Addendum No. 1 to MRID # 48542647, 48542650, 48542651, 48542652, 48542657, 48542658, 48542828, & 49223501

DER Study Title: Independent Laboratory Validation of BASF Analytical Method D1002: "Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS" (MRID 48542647), *etc.*

Guideline Number: 850.6100

PC Code: 138831

The study classifications for these eight environmental chemistry method (ECM) reports (attached below) are downgraded from acceptable to **unacceptable**.

- Reasons for changes:

None of these eight ECM reports have a supporting independent laboratory validation (ILV) report. ECM reports are unacceptable without an associated ILV report. MRIDs 48542647 and 48542828 are the only two ECM reports submitted regarding the same analytical method. However, the validations in these reports are not independent, as they were conducted at the same ADPEN Laboratories, Inc. facility and involved three of the same authors.

MRID	**Matrix**	**Analytes**
48542647	Soil	Cyflumetofen, A-2, B-3, B-1, AB-1 dimer
48542828	Soil	Cyflumetofen, A-2, B-3, B-1, AB-1 dimer
48542650	Water	Cyflumetofen
48542651	Water	B-1
48542652	Water	B-3
49223501	Water	AB-1, A-2, A-12
48542657	Sediment	AB-1
48542658	Algal medium	Cyflumetofen

Revised by: Gregory Orrick Gregory Orrick 2019.04.15 17:07:31 -04'00' Date: 4-15-19

Secondarily reviewed by: Faruque Khan Faruque A. Khan Date: 4-15-19

Volume 3

Annex B

Cyflumetofen

B.5 Methods of analysis

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B.5.1 Analytical methods for technical active substance and formulation analysis (Annex IIA 4.2, Annex IIIA 5.2)

B.5.1.1 Methods for the analysis of pure active substance in the active substance as manufactured (Annex II 4.2.1)

Report: II A 4.2.1/01
Reference Type: Study Report
Author(s): Saka M. 2004(b)
Year: 2004
Title: OK-5101 technical: Determination of purity and stability
Testing Laboratory: The Institute of Environmental Toxicology
4321, Uchimoriya-machi, Mitsukaido-shi,
Ibaraki 303-0043, Japan
Report Number: lET 01-5007
Owner Company: BASF
Company Study Number: BASF Reg. Doc. # OTSA-0021(EN)-FR(2)
MRID: 48542636
Report Date: 3 December 2004
Data Access: Data submitter is data owner
Data Protection Claimed: Yes
Guidelines: EPA 830.1550; EPA 830.1700; EPA 830.1750
GLP: Yes
PMRA # 2146738

Report: II A 4.2.1/02
Reference Type: Study Report
Author(s): Saka M. 2004(c)
Year: 2004
Title: Composition of components in OK-5101 technical (include validation of analytical methods)
Testing Laboratory: The Institute of Environmental Toxicology
4321, Uchimoriya-machi, Mitsukaido-shi,
Ibaraki 303-0043, Japan
Report Number: IET 03-5047
Owner Company: BASF
Company Study Number: BASF Reg. Doc. # OTSA-0167(EN)-FR
MRID: 48542605
Report Date: 9 June 2004
Data Access: Data submitter is data owner
Data Protection Claimed: Yes
Guidelines: EPA 830.1550; EPA 830.1700; EPA 830.1750; SANCO/3030/99 rev. 4 (11 July 2000)
GLP: Yes
PMRA # 2146673

Executive Summary
The purity of cyflumetofen technical (Lot No.: 01Dl) was determined to be 97.67% (w/w) by high performance liquid chromatograph (UV detection) using the internal standard method (internal standard: o-terphenyl).

In order to check the stability of cyflumetofen technical, it was stored under dark and cold conditions over 36 months and the active ingredient content was determined with time by HPLC (UV detection) using the internal standard method during the storage period to evaluate the stability.

Details of the analytical method used to determine active in cyflumetofen technical	
Method ID	Not stated

Details of the analytical method used to determine active in cyflumetofen technical	
Sample preparation	Accurately weigh ~ 25 mg of test substance into 100 mL volumetric flask, dissolve in acetonitrile, add 5 mL of the internal standard solution[1] and then dilute to volume with acetonitrile. Injection volume: 5 µL
Instrument	HPLC
Detector	UV at 220 nm
Column	L-column, 250 mm × 4.6 mm i.d.
Mobile phase (for LC)	Acetonitrile/water (70/30, v/v) Flow rate: 1.0 mL/min
Oven temperature	40°C
Quantitation	Internal standard method using *o*-terphenyl as internal standard Reference material: Cyflumetofen 99.92%, 98.46%
Retention time	Cyflumetofen 15.1 min *o*-terphenyl (IS) 18.1 min
Total run time	25 minutes
Chromatograms	Chromatograms of solvent blank, internal standard, standard and sample (with and without internal standard) were provided, which showed well resolved peaks.
[1] Internal standard preparation (3200 mg/L): accurately weigh 320 mg of *o*-terphenyl into a 100 mL volumetric flask, dissolve in acetonitrile and dilute to volume with acetonitrile.	

Linearity:
The standard solutions at 5 different concentrations of 150, 200, 250, 300 and 350 mg/L were injected into the HPLC to make the calibration curve. Linearity of the method was repeatedly proven over the range of 150-350 mg/L with a coefficient of variation over 0.999.

Specificity:
The HPLC-UV method was able to separate OK-5101 from impurities, solvent and internal standard (*o*-terphenyl). No peaks were observed close to the retention time of the active substance (OK-5101) peak. However, the study did not address specificity in compliance with SANCO/3030/99 but this data gap was covered by the results of study Saka (2004c) (BASF Reg. Doc. # OTSA-0167).

Accuracy:
Five replicate determinations were carried out of for each solution at 170, 250, and 330 mg/L (cyflumetofen analytical standard acetonitrile solution), and the recoveries of cyflumetofen were in the range of 100~101% (average, 100%), 100~101% (average, 100%), 100% (average, 100%), respectively.

Precision/repeatability:
The precision of the method was determined using the recovery data. The coefficients of variation (C.V.) for each level were calculated; the overall C.V. was 0.4%. The precision of the method was also determined by injecting 5 replicate samples of cyfumetofen technical. The mean active substance content was 97.67% with a coefficient of variation of 0.3%. Based on these findings the Horwitz-criteria for precision were met, since the calculated Horwitz value (%RSDr) of 1.3 was not exceeded by the experimental RSD of 0.3, measured in this study. Five replicates of the five batch samples were also analyzed for cyflumetofen; the relative standard deviations (RSDs) ranged from 0.2-0.6%.

Storage stability:
Content of the active ingredient of cyflumetofen technical was determined after storage of 0, 6, 12, 18, 24 and 36 months according to the above mentioned method. The ratio of each

content value to the initial content value was calculated as a percent remained (%) and rounded to whole number. The results are described below.

Storage stability of cyflumetofen under dark and cold storing conditions

Storage Period (months)	Date of Analysis	Mean Content[A] (%)	Percentage (%)
0	20-08-2001	97.67	100
6	20-02-2002	98.24	101
12	20-08-2002	98.04	100
18	20-02-2003	98.67	101
24	20-08-2003	98.48	101
36	20-08-2004	97.67	100

[A] mean recovery is based on 5 determinations, i.e. 1 determination per concentration

Conclusion:
The described HPLC method is sufficiently validated for linearity, accuracy and precision for the determination of cyflumetofen in the TGAI.

B.5.1.2 Applicability of existing CIPAC methods (Annex IIA 4.2.2)

Not relevant.

B.5.1.3 Description of analytical methods for the determination of impurities (Annex IIA 4.2.3)

Description in volume 4, confidential part.

B.5.1.4 Description of analytical methods for the determination of additives (Annex IIA 4.2.4)

Not relevant.

B.5.1.5 Enforcement analytical methodology (Annex IIA 4.2.5)

See 5.1.1

B.5.1.6 Inter-laboratory analytical methodology validation (Annex IIA 4.2.6)

Not required.

B.5.1.7 Storage stability of working solutions in analytical methodology (Annex IIA 4.2.7)

During the determination of accuracy, the samples for each level were stored in the autosampler tray after the first injection. After about 24 hours, a second injection was made for each sample and the recoveries and coefficients of variation were calculated. The range of the mean recoveries between the first and second injection was below 2%.

B.5.1.8 Analytical methods for the determination of the active substance in plant protection products (Annex IIIA 5.2)

An analytical method (including validation) has been developed for the determination of cyflumetofen (BAS 9210 I) in Nealta Miticide/Sultan Miticide (BAS 9210 2 I formulation).

Report: III A 5.2.1/01
Reference Type: Study report
Author(s): Brill J.H. 2011(a)

Year: 2011
Title: Validation of analytical method AFR0088/01: Determination of BAS 9210 I in BAS 9210 I SC formulations by reverse-phase HPLC Using UV Detection
Testing Laboratory: BASF Corporation, Crop Protection; 26 Davis Drive; Research Triangle Park, NC 27709
Report Number: 409943
Owner Company: BASF
Company Study Number: BASF Reg. Doc. # 2011/7005142
MRID: 48542905
Report Date: 04-Nov-2011
Data Access: Data submitter is data owner
Data Protection Claimed: Yes
PMRA # 2145855

Executive Summary
The study was conducted to validate analytical method AFR0088/01.

