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TRANSFLUTHRIN

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Document III-A
Section 4 Analytical Methods
Study Summaries
Active Substance

Rapporteur Member State: The Netherlands
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CA-report and Proposed Decision of The Netherlands in the context of the
Possible inclusion of Transfluthrin in Annex I of Council Directive 98/8/EC

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Document IIIA Analytical Methods for Detection and Identification

SECTION A4 (4.1/01) Analytical methods for the determination of pure active substance

BPD Data set IIA/ Annex Point IV.4.1

NAK 4455 (Transfluthrin) Technical Grade

Official use only

1 REFERENCE

1.1	**Reference**	Bissinger, H. (2002) Bayothrin technical - capillary gas chromatography Bayer AG Analytical Method 2201-0342301-02E Report No. VB1-2201-0342301-02E BES Ref. MO-04011186 Report date: 18 October 2002 Non-GLP. Unpublished. [*Method*] Bissinger, H. (2002) Validation of GLC method 2201-0342301-02 – Determination of a.i. in Bayothrin, industrial Bayer AG Report No. VB1-2201-0342301 BES Ref. MO-04-011186 Report date: 27 November 2002 Non-GLP. Unpublished [*Validation*] Bissinger, H.(2002) Validation of GLC method 2201-0342301-02E – Determination of active ingredient in Bayothrin, industrial Amendment of Report No. VB1-2201-0342301 Bayer CropScience Report No. VS1-2201-0342301 BES Ref. M-226183-01-1 Report date: 11 January 2006-02-21 Non-GLP. Unpublished [*Validation*]
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience
1.2.2	Companies with letters of access	
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 Directive 91/414/EEC, Annex 11 and III; Directive 96/46/EC Analytical Methods
2.2	**GLP**	No
2.3	**Deviation**	No

3 MATERIALS AND METHODS

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SECTION A4 (4.1/01)

BPD Data set IIA/ Annex Point IV.4.1

Analytical Methods for Detection and Identification

Analytical methods for the determination of pure active substance

NAK 4455 (Transfluthrin) Technical Grade

3.1	**Preliminary treatment**		
3.1.1	Enrichment	Samples of technical grade transfluthrin were homogenized in toluene and the active substance content determined by capillary gas chromatography using a flame ionisation detector. The quantitative evaluation of the transfluthrin content was carried out by use if an internal standard, diethyl phthalate (DEP).	
3.1.2	Cleanup	None	
3.2	**Detection**		
3.2.1	Separation method	Gas chromatography using quartz capillary column (Agilent, 30 m length, 0.53mm internal diameter, and 1.5µm film thickness), 300ºC detection port.	X
3.2.2	Detector	Flame ionization detector (FID)	
3.2.3	Standard(s)	Diethyl phthalate (internal standard).	
3.2.4	Interfering substance(s)	Chromatogram showed no interferences at the retention time for transfluthrin.	
3.3	**Linearity**		
3.3.1	Calibration range	9 working solutions in the range of 111.32 to 267.73 mg transfluthrin/20 mL internal standard. were used to determine linearity of detector response.	
3.3.2	Number of measurements	One measurement per concentration level.	
3.3.3	Linearity	Correlation coefficient $r^2 = 1.0000$	
3.4	**Specifity: interfering substances**	The retention time of the reference substance, M00381 (assay 98.3%), was identical to that of the technical grade sample, ranging from approximately 14.59 to 14.60 min under the conditions of the test.	
3.5	**Recovery rates at different levels**	9 concentrations within the working range, 55.7% to 133.9% with sample weight of 200 mg were determined and the recoveries ranged from 98.54% to 100.22% (Average = 99.57%.	
3.5.1	Relative standard deviation	RSD calculated from the above recovery range was 0.53% (n=9).	
3.6	**Limit of determination**	Limit of determination or detection of the active substance in the technical material is not meaningful.	
3.7	**Precision**		
3.7.1	Repeatability	A representative sample was determined 8 times by one operator using one instrument and the measured values ranged from 95.273% to 96.088% compared to a mean value of 95.722%. The RSD was 0.325%. The maximum Horwitz-Value RSD, 1.349 was >RSD, indicating acceptable repeatability of the method.	
3.7.2	Independent laboratory	Method validation was done internally.	

validation

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

After the technical material was dissolved in toluene and the internal standard, diethyl phthalate was added, the transfluthrin content was determined by capillary gas chromatography using flame ionisation detection.

4.2 Conclusion

Validation data given in Table A.4.1(01) -1 meet EU requirements in all respects. The method is linear in the range up to 134% of standard sample weight at active ingredient concentration of approximately 95% to 96%. The method is specific and precise, with an RSD of 0.53 % and there are no interferences.

