Section A4.1 Analytical Methods for Detection and Identification
Annex Point IIA4.1 **Technical product**

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A4.1/01:** Hxxxx Jxxxx (2002) Validation of HPLC-method SAMS 427-1 for the determination of Reg. No. 4060804 in technical flocoumafen. Bxxxx Axxxx, Lxxxx, Gxxxx, Report No. PCP06560, February 27, 2002 (unpublished). (BASF-Ref.: 2002/10046222) **A4.1/02:** Axxxx (undated) Determination of Flocoumafen in technical Material – Liquid chromatography method. Sxxxx Rxxxx Lxxxx., Sxxxx, Uxxxx, Report No. SAMS 427-1 (unpublished). (BASF-Ref.: FL-210-001) Remark: Reference A4.1/02 is the original method description, A4.1/01 the corresponding validation report. Therefore, these references are jointly reviewed in the current study summary for convenience.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	No	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	SANCO/3030/99 rev.4	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Detection**		
3.1.1	Separation method	Normal-phase HPLC, mobile phase: hexane/dichloromethane/acetic acid (70/30/0.5).	
3.1.2	Detector	DAD-detector, detection at 235 nm.	
3.1.3	Test substance	Flocoumafen, batch no. M02 Purity: not stated	
3.1.4	Reference substances	Flocoumafen, batch no. AC 12140-35 Purity: not stated	

Section A4.1 Analytical Methods for Detection and Identification
Annex Point IIA4.1 Technical product

3.2	**Linearity**	The determination is performed by external calibration. The detector response was linear for standard solutions in the concentration range of 0.4 to 0.63 mg/ml (r > 0.999).	X
3.3	**Specificity: interfering substances**	The method described in this study is suitable for the specific determination of Flocoumafen. Under the applied chromatographic conditions, the retention times were approx. 18.6 to 19.0 min. and 21.3 to 21.7 min. for the two isomers of Flocoumafen. No interfering blanks were observed.	X
3.4	**Recovery rates and relative standard deviations**	Accuracy was demonstrated by analysis of five subsets of technical Flocoumafen of one batch with known amounts of pure a.s. on the total amount of Flocoumafen. The recovery was 100.8%, with a relative standard deviation of 1.3%.	
3.5	**Limit of determination**	Not stated	
3.6	**Precision**	The precision (repeatability) of the method was determined by fivefold analysis of one subset of technical Flocoumafen, batch M02. Acceptability of the results of the relative standard deviation was assessed by application of the Horwitz equation. In each case the acceptable spread of the Horwitz equation was larger than the spread of the results obtained.	X

Concentration [mg/100 mL]	**Average recovery [%]**	**RSD [%]**	**n**
48.3	98.6	0.6	5

Concentration [mg/100 mL]	**%RSD$_R$**	**%RSD$_r$**	**RSD**	**RSD acceptable**
48.3	6.3	4.2	0.6	yes

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Normal-phase HPLC method for the determination of Flocoumafen in technical Flocoumafen.
4.2	**Conclusion**	The results of linearity, accuracy, precision and specificity demonstrate that the analytical method is suitable for the determination of Flocoumafen in technical Flocoumafen, according to SANCO/3030/99 rev. 4.
4.2.1	Reliability	2
4.2.2	Deficiencies	Description of the analytical determination is missing.

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	02 May 2005
Materials and Methods	(2.3) (3.2) (3.3) The calibration line consisted of only 4 points where 5 concentrations or more are required by the guideline. The occurrence or absence of interferences could not be assessed because no indication of retention times of known impurities was given. However, based on the structures of the impurities and the symmetric shape of the peaks of both isomers, specificity of the method is considered acceptable by the RMS.
Results and discussion	(3.6) The % of Flocoumafen in the technical material (98.6%) should be used to calculated the $\%RSD_R$ and $\%RSD_r$ (and not the concentration in the endsolution prior to analysis). The Table is revised to:

Flocoumafen in technical material [%]	**$\%RSD_R$**	**$\%RSD_r$**	**RSD**	**RSD acceptable**
98.6	2.004	1.342	0.6	yes

Conclusion	The results of linearity, accuracy, precision and specificity demonstrate that the analytical method (HPLC-UV) is suitable for the determination of Flocoumafen in technical Flocoumafen, according to SANCO/3030/99 rev. 4.
Reliability	1
Acceptability	Acceptable.
Remarks	-

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

Section A4.2/01, 02 **Analytical Methods for Detection and Identification in (a) soil**
Annex Point IIA4.2

Official use only

1 REFERENCE

1.1	**Reference**	**A4.2/01:** Kxxxx Exxxx, Kxxxx Jxxxx (1998) Determination of Flocoumafen in soil – validation of the method. Ixxxx Fxxxx Gxxxx, Hxxxx, Gxxxx, Report No. IF-95/14504-00, November 24, 1998 (unpublished). (BASF Ref.: FL-242-002). **A4.2/02:** Axxxx (undated) Determination of residues of WL108366 in soil – liquid chromatographic method. Sxxxx Rxxxx Lxxxx., Sxxxx, Uxxxx, Unpublished Report No. SAMS 450-1. (BASF-Ref.: FL-242-001). Remark: Reference A4.2/02 is the original method description, the study by Kxxxx and Kxxxx (A4.2/01) the corresponding validation report. Therefore, these references are jointly reviewed in the current study summary for convenience.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No A guideline was not available at the time the study was conducted, but the method is comparable to SANCO/825/00 rev. 6.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	Yes Four instead of five replicates were used at each fortification level. However, this does not affect the quality of the study.

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Residues of Flocoumafen were extracted with methanol/water (80/20). After filtration and evaporation, partition into n-hexane followed.
3.1.2	Clean-up	Following evaporation further clean-up was performed on NH_2 Bond Elut column. Residues were eluted with methyl-tert-butylether/ethanol/acetic acid (47.5/47.5/5).

