas. In severe cases, massive bleeding from internal organs could result in circulatory shock which may prove fatal. If the synthesis of the clotting factors is only partially inhibited, an increased bleeding tendency may not ensue.
5.5	**Diagnosis of intoxication**	Full screening for the presence of an increased bleeding tendency includes: bleeding time, platelet count, partial prothrombin time (PTT) and prothrombin test (PT). Clinical signs and symptoms are not suitable for assessment of the risk of bleeding in persons occupationally exposed to Flocoumafen since this may only occur when the circulating clotting factors have been reduced to 10-15% of the normal blood levels. A specific and appropriate test for medical surveillance of workers in manufacturing and formulation plants is the determination of inactive Factor II precursor.
5.6	**Treatment**	Treatment in mild cases of poisoning: 1. measurement of prothrombin time and haemoglobin level in venous blood 2. administration of 10 mg vitamin K_1 (phytomenadione) orally four times per day 3. daily check of response to therapy If prothrombin time has stabilised within normal limits for three days the daily oral dose may be reduced to 10 mg vitamin K_1 twice a day. Continued treatment may be required for a period of at least 60 days. Treatment of severe cases of poisoning: 1. measurement of prothrombin time and haemoglobin level in venous blood 2. immediately administration of 20 mg vitamin K_1 (phytomenadione) by slow intravenous injection 3. check of response to therapy after 3 hours: if there has been no reduction of prothrombin time, another 20 mg of vitamin K_1 should be given intravenously; if prothrombin time starts to decrease, oral administration of 10 mg vitamin K_1 four times per day should be given 4. daily check of prothrombin time If prothrombin time has stabilised within normal limits for three days the daily oral dose may be reduced to 10 mg vitamin K_1 twice a day. Continued treatment may be

Section A6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment

Annex Point IIA6.9.7

required for a period of at least 60 days.

In cases of severe bleeding, apart from vitamin K_1 medication and treatment of the hypovolaemic shock syndrome, the treatment may include blood component therapy. However, blood components are only to be used if the effect of vitamin K1 therapy cannot be awaited.

Subject to availability, the options are as follows:

1. Whole blood, which is considered to be a very poor component to use in view of both decay of pro-coagulants and of volume.
2. Blood plasma (cryosupernatant or fresh frozen), containing all the pro-coagulants, but the use carries a risk of circulatory overload.
3. Prothrombin complex concentrates
   These are concentrates of prothrombin and factors VII, IX and X and were developed to treat patients with deficiencies of the vitamin K1-dependent complex. These concentrates were considered to be particularly useful for rapid correction of haemostatis in patients with acute haemorrhage and may be preferable in patients at risk of volume overload. However, the use of prothrombin complex concentrates introduces a hazard because of possible contamination with hepatitis B- and human immunodeficiency virus and because of the danger of introducing thromboembolism in the recipient.

Following recovery from a severe case of poisoning surgical intervention to evacuate blood from e.g. joints, may be indicated.

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 May 2005
Materials and Methods	No comments.
Results and discussion	No comments.
Conclusion	No comments.
Reliability	0, since report is an advice, not a study report.
Acceptability	Acceptable.
Remarks	Advice is equal to advise given in A6.12.5/01, with the exception that within this advice the determination of inactive Factor II precursor is recommended.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.12.7
Annex Point IIA6.9.7

Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment
–Supportive data–

The following references are considered to contain additional information concerning the specific treatment in case of an accident or poisoning in children and are thus presented in tabular format as supportive data:

Reference	Title	System/methods	Results
A6.12.7/02 Kanabar D, Volans G (2002) THE LANCET 360, September 28, 2002, p963 (published)	Accidental superwarfarin poisoning in children – less treatment is better	Review article including experience with unintentional superwarfarin ingestions of children recorded at the National Poisons Unit for London (NPIS).	The authors concluded that single-substance ingestion of superwarfarins can be safely managed by observation alone without the need for gastric decontamination, laboratory work-up or prophylactic vitamin K. Procedures to empty the stomach in cases of childhood poisoning (i.e. syrup of ipecacuanha) should no longer be used. Gastric lavage and single-dose activated charcoal should be used within an hour of the suspected time of ingestion.
Cross-reference A6.12.2/01 Ingels M et al. (2002) Annals of Emergency Medicine 40 (1), July 2002, p 73-78 (published)	A prospective study of acute, unintentional, pediatric superwarfarin ingestions managed without decontamination.	545 patients younger than 6 years of age reported to poison control centres with acute unintentional superwarfarin ingestions; excluding patients who received gastrointestinal decontamination or prophylactic vitamin K. Forty-eight- to 96-hour prothrombin time or normalised ratio (INR) blood tests were recommended, and telephone contact was attempted at least 3 days after ingestion.	The authors concluded that children with acute unintentional superwarfarin ingestions of less than 1 box may be managed without gastric decontamination or prophylactic vitamin K. Laboratory testing for coagulophathy was recommended for cases involving clinically evident bleeding abnormalities only.
Cross-reference A6.12.2/02 Smolinske SC et al. (1989) PEDIATRICS 84 (3), September 1989, p 490-494 (published)	Superwarfarin poisoning in children: a prospective study.	110 patients younger than 12 years of age reported to a poison control centre with accidental superwarfarin ingestions; including patients who received dilution or gastrointestinal decontamination. No child received vitamin K. At least one prothrombin time ≥ 24 hours was recommended, and telephone follow-up was attempted.	Prothrombin times obtained 48 hours after ingestion were more likely to be prolonged than values obtained at 24 hours. The authors recommended the determination of prothrombin time values 24- and 48 hours after a child has ingested a superwarfarin.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2 May 2005
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.12.8 Prognosis following poisoning

Annex Point IIA 6.9.8

		Official use only
	Typical features of poisoning result from increased bleeding tendency and include: - minor poisoning: coagulation disturbance detected only by laboratory analyses, - moderate poisoning: coagulation disturbance resulting in haematomata, haematuria, blood in faeces or excessive bleeding from minor cuts or abrasions, gum bleeding, - severe poisoning: retroperitoneal haemorrhage, severe gastrointestinal bleeding, cerebrovascular accidents, massive haemorrhage (internal bleeding) resulting in shock. If anaemia or liver disease is present then the above features may be more severe and persistent and the poisoning may be more difficult to control (IPCS, EHC 175, ref. A5.4/03).	

	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 May 2005	
Reliability	0, not applicable. Above statement is derived from several studies and reports within the dossier of flocoumafen.	
Acceptability	Acceptable.	
Remarks	None.	
	COMMENTS FROM ...	
Date		
Materials and Methods		
Results and discussion		
Conclusion		
Reliability		
Acceptability		
Remarks		

Section A6.13 Toxic effects on livestock and pets

Annex Point IIIA6.2

Introduction

The following studies are available concerning toxic effects on livestock or pets, and were conducted assuming that the predominant risk is from ingestion of baits or of secondary poisoning:

(1) Acute oral toxicity was tested in Beagle dogs (A6.13/01), cats (A6.13/02), pigs (A6.13/03), goats (A6.13/07) and sheep (A6.13/08). The studies conducted with goats or sheep were provided as supportive data, since equivocal responses were observed. The following LD_{50} values were obtained: 0.075–0.25 mg/kg b.w. (Beagle dogs), > 10 mg/kg b.w. (cats) and approx. 60 mg/kg (pigs).

(2) The dietary LD_{50} value of Flocoumafen in laying hens following a 5-day treatment period and a 28-day observation period was determined to be 16.4 ppm (95% CI = 8.5–42.1 ppm), (A6.13/04). Birds which died during the study showed signs of haemorrhaging. No other treatment-related abnormalities were reported upon necropsy.