4.2.1 Reliability 1

4.2.2 Deficiencies No

Table A.4.1(01) -1 Validation data for the determination of transfluthrin in technical material

Method	Precision (repeatability) % RSD (n)	Linearity	Accuracy	Interference	Specificity
Capillary GC with FID detection	Results from determination of a representative sample 8 times ranged from 95.273% to 96.088% compared to a mean value of 95.722%. The RSD was 0.325%. Horwitz-Value RSD, 1.349, was >RSD, indicating acceptable repeatability of the method..	Single determinations at 9 levels showed method is linear up to 134% of standard sample weight, at active substance concentration of approximately 95% to 96%. r^2 = 1.0000	9 samples within the working range, 55.7% to 133.9% with sample weight of 200 mg were determined and the recoveries ranged from 98.54% to 100.22%. (Average = 99.57%; RSD = 0.53% (n=9).	Chromatograms demonstrate the lack of interference.	The retention time of the reference substance (M00381, assay 98.3%), was identical to that of the technical grade sample, ranging from approximately 14.59 to 14.60 min under the conditions 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	22-03-2007
Materials and methods	*3.2.1 Separation method:* Stationary phase = HP-1.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Document IIIA **Analytical Methods for Detection and Identification**

SECTION A4 (4.1/02) **Analytical methods for the determination of pure active substance**

BPD Data set IIA/ Annex Point IV.4.1

R/S Ratio of NAK 4455 Cis- and Trans Isomers

Official use only

	5 REFERENCE	
5.1	**Reference**	Dr. Reubke (2000a) R/S Ratio of Bayothrin (NAK 4455) Cis- and Trans Isomers (assay Chiral GLC) Bayer AG, Report No. 2005-0010901-00 E [BES Ref. M0-00-007975] Report date: May 19, 2000 Unpublished [*Method*] Dr. Reubke (2000b) Validation Report: Bayothrin Technical, R/S-ratio by Chiral GC Bayer AG, Report No. V01.01-2005-0010901E [BES Ref. M0-00-007977] Report date: May 19, 2000 Unpublished [*Validation*]
5.2	**Data protection**	Yes
5.2.1	Data owner	Bayer CropScience
5.2.2	Companies with letters of access	
5.2.3	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
	6 GUIDELINES AND QUALITY ASSURANCE	
6.1	**Guideline study**	Yes Validated according to EC Directive 91/414/EEC, Annex 11 and III
6.2	**GLP**	Yes
6.3	**Deviation**	No
	7 MATERIALS AND METHODS	
7.1	**Preliminary treatment**	
7.1.1	Enrichment	Samples were dissolved in dichloromethane and the R/S ratio determined by gas chromatography on chiral phase. Samples of technical grade material (batches 816779016, 816979003, 816979004, 816979005, 816979006) and batches of known isomers, 1R-trans isomer, M00381, 1R-cis isomer, NAK 4711, racemic mixture of cis- and trans-isomer, NAK 5014 were used in the analyses.

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BPD Data set IIA/ Annex Point IV.4.1

Analytical Methods for Detection and Identification

Analytical methods for the determination of pure active substance

R/S Ratio of NAK 4455 Cis- and Trans Isomers

7.1.2	Cleanup	None	
7.2	**Detection**		
7.2.1	Separation method	Capillary GC (Quartz capillary column, length: 26 m, I.D. 0.25 mm);	X
7.2.2	Detector	FID (flame ionisation detection), 220°C	
7.2.3	Standard(s)	Racemic cis/tran mixture or pure (~100%) Transfluthrin (external standard)	
7.2.4	Interfering substance(s)	None	
7.3	**Linearity**		
7.3.1	Calibration range	Solution of 1R-trans (98.7% R) and 1R-cis (88% R) isomers with mixed with racemic mixture of cis- and trans- isomers (trans/cis ratio 56.1%) in the range of 0% to 100% in five concentration levels, with three replicates for each level.	X
7.3.2	Number of measurements	Three measurements for each level	
7.3.3	Linearity	Linear regression analysis resulted in the calibration lines showing good linearity (R^2 for R/S ratios >99%).	
7.4	**Specifity: interfering substances**	Chromatograms showed separation of the isomers and no interfering substances.	
7.5	**Recovery rates at different levels**	The method is shown to be satisfactorily accurate from the specificity, linearity and precision results. The R/S ratios obtained from analysis of five batches of the technical were comparable to the corresponding optical rotation measurements. At the 95% confidence the average difference between the measurements was 0.82 ± 2.93.	
7.5.1	Relative standard deviation	RSD for the R/S ratio = 0.128% (see precision results)	
7.6	**Limit of determination**	The limits of quantification, calculated from the standard error of the estimate from linear regression, were 0.5% for the trans- isomer ratio, 0.1% for the cis- isomer ratio and 0.2% for the trans/cis/ ratio. This means, that the lowest quantifiable S-isomer content is 0.5% for trans- and 1% for cis-isomer.	
7.7	**Precision**		
7.7.1	Repeatability	Five solutions of three batches of Transfluthrin technical were each injected three times. R/S ratios could only be measured for the trans isomer as the trans/cis ratio was >98% in all three batches. The cis-isomer was present predominantly in the 1S-configuration. The standard deviations for the R/S ratio was ≤0.02% for the three batches and an overall precision (RSD) was estimated to be 0.128% (n=3).	
7.7.2	Independent laboratory validation	Method validation was done internally instead of by an independent laboratory.	