Section A4.2/01, 02 Analytical Methods for Detection and Identification in (a) soil
Annex Point IIA4.2

3.2	**Detection**		
3.2.1	Separation method	HPLC with a RP_{18}-column, mobile phase: acetonitrile/water/acetic acid (80:20:0.1). For confirmatory purposes different HPLC conditions can be used: CN-column, mobile phase: hexane/ethanol/acetic acid (95:5:0.1)	
3.2.2	Detector	Fluorescence-detector (E_x = 310 nm, E_m = 390 nm)	
3.2.3	Standard(s)	Flocoumafen: batch no.: AC 9745-35; purity: 97.1%; composition information: cis/trans: 53.6:43.2. Soil standards: 2.1 (soil) and 2.2 (loamy sand).	
3.3	**Linearity**		
3.3.1	Calibration range	The detector response for the analytical standards was found to be linear between 0.01–0.95 µg/ml.	
3.3.2	Number of measurements	The calibration curve was plotted based on seven different concentrations, each of them injected twice.	
3.3.3	Linearity	The equation of a typical standard calibration function for Flocoumafen was determined as $y = 9212229.1\,x + 24584.2;\ r > 0.999$ where y is the response in the chromatogram, and x the concentration of the substance [µg/ml].	
3.4	**Specificity: interfering substances**	The method is suitable for the specific determination of Flocoumafen. Under the chromatographic conditions used in this study, the retention times were about 3.2 min for cis-Flocoumafen and about 3.7 for trans-Flocoumafen. Blank control samples analysed gave no interfering signals (< 30% of LOQ).	
3.5	**Recovery rates and standard deviations at different levels**		

Soil	Fortification	Recovery	RSD	n
Sand				
	0.001 mg/kg	85 %	2.1 %	4
	0.01 mg/kg	91 %	5.1 %	4
	0.1 mg/kg	88 %	2.5 %	4
Loamy sand				
	0.001 mg/kg	85 %	2.6 %	4
	0.01 mg/kg	91 %	1.8 %	4
	0.1 mg/kg	82 %	7.4 %	4

3.6	**Limit of determination**	The limit of quantification (LOQ) is 0.1 mg/kg.	X
3.7	**Precision**		
3.7.1	Repeatability	The average recovery is in a range between 70–110% and the relative standard deviation is less than 20%.	
3.7.2	Independent laboratory validation	Not necessary.	

Section A4.2/01, 02 **Analytical Methods for Detection and Identification in (a) soil**
Annex Point IIA4.2

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of Flocoumafen were extracted with methanol/water, followed by partitioning into n-hexane and further clean-up on a NH_2 Bond Elut column. Determination was performed by HPLC with a fluorescence-detector.	
4.2	**Conclusion**	Average recoveries were in the range of 70–110% with relative standard deviations < 20%. No interfering blanks were observed. Therefore, this method fulfils the requirements of SANCO/825/00 rev.6 and can be used as an enforcement method for the determination of residues of Flocoumafen in soil.	X
4.2.1	Reliability	1	
4.2.2	Deficiencies	None	

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	20 September 2005
Materials and Methods	No comments.
Results and discussion	(3.6) The LOQ is 0.001 mg/kg.
Conclusion	Average recoveries (LOQ, 10x and 100x LOQ) were in the range of 70–110% with relative standard deviations < 20%. No interfering blanks were observed. This method fulfils the requirements of SANCO/825/00 rev.6 (except for the lack of validation data of the confirmatory method) and can be used as an enforcement method for the determination of residues of Flocoumafen in soil with a LOQ of 0.001 mg/kg.
Reliability	1
Acceptability	Acceptable.
Remarks	A confirmatory method was described in 4.2/02. No validation results for the confirmatory method were presented. A confirmatory method is however not listed in the guidance document on data requirements for active substances and biocidal products (vs 4.3.2, Oct 2000).

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

Section A4.2 Analytical Methods for Detection and Identification in (b) air

Annex Point IIA4.2

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	A method for the detection of residues in air is not submitted, since Flocoumafen is neither volatile nor intended to be sprayed or applied in any other way resulting in occurrence of Flocoumafen in air.	
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	04 January 2005
Materials and Methods	NA
Results and discussion	NA
Conclusion	A method for the detection of residues in air is not submitted, since Flocoumafen is neither volatile nor intended to be sprayed or applied in any other way resulting in occurrence of Flocoumafen in air.
Reliability	NA
Acceptability	Non-submission of data is accepted by the RMS.
Remarks	None.

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

Section A4.2/03 Analytical Methods for Detection and Identification in (c) water

Annex Point IIA4.2

Official use only

1 REFERENCE

1.1	**Reference**	**A4.2/03:** Xxxxx Bxxxx, Kxxxx Cxxxx (2002) BAS 322 I (Flocoumafen): Validation of method M 3490 for LC/MS determination and LC/MS/MS confirmation of BAS 322 I residues in ground water and surface water. Bxxxx Axxxx Rxxxx, Pxxxx, Uxxxx, Report No. RES 02-003, February 11, 2002 (unpublished). (BASF-Ref.: FL-123-014).
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 SANCO/825/00 rev. 6
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Residues of Flocoumafen were extracted with hexane. The hexane fraction was evaporated and redissolved in the mobile phase.
3.1.2	Clean-up	Further clean-up steps were not necessary since determination was performed by LC-MSD or LC-MS/MS.
3.2	**Detection**	
3.2.1	Separation method	Liquid chromatography: ODS-column, mobile phase: 0.1% acetic acid in water/ 0.1 % acetic acid in methanol, gradient.
3.2.2	Detector	MSD with electrospray liquid introduction interface, negative polarity, SIM mode for quantitation, monitored ion: m/z = 541 For confirmatory purposes: MS/MS conditions: atmospheric pressure ionization (API) system operated in the electrospray ionization mode. Ion transitions monitored: m/z 541 > 382.
3.2.3	Standard(s)	Flocoumafen, batch no. AC12140-35, purity: 99.4 %

Section A4.2/03 Analytical Methods for Detection and Identification in (c) water
Annex Point IIA4.2