(3) In addition, the elimination of radioactivity in excreta and total ^{14}C residues in eggs and in liver tissue was tested in laying hens receiving 1 or 4 mg/kg b.w./day of ^{14}C-Flocoumafen for five days followed by a 15-day postexposure period (A6.13/05). In both dose groups, about 68 % of the daily administered dose was eliminated via the excreta within 24 hours and the radioactive residues present in the livers accounted for less than 1 % of the cumulative dose. In contrast, about 74 to 79 % of a single administered dose retained in rats receiving a single oral dose of 0.14 mg/kg, with half of the dose retained located in the liver. Elimination via faeces and urine accounted for 23–26 % or less than 0.5 % of the administered dose, respectively (A6.2/02).

(4) Further, the effectiveness of vitamin K_1 therapy in Beagle dogs as an antidote to single exposure by Flocoumafen was shown (A6.13/06).

(5) Several references are considered to contain additional information concerning toxic effects on livestock and pets and are thus presented in tabular format as supportive data (A6.13/07–A6.13/15)

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	3 May 2005
Conclusion	Above summary represent the conclusions drawn for each individual study. For each study comments are given by the RMS for each study at the individual summaries.
Remarks	It should be noted that based on the product label bait should be placed in protected spaces, protected for children and animals. Furthermore, it is stated in Section B5 that baits should be deployed at protected baiting points, e.g. commercially available tamper-resistant bait containers, small pieces of piping, active rat and mouse holes, and other places to be covered by slates, boards or similar suitable material. The recommended bait boxes are inaccessible without use of a special key. When the product Storm BB is used according to the use instructions given in the product label, primary poisoning (from direct consumption of Storm BB) might occur only incidentally. However, secondary poisoning (from consumption of poisoned rodents) cannot be excluded.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.13 Acute oral toxicity to Beagle dogs

Annex Point IIIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.13/01:** Cxxxx Hxxxx, Bxxxx Pxxxx, Hxxxx Rxxxx, Hxxxx Rxxxx (1984) WL 108366 rodenticide – acute oral toxicity in Beagle dogs. Hxxxx Rxxxx Cxxxx, Hxxxx, Uxxxx, Report No. SLL 72/84757, November 12, 1984 (unpublished). (BASF-Ref.: FL-411-010)	
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 The conduct of the study was consistent to EU method B.1 (92/69/EEC) in all important aspects.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	X
3.1.1	Lot/Batch number	23.3.84.2	
3.1.2	Specification	Not stated in the report.	
3.1.3	Purity	Not stated in the report.	X
3.1.4	Description	White powder	
3.1.5	Stability	Not stated in the report.	
3.2	**Test animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	Huntingdon Research Centre	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 27 to 35 weeks (males), 28 to 39 weeks (females) Body weight: 9.6 to 11.1 kg (males), 9.2 to 11.0 kg (females)	

Section A6.13 Acute oral toxicity to Beagle dogs

Annex Point IIIA6.2

3.2.6	Number of animals per group	1 male and 1 female
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral (single)
3.3.1	Post-exposure period	28 days
3.3.2	Type	By gavage
3.3.3	Concentration	0.025, 0.075, 0.25 or 1.00 mg/kg b.w.
3.3.4	Vehicle	Corn oil
3.3.5	Concentration in vehicle	0.0005 to 0.02 % solution in corn oil
3.3.6	Total volume applied	5 ml/kg b.w.
3.3.7	Controls	Not applicable
3.4	**Examinations**	Clinical examinations (regularly throughout the study); Body weights (twice a week); Food consumption (daily); Gross pathology and organ weights upon necropsy.
3.5	**Method of determination of LD_{50}**	Not applicable
3.6	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.13- 1. Male and female dogs treated with 0.25 or 1.00 mg/kg b.w. of Flocoumafen were sacrificed non-scheduled for humane reasons between day 4 and day 9 after dose administration. Clinical signs recorded prior to sacrifice included retching, unsteady gait, laboured breathing, unwillingness to move and severe pallor of the gums. Slight losses in body weight associated with a reduction in food consumption were observed in high dose animals and females of the 0.25 mg/kg dose group prior to non-scheduled sacrifice. No adverse effects on body weight or food consumption were observed in the 0.025 and the 0.075 mg/kg b.w. dose groups. In addition, no treatment-related signs of toxicity were observed in these dose groups.

Section A6.13 Acute oral toxicity to Beagle dogs

Annex Point IIIA6.2

4.2	**Pathology**	Macroscopic post mortem examination of dogs sacrificed non-scheduled showed widespread haemorrhaging throughout the body which was found to be especially marked in the thoracic cavity for three of the four animals sacrificed non-scheduled and was associated with a general pale appearance of all tissues. No treatment-related abnormalities were recorded upon necropsy of dogs administered with 0.025 or 0.075 mg/kg b.w. Organ weights were within normally accepted limits for all animals tested.
4.3	**Other**	None
4.4	**LD_{50}**	Based on the non-scheduled sacrifices, the following range for the LD_{50} value is considered: 0.075–0.25 mg/kg b.w.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in Beagle dogs. Groups of 1 male and 1 female dog received 0.025 to 1.00 mg/kg b.w. of Flocoumafen in corn oil orally by gavage. Although not a guideline study, the method used was consistent to method B.1 (92/69/EEC) in all important aspects. No relevant deviations from the prescribed guideline were reported.
5.2	**Results and discussion**	All animals treated with 0.25 or 1.00 mg/kg b.w. of Flocoumafen were sacrificed non-scheduled for humane reasons between day 4 and day 9 after dose administration. Clinical signs recorded prior to sacrifice included retching, unsteady gait, laboured breathing, unwillingness to move and severe pallor of the gums. Macroscopic post mortem examination showed widespread haemorrhaging throughout the body which was found to be especially marked in the thoracic cavity for three of the four animals and was associated with a general pale appearance of all tissues. No treatment-related effects were observed in animals dosed with 0.025 or 0.075 mg/kg b.w..
5.3	**Conclusion**	Based on these results, the LD_{50} value can be expected in the range of 0.075 to 0.25 mg/kg b.w.
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	3 May 2005
Materials and Methods	3.1: The study was performed with WL108366 a former code number for flocoumafen.. 3.1.3: The purity of the test substance was as other used test material in A6.13 >90%.
Results and discussion	No comments.
Conclusion	In dogs, the oral LD_{50} of flocoumafen was found to be between 0.075 and 0.25 mg/kg bw.
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.13- 1: Acute toxicity in male and female beagle dogs.

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
1.00	2/2	day 8–9	Unsteady gait, unwilling to move, severe pallor of the gums, laboured breathing, blood passed from the nostrils, retching
0.25	2/2	day 4–5	Pale gums, laboured breathing, unwilling to move
0.075	0/2	–	–
0.025	0/2	–	Single incident of liquid faeces, no further reaction to treatment
LD_{50} value	0.075–0.25 mg/kg b.w.		