BASF method AFR0088/01 is a reverse phase HPLC method, using an MAC MOD HALO C18 column (50 mm x 4.6 mm, particle size = 2.7 µm) and UV detection at 230 nm. The mobile phase is a gradient mixture of water and acetonitrile, each with 0.05% acetic acid. The flow is 1.0 mL/min. The column temperature is 25 °C. The injection volume is 5 µL.

Five calibration standards with cyflumetofen concentrations ranging from 5.59 mg/25mL to 25.64 mg/25mL were used to validate the linearity of Method AFR0088/01. The response of cyflumetofen was shown to be linear over this concentration range.

The accuracy (recovery) of cyflumetofen determination in BAS 9210 2 I formulation was 100.06%. The cyflumetofen analysis reproducibility and injection precision were under 1.0% RSD. Method AFR0088/01 is valid for the assay of cyflumetofen in SC formulations.

Details of the analytical method used to determine cyflumetofen in Nealta Miticide/Sultan Miticide (BAS 9210 2 I)	
Method ID	AFR0088/01
Sample preparation	Accurately weigh ~ 80 mg of test substance into a 30 mL glass bottle. Add 1 mL of distilled water and gently swirl to disperse the contents. Add 1 mL of internal standard solution[1] and 23 mL of acetonitrile and mix well. Sonicate the samples for approximately 10 minutes. Filter a small aliquot through a 0.45 µm PTFE syringe filter.
Instrument	HPLC
Detector	UV at 230 nm
Column	MAC MOD HALO C18 column, 50 mm × 4.6 mm i.d., 2.7 µm
Mobile phase (for LC)	Time (min): % A % B 0.0 45 55 0.5 45 55 2.0 20 80 5.0 20 80 5.1 45 55 A = Water with 0.05% acetic acid B = Acetonitrile with 0.05% acetic acid Flow rate: 1.0 mL/min
Oven temperature	25°C

Details of the analytical method used to determine cyflumetofen in Nealta Miticide/Sultan Miticide (BAS 9210 2 I)	
Quantitation	Internal standard method using diethyl phthalate as internal standard Reference materials: Cyflumetofen 98.5% Diethyl phthalate 99.98% (Sigma-Aldrich)
Retention time	Cyflumetofen 3.65 min Diethyl phthalate 1.34 min
Total run time	5.5 minutes
Chromatograms	Representative chromatograms of the solvent blank, formulation blank, fortified formulation blank, standard and sample were provided. No interferences were observed around the peaks of interest.
[1] Internal standard preparation (diethyl phthalate, 11.12 mg/mL): Weigh 556.09 mg of diethyl phthalate into a 50 mL volumetric flask and dilute to the volume with acetonitrile.	

Linearity:
The linearity (0.224 mg/mL – 1.026 mg/mL) was established by showing that the plot of the peak area ratio versus the standard concentration ratio was a straight line. The linear regression curve was constructed using five levels of cyflumetofen with two injections per level for levels 1, 2, 4, and 5, and 13 injections for level 3. The linearity parameters were as follows:

Coefficient of correlation: 0.9995
Slope: 0.6659
Intercept: 0.0056

Specificity:
Chromatograms of the blank formulation, BAS 9210 2I, showed no indications of interferences due to other components at the particular retention time of the analyte and the internal standard.

Accuracy:
Known amounts of the cyflumetofen reference material were weighed into the blank formulation of BAS 9210 2I and analyzed according to the analytical method AFR0088/01. The three spiked solutions were prepared in levels according to the target concentration of the active in the test substance. The mean value for the recovery was 100.06% with a RSD of 0.32%.

Precision:
The precision of the method was determined by weighing five sub-samples of the test substance BAS 9210 2I Lot 1742-56 and analyzing them by HPLC under the AFR0088/01 conditions. The mean value was 18.8% with a RSD of 0.96%. In addition, a single solution (level 3 linearity standard) was injected 10 times, in order to measure the precision of the instrumentation.

Conclusion:
The analytical method AFR0088/01 is applicable to determine the content of cyflumetofen (BASF Reg. No. 5465430) in formulation type SC (e.g. in BAS 9210 2I) and was assessed to be acceptable for use as an enforcement analytical method.

B.5.1.9 Applicability of existing CIPAC methods (Annex IIIA 5.2.3)

Not relevant.

B.5.1.10 Analytical methods for the determination of relevant impurities which are of toxicological, ecotoxicological or environmental concern, in the preparation (Annex IIIA 5.2.4)

Not relevant.

B.5.1.11 Analytical methods for formulants or their constituents in the plant protection product (Annex IIIA 5.2.5)

Not relevant.

B.5.2 Analytical methods for the determination of residues (all components included in the residue definition proposed) (Annex IIA 4.3, Annex IIIA 5.3)

This section not reviewed by the PMRA chemistry reviewer.

B.5.3 Analytical methods for the determination of residues in soil, water, sediment and air (Annex IIA 4.4 to 4.7, Annex IIIA 5.4 to 5.7)

B.5.3.1 Soil (Annex IIA 4.4, Annex IIIA 5.4)

An analytical method has been developed for the determination of cyflumetofen and relevant metabolites in soils.

Report:	II A 4.4/05
Reference Type:	Study Report
Author(s):	Carter, M.L., Saha, M.G, Perez, S., and Miska, J.L.
Year:	2011
Title:	Validation of Analytical Method Number D1002: Validation of BASF Analytical Method D1002: 'Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil Using LC-MS/MS
Testing Laboratory:	ADPEN Laboratories, Inc.
Report Number:	416947
Owner Company:	BASF
Company Study Number:	BASF Reg. Doc. # 2011/7005073
MRID:	48542828
Report Date:	09 November 2011
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes
PMRA # 2146748	

Executive Summary

The objective of this study was to validate BASF Analytical Method D1002 for the analysis of residues of cyflumetofen [BAS 9210 I (5465430)] and its metabolites, A-2, B-3, B-1, and AB-1 dimer, in soil samples at a limit of quantitation (LOQ) of 0.01 ppm. The soil types included sandy loam (0–3"depth), silt loam (12–18" depth), loamy sand (high pH, 12–18" depth), and sandy loam (German soil).

This study was conducted in compliance with US Environmental Protection Agency (EPA) Good Laboratory Practices (GLP) standards, 40 CFR 160, EPA EFATE Guidelines Series 835.6100 (Terrestrial Field Dissipation), 860.1340 Residue Analytical Method, and EC Guidance documents; SANCO/3029/99 rev.4 (2000, pre-registration residue methods),and SANCO/825/00 rev.6 (2000, post-registration residue methods)

The analytical method has a limit of quantitation (LOQ) of 0.01 ppm for residues of cyflumetofen and its metabolites in all soil types. The method limit of detection (LOD) for residues of all analytes has been set at 0.002 ppm, or approximately 20% of the LOQ.

For validation, control samples were fortified and analyzed according to the established method validation guidelines. Each analytical set contained a reagent blank, two unfortified matrix controls, five matrix controls fortified LOQ, and five matrix controls fortified at 10× LOQ (0.1 ppm).

A brief description of the methodology for BASF Analytical method D1002 follows:

Bulk soil samples received from the field are homogenized with dry ice using a Fitzmill. An aliquot of the homogenized soil samples are further homogenized in a Retsch Ultra Centrifugal mill equipped with a 1.0 mm screen if necessary. The samples are stored frozen (< -5°C) before analysis.

Residues of cyflumetofen (BAS 9210 I), B-1, B-3, A-2, and AB-1 dimer are extracted from soil matrices with acetonitrile by vortexing, shaking, and centrifuging. Acetonitrile/water (60:40, v/v) is added and the above sample steps are repeated. The combined extract is diluted with 1:1 (v/v) with 0.1% formic acid in acetonitrile for the residue determination of cyflumetofen, B-1, and B-3. Similarly the extracts are diluted (1:1, v/v) with 0.1% formic acid in water and with acetonitrile, for the analysis of A-2 and AB-1 dimer, respectively. The residues are determined by LC-MS/MS.

Sample sizes used in the validation study were 0.1 g for all soil types except the German soil, which used a 5.0 g sample size. The aliquot and final dilution of the extract were adjusted accordingly for the determination of the residues.

The method validation was run successfully for all soil types and all analytes and LC-MS/MS ion transitions (primary and secondary) available for the method.

Overall mean recoveries of the analyses obtained from different soil types in the validation study were between 77–107% for all fortification levels. Overall relative standard deviations of recoveries for each analyte per soil type were between 1.4–17.8%.