3.2.4	Interfering substance(s)	No interfering substances are expected since determination was performed by quantitation of specific ion fragments of the active substance.	
3.3	**Linearity**		
3.3.1	Calibration range	The detector responses for the analytical standards were found to be linear in the range of 0.0005–0.004 µg/ml for LC-MSD and 0.001–0.008 µg/ml for LC-MS/MS.	
3.3.2	Number of measurements	The calibration curve was plotted based on four different concentrations, each of them injected twice.	
3.3.3	Linearity	The equations of a typical standard calibration function for Flocoumafen were determined as $y = 198.77x + 89.261;\ r > 0.99$ (LC-MSD) and $y = 15096x - 15479;\ r > 0.99$ (LC-MS/MS) where y is the response in the chromatogram, and x the concentration of the substance [pg].	X
3.4	**Specificity: interfering substances**	The method is suitable for the specific determination of residues of Flocoumafen in ground and surface water. Under the chromatographic conditions used in this study, the retention times were about 6.4 min for cis-Flocoumafen, 6.7 min for trans-Flocoumafen (LC-MSD) and about 7.5 min for Flocoumafen with the confirmatory method (LC-MS/MS). No interfering blanks were observed at the retention times of the monitoring ions.	
3.5	**Recovery rates and relative standard deviations at different levels**	**Ground water:** Fortification / Recovery / RSD / n *Enforcement method (LS-MSD)* 0.05 µg/l / 82 % / 5 % / 5 0.5 µg/l / 90 % / 3 % / 5 *Confirmatory method (LC-MS/MS)* 0.05 µg/l / 79 % / 5 % / 3 **Surface water:** Fortification / Recovery / RSD / n *Enforcement method (LS-MSD)* 0.05 µg/l / 81 % / 17 % / 5 0.5 µg/l / 105 % / 6 % / 5 *Confirmatory method (LC-MS/MS)* 0.05 µg/l / 78 % / 26 % / 3	
3.6	**Limit of determination**	The limit of quantification (LOQ) is 0.05 µg/l for ground and surface water for both methods (LC-MSD and LC-MS/MS).	X
3.7	**Precision**		
3.7.1	Repeatability	The method was successfully validated with five values at both fortification levels, with recoveries in the range from 70% to 110% and relative standard deviations below 20%. No interfering blanks were detected.	

Section A4.2/03 **Analytical Methods for Detection and Identification in (c) water**
Annex Point IIA4.2

3.7.2	Independent laboratory validation	Not necessary.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of Flocoumafen in ground and surface water were extracted with hexane. Determination was performed by LC-MSD. For confirmatory purposes LC-MS/MS can be used.
4.2	**Conclusion**	Average recoveries were in the range between 70 and 110% with relative standard deviations below 20%. Interfering blanks were not observed. Therefore the method fulfils the requirements of SANCO/825/00 rev. 6 and can be used as an enforcement method for the determination of residues of Flocoumafen in ground, surface and drinking water.
4.2.1	Reliability	1
4.2.2	Deficiencies	None

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

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	02 May 2005
Materials and Methods	No comments.
Results and discussion	(3.3.3) The second equation should read: y = 15096x + 15479 and x is the amount of substance injected [pg]. (3.6) The C.V. (n=3) is >20% for the LC-MS/MS method (confirmatory method) in surface water at 0.05 µg/L. The C.V. probably would have been ≤20% for n=5. Results for groundwater (n=3, 0.05 µg/L), resulted in a C.V. of 5%. Therefore, the proposed LOQ of 0.05 µg/L is accepted for the confirmatory method.
Conclusion	Average recoveries (LOQ and 10x LOQ) were between 70 and 110% with relative standard deviations below 20%. Interfering blanks were not observed. Therefore the method fulfils the requirements of SANCO/825/00 rev. 6 and can be used as an enforcement method for the determination of residues of Flocoumafen in ground and surface water with a LOQ of 0.05 µg/L. The method is also considered suitable for drinking water (based on the suitability of the method in surface and groundwater).
Reliability	1
Acceptability	Acceptable.
Remarks	None.

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

Section A4.2 Analytical Methods for Detection and Identification in (c) water

Annex Point IIA4.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A4.2/04:** Gxxxx Ixxxx (1993) Validation of an analytical method for the determination of residues of Flocoumafen (Storm) in water. Rxxxx Uxxxx Axxxx, Ixxxx, Sxxxx, Report No. 298315, November 04 1993 (unpublished). (BASF-Ref.: FL-243-002)	
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 Guideline compliance is not stated in the report, but the method is comparable to SANCO/825/00 rev. 6.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Three instead of five values were determined at each fortification level. However, this does not affect the quality of the study.	X
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Water samples were acidified with hydrochloric acid and extracted by partition into dichloromethane. The dichloromethane extracts were evaporated and redissolved in n-hexane.	
3.1.2	Clean-up	Further clean-up was performed by solid-phase extraction on a Bond Elut-Si column followed by a Bond Elut-NH_2 column. Residues of Flocoumafen were eluted with tert-butylmethyl ether/ethanol/acetic acid (95/95/10) and evaporated to dryness. The residues were dissolved in the mobile phase.	
3.2	**Detection**		
3.2.1	Separation method	High performance liquid chromatography (HPLC): RP_{18}-column; mobile phase: acetonitrile/water/acetic acid (80:20:0.1), isocratic.	
3.2.2	Detector	Fluorescence-detector (E_x = 310 nm, E_m = 390 nm)	

Section A4.2 Analytical Methods for Detection and Identification in (c) water
Annex Point IIA4.2