Section A6.13 Acute oral toxicity to cats

Annex Point IIIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.13/02:** Rxxxx Nxxxx, Cxxxx Dxxxx, Sxxxx Axxxx (1986) WL108366 – Acute oral toxicity to cats. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 81/851385, January 23, 1986 (unpublished). (BASF-Ref.: FL-411-011)	
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 The conduct of the study was similar to method B.1 (92/69/EEC).	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2.	X
3.1.1	Lot/Batch number	ST85/097	
3.1.2	Specification	As given in Section A2, apart from purity stated below.	
3.1.3	Purity	95.5%	
3.1.4	Description	Off-white powder	
3.1.5	Stability	Not stated in the report.	
3.2	**Test animals**		
3.2.1	Species	Cat	
3.2.2	Strain	Not stated in the report.	
3.2.3	Source	Interfauna Ltd., Houghton, UK (males); University of Manchester Medical School, Manchester, UK (females)	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 8–12 month Body weight: 2.82–3.45 kg (males), 2.55-2.66 kg (females)	

Section A6.13 Acute oral toxicity to cats

Annex Point IIIA6.2

3.2.6	Number of animals per group	1 male and 1 female
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Post-exposure period	21 days
3.3.2	Type	Gavage
3.3.3	Concentration	5, 10, 10 mg/kg
3.3.4	Vehicle	Corn oil
3.3.5	Concentration in vehicle	0.5, 1.0, 1.0 % w/v
3.3.6	Total volume applied	1 ml/kg b.w.
3.3.7	Controls	Not applicable
3.4	**Examinations**	Clinical signs (twice daily), Body weights (initial (day 0), day 3, 6, 9, 15 and 21), Food consumption (all food refusals were recorded), Haematology (prior to dosing, daily for the first 6 days after dosing, and every third day thereafter investigating the prothrombin times) Gross pathology upon necropsy.
3.5	**Method of determination of LD_{50}**	Estimation on basis of the mortalities observed
3.6	**Further remarks**	At first, one male and one female cat were dosed with 10 mg/kg of Flocoumafen. Since the female cat was found to be pregnant, two further groups of one male and one female receiving 5 or 10 mg/kg of Flocoumafen were investigated.

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.13- 2. At first, one male and one female cat were dosed with 10 mg/kg of Flocoumafen. On the fourth day after dosing the female cat showed subdued behaviour, inactivity, bloodstained discharge at the vulva, pale mucous membranes, slightly distended abdomen and dried and fresh blood was found on the pen floor and sleeping box. This animal was found dead on day 5. The male cat and all cats of further two groups administered with 5 or 10 mg/kg remained in good health throughout the study and no clinical signs of toxicity were noted. No treatment-related effects on body weight or food consumption were observed in surviving cats.

Section A6.13 Acute oral toxicity to cats
Annex Point IIIA6.2

4.2	**Prothrombin times**	In all cases prothrombin times increased after dosing with Flocoumafen, reaching maximum values on day 3 or 4. Prothrombin times in surviving cats decreased thereafter, reaching normal levels five to nine days after dosing. At one subsequent sampling time elevated prothrombin times occurred in three of the five cats, but were determined to be normal immediately before and after this sampling point.
4.3	**Pathology**	Gross necropsy of the female cat dying on day 5 revealed that this cat was pregnant (5 foetuses present; stage of gestation approximately 4 to 5 weeks). The uterus was reported to be grossly distended with free blood due to placental haemorrhage, which was considered to be the immediate cause of death. No other abnormalities were noted. No macroscopic abnormalities were observed in all surviving cats.
4.4	**Other**	None
4.5	**LD_{50}**	Based on the obtained results, the LD_{50} value is expected to be above the maximum tested dose: males, females: > 10 mg/kg b.w.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in cats. One male and one female cat received 10 mg/kg of Flocoumafen orally by gavage. Since the female cat was found to be pregnant, two further groups of one male and one female receiving 5 or 10 mg/kg of Flocoumafen were investigated. Although not a guideline study, the method used was similar to method B.1 (92/69/EEC). No relevant deviations to the prescribed guideline were reported.
5.2	**Results and discussion**	At first one male and one female cat were dosed with 10 mg/kg of Flocoumafen. On the fourth day after dosing the female cat showed subdued behaviour, inactivity, bloodstained discharge at the vulva, pale mucous membranes, slightly distended abdomen and dried and fresh blood was found on the pen floor and sleeping box. This animal was found dead on day 5. Gross necropsy revealed that this cat was pregnant (5 foetuses present; stage of gestation approximately 4 to 5 weeks). The uterus was reported to be grossly distended with free blood due to placental haemorrhage, which was considered to be the immediate cause of death. The male cat and all cats of further two groups administered with 5 or 10 mg/kg remained in good health throughout the study and no clinical signs of toxicity were noted. In addition, no treatment-related effects on body weight or food consumption were observed in surviving cats and no macroscopic abnormalities were observed. Temporary, not treatment-related elevations of prothrombin times were noted in all cats.
5.3	**Conclusion**	Based on these results, the LD_{50} value is expected to be above the maximum tested dose of 10 mg/kg b.w..
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	3 May 2005
Materials and Methods	3.1: The study was performed with WL108366 a former code number for flocoumafen.
Results and discussion	5.2: The applicant stated that temporary not-treatment related elevations of prothrombin times were noted in all cats. Elevations in prothrombin time (3 to 5 times normal levels) were treatment-related, but not dose related.
Conclusion	In cats, the oral LD_{50} of flocoumafen was found to be > 10 mg/kg bw. Increased prothrombin times were noted in animals dosed with 5 and 10 mg/kg bw.
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable.
Remarks	It should be noted that the oral LD_{50} for flocoumafen in cats is rather high when compared to the oral LD_{50}'s determined for rats, mice, rabbits, gerbils and dogs.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.13- 2: Acute toxicity in male and female cats

Dose [mg/kg]	Number of dead/ number investigated	Time of death	Observations
10	1*/2	day 5	subdued, inactive, dried fresh blood on the pen floor, bloodstained discharge at the vulva, pale mucous membranes, abdomen slightly distended
10	0/2	–	–
5	0/2	–	–
LD_{50} value	> 10 mg/kg		

* (animal was found to be pregnant)

Section A6.13 Acute oral toxicity in pigs

Annex Point IIIA6.2

Official use only

1 REFERENCE

1.1	**Reference**	**A6.13/03:** Rxxxx Nxxxx, Cxxxx Dxxxx, Sxxxx Axxxx (1985) WL 108366 – Acute oral toxicity to pigs. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No.: SLL 76/851108, June 28, 1985 (unpublished). (BASF-Ref.: FL-411-013)
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 The conduct of the study was similar to method B.1 (92/69/EEC).
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	SA/10308, SA/10377
3.1.2	Specification	As given in Section A2, apart from purity stated below.
3.1.3	Purity	> 97%
3.1.4	Description	Not stated
3.1.5	Stability	Not stated
3.2	**Test animals**	
3.2.1	Species	Pig
3.2.2	Strain	Large White hybrid pigs
3.2.3	Source	R. Beedles, Shadymoor, Dorrington, UK
3.2.4	Sex	Male (castrate) and female
3.2.5	Age/weight at study initiation	Age: not stated Body weight: 37–55 kg (males), 36–51 kg (females)

Section A6.13 Acute oral toxicity in pigs

Annex Point IIIA6.2

3.2.6	Number of animals per group	1 male and 1 female
3.2.7	Control animals	None
3.3	**Administration/ Exposure**	Oral
3.3.1	Post-exposure period	28 days
3.3.2	Type	By gavage
3.3.3	Concentration	10, 30, 60 or 90 mg/kg
3.3.4	Vehicle	Corn oil
3.3.5	Concentration in vehicle	1.0, 3.0, 6.0, 9.0 % w/v
3.3.6	Total volume applied	1 ml/kg b.w.
3.3.7	Controls	Not applicable
3.4	**Examinations**	Clinical examinations (twice daily), Body weight (initial, day 3, 7, 10, 14, 17, 21, 24 and 28), Food consumption (all feed refusals were recorded), Haematology (regularly investigating plasma prothrombin times), Gross pathology and liver weights upon necropsy
3.5	**Method of determination of LD_{50}**	Estimation on basis of the mortalities observed
3.6	**Further remarks**	None

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.13- 3. Both pigs of the low dose group and male pigs of the 30 and 60 mg/kg dose groups showed no treatment-related clinical signs. The female pig receiving 30 mg/kg showed subdued behaviour, reduced food consumption and intermittent bleeding from a cut on the right hind leg during day 6 to 13 after dosing. The animal appeared normal thereafter. In the 60 mg/kg dose group, the female appeared subdued and inactive eight days after dosing and a large haematoma at the site of blood sampling as well as slight bleeding at the ear tag were noted. The animal was found dead on day 9. Both animals in the high dose group were sacrificed non-scheduled after showing lethargy. Feed refusals (from 15 to 90% of feed offered) were noted for the female dosed at 30 mg/kg on days 6, 7, 10, 11 and 12 after dosing. All other pigs consumed all feed offered throughout the test period. Body weight changes were reported to be within normal limits, although overall weight gains in the low dose group were higher than those for surviving pigs in other treatment groups.