Matrix/Medium: Soil
Test Guideline: According to: U.S. EPA Residue Chemistry Guidelines:
OPPTS 835.6100 Terrestrial Field Dissipation,
OPPTS 860.1340 Residue Analytical Method,
SANCO/3029/99 rev. 4, SANCO/825/00 rev. 6
Deviations from Guideline: No
GLP Compliance: Yes

Methods:
Instrument/Detector: LC-MS/MS
Details on Method:
Instrument: Agilent 1200 HPLC
Detector: AB Sciex API 5500 Triple Quad

Summary Parameters for the Analytical Method Used for the Quantitation of Cyflumetofen (BAS 9210 I) and Metabolite Residues in Soils

Method ID	BASF method D1002				
Analyte(s)	Cyflumetofen (BAS 9210 I) and Metabolites A-2, B-3, B-1, AB-1				
Extraction Solvent/technique	Extracted from soil matrices by vortexing, shaking, and centrifuging with acetonitrile, followed by acetonitrile-water(60:40 v/v)				
Instrument	UPLC-MS/MS				
Detector for Method	**BAS 9210 I**	**B-1**	**B-3**	**A-2**	**AB-1**
Quantitation (m/z)	448.2 → 173.0	189.1 → 69.0	190.0 → 130.0	174.1 → 147.1	689.4 → 288.2
Confirmation (m/z)	448.2 → 145.1	189.1 → 145.1	190.0 → 102.0	174.1 → 117.1	689.4 → 268.2
Ionization Mode	Positive	Negative	Positive	Positive	Positive
Expected Retention Times[1]	~2.40	~1.88	~1.77	~1.80	~2.70
Standardization Method	Linear Regression				

Stability of Std Solutions	35 Days at refrigerated temperatures for fortification and calibration standards for BAS 9210 I, B-1, B-3, and A-2 (96 days for stock solutions). For AB-1, 1 day for stock, fortification and calibration standards.

[1] Expected retention times may vary using alternate methods. Using the methods for German soil, the retention times are ~6.41 for cyflumetofen, ~4.12 for B-1, ~3.35 for B-3, and ~5.18 for A-2.

LC conditions for the analysis of cyflumetofen, B-1 and B-3

Column: Acquity UPLC HSS T3, 50 mm × 2.1 mm, 1.8 µm
(Gemini C_{18}, 100 mm × 4.6 mm, 3.0 µm, for German soil)
Column temperature: 60°C (50°C for German soil)
Injection volume: 7.0 µL (20.0 µL for German soil)
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile
Gradient:

Time (min)	%A	%B
0.00	99.0	1.0
0.10	99.0	1.0
0.40	60.0	4.0
1.35	0.0	100
2.25	0.0	100
2.30	99.0	1.0
3.60	99.0	1.0

Flow rate: 500 µL/min

Gradient (for German soil):

Time (min)	%A	%B
0.00	95.0	5.0
0.10	95.0	5.0
0.60	60.0	40.0
2.80	25.0	75.0
7.00	0.0	100
7.50	0.0	100
7.60	95.0	5.0
10.60	95.0	5.0

Flow rate: 800 µL/min

LC conditions for the analysis of A-2

Column: Acquity UPLC BEH C_{18}, 50 mm × 2.1 mm, 1.7 µm
(Gemini C_{18}, 100 mm × 4.6 mm, 3.0 µm, for German soil)
Column temperature: 50°C
Injection volume: 40.0 µL
Mobile phase: A: 1% formic acid in water
B: 0.1% formic acid in acetonitrile
Gradient:

Time (min)	%A	%B
0.00	70.0	30.0
0.05	70.0	30.0
0.90	20.0	80.0
1.50	1.0	99.0
2.45	1.0	99.0
2.50	70.0	30.0
3.50	70.0	30.0

Flow rate: 600 µL/min

Gradient (for German soil):

Time (min)	%A	%B
0.00	70.0	30.0
0.10	70.0	30.0
3.00	20.0	80.0
4.00	1.0	99.0
5.00	1.0	99.0

5.10	70.0	30.0
8.00	70.0	30.0

Flow rate: 800 µL/min

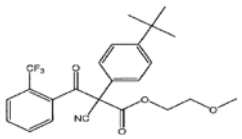
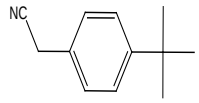
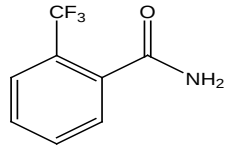
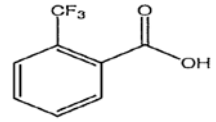
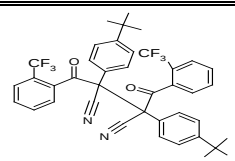
LC conditions for the analysis of AB-1 Dimer

Column: Acquity UPLC BEH C_{18}, 50 mm × 2.1 mm, 1.7 µm
Column temperature: 50°C
Injection volume: 20.0 µL
Mobile phase: A: 1% formic acid in water
B: 0.1% formic acid in acetonitrile
Gradient:

Time (min)	%A	%B
0.00	70.0	30.0
0.05	70.0	30.0
0.90	20.0	80.0
1.50	1.0	99.0
2.45	1.0	99.0
2.50	70.0	30.0
3.00	70.0	30.0

Flow rate: 500 µL/min

Test and Reference Items

Structural Formula	Codes	Reg. No.	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name							
	BAS 9210 I	5465430	400882-07-7	$C_{24}H_{24}F_3NO_4$	447.5 g/mol	006005	99.5%	08.03.12
	2-methoxyethyl-(*R*,*S*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl)propionate							
	A-2	133276	3288-99-1	$C_{12}H_{15}N$	173.3 g/mol	L84-64	98.5%	12.01.12
	(4-*tert*-butylphenyl)acetonitrile							
	B-3	4288294	360-64-5	$C_8H_6F_3NO$	189.13 g/mol	08721EB	99.9%	01.07.12
	2-(trifluoromethyl)benzamide							
	B-1	104415	433-97-6	$C_8H_5F_3O_2$	190.12 g/mol	MKBB8135	99.4%	12.07.11
	2-(trifluoromethyl)benzoic acid							
	AB-1 Dimer	5756389	NA	$C_{40}H_{34}F_6N_2O_2$	688.7 g/mol	L84-54	92.9%	12.01.12
	2,3-bis(4-tert-butylphenyl)-2,3-bis[2-(trifluoromethyl)benzoyl]butanedinitrile							

Results:

Linearity:
Good linearity was observed in the range of 0.125–10.0 ng/mL for all analytes with a coefficient of variation over 0.99.

Specificity:
The method determines cyflumetofen and its metabolites in soil matrices. There were no known interferences from soil components or from reagents, solvents and glassware used, except in B-1 analyses. The suggested LC-MS/MS conditions for the B-1 secondary ion transition (m/z 189.1 → 145.1) were not adequate for quantitation when injected with cyflumetofen and B-3. The B-1 metabolite, which is detected in negative ionization mode, elutes between two positive ionization periods and switching between polarities caused distorted peak shape in the secondary ion transition (m/z 189.1 → 145.1). Therefore, a separate chromatographic method is used for the analysis of B-1 for confirmatory purpose. No significant interferences were noted during analysis and no significant residues were found in reagent blanks or control samples. No other interferences were noted during analysis and no significant residues were found in reagent blanks or control samples.

Precision/repeatability:
The relative standard deviations (RSD, %) for all commodities and all fortification levels ranged from 1.4% to 17.8%. The detailed values are shown below.

Accuracy and precision:
The accuracy and precision of BASF Method D1002 is best represented by the recovery data generated during the validation. Summaries of the recovery results are shown below.

Limit of Quantification/Detection:
The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 ppm for cyflumetofen and its metabolites in all soil matrices. The limit of detection was set at 20 % (0.002 ppm) of the limit of quantitation. In addition, a minimum signal to noise ratio (S/N) of 3:1 was used for the lowest standard in the calibration curves.

Method Validation Recoveries for BAS 9210 I and its Metabolites in Fortified Soil Samples

Using LC-MS/MS, ABSciex API 5500				
Analyte	**Fortification Level (ppm)**	**N**	**Individual Recoveries (%)**	**Mean (%) ± %RSD (Std. Dev.)**
SANDY LOAM (0-3" DEPTH)				
Cyflumetofen	Primary Transition (m/z 448.2 → 173.0)			
	0.01	5	91, 90, 94, 97, 96	98 ± 6.7 (6.6)
	0.1	5	101, 109, 109, 100, 98	
	Overall	**10**	**Range, 90–109%**	
	Secondary Transition (m/z 448.2 → 145.1)			
	0.01	5	95, 98, 109, 89, 101	103 ± 6.6 (6.8)
	0.1	5	108, 108, 107, 109, 102	
	Overall	**10**	**Range, 95–109%**	
A-2	Primary Transition (m/z 174.1 → 147.1)			
	0.01	5	103, 90, 97, 87, 83	90 ± 7.7 (6.9)
	0.1	5	84, 84, 85, 87, 98	
	Overall	**10**	**Range, 83–103%**	
	Secondary Transition (m/z 174.1 → 117.1)			
	0.01	5	96, 72, 96, 97, 70	87 ± 14.6 (12.7)
	0.1	5	73, 76, 91, 100, 100	
	Overall	**10**	**Range, 70–100%**	
B-3	Primary Transition (m/z 190.0 → 130.0)			
	0.01	5	103, 106, 108, 102, 102	105 ± 2.5 (2.6)
	0.1	5	102, 106, 108, 108, 105	
	Overall	**10**	**Range, 102–108%**	
	Secondary Transition (m/z 190.0 → 102.0)			
	0.01	5	101, 100, 105, 104, 102	102 ± 3.2 (3.3)
	0.1	5	97, 100, 105, 108, 101	
	Overall	**10**	**Range, 97–108%**	

Table Continued, Footnotes Follow

Table (continued)