3.2.3	Standard(s)	Flocoumafen: batch no. 2104/001/90; purity: 97.8%; isomer ratio: cis/trans: 55:45)	
3.2.4	Interfering substance(s)	No interfering substances greater than 30% of LOQ were observed.	
3.3	**Linearity**		
3.3.1	Calibration range	The detector response was linear in a range from 0.005 to 0.055 µg/ml for the cis-isomer and from 0.0045 to 0.045 µg/ml for the trans isomer.	
3.3.2	Number of measurements	The calibration curve was plotted based on four different concentrations, each of them injected twice; the lowest concentration was injected five times.	
3.3.3	Linearity	The equations of typical standard calibration functions for Flocoumafen were determined as $\ln y = 1.019 \ln x - 15.571;\ r = 0.999$ (cis-isomer) and $\ln y = 1.014 \ln x - 14.893;\ r = 0.999$ (trans-isomer) where y is the response in the chromatogram and x the concentration of the substance [µg/ml].	
3.4	**Specificity: interfering substances**	The method is suitable for the specific determination of Flocoumafen. Under the chromatographic conditions used in this study, the retention times were about 6.2 min for cis-Flocoumafen and about 7.1 min for trans-Flocoumafen. Blank control samples analysed gave no interfering signals (< 30% of LOQ).	
3.5	**Recovery rates at different levels**		X

Fortification	Recovery	RSD	n
Tap water from Muelhausen (F)			
0.05 µg/l	91.6 %	1.8 %	3
0.2 µg/l	84.3 %	0.2 %	3
0.5 µg/l	82.3 %	0.1 %	3
Tap water from Rheinfelden (D)			
0.05 µg/l	79.6 %	2.8 %	3
0.2 µg/l	73.0 %	0.8 %	3
0.5 µg/l	64.0 %	3.4 %	3
Spring water from Buckten (CH)			
0.05 µg/l	86.5 %	0.8 %	3
0.2 µg/l	86.9 %	1.4 %	3
0.5 µg/l	79.4 %	1.3 %	10
Spring water from Grünholz (D)			
0.05 µg/l	71.2 %	4.2 %	3
0.2 µg/l	76.3 %	0.6 %	3
0.5 µg/l	79.7 %	0.3 %	3
EPTINGER mineral water from Eptinger (CH)			
0.05 µg/l	77.5 %	0.8 %	3
0.2 µg/l	71.3 %	0.6 %	3
0.5 µg/l	74.4 %	1.7 %	3

3.6	**Limit of determination**	The limit of quantification (LOQ) was 0.05 µg/l for each matrix.	X
3.7	**Precision**		

Section A4.2 **Annex Point IIA4.2**		**Analytical Methods for Detection and Identification in (c) water**	
3.7.1	Repeatability	The average recoveries were in the range from 70 to 110% (with one exception of lysimeter-effluent water from test lysimeter) and the relative standard deviations were below 20%.	X
3.7.2	Independent laboratory validation	Not necessary.	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Water samples were acidified and then extracted by partition with dichloromethane. Further clean-up was performed by solid-phase extraction on a Bond Elut-Si column and then on a Bond Elut-NH_2 column. Determination was performed by HPLC with a fluorescence detector. The part of the study dealing with lysimeter water was omitted from this summary due to lack of relevance.	
4.2	**Conclusion**	The average recoveries were in the range of 70 to 110 % with relative standard deviations below 20%. Interfering blanks were not observed. Therefore, the method fulfils the requirements of SANCO/825/00 rev. 6. as a confirmatory method for the determination of residues of Flocoumafen in ground, surface and drinking water.	X
4.2.1	Reliability	1	
4.2.2	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

02 May 2005

Materials and Methods

(2.3) Only one water sample was spiked per concentration level and processed. The final extract was injected three times. Hence, for assessment of precision/accuracy (per water sample), n=1 applies (and not n=3). The 5 drinking waters were combined by the RMS to assess recovery and precision/accuracy for drinking water.

Results and discussion

(3.5) No RSD (C.V.) can be calculated as n=1 (see remark above). The table is revised as follows:

Fortification	Recovery	RSD	n
Tap water from Muelhausen (F)			
0.05 µg/l	91.6 %	NA	1
0.2 µg/l	84.3 %	NA	1
0.5 µg/l	82.3 %	NA	1
Tap water from Rheinfelden (D)			
0.05 µg/l	79.6 %	NA	1
0.2 µg/l	73.0 %	NA	1
0.5 µg/l	64.0 %	NA	1
Spring water from Buckten (CH)			
0.05 µg/l	86.5 %	NA	1
0.2 µg/l	86.9 %	NA	1
0.5 µg/l	79.4 %	NA	1
Spring water from Grünholz (D)			
0.05 µg/l	71.2 %	NA	1
0.2 µg/l	76.3 %	NA	1
0.5 µg/l	79.7 %	NA	1
EPTINGER mineral water from Eptinger (CH)			
0.05 µg/l	77.5 %	NA	1
0.2 µg/l	71.3 %	NA	1
0.5 µg/l	74.4 %	NA	1
Lysimeter water from Ittingen (CH)			
0.05 µg/l	45.5 %	NA	1
0.2 µg/l	29.5 %	NA	1
0.5 µg/l	34.8 %	NA	1

(3.6) When combining the 5 drinking waters, average recoveries of 81.3, 78.4 and 76.0 were calculated by the RMS at 0.05, 0.2 and 0.5 µg/L, respectively. C.V.s (n=5) were 9.8, 8.8 and 9.6%, respectively. Therefore, the LOQ of 0.05 µg/L for drinking water is acceptable.

(3.7.1) C.V.s were calculated for each level for the combined drinking waters (n=5) and were <20%.

Conclusion

(4.3) The method (HPLC-fluorescence) can be accepted as a confirmatory method for the analysis of Flocoumafen residues in groundwater and drinking water with a LOQ of 0.05 µg/L. Suitability for surface water was not demonstrated and therefore not accepted by the RMS.

Reliability	1.
Acceptability	Acceptable.
Remarks	The method is not accepted for surface water.