Section A6.13 Acute oral toxicity in pigs

Annex Point IIIA6.2

4.2	**Prothrombin times**	Both pigs of the low dose group showed increased prothrombin times three days after dosing which decreased thereafter. After dosing at 30 mg/kg, the highest prothrombin times were measured 6 days after dosing. A decrease to normal levels by day 21 was observed thereafter. Similar results were recorded for the surviving animal of the 60 mg/kg dose group. In animals which died or were sacrificed non-scheduled, markedly increased prothrombin times were observed prior to death.
4.3	**Pathology**	Upon necropsy of pigs which died or were sacrificed non-scheduled, extensive haemorrhage was noted, particularly in association with the jugular furrow at the site of blood sampling. Except for a subcutaneous haematoma noted in one animal, no gross pathological changes were observed in any of the five surviving pigs examined upon study termination.
4.4	**Other**	None
4.5	**LD_{50}**	The results indicate that the LD_{50} value is approximately 60 mg/kg.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of Flocoumafen was tested in Large White hybrid pigs. Although not a guideline study, the method used was similar to method B.1 (92/69/EEC). No relevant deviations to the prescribed guideline were reported.
5.2	**Results and discussion**	No treatment-related effects apart from temporary changes in prothrombin times were noted in both animals of the low dose group. At 30 mg/kg, minimal adverse clinical signs occurred in one animal only and elevations in prothrombin times were more marked and of longer duration. In one pig dosed at 60 mg/kg and both animals dosed at 90 mg/kg severe clinical toxicity and death occurred following a marked increase in prothrombin times. Necropsy of pigs which died or were sacrificed non-scheduled revealed extensive haemorrhage particularly in association with the jugular furrow at the site of blood sampling.
5.3	**Conclusion**	These results indicate a LD_{50} value of approximately 60 mg/kg.
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	3 May 2005
Materials and Methods	3.1: The study was performed with WL108366 a former code number for flocoumafen.
Results and discussion	No comments.
Conclusion	In pigs, the oral LD_{50} of flocoumafen was found to be approximately 60 mg/kg bw. A dose-related increase in prothrombin times was noted in animals at all dose levels (10, 30, 60 and 90 mg/kg bw).
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.13- 3: Acute toxicity in male and female pigs

Dose [mg/kg]	**Number of dead/ number investigated**	**Time of death**	**Observations**
10	0/2	–	–
30	0/2	–	Subdued behaviour, intermittent bleeding from a cut on the right hind leg
60	1/2	day 9	Subdued and inactive behaviour, large haematoma at the site of blood sampling, slight bleeding at the ear tag
90	2/2	day 6–7	Inactive, lethargic, unable to stand
LD_{50} value	approximately 60 mg/kg		

Section A6.13 Dietary toxicity of Flocoumafen to laying hens

Annex Point IIIA6.2

Official use only

1 REFERENCE

1.1	**Reference**	**A6.13/04:** Hxxxx Bxxxx, Rxxxx Mxxxx (1990) The dietary toxicity of WL 108366 to broiler chickens and laying hens. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 156/891981, June 1, 1990 (unpublished). (BASF-Ref.: FL-505-018)
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 The conduct of the study was similar to OECD 205 (1984), with the exception that the two control groups contained less than 10 birds each and that four instead of five treatment levels were investigated.
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	5003
3.1.2	Specification	As given in Section A2, apart from purity stated below.
3.1.3	Purity	> 90%
3.1.4	Description	Off-white powder
3.1.5	Stability	Not stated in the report.
3.2	**Test animals**	
3.2.1	Species	*Gallus domesticus*
3.2.2	Strain	ISA Brown
3.2.3	Source	Elemby Farm Eggs Ltd., Peterborough, UK
3.2.4	Sex	Female

Section A6.13 Dietary toxicity of Flocoumafen to laying hens

Annex Point IIIA6.2

3.2.5	Age/weight at start of the 14-day pre-treatment period	Age: approx. 30 weeks Body weight: 1805–2190g
3.2.6	Number of animals per group	5 females
3.2.7	Control animals	Yes, two groups of 5 females each.
3.3	**Administration/ Exposure**	Oral
3.3.1	Exposure period	5 days
3.3.2	Post-exposure period	28 days
3.3.3	Type	Dietary
3.3.4	Concentration	1.5, 5.0, 15.0 or 50.0 ppm (two groups of 5 hens per dose level)
3.3.5	Vehicle	Flocoumafen was dissolved in acetone and mixed with the basal diet.
3.3.6	Concentration in vehicle	Not stated in the report.
3.3.7	Total volume applied	Not stated in the report.
3.3.8	Controls	Yes, 10 individuals fed on blank diet, group allocation as specified under 3.3.4.
3.4	**Examinations**	Clinical examinations and mortality (daily) Number of eggs laid per group (daily) Body weight (pre-treatment and on days 5, 12, 19, 26 and 33) Group food consumption (daily during dosing and weekly thereafter) Gross pathology upon necropsy
3.5	**Method of determination of LD_{50}**	Probit analysis using maximum likelihood program, Lawes Agricultural Trust, 1985
3.6	**Further remarks**	An additional study with broiler chicks (strain Cobb) was performed prior to the described study with laying hens. Groups of 9–10 chicks received a single dose of 50, 100 or 150 ppm of Flocoumafen. Four chicks out of ten dosed with 50 ppm, eight chicks out of nine dosed with 100 ppm and five chicks out of nine dosed with 150 ppm of Flocoumafen died during the study. Dose levels for the dietary study with laying hens were selected on basis of these results. Samples of eggs, liver, breast muscle, leg muscle, abdominal fat and skin and fat were stored at –20°C prior to despatch to Bxxxx Rxxxx Lxxxx for determination of Flocoumafen residues. The results were not given in this reference and the report by Bxxxx Rxxxx Lxxxx is not available. However, the results are cited in reference A6.13/10.

Section A6.13 **Dietary toxicity of Flocoumafen to laying hens**
Annex Point IIIA6.2

4 RESULTS

4.1	**Clinical signs**	Mortalities and observations are presented in Table A6.13- 4. Mortalities occurred between day 3 and day 8. Most animals remained healthy throughout the study. One hen dosed with 5.0 ppm of Flocoumafen showed swollen eyes and subdued behaviour prior to death. Two animals of the 15.0 ppm group and one animal of the 50.0 ppm were subdued and unsteady.
4.2	**Egg production**	A slight reduction in the number of eggs laid per bird was evident in the 50 ppm group during the 28 day observation period. No treatment-related effects on mean egg weights were observed.
4.3	**Pathology**	Birds which died during the study showed signs of haemorrhaging. No abnormalities were observed in animals sacrificed upon study termination, with exception of a broken right leg in a control animal. In addition, a liver abnormality in one bird of the 1.5 mg/kg dose group was reported in reference A6.13/10.
4.4	**Other**	Body weights decreased during the 5-day treatment period in all groups receiving Flocoumafen in the diet, with the largest decrease observed in the highest dose group. No evidence of any treatment-related effect during the post-treatment period was noted. No treatment-related effect on food consumption was recorded during the study.
4.5	**LC_{50} (dietary)**	16.4 ppm (95% CI = 8.5–42.1 ppm)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The dietary toxicity of Flocoumafen to laying hens was determined following a 5-day treatment period and a 28-day observation period. Although not a guideline study, the method used was similar to OECD 205 (1984), with the exception that the two control groups contained less than 10 birds each and that four instead of five treatment levels were investigated.
5.2	**Results and discussion**	Mortalities occurred between day 3 and day 8. Most animals remained healthy throughout the study. Clinical signs observed during the study included swollen eyes, subdued behaviour and three animals appeared unsteady. A slight reduction in the number of eggs laid per bird was evident in the 50 ppm group during the 28 day observation period. No treatment-related effects on mean egg weights were observed. No treatment-related abnormalities were observed in animals sacrificed upon study termination. Birds which died during the study showed signs of haemorrhaging. Body weights decreased during the 5-day treatment period in all groups receiving Flocoumafen in the diet, with the largest decrease observed in the highest dose group. No evidence of any treatment-related effect during the post-treatment period was noted. No treatment-related effect on food consumption was recorded during the study.