Using LC-MS/MS, ABSciex API 5500				
Analyte	**Fortification Level (ppm)**	**N**	**Individual Recoveries (%)**	**Mean (%) ± %RSD (Std. Dev.)**
SANDY LOAM (0-3" DEPTH) (CONTINUED)				
B-1	Primary Transition (m/z 189.1 → 69.0)			
	0.01	5	68[1], 97, 102, 87, 92	97 ± 12.9 (12.5)
	0.1	5	100, 101, 97, 114[1], 108	
	Overall	**10**	**Range, 68–114%**	
	Primary Transition (m/z 189.1 → 69.0) Confirmation			
	0.01	5	92, 90, 110, 102, 109	104 ± 8.6 (8.9)
	0.1	5	99, 107, 110, 103, 120[1]	
	Overall	**10**	**Range, 90–120%**	
	Secondary Transition (m/z 189.1 → 145.1) Confirmation			
	0.01	5	88, 98, 104, 104, 109	105 ± 7.8 (8.2)
	0.1	5	102, 110, 109, 119[1], 108	
	Overall	**10**	**Range, 88–119%**	
AB-1 Dimer	Primary Transition (m/z 689.4 → 288.2)			
	0.01	5	104, 107, 107, 107, 108	103 ± 5.2 (5.4)
	0.1	5	100, 96, 106, 98, 94	
	Overall	**10**	**Range, 94–108%**	
	Secondary Transition (m/z 689.4 → 268.2)			
	0.01	5	97, 105, 100, 108, 105	100 ± 7.1 (7.1)
	0.1	5	85, 97, 107, 94, 101	
	Overall	**10**	**Range, 85–108%**	
SILT LOAM (12-18" DEPTH)				
Cyflumetofen	Primary Transition (m/z 448.2 → 173.0)			
	0.01	5	95, 95, 78, 88, 96	93 ± 7.6 (7.1)
	0.1	5	91, 94, 106, 98, 93	
	Overall	**10**	**Range, 78–106%**	
	Secondary Transition (m/z 448.2 → 145.1)			
	0.01	5	104, 107, 98, 105, 107	101 ± 5.7 (5.7)
	0.1	5	95, 96, 108, 97, 93	
	Overall	**10**	**Range, 93–107%**	
A-2	Primary Transition (m/z 174.1 → 147.1)			
	0.01	5	104, 130[1], 100, 110, 101	107 ± 12.7 (13.6)
	0.1	5	103, 132[1], 92, 94, 107	
	Overall	**10**	**Range, 92–132%**	
	Secondary Transition (m/z 174.1 → 117.1)			
	0.01	5	91, 93, 136[1], 102, 109	103 ± 13.9 (14.4)
	0.1	5	102, 92, 87, 110, 113[1]	
	Overall	**10**	**Range, 87–136%**	
B-3	Primary Transition (m/z 190.0 → 130.0)			
	0.01	5	99, 99, 101, 99, 102	100 ± 1.4 (1.4)
	0.1	5	99, 98, 101, 100, 98	
	Overall	**10**	**Range, 98–102%**	
	Secondary Transition (m/z 190.0 → 102.0)			
	0.01	5	103, 101, 100, 103, 105	102 ± 2.8 (2.8)
	0.1	5	99, 105, 101, 103, 96	
	Overall	**10**	**Range, 96–105%**	
B-1	Primary Transition (m/z 189.1 → 69.0)			
	0.01	5	70, 116[1], 94, 81, 87	99 ± 17.2 (17.0)
	0.1	5	104, 101, 99, 128[1], 108	
	Overall	**10**	**Range, 70–128%**	
	Primary Transition (m/z 189.1 → 69.0) Confirmation			
	0.01	5	106, 83, 99, 87, 93	89 ± 11.0 (9.9)
	0.1	5	85, 99, 81, 89, 73	
	Overall	**10**	**Range, 73–106%**	
	Secondary Transition (m/z 189.1 → 145.1) Confirmation			
	0.01	5	95, 93, 94, 82, 91	93 ± 6.5 (6.0)
	0.1	5	102, 89, 92, 102, 89	
	Overall	**10**	**Range, 82–102%**	
AB-1 Dimer	Primary Transition (m/z 689.4 → 288.2)			
	0.01	5	97, 106, 99, 94, 91	96 ± 5.1 (4.9)
	0.1	5	91, 101, 92, 96, 98	
	Overall	**10**	**Range, 91–106%**	
	Secondary Transition (m/z 689.4 → 268.2)			
	0.01	5	90, 105, 99, 94, 94	96 ± 8.5 (8.2)
	0.1	5	87, 113[1], 95, 97, 87	
	Overall	**10**	**Range, 87–113%**	

Table Continued, Footnotes Follow

Table (continued)

Using LC-MS/MS, ABSciex API 5500				
Analyte	**Fortification Level (ppm)**	**N**	**Individual Recoveries (%)**	**Mean (%) ± %RSD (Std. Dev.)**
LOAMY SAND (HIGH PH, 12-18" DEPTH)				
Cyflumetofen	Primary Transition (m/z 448.2 → 173.0)			
	0.01	5	91, 96, 96, 99, 97	102 ± 8.2 (8.3)
	0.1	5	106, 101, 120[1], 108, 106	
	Overall	**10**	**Range, 91–120%**	
	Secondary Transition (m/z 448.2 → 145.1)			
	0.01	5	96, 100, 101, 103, 93	101 ± 6.0 (6.1)
	0.1	5	97, 102, 103, 99, 115[1]	
	Overall	**10**	**Range, 93–115%**	
A-2	Primary Transition (m/z 174.1 → 147.1)			
	0.01	5	91, 100, 110, 96, 106	100 ± 7.8 (7.8)
	0.1	5	101, 101, 88, 113[1], 95	
	Overall	**10**	**Range, 88–113%**	
	Secondary Transition (m/z 174.1 → 117.1)			
	0.01	5	71, 116, 107, 109, 96	93 ± 17.8 (16.6)
	0.1	5	98, 72, 94, 95, 71	
	Overall	**10**	**Range, 71–116%**	
B-3	Primary Transition (m/z 190.0 → 130.0)			
	0.01	5	99, 100, 101, 103, 102	104 ± 3.1 (3.2)
	0.1	5	104, 106, 108, 108, 106	
	Overall	**10**	**Range, 99–108%**	
	Secondary Transition (m/z 190.0 → 102.0)			
	0.01	5	98, 101, 101, 100, 101	103 ± 3.5 (3.6)
	0.1	5	103, 106, 104, 109, 107	
	Overall	**10**	**Range, 98–109%**	
B-1	Primary Transition (m/z 189.1 → 69.0)			
	0.01	5	103, 95, 100, 97, 103	102 ± 3.9 (4.0)
	0.1	5	102, 106, 109, 99, 103	
	Overall	**10**	**Range, 95–109%**	
	Primary Transition (m/z 189.1 → 69.0) Confirmation			
	0.01	5	98, 98, 103, 100, 104	103 ± 3.9 (4.0)
	0.1	5	102, 101, 102, 109, 110	
	Overall	**10**	**Range, 98–110%**	
	Secondary Transition (m/z 189.1 → 145.1) Confirmation			
	0.01	5	97, 97, 102, 102, 102	102 ± 3.2 (3.3)
	0.1	5	104, 106, 105, 105, 104	
	Overall	**10**	**Range, 97–106%**	
AB-1 Dimer	Primary Transition (m/z 689.4 → 288.2)			
	0.01	5	101, 114[1], 106, 94, 100	97 ± 9.6 (9.3)
	0.1	5	102, 89, 83, 93, 87	
	Overall	**10**	**Range, 83–114%**	
	Secondary Transition (m/z 689.4 → 268.2)			
	0.01	5	103, 115[1], 101, 106, 97	96 ± 11.2 (10.7)
	0.1	5	84, 90, 83, 93, 86	
	Overall	**10**	**Range, 83–115%**	
SANDY LOAM (GERMAN SOIL SAMPLES)				
Cyflumetofen	Primary Transition (m/z 448.2 → 173.0)			
	0.01	5	95, 101, 101, 89, 100	93 ± 11.0 (10.2)
	0.1	5	82, 80, 83, 112[1], 91	
	Overall	**10**	**Range, 79–112%**	
	Secondary Transition (m/z 448.2 → 145.1)			
	0.01	5	110, 106, 112[1], 88, 93	97 ± 12.7 (12.2)
	0.1	5	82, 78, 93, 110, 95	
	Overall	**10**	**Range, 82–112%**	

Table Continued, Footnotes Follow

Table (continued)