8 APPLICANT'S SUMMARY AND CONCLUSION

8.1 Materials and methods

After diluting with a suitable solvent (dichloromethane), the R/S ratio was determined by gas chromatography on chiral phase using an FID detector.

8.2 Conclusion

Validation data are given in Table A4.1(02) -1 and meet EU requirements in all respects. The method is linear in the range up to 100% of standard sample weight. The method is specific and precise, with an RSD of 0.128% for the R/S ratio determinations and there are no interferences.

8.2.1 Reliability 1

8.2.2 Deficiencies None

Table A4.1(02) -1 Validation data for the determination of R/S ratioof transfluthrin cis- and trans isomers

Method	Precision (repeatability)* % RSD (n)				Linearity	Interference	Specificity
GC-FID (2005-010901-00 E)	Batch	1	2	3	Determinations in triplicate at 5 levels showed method is linear up to 100% of standard sample weight. unpublished Linear regression analysis resulted in the calibration lines showing good linearity (R^2 for R/S ratios >99%).	Chromatograms demonstrate the lack of interference.	Chromatograms showed separation of the isomers at various retention times.
	Mean	99.04	99.28	98.83			
	Std	0.14	0.10	0.14			
	RSD	0.14	0.10	0.14			
	Overall RSD	0.128% (n=2)					
	* R/S ratios could only be measured for trans isomer since the tranns/cis ratio was >98% in all three batches.						

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22-03-2007
Materials and methods	*3.2.1 Separation method:* Length of column is 25 m, not 26 m. *3.3.1 Calibration range:* '(98.7% R)' and '(88% R)' are considered to be 98.7% R/S trans and 88% R/S cis, respectively.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Document IIIA Analytical methods for the active substance in Soil

SECTION A4 (4.2/01)

BPD Data set IIA/ Annex Point IV.4.2 Transfluthrin residues in soil

Official use only

	9 REFERENCE	
9.1	**Reference**	Weeren, R and Pelz, S (2001) Validation of DFG Method S 19 (extended Revision) for the Determination of Residues of Transfluthrin in Soil., Dr. Specht and Partner Chemische Laboratorien GmbH Bayer AG Report No. BAY-00106V Az G01-0009 [BES Ref. M0-01-009826] Report date: April 30, 2001 [*Validation*] Unpublished
9.2	**Data protection**	Yes
9.2.1	Data owner	Bayer CropScience
9.2.2	Companies with letters of access	
9.2.3	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
	10 GUIDELINES AND QUALITY ASSURANCE	
10.1	**Guideline study**	Yes EC Directive 91/414/EEC, Annex 11 and III Guideline document SANCO/825/00 rev.6 of 20/06/00 of the European Commission; BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998
10.2	**GLP**	Yes
10.3	**Deviations**	No
	11 MATERIALS AND METHODS	
11.1	**Preliminary treatment**	
11.1.1	Enrichment	DFG Method S 19 (extended version) was validated for determination of transfluthrin residues in soil samples (LUFA Speyer standard soil 2.2). Extraction was performed according to module E 2, which consisted of extraction of 'LUFA Speyer standard 2.2' soil samples with acetone after adding water, maintaining the acetone/water ratio at 2/1 (v/v). Liquid-liquid partitioning was performed with a solution of ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride and after repeated mixing, excess water was separated. The residue remaining after evaporation of an aliquot of the organic phase was cleaned up by

		gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. The residue containing fraction was concentrated and after further cleanup with supplemental silica gel mini column chromatography, analysed for transfluthrin by capillary gas chromatography using electron capture detection (module D 1). Confirmation was done by gas chromatography using mass selective detection (MSD) (module D 4). Control samples of soil were analysed in duplicate and fortified samples of soil were analysed in quintuplicate for each fortification level.
11.1.2	Cleanup	Cleanup was first done by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane as eluent. The extract obtained was further cleaned using silica gel mini column chromatography.
11.2	**Detection**	
11.2.1	Separation method	Primary: 30 m fused silica capillary column DB1(J&W), internal diameter, 0.25 mm and film thickness of 0.25 µm Retention time for transfluthrin was ~ 18.7 min. Confirmatory: 30 m fused silica capillary column DB-5 MS (J&W), internal diameter, 0.25 mm and film thickness of 0.25 µm Selected ions for quantitation: m/z 163 and verification: m/z 165, m/z 335. Retention time for transfluthrin was ~ 11.8 min.
11.2.2	Detector	Electron capture detector (ECD), 300ºC (primary) Mass selective detector (MSD), 280ºC (confirmatory)
11.2.3	Standard(s)	Transfluthrin (in hexane), 0.0234 µg/ml and 0.234 µg/ml
11.2.4	Interfering substance(s)	None
11.3	**Linearity**	
11.3.1	Calibration range	Individual determinations ranging from 0.005 µg/ml to 2.00 µg/ml
11.3.2	Number of measurements	Control samples of soil were analysed in duplicate and fortified samples of soil were analysed in quintuplicate for each fortification level.
11.3.3	Linearity	Correlation coefficient $r^2 = 0.9997$
11.4	**Specifity: interfering substances**	No significant interferences from the sample matrix were detected in any of the samples at the retention time corresponding to transfluthrin.
11.5	**Recovery rates at different levels**	Samples of were fortified with transfluthrin at two different fortification levels (0.005 and 0.05 µg/l) and analysed using the primary and confirmatory methods described above. Recoveries were calculated from measured and theoretical compound concentrations. Results are given in Table 4.2.1-1 below.
11.5.1	Relative standard deviation	See Table 4.2.1-1 below for the primary method and 4.2.1-2 for the confirmatory test.