Section A6.13 Dietary toxicity of Flocoumafen to laying hens

Annex Point IIIA6.2

5.3	**Conclusion**	The dietary LC_{50} value of Flocoumafen to laying hens following a 5-day treatment period and a 28-day observation period was determined to be 16.4 ppm (95% CI = 8.5–42.1 ppm).	
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	3 May 2005
Materials and Methods	3.1: The study was performed with WL108366 a former code number for flocoumafen. The stability in hen diet is principally equivalent to the basic material for rodenticide baits: cereals. 3.1.5: Results of analyses of test substance in the diet were not reported. Therefore, there is no evidence that the concentration of the test substance in diet has been satisfactorily maintained in the diet.
Results and discussion	No comments.
Conclusion	In laying hens, the oral LC_{50} of flocoumafen was found to be approximately 16.4 ppm. A dose-related increase in prothrombin times was noted in animals at all dose levels (10, 30, 60 and 90 mg/kg bw).
Reliability	3, see acceptability.
Acceptability	Acceptable.
Remarks	None.

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

Table A6.13- 4: 5-day dietary toxicity in laying hens.

Dose (ppm)	Number of dead/ number investigated	Time of death	Observations
0	0/10	–	–
1.5	0/10	–	–
5.0	3/10	Day 4–8	Subdued, swollen eyes
15.0	4/10	Day 4–6	Subdued, unsteady
50.0	8/10	Day 3–7	Subdued, unsteady
LD_{50} value	16.4 ppm (95% CI = 8.5–42.1 ppm)		

Section A6.13 **Annex Point IIIA6.2**	**Elimination of radioactivity in excreta and total ^{14}C residues in eggs and in liver tissue of laying hens.**

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.13/05:** Hxxxx Kxxxx (1988) Fate of ^{14}C-WL108366 fed to laying hens at a rate of 1 mg and 4 mg per kg per day for 5 days: elimination of radioactivity in excreta and total ^{14}C-residues in eggs and in liver tissue. Sxxxx Rxxxx Lxxxx, Sxxxx, Uxxxx, Report No.: SBGR.87.079 February 11, 1988 (unpublished). (BASF-Ref.: FL-440-007)	
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 EPA Pesticide Assessment Guideline 171-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No deviations were reported.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section A2, radio-labelled.	X
3.1.1	Lot/Batch number	Not stated.	
3.1.2	Specification	As given in Section A2, apart from purity stated below.	X
3.1.3	Purity	Radiochemical purity: 99.3 % (sample 1), 98.9% (sample 2)	X
3.1.4	Description	Not stated.	
3.1.5	Stability	^{14}C-Flocoumafen was shown to be stable for 7 days at 4°C in the dark when applied to avian laboratory diet and stored in gelatine capsules in a previous study.	
3.1.6	Radiolabelling	^{14}C	X
3.2	**Test animals**		
3.2.1	Species	Domestic laying hens	
3.2.2	Strain	Brown hybrid egg laying strain – ISA	
3.2.3	Source	Elemby Farm Eggs, Peterborough, UK	
3.2.4	Sex	Female	

Section A6.13 **Elimination of radioactivity in excreta and total ^{14}C residues in eggs and in liver tissue of laying hens.**
Annex Point IIIA6.2

3.2.5	Age/ weight at study initiation	Age: approx. 30 weeks Body weight: 1750–2370 g	
3.2.6	Number of animals per group	5	
3.2.7	Control animals	Yes, containing two birds.	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Type	Dietary via gelatine capsule	
3.3.2	Concentration of test substance	1 or 4 mg/kg b.w./day	
3.3.3	Specific activity of test substance	20.52 µCi/mg (sample 1), 5.34 µCi/mg (sample 2)	
3.3.4	Total volume applied	One capsule containing 0.5 g layer diet plus test substance dissolved in acetone	
3.3.5	Solvent	Acetone	
3.3.6	Exposure period	5 days	
3.3.7	Post-exposure period	15 days	
3.3.8	Samples (sampling time)	Eggs (collected daily throughout the study) Excreta (collected 24-hourly throughout the study) Liver and carcass (upon study termination)	
3.3.9	Control	Capsule containing 0.5 g layer diet plus acetone.	
		4 RESULTS	X
4.1	**Toxic effects, clinical signs**	Four out of five birds dosed with 1 mg/kg/day and three out of five birds dosed with 4 mg/kg/day died during the study. All birds showed signs of haemorrhaging prior to death, indicating that the deaths were treatment-related. No treatment-related abnormalities were found in animals sacrificed upon study termination. Mortalities and clinical observations are summarised in Table A6.13- 5. There was no evidence of treatment-related differences in body weight or body weight change. Birds receiving 1 or 4 mg/kg/day of Flocoumafen consumed less food than control birds throughout the study. During the post-exposure period, the number of eggs laid per bird per day and total egg weight per bird per day were reduced in the 4 mg/kg/day group.	
4.2	**Residues in the liver**	For deceased animals in the low and high dose groups, hepatic residues were in the range of 0.95 to 2.16 and 2.12 to 6.91 µg/g, respectively. Animals surviving until study termination showed slightly lower radioactive residues in the liver. At both dose levels, radioactivity present in the liver accounted for less than 1% of the cumulative administered dose.	

Section A6.13 **Elimination of radioactivity in excreta and total ^{14}C residues in eggs and in liver tissue of laying hens.**
Annex Point IIIA6.2