Using LC-MS/MS, ABSciex API 5500				
Analyte	**Fortification Level (ppm)**	**N**	**Individual Recoveries (%)**	**Mean (%) ± %RSD (Std. Dev.)**
SANDY LOAM (GERMAN SOIL SAMPLES) (CONTINUED)				
A-2	Primary Transition (m/z 174.1 → 147.1)			
	0.01	5	110, 102, 108, 101, 114[1]	102 ± 7.3 (7.5)
	0.1	5	97, 96, 95, 105, 90	
	Overall	**10**	**Range, 90–114%**	
	Secondary Transition (m/z 174.1 → 117.1)			
	0.01	5	98, 84, 83, 105, 105	93 ± 13.4 (12.4)
	0.1	5	87, 112[1], 71, 93, 92	
	Overall	**10**	**Range, 71–112%**	
B-3	Primary Transition (m/z 190.0 → 130.0)			
	0.01	5	100, 102, 101, 92, 95	97 ± 5.0 (4.9)
	0.1	5	88, 97, 97, 105, 96	
	Overall	**10**	**Range, 88–105%**	
	Secondary Transition (m/z 190.0 → 102.0)			
	0.01	5	97, 98, 97, 98, 92	96 ± 3.2 (3.1)
	0.1	5	91, 95, 96, 101, 96	
	Overall	**10**	**Range, 91–101%**	
B-1	Primary Transition (m/z 189.1 → 69.0)			
	0.01	5	97, 103, 101, 97, 104	99 ± 2.5 (2.5)
	0.1	5	99, 97, 100, 98, 98	
	Overall	**10**	**Range, 97–104%**	
	Secondary Transition (m/z 189.1 → 145.1)			
	0.01	5	97, 101, 103, 99, 103	98 ± 3.0 (2.9)
	0.1	5	97, 98, 93, 97, 98	
	Overall	**10**	**Range, 93–103%**	
AB-1 Dimer	Primary Transition (m/z 689.4 → 288.2)			
	0.01	5	70, 79, 70, 71, 78	77 ± 7.3 (5.6)
	0.1	5	82, 87, 78, 81, 73	
	Overall	**10**	**Range, 70–87%**	
	Secondary Transition (m/z 689.4 → 268.2)			
	0.01	5	77, 76, 76, 83, 70	78 ± 7.6 (5.9)
	0.1	5	75, 88, 79, 85, 71	
	Overall	**10**	**Range, 70–88%**	

[1] Recovery result statistically determined not to be an outlier. Result was included in statistical data.

Conclusion:
The overall mean recoveries of cyflumetofen, and its metabolites, A-2, B-3, B-1, and AB-1 dimer in different soil types (sandy loam (0–3"depth), silt loam (12–18" depth), loamy sand (high pH, 12–18" depth), and sandy loam (German soil) were between 77 and 107%. Relative standard deviations of the overall recovery values for each matrix were less than 18% which demonstrates the accuracy and precision of BASF Analytical Method D1002. This study shows that BASF Analytical Method D1002 is suitable for determining residues of cyflumetofen, A-2, B-3, B-1, and AB-1 dimer at a level as low as 0.01 ppm. One set of 13 samples can be completed in nine hours, if no complications are encountered. This does not include instrument analysis time. The method was assessed to be acceptable as a post registration monitoring method.

PRIMARY REVIEWER`S REMARKS (USEPA-EFED)

No deficiencies were noted. The environmental chemistry method appears as Appendix C of MRID 48542828.

PRIMARY REVIEWER`S CONCLUSION (USEPA-EFED)

This study is classified as **Acceptable**.

Additional method validation was conducted using Analytical Method D1002 on a silt loam soil sample with high water content (> 20% moisture) at 24-30 inches.

Report:	II A 4.4/06
Reference Type:	Study Report
Author(s):	Perez, S., Miska, J.L. and Saha, M.
Year:	2011
Title:	Independent Laboratory Validation of BASF Analytical Method D1002: "Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS
Testing Laboratory:	ADPEN Laboratories, Inc.
Report Number:	379626
Owner Company:	BASF
Company Study Number:	BASF Reg. Doc. # 2011/7005075
MRID:	48542647
Report Date:	19 October 2011
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes
PMRA # 2146749	
Matrix/Medium:	Soil
Test Guideline: According to:	U.S. EPA Ecological Effects Test Guidelines: OPPTS 835.6100 (Terrestrial Field Dissipation), OPPTS 850.7100, Data Reporting Guideline for Environmental Chemistry Methods (ECM) OPPTS 860.1340, Residue Analytical Method
Deviations from Guideline:	No
GLP Compliance:	Yes

Summary Parameters for the Analytical Method Used for the Quantitation of Cyflumetofen and Metabolite Residues in Soils

Method ID	BASF method D1002				
Analyte(s)	BAS 9210 I and Metabolites A-2, B-3, B-1, AB-1				
Extraction Solvent/technique	Extracted from soil matrices by vortexing, shaking, and centrifuging with acetonitrile, followed by acetonitrile-water(60:40 v/v)				
Instrument	UPLC-MS/MS				
Detector for Method	**BAS 9210 I**	**B-1**	**B-3**	**A-2**	**AB-1 Dimer**
Quantitation (m/z)	448.2 → 173.0	189.1 → 69.0	190.0 → 130.0	174.1 → 147.1	689.4 → 288.2
Confirmation (m/z)	448.2 → 145.1	189.1 → 145.1	190.0 → 102.0	174.1 → 117.1	689.4 → 268.2
Ionization Mode	Positive	Negative	Positive	Positive	Positive
Expected Retention Times	2.51	2.01	1.85	1.74	2.80
Standardization Method	Linear Regression				
Stability of Std Solutions	35 Days at refrigerated temperatures for fortification and calibration standards for BAS9210I, B-1, B-3, and A-2 (96 days for stock solutions). For AB-1, 1 day for stock, fortification and calibration standards.				

Validation results show the mean recoveries were within the 70 to 120% range and the relative standard deviations (RSD, %) for all commodities and all fortification levels ranged from 2.0% to 17.3%. The detailed values are shown below.

Validation summary results of method D1002: for Cyflumetofen in soil matrices

Summary of Recoveries (%) in Soil						
Analyte	**Fortification Levels (ppm)**	**N**	**Recovery (%)**	**Average Recovery**	**Standard Deviation**	**% RSD[1]**
Cyflumetofen	Primary[2] (m/z 448.2 → 173.0) using LC-MS/MS Method A, AB Sciex 5500					
	0.01	5	99, 107, 97, 102, 113	103	6.4	6.2
	0.10	5	74, 70, 78, 76, 80	76	3.5	4.7
	Overall	**10**	**Range, 70–113**	90	15.5	17.3
	Secondary[3] (m/z 448.2 → 145.1) using LC-MS/MS Method A, AB Sciex 5500					

Summary of Recoveries (%) in Soil						
Analyte	**Fortification Levels (ppm)**	**N**	**Recovery (%)**	**Average Recovery**	**Standard Deviation**	**% RSD[1]**
	0.01	5	110, 105, 91, 108, 107	104	7.6	7.3
	0.10	5	77, 83, 72 ,71, 90	78	8.1	10.3
	Overall	**10**	**Range, 71–110**	91	15.4	16.9
B-1	Primary[2] (m/z 189.1 → 69.0) using LC-MS/MS Method A, AB Sciex 5500					
	0.01	5	100, 101, 109, 99, 93	100	5.6	5.6
	0.10	5	82, 73, 85, 81, 80	80	4.2	5.3
	Overall	**10**	**Range, 73–109**	90	11.5	12.8

Table Continued, Footnotes Follow

Table (continued)

Summary of Recoveries (%) in Soil						
Analyte	**Fortification Levels (ppm)**	**N**	**Recovery (%)**	**Average Recovery**	**Standard Deviation**	**% RSD[1]**
B-3	Primary[2] (m/z 190.0 → 130.0) using LC-MS/MS Method A, AB Sciex 5500					
	0.01	5	105, 103, 106, 100, 104	104	2.3	2.2
	0.10	5	85, 83, 85, 79, 85	83	2.8	3.3
	Overall	**10**	**Range, 79–106**	93	11.0	11.7
	Secondary[3] (m/z 190.0 → 102.0) using LC-MS/MS Method A, AB Sciex 5500					
	0.01	5	105, 98, 101, 99, 107	102	3.9	3.8
	0.10	5	82, 77, 74, 78, 85	79	4.4	5.5
	Overall	**10**	**Range, 74–107**	90	12.7	14.0
A-2	Primary[2] (m/z 174.1 → 147.1) using LC-MS/MS Method B, AB Sciex 5500					
	0.01	5	94, 86, 85, 80, 100	89	7.8	8.7
	0.10	5	72, 78, 78, 73, 79	76	3.2	4.2
	Overall	**10**	**Range, 72–100**	83	8.9	10.8
	Secondary[3] (m/z 174.1 → 117.1) using LC-MS/MS Method B, AB Sciex 5500					
	0.01	5	99, 102, 98, 106, 97	100	3.7	3.7
	0.10	5	85, 77, 90, 95, 88	87	6.5	7.5
	Overall	**10**	**Range, 77–106**	94	8.7	9.3
AB-1 Dimer	Primary[2] (m/z 689.4 → 288.2) using LC-MS/MS Method C, AB Sciex 5500					
	0.01	5	119, 115, 117, 110, 115	115	3.3	2.8
	0.10	5	83, 88, 98, 86, 77	86	7.6	8.8
	Overall	**10**	**Range, 77–119**	101	16.2	16.1
	Secondary[3] (m/z 689.4 → 268.2) using LC-MS/MS Method C, AB Sciex 5500					
	0.01	5	116, 115, 120, 119, 115	117	2.4	2.0
	0.10	5	89, 89, 97, 92, 81	90	5.9	6.6
	Overall	**10**	**Range, 81–120**	103	15.1	14.6

[1] Relative Standard Deviation = (Standard Deviation / Average Recovery) × 100.
[2] The primary ion transition for most soils, which would typically be used for quantification of residues.
[3] The secondary ion transition for most soils, which would typically be used for confirmation purposes.