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

Section A4.2/05 **Analytical Methods for Detection and Identification**
Annex Point IIA4.2 **(d) animal and human body fluids and tissues**

			Official use only
		1 REFERENCE	
1.1	**Reference**	**A4.2/05:** Xxxxx Bxxxx, Kxxxx Cxxxx (2002) BAS 322 I (Flocoumafen): Validation of method M 3508 for LC/MS determination and LC/MS/MS confirmation of BAS 322 I residues in urine, blood and liver. Bxxxx Axxxx Rxxxx, Pxxxx, Uxxxx, Report No. RES 02-008, February 11, 2002 (unpublished). (BASF-Ref.: FL-123-015)	
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 SANCO/825/00 rev. 6	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Urine: Residues of Flocoumafen were extracted by solid phase chromatography onto a C_{18} cartridge, following by elution with methanol. Blood: Residues of Flocoumafen were extracted with acetonitrile. Liver: Residues of Flocoumafen were extracted with 50% of dichloromethane in acetone.	X
3.1.2	Clean-up	Urine and blood: no further clean-up was performed. Liver: After evaporating the extract was cleaned up by solid phase chromatography on a Bond Elut CN-U cartridge. Residues of Flocoumafen were eluted with 30% ethyl acetate in hexane.	
3.2	**Detection**		
3.2.1	Separation method	Liquid chromatography: ODS-column, mobile phase: 0.1% acetic acid in water/0.1 % acetic acid in methanol, gradient	

Section A4.2/05 Analytical Methods for Detection and Identification
Annex Point IIA4.2 **(d) animal and human body fluids and tissues**

3.2.2	Detector	MSD with electrospray liquid introduction interface, negative polarity, SIM mode for quantitation, monitored ion: m/z = 541 For confirmatory purposes: MS/MS conditions: atmospheric pressure ionization (API) system operated in the electrospray ionization mode; ion transitions monitored: m/z 541 > 382	
3.2.3	Standard(s)	Flocoumafen, batch no. AC12140-35, purity: 99.4%	
3.2.4	Interfering substance(s)	No interfering substances may be expected, since determination was performed by quantitation of specific ion fragments of the active substance.	
3.3	**Linearity**		
3.3.1	Calibration range	The detector responses for the analytical standards were found to be linear in the range of 0.0005–0.004 µg/ml for LC-MSD and LC-MS/MS.	
3.3.2	Number of measurements	The calibration curve was plotted by four different concentrations, each of them injected twice.	
3.3.3	Linearity	The equations of typical standard calibration functions for Flocoumafen were determined as $y = 384.9x + 147.5;\ r > 0.99$ (LC-MSD) and $y = 80592x - 77972;\ r > 0.99$ (LC-MS/MS) where y is the response in the chromatogram, and x the amount of substance injected [pg].	
3.4	**Specificity: interfering substances**	The method is suitable for specific determination of residues of Flocoumafen in urine, blood and liver. Under the chromatographic conditions used in this study the retention times were about 6.2 min for cis-Flocoumafen, 6.6 min for trans-Flocoumafen (LC-MSD) and about 7.1 min for Flocoumafen using the confirmatory method (LC-MS/MS). No interfering blanks were observed at the retention times of the monitoring ions.	X

Section A4.2/05 **Analytical Methods for Detection and Identification**
Annex Point IIA4.2 **(d) animal and human body fluids and tissues**

3.5	**Recovery rates at different levels**	**Urine:**	

Fortification	Recovery	RSD	n
Enforcement method (LS-MSD)			
0.005 mg/l	90%	4%	5
0.05 mg/l	87%	5%	5
Confirmatory method (LC-MS/MS)			
0.005 mg/l	81%	11%	3

Blood:

Fortification	Recovery	RSD	n
Enforcement method (LS-MSD)			
0.005 mg/l	101%	4%	5
0.05 mg/l	80%	5%	5
Confirmatory method (LC-MS/MS)			
0.005 mg/l	78%	10%	3

Liver:

Fortification	Recovery	RSD	n
Enforcement method (LS-MSD)			
0.005 mg/kg	89%	5%	5
0.05 mg/kg	81%	4%	5
Confirmatory method (LC-MS/MS)			
0.005 mg/kg	76%	13%	3

3.6	**Limit of determination**	The limit of quantification (LOQ) is 0.005 mg/kg for liver and 0.005 mg/l for blood and urine.	
3.7	**Precision**		
3.7.1	Repeatability	The method was successfully validated with five values at both fortification levels, with recoveries in the range from 70% to 110% and relative standard deviations below 20%.	
3.7.2	Independent laboratory validation	Not necessary.	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of Flocoumafen in liver were extracted with 50% of dichloromethane in acetone, followed by further clean-up on a Bond Elut CN-U cartridge. Residues of Flocoumafen in urine were extracted by solid phase chromatography onto a C_{18} cartridge and residues in blood were extracted with acetonitrile. Determination was performed by LC-MSD. For confirmatory purposes LC-MS/MS can be used.	X
4.2	**Conclusion**	Average recoveries were in the range of 70 to 110% with relative standard deviations below 20%. Interfering blanks were not observed. Therefore, the method fulfils the requirements of SANCO/825/00 rev. 6 and can be used as an enforcement method for the determination of residues of Flocoumafen in animal and human body fluids and tissues.	

Section A4.2/05 **Annex Point IIA4.2**	**Analytical Methods for Detection and Identification** **(d) animal and human body fluids and tissues**

4.2.1	Reliability	1
4.2.2	Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	02 May 2005
Materials and Methods	(3.1.1) (4.1) liver: it is not clear to the RMS whether 50% dichloromethane in hexane (p.64 report) or in acetone (p.55 report) was used. Applicant: it appears that DCM in hexane is an error. DCM in acetone is correct.
Results and discussion	(3.4) Control chromatograms of urine, blood and liver did show small interferences (always <30% of LOQ).
Conclusion	Average recoveries (LOQ and 10x LOQ) were in the range of 70 to 110% with relative standard deviations below 20%. Interfering blanks (>30% LOQ) were not observed. Therefore, the method fulfils the requirements of SANCO/825/00 rev. 6 and can be used as an enforcement method for the determination of residues of Flocoumafen in animal and human body fluids and tissues at LOQs of 0.005 mg/kg for liver and 0.005 mg/L for blood and urine.
Reliability	1
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2/06 **Analytical Methods for Detection and Identification in**
Annex Point IIA4.2 **(d) Animal and Human Body Fluids and Tissues**