4.3	**Residues in eggs**	Radioactive residues in the whites of eggs were below the limit of detection for the low dose treatment and in surviving high dose animals. Residues in eggs laid during the treatment period of the high dose group were ≤ 0.05 µg/g. In low dose egg yolks, radioactive residues were first detected 2 days after commencement of the study and were observed to increase with days of treatment, until death. In the one surviving hen, maximum residues in the egg yolk were seen at 10 days after commencement of the study, this residue constituted 0.18 % of the cumulative administered dose. The highest residue detected in high dose animals was measured in eggs laid on days 10 and 12 and accounted for 0.06 % of the cumulative administered dose. About 40 % of radioactive residues found in egg yolk were found to be the unchanged parent compound.
4.4	**Elimination**	A mean daily elimination of 71 % or 67 % of the daily administered radioactivity was observed in excreta of the low and high dose groups, respectively. During the post-exposure period a steady decrease in the amount of radioactivity eliminated via excreta was observed in animals surviving to termination of the study.
4.5	**Recovery of labelled compound**	Not stated.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The elimination of radioactivity in excreta and total ^{14}C residues in eggs and in liver tissue were examined in laying hens receiving 1 or 4 mg/kg b.w./day of ^{14}C-Flocoumafen for five days followed by a 15-day post-exposure period. The study was conducted according to the EPA Pesticide Assessment guideline 171-4. No deviations from the guideline were reported.
5.2	**Results and discussion**	In both dose groups, about 68 % of the daily administered dose was eliminated via the excreta within 24 hours. Radioactivity present in the liver accounted for less than 1% of the cumulative administered dose. Radioactive residues in the whites of eggs were below the limit of detection for the low dose treatment and in surviving high dose animals. Residues in eggs laid during the treatment period of the high dose group were ≤ 0.05 µg/g. Maximum residues found in egg yolk accounted for 0.18 % or 0.06 % of the cumulative administered dose for the low dose and high dose group, respectively. About 40 % of radioactive residues found in egg yolk were found to be the unchanged parent compound.
5.3	**Conclusion**	
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	3 May 2005
Materials and Methods	3.1: The study was performed with WL108366 a former code for flocoumafen. 3.1.2: The isomer composition (cis:trans)of non-labelled test substance was 57:43. The isomer composition (cis:trans) of sample 1 was 57:43 and of sample 2 58:42. 3.1.3: The chemical purity of non-labelled test substance was 94.9%. 3.1.6: Coumarin-U-C^{14} label 3.3.8: Analysis of radioactivity in eggs, excreta and liver was performed y oxygen combustion and LSC. 3.3.10, Observations: clinical signs and mortality (daily), body weights (day -3, 0 and at termination), food consumption (daily from day -3), gross pathology upon necropsy.
Results and discussion	No comments.
Conclusion	After oral administration of 1 or 4 mg/kg bw/day to laying hens for 5 days, mortality and haemorrhaging were noted in both dose groups. Decreased number of eggs laid per bird per day and total egg eight per bird per day were noted at 4 mg/kg bw/d. In both dose groups, about 68 % of the daily administered dose was eliminated via the excreta within 24 hours. Radioactivity present in the liver accounted for less than 1% of the cumulative administered dose. Radioactive residues in the whites of eggs were below the limit of detection for the low dose treatment and in surviving high dose animals. Residues in eggs laid during the treatment period of the high dose group were ≤ 0.05 µg/g. Maximum residues found in egg yolk accounted for 0.18 % or 0.06 % of the cumulative administered dose for the low dose and high dose group, respectively. About 40 % of radioactive residues found in egg yolk were found to be the unchanged parent compound.
Reliability	2, since at the time of the study conduct, GLP was not compulsory.
Acceptability	Acceptable.
Remarks	None.

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Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.13- 5: Mortalities occurring after 5 days of dietary administration with Flocoumafen

Dose [mg/kg/day]	**Number of dead/ number investigated**	**Time of death**	**Observations**
0	0/2	–	Wet excreta on days 13 to 19
1	4/5	Day 4–8	Blood on the beak, blood on the cage and excreta tray, subdued behaviour
4	3/5	Day 3–5	Blood around the mouth, blood on the cage and excreta tray, vomiting a small amount of blood

Section A6.13 Effectiveness of vitamin K_1 therapy in Beagle dogs as an antidote to single exposure by Flocoumafen

Annex Point IIIA6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A6.13/06:** Bxxxx Pxxxx, Cxxxx Jxxxx, Bxxxx Dxxxx, Cxxxx Dxxxx (1989) An investigative study of the effectiveness of vitamin K_1 therapy as an antidote to single exposure intoxication by WL 108366. Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx. Report No. SLL 137/89474, October 9, 1989 (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 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 No guidelines available.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section A2.	X
3.1.1	Lot/Batch number	5003	
3.1.2	Specification	Not stated	
3.1.3	Purity	Not stated	X
3.1.4	Description	Off-white powder	
3.1.5	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Tested antidote**	Vitamin K_1 (i) Konakion 10 tablets, batch no.: 750592 (ii) Konakion 10 1.1 ml ampoules, batch no.: 287047	
3.3	**Test animals**		
3.3.1	Species	Dog	
3.3.2	Strain	Beagle	
3.3.3	Source	Interfauna UK Ltd., Huntingdon, UK	
3.3.4	Sex	Male and female	

Section A6.13 **Annex Point IIIA6.2**		**Effectiveness of vitamin K_1 therapy in Beagle dogs as an antidote to single exposure by Flocoumafen**	
3.3.5	Age/weight at study initiation	Age: Group I: 33 weeks, Group II and III: 41–44 weeks Body weight: Group I: 10.7 kg (male), 8.9 kg (female); Group II and III: 11.6–13.9 kg (male), 11.8–13.5 kg (female)	
3.3.6	Number of animals per group	1 male; 1 female (group I); 2 males; 2 females (group II, group III)	
3.4	**Administration/ Exposure**		
3.4.1	Phase I	All dogs received a single dose of Flocoumafen tech. of 0.50 mg/kg formulated as a 0.010 % solution in corn oil at a dosage volume of 5.0 ml/kg.	
3.4.2	Phase II	Animals in group I did not receive any therapy (positive control). Therapy with vitamin K_1 was commenced without delay for any animal in group II or III that showed signs of intoxication. Subcutaneous injections once each day were administered during the first 7 days of therapy. On days 8 to 35 of therapy the test compound was administered orally by tablet following the regime outlined below: Days 8–21 of therapy Group II: 2.0 mg/kg Group III: 5.0 mg/kg Days 22–28 of therapy Group II: 1.0 mg/kg Group III: 2.5 mg/kg Days 29–35 of therapy Group II: 0.5 mg/kg Group III: 1.25 mg/kg In addition, if any animal did not show response to the vitamin K_1 therapy and was thought to be unlikely to survive, a blood transfusion was given.	X
3.5	**Examinations**	Clinical signs (regularly throughout the study), Body weights (twice a week pre-dosing and during dosing daily before administration), Food consumption (daily) Haematology (twice weekly investigating prothrombin time and activated partial thromboplastin time) Gross pathology (for group I animals only)	
3.6	**Further remarks**	None	

Section A6.13
Annex Point IIIA6.2

Effectiveness of vitamin K_1 therapy in Beagle dogs as an antidote to single exposure by Flocoumafen

4 RESULTS

4.1 Phase I

The female dog receiving a single dose of 0.5 mg Flocoumafen/kg b.w. showed in-appetence, pale gums and subdued behaviour 5 days after dosing. Post mortem examinations after sacrifice for humane reasons showed free blood in the abdominal cavity and massive haemorrhages.

In contrast to the female dog the male showed no clinical signs of intoxication after the single dose despite showing elevated clotting times. Thus, a second dose of 0.5 mg Flocoumafen/kg b.w. was administered. On day 6, in-appetence, pale gums and laboured breathing was observed. Gross pathology revealed free blood in the thoracic cavity and massive blood clots.

Clotting times were elevated on day 5 after the first (both dogs) and the second dose (male dog only).

4.2 Phase II

Clinical signs observed included subdued behaviour, in-appetence, pale gums, ears and conjunctiva, erratic respiration, slight coldness of the extremities and haemorrhage in the conjunctiva.

Since one female of group II, one female of group III and one male of group III did not show any clinical signs following administration of Flocoumafen, a second dose of 0.5 mg Flocoumafen/kg b.w. was administered about 7 weeks after the first dose. One male of group III did not show any clinical signs after this second dose, and thus did not receive vitamin K_1 therapy.

It was necessary to administer whole-blood transfusion in addition to the vitamin K_1 therapy for 2 animals each of group II and III. All animals of group II and III recovered from intoxication by Flocoumafen.

Generally the prothrombin time and the activated partial thromboplastin time were elevated at the onset of clinical signs and decreased to pre-dose levels after about 6–12 days of therapy.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effectiveness of vitamin K_1 therapy as an antidote to single exposure intoxication by Flocoumafen was investigated in Beagle dogs. Therapy with vitamin K_1 was commenced when dogs showed clinical signs of intoxication and was continued for 35 days.