Document IIIA Analytical methods for the active substance in Soil

SECTION A4 (4.2/01)

BPD Data set IIA/ Annex Point IV.4.2 Transfluthrin residues in soil

11.6	**Limit of determination**	The LOQ was 0.005 mg/kg. The limit of detection LOD, was estimated from the lowest calibration standard to be 0.001 mg/kg.
11.7	**Precision**	
11.7.1	Repeatability	Results for repeatability of recovery are given in Table 4.2.1-1 below.
11.7.2	Independent laboratory validation	ILV was not undertaken in this study. Primary validation was performed strictly according to guidelines and all results were well within acceptable limits. The method uses commonly available reagents and techniques and has been shown to be suitable for monitoring.

12 APPLICANT'S SUMMARY AND CONCLUSION

12.1	**Materials and methods**	DFG Method S 19 (extended version) was validated for determination of transfluthrin residues in soil samples (LUFA Speyer standard soil 2.2). Soil samples were extracted with acetone, followed by liquid-liquid partitioning with a solution of ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride. After repeated mixing, excess water was separated. Clean-up was performed by gel permeation chromatography followed by silica gel mini column chromatography. Transfluthrin residues were determined by capillary gas chromatography using electron capture detection. Confirmation was done by gas chromatography using mass selective detection (MSD). Control samples were analysed in duplicate and fortified samples of soil were analysed in quintuplicate for each fortification level..
12.2	**Conclusion**	Validation data given in Tables A4.2(01) -1 and A4.2(01) -2 meet EU requirements in all respects. The method is linear in the concentration range of 0.005 µg/ml to 2.00 µg/ml transfluthrin. The method is specific and precise, with an RSD 4.1% (n=10) and there are no interferences.
12.2.1	Reliability	1
12.2.2	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	06-06-2007
Materials and methods	- GLP statement and quality assurance statement were not signed. Please provide signed statements and a signed report. - In Appendix IV of the study report representative chromatograms should be displayed, but they are not. Please provide the missing figures (1-8) The report was re-submitted, including signed quality control forms and the missing chromatograms.
Conclusion	Acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	None.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4.2-01 Validation data for the determination of transfluthrin in soil

Fortification level (mg/kg)	**Accuracy % recovery**	**Precision % RSD (n)**	**Linearity**	**Interference**	**Specificity**
0.005	81, 87, 82, 84, 88		Single determinations at conc. Range 0.005 –2.0 µg/ml transfluthrin. r^2 = 0.9997	None. Chromatograms demonstrated the lack of interference.	No significant interferances from the sample matrix were detected. Compound confirmation performed using GC-MSD proved the peak identity within the acceptable range.
Mean	84 ± 3.0	3.6 (n = 5)			
0.05	83, 81, 78, 81, 88				
Mean	82 ± 3.7	4.5 (n = 5)			
Overall	83 ± 3.4	4.1 (n=10)			

Confirmatory method:

For confirmation, one control and one fortified sample of each level were analysed by capillary gas chromatogrpahy with mass selective detection (MSD).

Table A4.2-02 Results of confirmatory test for residues of transfluthrin in soil samples

Fortification Level	Recoveries (%)	
mg/kg	MSD single values	Corresponding ECD single values
0.005	97	81
0.05	87	83

Document IIIA Analytical methods for the active substance in Air

SECTION A4 (4.2/02)

BPD Data set IIA/ Annex Point IV.4.2 Transfluthrin residues in air samples

Official use only

13 REFERENCE

13.1	**Reference**	Class, T (2005) Analytical Method for the Determination of Transfluthrin in Air , PTRL Europe, Helmholtzstr. 22, Science Park, D-89081, Ulm, Germany Bayer AG Report No. P/B 911G [BES Ref. M0-05-010149] Report date : June 27, 2005 [*Method and ILV*] Unpublished
13.2	**Data protection**	Yes
13.2.1	Data owner	Bayer CropScience
13.2.2	Companies with letters of access	
13.2.3	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

14 GUIDELINES AND QUALITY ASSURANCE

14.1	**Guideline study**	Yes BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998 EU Directive 91/414/EEC Annex II (Part A, Section 4.2), as amended by Commission Directive 96/46/EC EC Guidance Document on residue analytical methods, SANCO/825/00 rev. 7 17/03/04
14.2	**GLP**	Yes
14.3	**Deviations**	No