A separate chromatographic method is used for the analysis of B-1 for confirmatory purpose. The recovery results using this confirmatory method are shown below:

Validation summary results for analysis of B-1 for confirmatory purpose

Additional Analysis for B-1 Recoveries (%) in Soil

Analyte	Spike Level (ppm)	N	Recovery (%)	Average Recovery	Standard Deviation	% RSD[1]
B-1	Primary[2] (m/z 189.1 → 69.0) using LC-MS/MS, AB Sciex 5500					
	0.01	5	92, 97, 98, 96, 102	97	3.5	3.6
	0.10	5	84, 79, 84, 83, 82	82	2.2	2.6
	Overall	**10**	**Range, 79–102**	90	8.2	9.1
	Secondary[3] (m/z 189.1 → 145.1) using LC-MS/MS, AB Sciex 5500					
	0.01	5	98, 98, 96, 99, 106	99	3.8	3.9
	0.10	5	77, 81, 82, 77, 84	80	3.2	4.0
	Overall	**10**	**Range, 77–106**	90	10.6	11.9

[1] Relative Standard Deviation = (Standard Deviation / Average Recovery) × 100.
[2] The primary ion transition for most soils, which would typically be used for quantification of residues.
[3] The secondary ion transition for most soils, which would typically be used for confirmation purposes.

Conclusion:
The results from the method validation study demonstrated that BASF Analytical Method D1002 can be performed successfully on soil samples for cyflumetofen and its four metabolites, A-2, B-3, B-1, and AB-1 dimer, down to a level of 0.01 ppm with limit of detection at 0.002 ppm. This method was assessed to be acceptable as a post registration monitoring method.

PRIMARY REVIEWER`S REMARKS (USEPA-EFED)

No deficiencies were noted. The environmental chemistry method appears as Appendix F of MRID 48542647.

PRIMARY REVIEWER`S CONCLUSION (USEPA-EFED)

This study is classified as **Acceptable**.

B.5.3.2 Water (Annex IIA 4.5, Annex IIIA 5.5)

An analytical method has been developed for the determination of cyflumetofen in water.

Report: II A 4.5/01
Reference Type: Study Report
Author(s): Baltussen E.
Year: 2007
Title: Development and validation of an analytical method for the determination of OK-5101 residues in water
Testing Laboratory: Notox B.V.
Report Number: OTSUKA Study No. 475741
Owner Company: BASF
Company Study Number: BASF Reg. Doc. # OTSA-0326-FR
MRID: 48542650
Report Date: 10 May 2007
Data Access: Data submitter is data owner
Data Protection Claimed: Yes
PMRA # 2146756

Executive Summary
The purpose of the study was to develop and validate a method for the quantitative analysis of cyflumetofen residues in water (surface, tap and ground). A method based on high performance liquid chromatographic (HPLC) coupled to tandem mass spectrometric (MS/MS) detection for quantitative analysis of the test substance was developed.

This study was conducted in compliance with EPA OPPTS 860.1400, Commission Directive 96/46/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. EEC publication no L214/18. 1996.

In addition, the "EC Guidance document on residue analytical methods SANCO/825/00 rev 6" (20/06/00) was consulted.

The limit of quantification was set at 0.1 µg/L and was validated with a mean accuracy of 100% and a precision of 7.8%, therefore the LOQ of 0.1 µg/L is justified for residues of cyflumetofen in all water types.

For validation, surface water samples were fortified with cyflumetofen to a final concentration of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ).

A brief description of the methodology follows:

Surface water samples were fortified with cyflumetofen to a final concentration of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ). The residues of cyflumetofen in water samples (8 mL) are diluted with 0.5% formic acid in methanol (2 mL) and analyzed by HPLC-MS/MS operated in the positive ion mode and monitoring MS/MS ion transition 448.3→173.1 amu. Calibration solutions consisted of 80/20/0.1 (v/v/v) Milli-Q water/methanol/formic acid. The samples were subsequently analyzed with a HPLC method coupled to tandem mass spectrometric (MS/MS) detection.

Overall mean recoveries of the analyses obtained from surface water in the validation study were between 70-110% for all fortification levels. The relative standard deviation was < 20% and the analytical method was accepted for the analysis of the test substance in surface water in the concentration range 0.100 - 1.00 pg/L.

Matrix/Medium:	Water
Test Guideline: According to:	EPA OPPTS 860.1400, European Economic Community (EEC), Commission Directive 96/46/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. EEC publication no L214/18. 1996. In addition, the "EC Guidance document on residue analytical methods SANCO/825/00 rev 6" (20/06/00) was consulted.
Deviations from Guideline:	No
GLP Compliance:	Yes

Method:

Instrument/Detector:	HPLC-MS/MS
Details on Method:	
Instrument:	HPLC
Detector:	AB Sciex API 3000 mass spectrometer

LC conditions for the analysis of cyflumetofen

Column:	Water Symmetry C_{18}, 50 mm × 2.1 mm, 3.5 µm
Guard column:	Water Symmetry C_{18}, 10 mm × 2.1 mm, 3.5 µm
Mobile phase:	A: Milli-Q-water with 0.1% formic acid B: Methanol with 0.1% formic acid

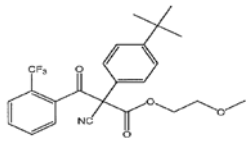
Gradient:

Time (min)	%A	%B
0.00	80	20
2.00	80	20
5.00	0	100
9.00	0	100
9.01	80	20
12.0	80	20

Flow rate: 400 µL/min

Retention time:	Cyflumetofen (OK-5101) 8.4 min

Test and Reference Items

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name						
	OK-5101	400882-07-7	$C_{24}H_{24}F_3NO_4$	447.5 g/mol	01H1	98.4%	05.13.2008
	2-methoxyethyl-(*R*,*S*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl)propionate						

Results:

Linearity:
Good linearity was observed in the range of 0.125–10.0 ng/mL for all analytes. Linearity of the method was determined at a range of 0.0500 to 2.00 µg/L (in solution) and had a correlation coefficient of > 0.99.

Specificity:
According to SANCO/825/00, MS/MS detection is considered to be a highly specific method. Three fragment ions with a m/z >100 were used. Based on these results the described method is specific for the analysis of cyflumetofen in tap, surface and groundwater. The response of the three blank matrices surface, tap and groundwater did not interfere for more than 30% with the response of cyflumetofen in surface water at the LOQ level.

Accuracy:
Accuracy for the surface water method was determined by evaluating the mean recoveries of multiple injections (n=6) at the 0.1 and 1.0 µg/L level. Mean recoveries were 100% and 92%, respectively, with mean coefficients of variation of 7.8% and 3.8%. Individual recoveries ranged from 93% to 114% (LOQ) and 90% to 98% (10×LOQ). It is anticipated that results are also applicable for tap and groundwater.

Accuracy of analysis method for cyflumetofen at LOQ and 10×LOQ in surface water

Fortification level (µg/L)	Individual values (%)	Mean accuracy (%)	Coefficient of variation (%)
0.1	93, 93, 102, 114, 100, 100	100	7.8
1.0	90, 92, 92, 90, 71[A], 98	92	3.8
Overall (min-max)	90-114	97	7.5

[A]: outlier determined by Dixon's Q-test

Precision/repeatability:
The criteria for precision (repeatability), as laid out in SANCO/825/00, were met, since the relative standard deviation (CV) was well below 20%

Reproducibility:
The experiment included a sufficient number of replicates at the fortification levels tested to demonstrate the reproducibility of the method.

Limit of Quantification/Detection:
The limit of quantification was set at 0.1 µg/L and was validated with a mean accuracy of 100% and a precision of 7.8%, therefore the LOQ of 0.1 µg/L is justified.

Matrix effect:
Matrix effect of surface water was determined by analyzing samples of cyflumetofen in surface water in duplicate at the LOQ and 10× LOQ level. The mean recoveries of the processed matrix effect samples were 92% and 79%, respectively, and are well within the acceptance criteria of 70%-110%. It is anticipated that results are also applicable for tap and groundwater.

Conclusion:
A method based on high performance liquid chromatographic (HPLC) coupled to a tandem mass spectrometric (MS/MS) detection for quantitative analysis of cyflumetofen in water was developed and validated. The target LOQ level for the method was 0.100 µg/L, the 10×LOQ level corresponded to 1.0 µg/L. The results of the development and validation of an analytical method for the analysis of cyflumetofen in water are given below.

	Result	Criterion	Conclusion
Specificity	specific	<30% LOQ	Pass
Linearity	r = 0.994	r > 0.99	Pass
Precision	7.8 and 3.8%	< 20%	Pass
Accuracy	100 and 92%	70-110%	Pass
Limit of quantification	0.1 µg/L	--	Pass
Matrix effect	92 and 79%	70-110%	Pass
Stability of the chromatographic system and	18 and 19% (Over a 32.7 hour time period)	<20%	Pass
end solution	14 and 15%		
Stability standard solution	--	<10%	Pass

Based on the above findings, the described HPLC method is sufficiently validated and suitable for the determination of the pure active substance, cyflumetofen, in water at an LOQ of 0.1 µg/L. The determined LOQ is sufficiently low to cover the requirements for surface water (i.e. no impact on non-target organisms, Annex VI, 91/414/EEC). The method was assessed to be acceptable as a post registration monitoring method.

PRIMARY REVIEWER`S REMARKS (USEPA-EFED)

No deficiencies were noted.