Section A6.13 Effectiveness of vitamin K_1 therapy in Beagle dogs as an antidote to single exposure by Flocoumafen

Annex Point IIIA6.2

5.2	**Results and discussion**	Clinical signs observed included subdued behaviour, in-appetence, pale gums, ears and conjunctiva, erratic respiration, slight coldness of the extremities and haemorrhage in the conjunctiva. No apparent difference in the response to the vitamin K_1 therapy was obtained for group II and III. It was necessary to administer a whole-blood transfusion in addition to the vitamin K_1 therapy for 2 animals of each group. All animals recovered from intoxication by Flocoumafen after the therapy. Generally the prothrombin time and the activated partial thromboplastin time were elevated at the onset of clinical signs and decreased to pre-dose levels after about 6–12 days of therapy.
5.3	**Conclusion**	Based on the results of this study, it can be concluded that the therapeutic regime followed for anticoagulant poisoning was effective and suitable for dogs intoxicated with Flocoumafen. It was considered to be advisable to give a blood transfusion of approx. 150 to 200 ml whole fresh blood to any animal that does not show rapid resolution of clinical signs following the first subcutaneous injection of vitamin K_1 together with a second subcutaneous dose of the antidote.
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	3 May 2005
Materials and Methods	3.1: The study was performed with WL108366 a former code for flocoumafen. 3.1.3: The purity of the test substance was 97.6%.3.4.2: Group II received subcutaneous injections of 2 mg/kg, Group III received subcutaneous injections of 5 mg/kg. 3.5: PTT was determined by Quick's method. APTT was determined by the method of Proctor and Rapaport.
Results and discussion	No comments.
Conclusion	The effectiveness of vitamin K1 therapy as an antidote to single exposure intoxication by Flocoumafen was investigated in Beagle dogs. After single oral administration of 0.5 mg/kg bw, dogs showed clinical signs including pale gumas, ears and conjunctiva, erratic respirations and haemorrhage in the conjunctiva. PTT and APTT were increased within 5 days after dosing. Therapy with vitamin K_1 was commenced without delay for any animal in group II or III that showed signs of intoxication. Subcutaneous injections of 2 or 5 mg/kg once each day were administered during the first 7 days of therapy. On days 8 to 35 of therapy the test compound was administered orally by tablet by the following regime. On days 8–21 of therapy, group II received 2.0 mg/kg and group III received 5.0 mg/kg. On days 22–28 of therapy group II received 1.0 mg/kg and group III received 5 mg/kg. On days 29–35 of therapy group II received 0.5 mg/kg and group III received 1.25 mg/kg. Treatment with vitamin K_1 resulted in decreased PTT and APTT to pre-dose levels within 6-12 days of therapy. For two animals of different therapy regimes, blood transfusion (appr. 150 to 200 ml whole frsh blood) was considered necessary in addition to the vitamin K_1 therapy.
Reliability	3, see acceptability. When data on the purity of the test substance are available, reliability is 1.
Acceptability	Acceptable.
Remarks	None.
COMMENTS FROM ...	
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.13 Toxic effects on livestock and pets

Annex Point IIIA6.2 Supportive data

The following references are considered to contain additional information concerning toxic effects on livestock and pets and are thus presented in tabular format as supportive data (all studies were non-GLP studies):

Reference	Title	System	Results
A6.13/07: Rxxxx Nxxxx, Cxxxx Dxxxx, Rxxxx Vxxxx, Cxxxx Dxxxx (1987) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No.: SLL 89/861037, January 21, 1987 (unpublished). (BASF-Ref.: FL-411-016)	Acute oral toxicity of WL 108366 to goats.	Male and female goats (1 per sex and group)	Groups of one male and one female received a single dose of 10, 30 or 60 mg/kg of Flocoumafen in corn oil. One animal dosed at 30 mg/kg showed severe signs of toxicity and was sacrificed non-scheduled. However, no severe signs of toxicity or mortality occurred in the high dose group. prothrombin times and activated partial thromboplastin times were elevated in all surviving animals and reached maximum levels at 6–12 or 6–15 days post-treatment, respectively.
A6.13/08: Rxxxx Nxxxx, Cxxxx Dxxxx, Rxxxx Vxxxx, Cxxxx Dxxxx (1987) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No.: SLL 88/861036 January 20, 1987 (unpublished). (BASF-Ref.: FL-411-012)	WL 108366 – Acute oral toxicity to sheep.	Male and female sheep of Clun x Suffolk cross (1 per sex and group)	Groups of one male and one female received a single dose of 5, 10 or 20 mg/kg of Flocoumafen in corn oil. One animal dosed at 10 mg/kg died. However, animals of the high dose group showed no severe signs of toxicity.
A6.13/09: Rxxxx Nxxxx, Fxxxx Cxxxx, Bxxxx Mxxxx (1985) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No.: SLL 71BT/8592, March 12, 1985 (unpublished). (BASF-Ref.: FL-505-010)	The short-term cumulative dietary toxicity of WL108366 to the domestic chicken.	Domestic chicken, strain: Light Sussex (5 per sex and group)	Groups of 5 male and 5 female chicken received 0, 50 or 200 ppm of Flocoumafen in the diet for 5 days followed by a 21-day observation period. No control animals died during the study. Nine animals treated with 50 ppm and all animals treated with 200 ppm died between day 2 and 7. LD_{50} < 50 ppm

Section A6.13 Toxic effects on livestock and pets

Annex Point IIIA6.2 Supportive data

A6.13/10: Eadsforth CV, Gray A, Huckle KR, Inglesfield C (1993) Pestic. Sci. 38, 17–25 (published). (BASF-Ref.: FL-905-069)	The dietary toxicity of Flocoumafen to hens: elimination and accumulation following oral administration.	Domestic laying hens (ISA Brown)	Published results of references A6.13/04 and A6.13/05. The following additional information was included in the abstract: Livers of birds which received doses of Flocoumafen between 5 and 50 mg/kg had concentrations of Flocoumafen that were independent of dose. The data indicate the presence of a saturable high-affinity Flocoumafen binding site in hen liver, with similar characteristics and capacity to that of the quail and the rat. Residues of Flocoumafen in samples of breast and leg muscle were low in all exposure groups. Higher, dose-related residues were found in samples of abdominal fat and skin-associated fat and there was clear evidence of dose-related transfer of residues into eggs.
A6.13/11: Bxxxx (1998) Uxxxx dxxxx pxxxx, Mxxxx x'Exxxx, Fxxxx, Report No. 97-02 (unpublished). (BASF-Ref.: FL-490-001)	Clinical trial: evaluation of the efficacy of vitamin K_1 in the treatment of poisoning in dogs with flocoumafen, anticoagulant rodenticide.	Beagle dogs (male)	Vitamin K_1 therapy was investigated after intoxication by Flocoumafen 0.5% premix. Exposure to 0.48 mg Flocoumafen/kg b.w. resulted in increased prothrombin times for all dogs. All dogs displayed bleeding from venipuncture sites and additionally some dogs also displayed bleeding from small cuts and from the gastrointestinal tract. The therapy with vitamin K_1 included two intravenous injections 12 hours apart at 5 mg/kg/day followed by daily oral administration at 5 mg/kg/day for 28 consecutive days. It was concluded that this treatment regimen was effective under the experimental conditions.
A6.13/12: Anonymous (undated) Leaflet by consortium of rodenticide manufacturers (published). (BASF-Ref.: FL-190-005)	Treatment of anticoagulant rodenticide poisoning – Advice to veterinarians	Animals exposed to Flocoumafen by consumption of bait or secondary poisoning	It was advised to administer vitamin K_1 (phytomenadione) by intravenous injection to exposed animals. Once prothrombin time has returned to normal, oral dosing of vitamin K_1 (2 to 5 mg/kg b.w.) should be started and continued for three to four weeks without interruption even if symptoms have regressed. Finally, 24 to 48 hours after the antidote has been withdrawn prothrombin time should be determined and treatment be continued for a further two to three weeks if the prothrombin time is elevated.