15 MATERIALS AND METHODS

15.1	**Preliminary treatment**	
15.1.1	Enrichment	The method describes the determination of transfluthrin in air by gas chromatography with specific mass spectrometric detection (GC/MS). Air is drawn through XAD adsorption tubes at about 1 L/min for 6 hours (total air sampling volume = 0.4 m^3). Subsequently, the adsorption material is extracted with acetone and the extract analysed by GC/MS, monitoring three fragment ions with m/z of >100. The method was validated for determination of transfluthrin in ambient and in warm (35ºC), humidified air (relative humidity >80%). Method validation was performed by an independent laboratory, PTRL, Europe.
15.1.2	Cleanup	None

Document IIIA Analytical methods for the active substance in Air

SECTION A4 (4.2/02)

BPD Data set IIA/ Annex Point IV.4.2 Transfluthrin residues in air samples

15.2	**Detection**	
15.2.1	Separation method	Fused silica capillary column: 30 m length, 0.32 mm i.d., 0.25 µm film thickness Stationary phase: 5% phenyl 95% dimethylpolysiloxane
15.2.2	Detector	Electron impact (EI) mass spectrometric detection. Ion trap selected ion storage (SIS) mode monitoring the intense 163 *m/z* fragment ion for primary quantification, and the 127 *m/z* and 143 *m/z* fragment ions for quantitative confirmation (as sum).
15.2.3	Standard(s)	External standard (Transfluthrin, retention time, approx. 6.7min)
15.2.4	Interfering substance(s)	There were no interfering substances as shown by the blank chromatograms.
15.3	**Linearity**	
15.3.1	Calibration range	4 ng/mL to 2000 ng/mL (4, 20, 40, 100, 200, 500 and 2000 ng/mL)
15.3.2	Number of measurements	2 determinations with duplicate samples per determination
15.3.3	Linearity	Correlation coefficient , r = > 0.998
15.4	**Specifity: interfering substances**	Quantification was performed using either the most intense fragment ion observed at 163 *m/z*, or the sum of the less intensive fragment ions observed at 127 and 143 *m/z*, all present in the EI mass spectrum of transfluthrin. The chromatograms of the control samples showed no signals (<0.05 ug/m^3) at the retention time of transfluthrin, with the exception of one blank extract, which gave a contamination signal of approx. 0.1 ug/m^3 (<30% of LOQ).
15.5	**Recovery rates at different levels**	One portion of sampling cartridges was fortified with transfluthrin at the LOQ or at 10-fold LOQ. Subsequently, sampling of air was performed for 6 hours with ambient or with warm, humidified air (35ºC, 87 to 100% r.h.). Five replicate samples were analysed at each fortification level. Average recoveries for both fortification levels and for both sampling conditions ranged between 102% and 109%. Breakthrough in the back portion of the adsorption tubes was always <5% of the amount fortified on the front portion. Extraction efficiencies and storage stability for 5 days at room temperature was demonstrated with average recoveries of 76% to 109% (See Table 4.2 (02)– 1)
15.5.1	Relative standard deviation	Relative standard deviations were always ≤9%, except for extraction efficiency, where the overall relative standard deviations ranged from 15% to 17% (n=7 in each case).
15.6	**Limit of determination**	Limit of quantification (LOQ) = 0.5 µg/m^3 Limit of detection (LOD) = ≤ 0.05 µg/m^3
15.7	**Precision**	
15.7.1	Repeatability	Above retention efficiency and recovery data confirm precision of method.

Document IIIA Analytical methods for the active substance in Air

SECTION A4 (4.2/02)

BPD Data set IIA/ Annex Point IV.4.2 Transfluthrin residues in air samples

15.7.2	Independent laboratory validation	Method validation in this study was performed by an independent laboratory, PTRL, Europe.

16 APPLICANT'S SUMMARY AND CONCLUSION

16.1	**Materials and methods**	The method describes the determination of transfluthrin in air by gas chromatography with specific mass spectrometric detection (GC/MS). Air is drawn through XAD adsorption tubes at about 1 L/min for 6 hours (total air sampling volume = 0.4 m^3). Subsequently, the adsorption material is extracted with acetone and the extract analysed by GC/MS, monitoring three fragment ions with m/z of >100. The method achieves a limit of quantification (LOQ) of 0.5 µg/m^3. The method was validated for determination of transfluthrin in ambient and in warm (35ºC), humidified air (relative humidity >80%).
16.2	**Conclusion**	Based on the above results, an analytical method for the determination of transfluthrin in air samples using highly selective GC/MS determination (including three fragment ions of quantification and confirmation) has been successfully validated with a limit of quantification of 0.5 µg/m^3.
16.2.1	Reliability	1
16.2.2	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	6-06-2007
Materials and methods	Applicant's version is adopted.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2 (02)- 1.Summary of recovery results

Type of experiment	Fortified µg	Ave C_{Air} µg/m³	Recoveries using 163 *m/z* ion		Recoveries using 127+143 *m/z* ions		n
			Average	RSD	Average	RSD	
Extractability	0.20	--	76%	Not applicable	76%	Not applicable	2
	2.0	--	109%		98%		2
	20	--	105%	4%	103%	7%	3
	Overall		95%	17%	92%	15%	7
Storage stability, RT:							
2 days storage	20	--	104%		106%	--	2
5 days storage	20	--	107%		106%	--	2
	Overall		105%	4%	106%	1%	4
Ambient air sampling	0.20	0.49	106%	3%	109%	2%	5
	2.0	4.8	102%	4%	103%	5%	5
	Overall		104%	4%	106%	5%	10
Air sampling at warm, humid conditions	0.20	0.49	104%	5%	105%	9%	5
	2.0	4.3	106%	5%	106%	4%	5
	Overall		105%	5%	106%	6%	10