Official use only

1 REFERENCE

1.1	**Reference**	**A4.2/06:** Dxxxx Axxxx (1994) Development of a method for the analysis of regurgitated raptor pellets for residues of coumarin based rodenticides. Sxxxx Rxxxx Lxxxx., Sxxxx, Uxxxx, Report No. SBGR.91.248, February 1994 (unpublished). (BASF Ref.: FL-245-009)
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 A guideline was not available at the time the study was conducted, but the method is comparable to SANCO/825/00 rev. 6, with the deviations specified below.
2.2	**GLP**	No
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Residues of Flocoumafen were twice extracted with acetone/chloroform (1:1).
3.1.2	Clean-up	Column Chromatography on a Bond Elut-NH_2. Residues were eluted with methyl-t-buthyl ether/acetic acid (9+1). Remark: It is noted that clean-up is also feasible using a Bond Elut-Si column, but this method is not reviewed in this study summary due the low recovery rates for Flocoumafen (for details see the original report).

Section A4.2/06 Analytical Methods for Detection and Identification in
Annex Point IIA4.2 (d) Animal and Human Body Fluids and Tissues

3.2	**Detection**		
3.2.1	Separation method	HPLC with a RP_{18}-column, mobile phase: acetonitrile/water/acetic acid (75:25:0.2). For confirmatory purposes different HPLC conditions can be used: Normal-phase column, mobile phase: hexane/ethanol/acetic acid (95:5:0.2)	
3.2.2	Detector	Fluorescence-detector (E_x = 310 nm, E_m = 390 nm)	
3.2.3	Standard(s)	Flocoumafen: batch no.: 003/87; purity: 97.1%.	
3.3	**Linearity**		
3.3.1	Calibration range	Not stated	
3.3.2	Number of measurements	See 3.5	
3.3.3	Linearity	Not stated	
3.4	**Specificity: interfering substances**	The method is suitable for the specific determination for residues of Flocoumafen in regurgitated raptor pellets. Under the chromatographic conditions used in this study, the retention times were about 12.4 min for cis-Flocoumafen and about 14.6 for trans-Flocoumafen. For the confirmatory method on normal-phase HPLC the retention times were about 12.4 min. for cis-Flocoumafen and 19.1 min for trans-Flocoumafen. Blank control samples analysed gave no interfering signals (< 30% of LOQ).	
3.5	**Recovery rates and standard deviations at different levels**	Fortification / Recovery / RSD / n *Cis-Flocoumafen* 0.25 mg/kg 89 % 11 % 3 0.5 mg/kg 76 % 14 % 5 *Trans-Flocoumafen* 0.25 mg/kg 100 % 7 % 3 0.5 mg/kg 72 % 11 % 5	X
3.6	**Limit of determination**	The limit of quantification (LOQ) is 0.25 mg/kg.	X
3.7	**Precision**		
3.7.1	Repeatability	The average recovery is in the range 70–110 % and the relative standard deviation is less than 20 %.	
3.7.2	Independent laboratory validation	Not necessary.	

Section A4.2/06 **Analytical Methods for Detection and Identification in**
Annex Point IIA4.2 **(d) Animal and Human Body Fluids and Tissues**

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of Flocoumafen were extracted with acetone/chloroform, followed by clean-up on a Bond Elut-NH_2. Determination was performed by HPLC with a fluorescence-detector.	
4.2	**Conclusion**	Average recoveries were in the range of 70–110 % with relative standard deviations < 20 %. No interfering blanks were observed. Therefore, this method can be used as an enforcement method for the determination of residues of Flocoumafen in raptor pellets.	
4.2.1	Other Conclusions	Although this study is not strictly required according to the BPD and does not deal with "body fluids and tissues" *sensu strictu*, it is submitted since the method is referred to in monitoring studies on secondary poisoning.	
4.2.2	Reliability	1	X
4.2.3	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	02 May 2005
Materials and Methods	No comments.
Results and discussion	(3.5) No validation results for the confirmatory method are given. (3.6) The number of accuracy determinations was 3 instead of 5 at the LOQ. The proposed LOQ was accepted by the RMS because all individual recoveries at the LOQ and accuracy results at 2xLOQ were adequate.
Conclusion	Average recoveries (LOQ and 2xLOQ) were in the range of 70–110 % with relative standard deviations < 20 %. No interfering blanks were observed. Therefore, this method can be used for the determination of residues of Flocoumafen in raptor pellets at a LOQ of 0.25 mg/kg.
Reliability	2
Acceptability	Acceptable.
Remarks	The reliability was lowered to 2 because the number of spiked samples at the LOQ was 3 instead of 5 (current guideline recommendation) and the linearity results were not reported.

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

Section A4.3
Annex Point IIIA4.1

Analytical methods including recovery rates and the limits of determination of the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant
– food of plant and animal origin –

Official use only

1 REFERENCE

1.1	**Reference**	**A4.3/01:** Txxxx Gxxxx (2005) Validation of analytical methodology to determine rodenticides in food matrices. Cxxxx Sxxxx Lxxxx, Sxxxx Hxxxx, Yxxxx, Uxxxx, Report no. PGD-180, June 16, 2005 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Activa s.r.l. BASF AG Bell Laboratories Inc. Hentschke + Sawatzki KG Liphatec SAS PelGar International Ltd. Rentokil Initial PLC Sorex Ltd. Syngenta
1.2.2	Companies with letter of access	See above
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I of Directive 98/8/EC.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	SANCO/825/00 rev.7 (17 March 2004)
2.2	**GLP**	Yes
2.3	**Deviations**	None

Section A4.3
Annex Point IIIA4.1

Analytical methods including recovery rates and the limits of determination of the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant
– food of plant and animal origin –

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

Cucumber:

Extraction of the homogenised sample with ethyl acetate under presence of sodium sulphate in a ratio of 2:1. The extract is separated by pouring through a funnel with a non-absorbent cotton-wool plug and sodium sulphate. An aliquot of the extract is evaporated to dryness under nitrogen, then re-dissolved in acetone, with subsequent addition of 2-butylamine (4 % of acetone volume).