PRIMARY REVIEWER`S CONCLUSION (USEPA-EFED)

This study is classified as **Acceptable**.

An analytical method has been developed for the determination of metabolite B-1 in water.

Report: II A 4.5/02
Reference Type: Study Report
Author(s): Oudhoff K.A
Year: 2009
Title: Development and validation of an analytical method for the analysis of residues of B-1 in water
Testing Laboratory: Notox B.V.
Report Number: OTSUKA Study No. 490990
Owner Company: BASF
Company Study Number: BASF Reg. Doc. # 2009/1126660
MRID: 48542651
Report Date: 30 June 2009
Data Access: Data submitter is data owner
Data Protection Claimed: Yes
PMRA # 2146754

Executive Summary
The purpose of the study was to develop and validate a method for the quantitative analysis of B-1 residues in water (surface, tap and ground). A method based on ultra high performance liquid chromatographic (UPLC) coupled to tandem mass spectrometric (MS/MS) detection for quantitative analysis of the test substance was developed.

This study was conducted in compliance with EPA OPPTS 860.1400, European Community (EC), Commission Directive 96/46/EC of 16 July 1996 amending Council Directive 91/414/EEC concerning the Placing of Plant Protection Products on the Market, Official Journal of the European Communities no. L214, August 23, 1996 and European Commission, SANCO/825/00 revision 7: Guidance Document on Residue Analytical Methods, March 17, 2004.

The limit of quantification was set at 0.1 µg/L and was validated with a mean accuracy of 100% and a precision of 7.8%, therefore the LOQ of 0.1 µg/L is justified for residues of B-1 in all water types.

For validation, surface water, groundwater and drinking water samples were fortified with B-1 to a final concentration of 0.1 µg/L (LOQ) and 1.0 µg/L (10xLOQ).

A brief description of the methodology follows:

Surface water, groundwater and drinking water samples were fortified with B-1 to a final concentration of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ). The residues of B-1 in water samples are diluted with acetonitrile containing 1% formic acid (9:1,v:v), filtered (if surface or groundwater), and analyzed by UPLC-MS/MS operated in the positive ion mode and monitoring MS/MS ion transition *m/z* 189 → *m/z* 145.

Calibration solutions consisted of 10/90 (v/v) acetonitrile/ water containing 0.1% formic acid. The samples were subsequently analyzed with a HPLC method coupled to tandem mass spectrometric (MS/MS) detection.

Overall mean recoveries at LOQ and 10×LOQ for drinking water were 87 and 98%, for ground water 92 and 102% and for surface water 101 and 99%, respectively. The relative standard deviation was < 20% and acceptable for all water types.

Matrix/Medium: Water
Test Guideline: According to: EPA OPPTS 860.1400, EEC 96/46, 91/414/EEC and SANCO 825/00 rev 7..
Deviations from Guideline: No
GLP Compliance: Yes (laboratory certified by Food and Consumer Product Safety Authority (VWA), Den Haag, The Netherlands)

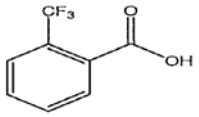
Method:
Instrument/Detector: UPLC-MS/MS
Details on Method:
Instrument: Acquity UPLC
Detector: AB Sciex API 5000 mass spectrometer

LC conditions for the analysis of B-1

Column:	Acquity UPLC BEH Shield RP-18, 50 mm × 2.1 mm, 1.7 µm
Column temperature:	40°C
Injection volume:	20 µL
Mobile phase:	A: 2.5 mM ammonium formate in 95/5 (v/v) acetonitrile/water with 0.1% formic acid B: 2.5 mM ammonium formate in 5/95 (v/v) acetonitrile/water with 0.1% formic acid Isocratic: 25/75 (v/v) A/B Flow rate: 0.8 mL/min
Retention time:	B-1 0.76 min

Test and Reference Items

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name: 2-(trifluoromethyl)benzoic acid						

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name: 2-(trifluoromethyl)benzoic acid						
	B-1	433-97-6	$C_8H_5F_3O_2$	190.12 g/mol	01107BH	99.7%	01.28.10

Results:

Linearity:
Linearity of the method was determined at a range of 0.050 to 1.50 µg/L (in solution) and had a correlation coefficient of > 0.99.

Specificity:
According to SANCO/825/00, MS/MS detection is considered to be a highly specific method. The response of the blank matrices surface, tap and groundwater did not interfere for more than 30% with the response of B-1 in surface water at the LOQ level.

Accuracy:
Accuracy for the drinking, ground and surface water method was determined by evaluating the mean recoveries of multiple injections (n=5) at the 0.1 and 1.0 µg/L level. Mean recoveries at LOQ and 10× LOQ for drinking water were 87 and 98%, for ground water 92 and 102% and for surface water 101 and 99%, respectively.

Precision/repeatability:
The criteria for precision (repeatability), as laid out in SANCO/825/00, were met, since the relative standard deviation (CV) was well below 20%.

Accuracy of analysis method for B-1 at LOQ and 10×LOQ in surface water

Sample	Fortification Level (µg/L)	Individual Values (%)	Mean Accuracy (%)	Coefficient of Variation (%)
Drinking water	0.1	90, 85, 82, 85, 91	87	4.3
	1.0	100, 97, 99, 96, 99	98	1.7
	Overall (min-max)	82-100		
Ground water	0.1	97, 89, 88, 91, 94	92	4.1
	1.0	106, 103, 101, 101, 100	102	2.6
	Overall (min-max)	88-106		
Surface water	0.1	102, 106, 100, 95, 100	101	3.9
	1.0	100, 98, 99, 98, 101	99	1.4
	Overall (min-max)	95-106		
mean	0.1		93.0	7.47
	1.0		99.8	2.45

Reproducibility:
The experiment included a sufficient number of replicates at the fortification levels tested to demonstrate the reproducibility of the method.

Limit of Quantification/Detection:
The limit of quantification was set at 0.1 µg/L and was validated with a mean accuracy of 93.0% and a precision of 7.47%, therefore the LOQ of 0.1 µg/L is justified. The limit of detection was set at 0.01 µg/L (based on 3 times the signal-to-noise ratio).

Conclusion:
A method based on ultra high performance liquid chromatographic (UPLC) coupled to a tandem mass spectrometric (MS/MS) detection for quantitative analysis of B-1 in water was developed and validated. The target LOQ level for the method was 0.100 µg/L, the 10×LOQ

level corresponded to 1.0 µg/L. The results of the development and validation of an analytical method for the analysis of B-1 in water are given below.

	Result	Criterion	Conclusion
Specificity	specific	<30% LOQ	Pass
Linearity	r = 0.998	r > 0.99	Pass
Precision	4.3 and 1.7% (Drinking water) 4.1 and 2.6% (Ground water) 3.9 and 14% (Surface water	< 20%	Pass
Accuracy	87 and 98% (Drinking water) 92 and 102% (Ground water) 101 and 99% (Surface water	70-110%	Pass
Limit of quantification	0.1 µg/L	--	Pass
Stability of the chromatographic system and end solution		Stable	

Based on the above findings, the described HPLC method is sufficiently validated and suitable for the determination of the metabolite, B-1 in water at an LOQ of 0.1 µg/L. The determined LOQ is sufficiently low to cover the requirements for drinking, ground and surface water. The method was assessed to be acceptable as a post registration monitoring method.

PRIMARY REVIEWER`S REMARKS (USEPA-EFED)

No deficiencies were noted.

PRIMARY REVIEWER`S CONCLUSION (USEPA-EFED)

This study is classified as **Acceptable**.

An analytical method has been developed for the determination of metabolite B-3 in water.

Report:	II A 4.5/03
Reference Type:	Study Report
Author(s):	Oudhoff K.A
Year:	2009
Title:	Development and validation of an analytical method for the analysis of residues of B-3 in water
Testing Laboratory:	Notox B.V.
Report Number:	OTSUKA Study No. 490989
Owner Company:	BASF
Company Study Number:	BASF Reg. Doc. # 2009/1126661
MRID:	48542652
Report Date:	30 June 2009
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes
PMRA # 2146755	

Executive Summary

The purpose of the study was to develop and validate a method for the quantitative analysis of B-3 residues in water (surface, tap and ground). A method based on ultra high performance liquid chromatographic (UPLC) coupled to tandem mass spectrometric (MS/MS) detection for quantitative analysis of the test substance was developed.

This study was conducted in compliance with EPA OPPTS 860.1400, European Community (EC), Commission Directive 96/46/EC of 16 July 1996 amending Council Directive 91/414/EEC concerning the Placing of Plant Protection Products on the Market, Official Journal of the European Communities no. L214, August 23, 1996 and European Commission, SANCO/825/00 revision 7: Guidance Document on Residue Analytical Methods, March 17, 2004.

The limit of quantification was set at 0.1 µg/L and was validated with a mean accuracy of 93.8% and a precision of 3.7%, therefore the LOQ of 0.1 µg/L is justified for residues of B-3 in all water types.

For validation, surface water, groundwater and drinking water samples were fortified with B-3 to a final concentration of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ).

A brief description of the methodology follows:

Surface water, groundwater and drinking water samples were fortified with B-3 to a final concentration of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ). The residues of B-3 in water samples are diluted with acetonitrile (9:1,v:v), filtered (if surface or groundwater), and analyzed by UPLC/MS/MS operated in the positive ion mode and monitoring MS/MS ion transition *m/z* 190.1 → *m/z* 130.0.