Document IIIA Analytical methods for the active substance in Water

SECTION A4 (4.2/03)

Annex Point IIA 4.2 & IIIA-IV.2 Transfluthrin Residues in Water

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17 REFERENCE

17.1	**Reference**	Krebber, R & Braune, M (2006) Analytical Method 01026 for the Determination of Transfluthrin in Drinking and Surface Water by GC-MS. Bayer CropScience AG Report: MR-06/174 [BES Ref. M-280731-01-1] Report date: November, 27th 2006 [*Method and validation]* Unpublished.
17.2	**Data protection**	Yes
17.2.1	Data owner	Bayer CropScience
17.2.2	Companies with letters of access	None
17.2.3	Criteria for data protection	Data submitted to the RMS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

18 GUIDELINES AND QUALITY ASSURANCE

18.1	**Guideline study**	Yes EU Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 of March 17th 2004. BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998 EU-Commission Directive 96/46/EC amending Council Directive 91/414 of 16th July 1996
18.2	**GLP**	Yes
18.3	**Deviations**	No

19 MATERIALS AND METHODS

19.1	**Preliminary treatment**	
19.1.1	Enrichment	Water samples are added with methanol. Transfluthrin is extracted from water samples by liquid-liquid extraction with dichloromethane. The organic phase is evaporated to a final volume of ca. 2 mL, the residue is transferred into test tubes and a spattle spike of LiChrosorb RP18 is added. After evaporation to dryness the residue is reconstituted in ethyl acetate and filled into GC vials by filtering through single use syringe filter.
19.1.2	Cleanup	None

Document IIIA Analytical methods for the active substance in Water

SECTION A4 (4.2/03)

Annex Point IIA 4.2 & IIIA-IV.2 Transfluthrin Residues in Water

19.2	**Detection**		
19.2.1	Separation method	Identification and quantitative determination is done by means of GC-MS. For selection the chlorine isotopic pattern of the fragment ions of the analyte were used. The first ion is the target ion with the mass 207, the second ion is the first confirmatory ion with the mass 209 and the third ion is second confirmatory ion with the mass 211. .	
19.2.2	Detector	Mass selective detector (MSD)	
19.2.3	Standard(s)	Transfluthrin external standard	
19.2.4	Interfering substance(s)	None	
19.3	**Linearity**		
19.3.1	Calibration range	In solvent and matrix the mass selective detector showed linear response in the concentration range of 1 µg/L to 100 µg/L	
19.3.2	Number of measurements	10/fortification level	
19.3.3	Linearity	Correlation coefficient $r \geq 0.9972$ (1/x weighted) for all masses.	
19.4	**Specifity: interfering substances**	No significant interferences from the sample matrix were detected in any of the samples at the retention time corresponding to transfluthrin.	X
19.5	**Recovery rates at different levels**	The mean recovery for transfluthrin (0.05 µg/L) of the target ion (m/z 207) was 103% for the first confirmatory ion (m/z 209) 101%, and for the second confirmatory ion (m/z 211) 108%. The mean recovery for transfluthrin (0.5 µg/L) of the target ion (m/z 207) was 93%, for the first confirmatory ion (m/z 209) 93%, and for the second confirmatory ion (m/z 211) 95% (see Table 2 to Table 4).	
19.5.1	Relative standard deviation	The relative standard deviations on recoveries were between 10.3% - 13.1% at 0.05 µg/L concentrations of transfluthrin. The relative standard deviation on recoveries were between 15.0% - 15.7% at 0.5 µg/L concentration of transfluthrin.	
19.6	**Limit of determination**	The limit of detection of the method was 0.01 µg/l. The limit of quantitation of the method was 0.05 µg/l.	
19.7	**Precision**		
19.7.1	Repeatability	The repeatability is given as the relative standard deviation of the recovery rates. 5 samples were used for each fortification level. Each sample was injected in duplicate and analysed for each ion. In each of the tables 2-4, 10 values per fortification level are reported.	
19.7.2	Independent laboratory validation	ILV was not undertaken in this study. Validation was performed strictly according to guidelines and all results were well within acceptable limits. The method uses commonly available reagents and techniques and has been shown to be suitable for monitoring.	