Wheat:

Extraction of the finely ground sample with ethyl acetate and water in a ratio of 7.5:1. The extract is separated by centrifugation and pouring through a funnel with a non-absorbent cotton-wool plug.

Meat:

50 g of anhydrous sodium sulphate are added to 10 g of meat (cut in small pieces) and ground with a pestle until a free-running dry homogeneous powder is obtained. 100 ml dichloromethane:acetone (1:1 v/v) are added and the mixture shaken 2 hours. The extract is separated by through a fluted filter paper, evaporated to < 1 ml volume and taken in approx. 10 ml GPC solvent (cyclohexane/ethyl acetate 50:50, v/v).

Oil seed rape:

25 g of oil seed rape are homogenised in 60 ml acetone and filtered through Whatman no. 1 filter paper. The extract is evaporated to < 50 ml volume. To a 20 ml aliquot of the extract, 200 µl of 2-butylamine are added.

Lemon:

Lemon is homogenised in a food processor in the presence of solid CO2. A 30 g sample is mixed with 60 ml ethyl acetate and 30 g sodium sulphate, homogenised, and the extract separated by pouring through a funnel with a non-absorbent cotton-wool plug and sodium sulphate. A 20 ml aliquot is shaken (four repetitions) with 10 ml water in a separating funnel and the water phase discarded, respectively. The ethyl acetate phase is evaporated to dryness, and the residue re-dissolved in 5 ml acetone, with subsequent addition of 200 µl 2-butylamine.

Section A4.3
Annex Point IIIA4.1

Analytical methods including recovery rates and the limits of determination of the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant
– food of plant and animal origin –

3.1.2	Cleanup	Cucumber: The acetone solution is loaded on a pre-conditioned SPE cartridge. After washing with acetone, the cartridge is eluted with methanol (fraction A). Fraction A is intended for determination of alphachloralose using this multi-residue method and will not be considered further. Fraction B, containing Flocoumafen, is eluted with ethanol containing 2 % (v/v) formic acid. Fraction B is then evaporated to dryness and the residue re-dissolved in methanol containing 0.4 µg/ml coumatetralyl as internal standard. Wheat: A 40 ml aliquot of the extract is evaporated to < 1 ml volume, then mixed with GPC solvent (cyclohexane/ethyl acetate 50:50, v/v). GPC (245 × 25 mm column, S-X3 resin, flow rate 5 ml/min) eluate fraction 80–160 ml is collected. The eluate is evaporated to approx. 1 ml volume, taken in ethyl acetate, and evaporated to dryness. The residue is re-dissolved in methanol containing 0.4 µg/ml coumatetralyl and diphacinone (relevant for chlorophacinone analysis only), respectively, as internal standards. Meat: GPC (245 × 25 mm column, S-X3 resin, flow rate 5 ml/min) eluate fraction 80–160 ml is collected. The eluate is evaporated to approx. 1 ml volume, taken in ethyl acetate, and evaporated to dryness. The residue is re-dissolved in methanol containing 0.4 µg/ml coumatetralyl and diphacinone (relevant for chlorophacinone analysis only), respectively, as internal standards. Oil seed rape: The acetone extract is loaded on a pre-conditioned SPE cartridge. After washing with acetone, the cartridge is eluted with methanol (fraction A). Fraction A is intended for determination of alphachloralose using this multi-residue method and will not be considered further. Fraction B, containing Flocoumafen, is eluted with ethanol containing 2 % (v/v) formic acid. Fraction C, obtained by elution with 0.12 M HCl in ethanol, is intended for determination of chlorophacinone using this multi-residue method and will not be considered further. Fraction B is then evaporated to dryness and the residue re-dissolved in methanol containing 0.4 µg/ml coumatetralyl as internal standard. Lemon: The acetone extract is loaded on a pre-conditioned SPE cartridge. After washing with acetone, the cartridge is eluted with methanol (fraction A). Fraction A is intended for determination of alphachloralose using this multi-residue method and will not be considered further. Fraction B, containing Flocoumafen, is eluted with ethanol containing 2 % (v/v) formic acid. Fraction C, obtained by elution with 0.12 M HCl in ethanol, is intended for determination of chlorophacinone using this multi-residue method and will not be considered further. Fraction B is then evaporated to dryness and the residue re-dissolved in methanol containing 0.4 µg/ml coumatetralyl as internal standard.

Section A4.3
Annex Point IIIA4.1

Analytical methods including recovery rates and the limits of determination of the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant
– food of plant and animal origin –

3.2	**Detection**	
3.2.1	Separation method	All matrices: Liquid chromatography: Reversed-phase column (phenyl-hexyl, 5 µm) Mobile phase: Solvent A: Water containing 10 mM ammonium acetate Solvent B: Methanol Gradient: Time (min) / % A / % B 0 / 80 / 20 5 / 15 / 85 17.5 / 15 / 85 18 / 80 / 20 25 / 80 / 20 Flow rate: 0.2 ml/min Retention time of Flocoumafen: approx. 13.6 min.
3.2.2	Detector	All matrices: MS/MS-detector with turboionspray negative ionisation. Ions monitored: 541 → 161 m/z (quantifier) and 541 → 289 m/z (qualifier).
3.2.3	Standard(s)	All matrices: Internal standard: Coumatetralyl
3.2.4	Interfering substance(s)	No interfering substances observed.
3.3	**Linearity**	
3.3.1	Calibration range	For all matrices: 0.03–1.2 µg/ml
3.3.2	Number of measurements	All matrices: Four concentrations, measured in duplicate
3.3.3	Linearity	All matrices (range): Coefficient of determination: r^2 = 0.9376–0.9975 An individual calibration curve is only given for cucumber (r^2 = 0.9969) which is, however, considered as representative.
3.4	**Specificity: interfering substances**	The method enables the specific determination of Flocoumafen in five representative matrices of foodstuff of plant and animal origin. The method is highly specific, since MS/MS-detection was used for identification and quantification. No interfering substances were observed at the retention time of the analyte.