Section A6.13 Toxic effects on livestock and pets

Annex Point IIIA6.2 Supportive data

A6.13/13: Anonymous (undated); Leaflet by Shell Agriculture (published). (BASF-Ref.: FL-190-002)	Storm – advice to veterinarians	Animals exposed to Flocoumafen by consumption of bait or secondary poisoning	Fresh whole blood transfusion (10–15 ml/kg) accompanied by parental, subcutaneous or intramuscular administration of vitamin K_1 (2–5 mg/kg b.w./day) was recommended for animals exhibiting advanced clinical signs of intoxication. After up to two weeks of parental administration, oral administration of vitamin K_1 was recommended for a further period of up to four weeks. For animals exhibiting early signs of intoxication, no blood transfusion was recommended.
A6.13/14: Hxxxx Rxxxx, Bxxxx Pxxxx, Fxxxx Sxxxx, Bxxxx Dxxxx, Cxxxx Dxxxx (1987) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 87/861452 May 11, 1987 (unpublished). (BASF-Ref.: FL-420-004)	WL 108366 palatability/oral toxicity in Beagle dogs.	Beagle dogs (Four males and four females)	Apart from one animal the dogs did not find the wax blocks attractive to eat. There was no evidence of the dogs discriminating against the placebo wax blocks when incorporated into the daily food ration. Groups of male and female dogs received wax blocks or grain bait containing 50 ppm of Flocoumafen incorporated into the diet once a week for up to 3 or 6 weeks, respectively. No significant differences were observed following treatment with wax blocks or grain bait. No macroscopic findings apart from haemorrhages were reported upon necropsy.
A6.13/15: Hxxxx Rxxxx, Bxxxx Pxxxx, Fxxxx Sxxxx, Bxxxx Dxxxx (1987) Hxxxx Rxxxx Cxxxx Lxxxx, Hxxxx, Uxxxx, Report No. SLL 111-G/861427 (unpublished). (BASF-Ref.: FL-470-033)	An investigative study of the palatability of Bitrex treated wax blocks and untreated wax blocks to Beagle dogs.	Beagle dogs (male and female)	It was concluded that untreated wax and wax containing Bitrex at levels of 1 and 10 ppm were equally palatable to the Beagle dogs.

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	3 May 2005
Conclusion	The presentation of the above studies as supportive data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Conclusion	
Remarks	

Section A6.14 Other tests related to the exposure of humans

Annex Point IIIA11.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Other tests related to the exposure of humans are not considered to be required, since the TNG on additional data requirements expressly requires such information for example only under the following prerequisites: (i)·in the case of toxicity of degradation products, by-products and reaction products related to human exposure (ii) in the case of toxic effects of substances generated from an active substance, other than mammalian metabolites, in the normal use of a biocidal product (iii) for product types that may involve relevant human exposure due to reaction products with water when the substance is used for human hygiene purposes or reaction products with water or other materials released in water or air when the substance is used for the treatment of bathing waters, for example. However, none of these considerations apply to the product type 14 (rodenticides) and especially not to Flocoumafen, which is why any such tests are 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	9 May 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substances and biocidal products, tests related to the exposure of humans are required in case of 1) toxicity of degradation products, by-products and reaction products related to human exposure; 2) toxic substances generated from the active substance, other than mammalian metabolites, in normal use of the biocidal product and 3) product type 1, 2, 5, 6, 7, 8, 9, 10 and 18. For flocoumafen, above criteria do not apply.
Conclusion	Non-submission of data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 6.15.1–6 Food and feedingstuffs

Annex Point IIIA 6.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	(i) The submission of data on residues in food and feedingstuffs is not considered to be required for lack of exposure, since the intended use as a rodenticide and the related baiting practices as well as the properties of the formulated rodenticide products are in no way associated with any potential for contamination of food and feedingstuffs. In chapter 3, points A6.15.1–6.15.5 of the TNsG on data requirements the submission of such data is only requested in the case that "the active substance is to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedingstuff for livestock is prepared, consumed or stored." However, in the case of rodenticides, such a use is in fact an application governed under the plant protection directive 91/414. In contrast, the use applied for in this dossier is a strict biocidal use, which intrinsically excludes use in storage protection of agricultural goods. (ii) Data to be submitted under point 6.15.1–6.15.6 are not part of the mandatory "common core data" set for active substances, but instead represent either product-type specific "additional data requirements" as further specified in chapter 2.5 of the TNsG on data requirements, or further additional data requirements as set forth in chapter 3 of the TNsG on data requirements. To this, it is initially noted that for rodenticides such as Flocoumafen, such additional data are not required according to chapter 2.5. (iii) For the reasons above, a summary under point A6.15.6 similarly does not need to be 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	9 May 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substances and biocidal products, data on residues in food and feeding stuff are considered necessary if 1) the active substance is to be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feedingstuff for livestock is prepared, consumed or stored. Considering the instructions for use in section B.5 and the product label of Storm BB, contamination of food and feeding stuff can be excluded.
Conclusion	Acceptable
Remarks	None.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 6.16
Annex Point IIIA 6.3.5, 11.2

Any other test related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	In chapter 3 of the TNsG on additional data requirements it is stated that it may be required under point A6.16 to submit other tests related to the exposure of the active substance to humans in its proposed biocidal products that are considered necessary. Further, it is said that an expert judgement for suitable tests and reasoned case is needed to decide whether such additional studies are required. The document then refers to chapter 1.2, point 4, where it also says: The data requirements have been specified in as much detail as possible. However, in certain cases **expert judgement** by the applicant and by the competent authority may be necessary in order to assess, for instance, whether an additional study is needed or on which organism or under which conditions a test should be performed. The applicant hereby states that a dossier is submitted which is largely congruent with the one currently under evaluation in the context of Directive 91/414 for the inclusion of Flocoumafen in Annex I. In fact, there have been some additions to the underlying data base dossier in order to address biocide-specific issues. Since the applicant is lead to believe that the data base is adequate to conclude on completeness for the purpose of inclusion in Annex I of Directive 91/414, and since all essential data requirements have also been met concerning Directive 98/8, the applicants does not at this time see any need for further data submissions. However, this does not preclude that if in the course of the evaluation of the dossier, in exercising expert judgement by the rapporteur, the need for further testing arises, the applicant will readily enter into discussion on the requirement of such testing, the type of data requirement, and in the refinement of protocols etc.	
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	9 May 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substances and biocidal products, other tests related to the exposure of the active substance to humans are considered necessary based on expert judgement. In case of flocoumafen, the proposed use of Storm BB and the available data in the dossier, additional data related to the exposure of humans are not considered necessary.
Conclusion	Non-submission of data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 6.17
Annex Point IIIA 6.6

If the active substance is to be used in products for action against plants then tests to assess toxic effects to metabolites from treated plants, if any, where different from those identified in animals shall be required

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	In chapter 3 of the TNsG on additional data requirements it is stated under point A6.17 that the submission tests to assess toxic effects to metabolites from treated plants may be required, if the active substance is to be used in products for action against plants. However, since Flocoumafen is exclusively used as a rodenticide, the conduct of such tests is obviously not relevant.	
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	9 May 2005
Evaluation of applicant's justification	According to the guidance on data requirements for active substances and biocidal products, tests to access toxic effects of metabolites from treated plants (where different from those identified in animals) are required when the active substance is to be used in products for action against plants. Since the instruction of use of Storm BB does not include use against plants, additional data are not considered necessary.
Conclusion	Non-submission of data is accepted.
Remarks	None.
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	