Document IIIA Analytical methods for the active substance in Water

SECTION A4 (4.2/03)

Annex Point IIA 4.2 & IIIA-IV.2 Transfluthrin Residues in Water

20 APPLICANT'S SUMMARY AND CONCLUSION

20.1	**Materials and methods**	This method describes the determination of transfluthrin in drinking and surface water using gas chromatography - mass selective detection (GC-MS) and provides validation data for three ion masses.
20.2	**Conclusion**	The method was validated for the determination of transfluthrin in drinking and surface water to meet EU requirements in all respects. The method is linear in the concentration range of 0.01 µg/L to 1.0 µg/L transfluthrin and there are no interferences at the retention time corresponding to transfluthrin.
20.2.1	Reliability	1
20.2.2	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	27-03-2008
Materials and methods	Applicant's version is adopted *3.4 Specificity:* Retention time of transfluthrin is approximately 7.5 min.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	acceptable
Remarks	A study report on the determination of transfluthrin in drinking water and test water from aquatic toxicity tests by GLC with on-line solid phase microextraction (König, T (1998), Method 00512, BES Ref. MO-99-018150) is also available, but a DocIIIA study summary is missing. However, since a valid method is available to analyse the active substance in drinking water and in surface water, the summary for this study report is not required.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 2: Recoveries for Transfluthrin (Target Ion, m/z 207)

Crop	Fortification level (µg/L)	Recoveries (%) – single values					Mean per FL (%)	RSD* (%)	Mean overall (%)	RSD overall (%)
Surface water	0.05	99	105	128	114	102	103	12.5		
		106	96	98	78	100				
Surface water	0.5	79	82	87	116	108	93	15.0	98	14.4
		85	95	106	73	94				

*RSD = Relative Standard Deviation

Table 3: Recoveries for Transfluthrin (1st Confirmatory Ion, m/z 209)

Crop	Fortification level (µg/L)	Recoveries (%) – single values					Mean per FL (%)	RSD* (%)	Mean overall (%)	RSD overall (%)
Surface water	0.05	96	104	127	112	101				
		104	94	97	75	100	101	13.1		
Surface water	0.5	79	82	87	116	108				
		85	95	106	73	95	93	15.0	97	14.4

*RSD = Relative Standard Deviation

Table 4: Recoveries for Transfluthrin (2nd Confirmatory Ion, m/z 211)

Crop	Fortification level (µg/L)	Recoveries (%) – single values					Mean per FL (%)	RSD* (%)	Mean overall (%)	RSD overall (%)
Surface water	0.05	100	107	120	105	95				
		100	120	121	94	121	108	10.3		
Surface water	0.5	82	84	89	122	110				
		87	98	110	74	97	95	15.7	102	14.2

*RSD = Relative Standard Deviation

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SECTION A4 (4.2/04)

Analytical methods for the active substance in animal and human body fluids and tissue

BPD Data set IIA/ Annex Point IV.4.2

Transfluthrin residues in plasma

Official use only

21 REFERENCE

21.1	**Reference**	Gries, W (2000) Determination of Transfluthrin in Plasma, Bayer AG Medical Sciences Institute of Biological Monitoring - Method SPE with Oasis HLB, Auto Spec, NCI mode. Bayer AG. Bayer AG Report No. 2005-0007201-97 E [BES Ref: M0-03-011204] Report date: September 28, 2000 [*Method and Validation]* Unpublished
21.2	**Data protection**	Yes
21.2.1	Data owner	Bayer CropScience
21.2.2	Companies with letters of access	
21.2.3	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

22 GUIDELINES AND QUALITY ASSURANCE

22.1	**Guideline study**	Yes EC Directive 91/414/EEC, Annex 11 and III BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998
22.2	**GLP**	No
22.3	**Deviations**	No

23 MATERIALS AND METHODS

23.1	**Preliminary treatment**	
23.1.1	Enrichment	Transfluthrin in plasma samples is determined by GC-MS with negative chemical ionisation. The sample is diluted with water, transferred to a conditioned Oasis column and allowed to trickle in slowly under atmospheric pressure. After rinsing with water, centrifuging, and drying the column in N_2, it is rinsed with hexane and transfluthrin is eluted into a test tube with hexane:dichloromethane (1:1, v:v). After evaporating to dryness in N_2, the sample is taken up in toluene.
23.1.2	Cleanup	None
23.2	**Detection**	
23.2.1	Separation method	GC: Column: DB 1 30 mm x 0.25 mm x 0.1 µm or DB 5 30 mm x 0.25 mm x 0.1 µm; 300ºC

Document IIIA
Analytical methods for the active substance in animal and human body fluids and tissue

SECTION A4 (4.2/04)

BPD Data set IIA/ Annex Point IV.4.2 Transfluthrin residues in plasma

23.2.2	Detector	SMS (Single Mass Selective) MS detection; Target mass, m/z of transfluthrin = 206.9980
23.2.3	Standard(s)	Fenvalerate (internal standard)
23.2.4	Interfering substance(s)	Interference with cyfluthrin was noted.
23.3	**Linearity**	
23.3.1	Calibration range	Linearity was checked for transfluthrin in the range of 0.1 and 1.0 µg/l.
23.3.2	Number of measurements	5
23.3.3	Linearity	Linearity r^2 = 0.9996
23.4	**Specifity: interfering substances**	Contamination of samples, particularly with cyfluthrin, was observed.
23.5	**Recovery rates at different levels**	1 µg/l = >90%
23.5.1	Relative standard deviation	1 µg/l = 14.6% (n=5) 0.1 µg/l = 14.2% (n=5)
23.6	**Limit of determination**	The limit of quantification of the method was 10 ng/l plasma The limit of detection was 5 ng/l plasma (theoretical)
23.7	**Precision**	
23.7.1	Repeatability	1 µg/l : RSD = 14.6% (n=5) 0.1 µg/l : RSD = 14.2% (n=5)
23.7.2	Independent laboratory validation	This report includes validation data from studies conducted internally.