Section A4.3
Annex Point IIIA4.1

Analytical methods including recovery rates and the limits of determination of the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant
– food of plant and animal origin –

3.5	**Recovery rates at different levels**	The average recovery rates and relative standard deviations complied with the acceptance criteria of SANCO/825/00 rev.7 (70–110%, RSD ≤ 20%) in all matrices at all fortification levels, except with meat at the higher fortification level of 0.1 mg/kg, where mean recovery was only 66 %, and oil seed rape at 0.1 mg/kg, where mean recovery was 122 %. Details are presented in Table A4.3- 1. For the applicant's opinion regarding the two cases of deviation from SANCO requirements (from which rodenticides are nevertheless explicitly exempted) please refer to chapter 4.2 below.
3.5.1	Relative standard deviation	≤ 20% in all matrices at all fortification levels, except for meat at the higher fortification level of 0.1 mg/kg, where the RSD was 30 %. Details are presented in Table A4.3- 1. For the applicant's opinion regarding the high RSD in one case please refer to chapter 4.2 below.
3.6	**Limit of determination**	LoQ = 0.01 mg/kg for all matrices
3.7	**Precision**	
3.7.1	Repeatability	The repeatability was assessed on the basis of the relative standard deviations, which were generally ≤ 20%, except with meat at the higher fortification level of 0.1 mg/kg, where the RSD was 30 %.
3.7.2	Independent laboratory validation	Not required

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	A multi-residue method for the determination of the rodenticide active substances Alphachloralose, Brodifacoum, Bromadiolone, Chlorophacinone, Difenacoum, Difethialone, Flocoumafen, and Warfarin in cucumber, wheat, meat, oil seed rape, and lemon was developed and validated. Matrices can be extracted with ethyl acetate (cucumber, wheat, lemon), dichloromethane:acetone (1:1 v/v) (meat), or acetone (oil seed rape). Clean-up was performed using SPE cartridges or by GPC, depending on the matrix. Determination is performed by LC-MS/MS using a reversed-phase phenyl-hexyl column with methanol and 10 mM ammonium acetate in water as the mobile phase (gradient mode), with monitoring of substance specific transitions.

Section A4.3
Annex Point IIIA4.1

Analytical methods including recovery rates and the limits of determination of the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant
– food of plant and animal origin –

4.2	**Conclusion**	The method is specific for the determination of residues of Flocoumafen in five representative food matrices. The method is even highly specific since MS/MS-detection is used for identification and quantification. No interfering substances occurred. The average recovery rates were between 70 and 110 % with relative standard deviations below 20%, with the exceptions presented in chapter 3.5 above. The limit of quantification was established at 0.01 mg/kg for all matrices. With two matrix/fortification level combinations (meat and oil seed rape at 0.1 mg/kg), the formal requirements of SANCO/825/00 rev.7 regarding mean recovery and/or RSD are not fulfilled. It is important to note, however, that the method is a multi-residue method by nature, allowing determination of 8 different rodenticide active substances from the same sample extract. This inevitably compromises the choice of suitable extracting agents, clean-up procedures etc. Possible improvements in quantification would probably have required complex and expensive clean-up stages which may well have been very matrix and substance specific. Moreover, it should be noted in this context that SANCO/825/00 rev.7 applies to plant protection active substances only and the purpose of the guideline is specification of criteria for verifying compliance of food commodities with MRLs. MRLs do, however, not apply to rodenticides and they are explicitly exempted from the provisions of SANCO/825/00 rev.7. Most importantly, the sensitivity and specificity of the employed methods allow detection and quantification of all 8 analytes at a LoQ of 0.01 mg/kg in all representative matrices. This result should outweigh any potential shortcomings in recoveries or RSD occurring only at two matrix/fortification level combinations. In conclusion, where formal guideline criteria may not be fulfilled, the method can still be used as a monitoring method, especially since MRLs for Flocoumafen do not exist, provided that an estimate of the precision has been made. In all other cases, the method can be used as an enforcement and confirmatory method for the determination of residues of Flocoumafen in food commodities.
4.2.1	Reliability	1
4.2.2	Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	September 9th, 2008
Materials and Methods	Linearity: 4 samples, injected twice. Correlation was linear (y = 3.1889x) Accuracy: recoveries at LOQ for oil seed rape were acceptable, but not at 10x LOQ. This is not considered a problem. The same counts for meat at 10x LOQ, where recoveries were slightly too low (66%) with high RSD. These deficiencies are considered minor.
Results and discussion	For LC-MS/MS methods no confirmatory method is required. Specificity, linearity, accuracy and repeatability is sufficient.
Conclusion	The method submitted suitable for the determination of flocoumafen residues in meat, wheat, lemon, cucumber and rape seed at a LOQ of 0.01 mg/kg.
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A4.3- 1: Recovery rates for the determination of Flocoumafen in food matrices of plant and animal origin by LC-MS/MS, based on monitoring of the mass transition 541 → 161.

Matrix	Fortification level [mg/kg]	n	Recovery	
			Range[%]	Mean [%] ± RSD
Cucumber				
	0.01*	5	90–106	97.2 ± 6.19 %
	0.1	5	88–101	94.1 ± 6.30 %
Wheat				
	0.01*	5	104–120	109 ± 6.33 %
	0.1	5	66–86	79.2 ± 10.1 %
Meat				
	0.01*	5	64–87	75.2 ± 10.7 %
	0.1	5	44–92	66.0 ± 30.0 %
Oil seed rape				
	0.01*	5	76–93	83.7 ± 8.45 %
	0.1	5	110–135	122 ± 8.54 %
Lemon				
	0.01*	5	79–90	83.4 ± 4.99 %
	0.1	5	67–97	83.0 ± 13.7 %

*) limit of quantification
n: number of determinations
RSD: relative standard deviation