Document IIIA

Analytical methods for the active substance in animal and human body fluids and tissue

SECTION A4 (4.2/04)

BPD Data set IIA/ Annex Point IV.4.2

Transfluthrin residues in plasma

24 APPLICANT'S SUMMARY AND CONCLUSION

24.1 Materials and methods

The method describes the determination of transfluthrin in plasma by GC-MS with negative chemical ionisation. The sample is diluted with water, transferred to a conditioned Oasis column and allowed to trickle in slowly under atmospheric pressure. After rinsing with water, centrifuging, and drying the column in N_2, it is rinsed with hexane and transfluthrin is eluted into a test tube with hexane:dichloromethane (1:1, v:v). After evaporating to dryness in N_2, the sample is taken up in toluene.

24.2 Conclusion

The method was validated for the determination of transfluthrin in plasma. The method is linear in the concentration range of 0.1 µg/l to 1.0 µg/l transfluthrin and with recovery rate ~90%. The RSD at both levels was ~ 14%. Although within acceptable level, this high coefficient of variation is probably due to the fact that the fenvalerate used as internal standard is not an optimal/universal internal standard

24.2.1 Reliability: 1

24.2.2 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	*25-09-2007*
Materials and methods	Method is not evaluated. Analytical methods for bodyfluids are considered not relevant/are not needed, because the active substance is not classified as toxic or highly toxic.
Conclusion	Method is not evaluated. Analytical methods for bodyfluids are considered not relevant/are not needed, because the active substance is not classified as toxic or highly toxic.
Reliability	n.a.
Acceptability	Method is not evaluated. Analytical methods for bodyfluids are considered not relevant/are not needed, because the active substance is not classified as toxic or highly toxic.
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Document IIIA **SECTION A4 (4.3)** **BPD Data set IIA/** **Annex Point IIIA-IV.1**	**Analytical methods for the active substance in/on Food or Feedstuffs**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [✓]**	**Technically not feasible [ ]** **Scientifically unjustified [✓]** **Other justification [ ]**	
Detailed justification:	The proposed uses of transfluthrin are for non-professional indoor use, either as a vapouriser (Raid Portable Electric) or as a disc product designed to treat cupboards, closets and wardrobes (Turbo 4 Seasons), or both indoor and out door use as a mosquito coil (Baygon Mosquito Coil). The estimation of potential exposure of the active substance to humans through diet and other means has been carried out (Documents IIB-1 and IIB-2, section 3.2 and Document IIIA, section 6.15), taking into account the frequency and duration of use, the emission rate of the active substance from the product, assuming that the airborne fraction of emitted residues is 100% and using standard room volume and ventilation rates. Worst case intakes were from the use of Raid Portable Electric. Calculated intakes from worst to extreme worst case scenarios were negligible: for a 10 kg toddler: 5.2×10^{-7} - 3.9×10^{-5} mg/kg bw/day for a 60 kg adult: 8.5×10^{-8} - 6.4×10^{-6} mg/kg bw/day Comparisons of estimated intakes from potential contaminated food with the proposed ADI (Acceptable Daily Intake) and ARfD (Acute Reference Dose) were performed: *Chronic exposure*: An adult would need to consume 1,562 – 117,647 sandwiches per day and a toddler would need to consume 256 – 19,231 sandwiches per day to achieve intakes equivalent to the ADI. *Acute Exposure*: In order to achieve the acute reference dose, an adult would need to consume 26,562 – 2,000,000 sandwiches per day and a toddler would need to consume 4359 – 326,923 sandwiches per day. A toddler or adult could not eat this number of sandwiches in a day and therefore the risk to consumers was considered to be acceptable In conclusion: In summary, the amateur indoor use of transfluthrin in the vapouriser, with subsequent deposition and transference of residues from room surfaces to foodstuffs (sandwich of 150 cm^2 surface area), results in negligible potential residue levels in food which do not pose a risk to consumers. The need for an analytical method to determine residues in food and feedingstuffs is therefore considered to be scientifically unjustified.	
Undertaking of intended	Not applicable	

Document IIIA **SECTION A4 (4.3)** **BPD Data set IIA/** **Annex Point IIIA-IV.1**	**Analytical methods for the active substance in/on Food or Feedstuffs**
data submission [ ]	
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*25-09-2007*
Evaluation of applicant's justification	The applicant's conclusion that potential residue levels in food are negligible is considered acceptable, based on calculations in Doc IIIA 6.15. Therefore, analytical methods for food/feed are considered not relevant/are not needed.
Conclusion	Analytical methods for food/feed are considered not relevant/are not needed, because calculations show that potential residue levels in food will be negligible (see Doc IIIA 6.15).
Remarks	None.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	