Calibration solutions consisted of 10/90 (v/v) acetonitrile/ water. The samples were subsequently analyzed with a HPLC method coupled to tandem mass spectrometric (MS/MS) detection.

Overall mean recoveries at LOQ and 10×LOQ for drinking water were 95 and 87%, for ground water 90 and 85% and for surface water 96 and 88%, respectively. The relative standard deviation was < 20% and acceptable for all water types.

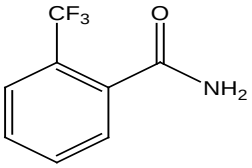
Matrix/Medium: Water
Test Guideline: According to: EPA OPPTS 860.1400, EEC 96/46, 91/414/EEC and SANCO 825/00 rev 7..
Deviations from Guideline: No
GLP Compliance: Yes (laboratory certified by Food and Consumer Product Safety Authority (VWA), Den Haag, The Netherlands)

Method:
Instrument/Detector: UPLC-MS/MS
Details on Method:
Instrument: Acquity UPLC
Detector: AB Sciex API 5000 mass spectrometer

LC conditions for the analysis of B-3
Column: Acquity UPLC BEH Shield RP-18, 100 mm × 2.1 mm, 1.7 µm
Column temperature: 40°C
Injection volume: 10 µL
Mobile phase: A: 95/5 (v/v) acetonitrile/water with 0.1% formic acid
B: 5/95 (v/v) acetonitrile/water with 0.1% formic acid
Isocratic: 20/80 (v/v) A/B
Flow rate: 0.5 mL/min
Retention time: B-3 1.3 min

Test and Reference Items

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name: 2-(trifluoromethyl)benzamide						

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name: 2-(trifluoromethyl)benzamide						
	B-3	360-64-5	$C_8H_6F_3NO$	189.13 g/mol	MA080115	99.1%	01/20/2011

Results:

Linearity:
Linearity of the method was determined at a range of 0.050 to 1.50 µg/L (in solution) and had a correlation coefficient of > 0.99.

Specificity:
According to SANCO/825/00, MS/MS detection is considered to be a highly specific method. The response of the blank matrices surface, tap and groundwater did not interfere for more than 30% with the response of B-3 in surface water at the LOQ level.

Accuracy:
Accuracy for the drinking, ground and surface water method was determined by evaluating the mean recoveries of multiple injections (n=5) at the 0.1 and 1.0 µg/L level. Mean recoveries at LOQ and 10×LOQ for drinking water were 95 and 87%, for ground water 90 and 85% and for surface water 96 and 88%, respectively.

Precision/repeatability:
The criteria for precision (repeatability), as laid out in SANCO/825/00, were met, since the relative standard deviation was well below 20%.

Accuracy of analysis method for B-3 at LOQ and 10xLOQ in surface water

Sample	Fortification Level (µg/L)	Individual Values (%)	Mean Accuracy (%)	Coefficient of Variation (%)
Drinking water	0.1	93, 99, 94, 97, 92	95	3.1
	1.0	88, 86, 88, 87, 86	87	1.2
	Overall (min-max)	86-99		
Ground water	0.1	89, 89, 92, 90, 90	90	1.5
	1.0	84, 83, 85, 85, 88	85	2.1
	Overall (min-max)	83-92		
Surface water	0.1	98, 99, 95, 95, 95	96	2.1
	1.0	90, 88, 88, 87, 89	88	0.96
	Overall (min-max)	87-99		
Mean	0.1		93.8	3.7
	1.0		86.8	2.2

Reproducibility:
The experiment included a sufficient number of replicates at the fortification levels tested to demonstrate the reproducibility of the method.

Limit of Quantification/Detection:
The limit of quantification was set at 0.1 µg/L and was validated with a mean accuracy of 93.8% and a precision of 3.7%, therefore the LOQ of 0.1 µg/L is justified. The limit of detection was set at 0.001 µg/L (based on 3 times the signal-to-noise ratio).

Conclusion:
A method based on ultra high performance liquid chromatographic (UPLC) coupled to a tandem mass spectrometric (MS/MS) detection for quantitative analysis of B-3 in water was

developed and validated. The target LOQ level for the method was 0.100 µg/L , the 10×LOQ level corresponded to 1.0 µg/L. The results of the development and validation of an analytical method for the analysis of B-3 in water is given below.

	Result	Criterion	Conclusion
Specificity	specific	<30% LOQ	Pass
Linearity	r = 0.996	r > 0.99	Pass
Precision	3.1 and 1.2 (Drinking water) 1.5 and 2.1 (Ground water) 2,1 and 0,96 (Surface water)	< 20%	Pass
Accuracy	95 and 87% (Drinking water) 90 and 85% (Ground water) 96 and 88% (Surface water)	70-110%	Pass
Limit of quantification	0.1 µg/L	--	Pass
Stability of the chromatographic system and end solution		Stable	

Based on the above findings, the described HPLC method is sufficiently validated and suitable for the determination of the metabolite B-3 in water at an LOQ of 0.1 µg/L. The determined LOQ is sufficiently low to cover the requirements for drinking, ground and surface water. The method was assessed to be acceptable as a post registration monitoring method.

PRIMARY REVIEWER`S REMARKS (USEPA-EFED)

No deficiencies were noted.

PRIMARY REVIEWER`S CONCLUSION (USEPA-EFED)

This study is classified as **Acceptable**.

An analytical method has been developed for the determination of cyflumetofen in laboratory freshwater and saltwater algal nutrient medium (SWAM).

Report:	II A 4.6/02
Reference Type:	Study Report
Author(s):	Leak T. 2011(b)
Year:	2011
Title:	Validation of test solution preparations and analytical methods for use in the determination of BAS 9210-I in various media used in environmental toxicity studies (Including final report amendment)
Testing Laboratory:	ABC Laboratories
Report Number:	ABC study numbers 379967 and 65234
Owner Company:	BASF
Company Study Number:	BASF Reg. Doc. # 2011/7005329
MRID:	48542658
Report Date:	January 25, 2011
Amendment Date:	October 31, 2011
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes
PMRA # 2210997	

Executive Summary

The aim of the study was to develop and to validate the quantitative analysis of cyflumetofen in laboratory freshwater and saltwater algal nutrient medium (SWAM); a HPLC method coupled to tandem mass spectrometric detection (HPLC-MS/MS) was developed and validated for pre-registration purposes which can be used as a post-registration method.

The freshwater was prepared by blending naturally hard well water with well water that was demineralized by reverse osmosis. These waters were blended to yield a total hardness of approximately 130 to 160 mg $CaCO_3$/L.

Saltwater algal nutrient medium (SWAM) was prepared by the addition of appropriate reagent grade salts to filtered synthetic seawater. The synthetic seawater was prepared by adding a commercial salt mix (Marinemix manufactured by Wiegandt GmbH) to autoclaved ABC reagent water until salinity reached approximately 30 ± 2‰. ABC reagent water is produced by passing reverse-osmosis water through a series of deionization tanks, a laboratory water purification system consisting of carbon, de-mineralization, and organic adsorption cartridges, and then through a 0.2-µm filter. The synthetic seawater was then passed through 0.45-µm Millipore® filters. After preparation, the medium was pH-adjusted to 8.0 ± 0.1 using 0.1 N HCl. Final salinity of the prepared medium was 29.7‰ as measured with a WTW Cond 330i conductivity/salinity meter.

This study was conducted in compliance with EPA 835.1220; EPA 835.1230.

Test procedures followed the ABC test protocol entitled, “Validation of Test Solution Preparations and Analytical Methods for Use in the Determination of BAS 9210-I in Various Media Used in Environmental Toxicity Studies,” with amendment.

The samples were analyzed for cyflumetofen by LC/MS/MS with turbo ion spray in the positive ion mode monitoring ion transitions m/z 448.10 → m/z 173.10.

Based on the above findings, the described HPLC-MS/MS method for recovering cyflumetofen from laboratory freshwater and saltwater algal nutrient medium were determined to be acceptable. The Method Detection Limit (MDL) was determined to be 0.0314 µg/L and the Practical Quantitation Limit (PQL) was determined to be 0.157 µg/L.

Matrix/Medium: Fresh and Salt water
Test Guideline: According to: EPA 835.1220; EPA 835.1230
Deviations from Guideline: No
GLP Compliance: Yes

Method Validation in Freshwater:
Nine 5 mL volumes of laboratory freshwater were collected and transferred to 15 mL Culture tubes on 26 April 2010. Three low validation spike samples (0.485 µg/L) were fortified with 0.080 mL of a 0.0303 µg a.i./mL stock solution in 1% formic acid in methanol. Three high validation spike samples (18.2 µg/L) were fortified with 0.090 mL of a 1.01 µg a.i./mL stock solution in 1% formic acid in methanol. Three replicates were prepared for the control (matrix blank).

The 5 mL samples were diluted with 0.1% formic acid in methanol to produce an analyte concentration that was within the range of the standard curve. The samples were capped and vortexed to mix prior to analysis using high performance liquid chromatographic/mass spectrometry (HPLC/MS/MS).

Method Validation in Saltwater Algal Nutrient Medium (SWAM):
Nine 5 mL volumes of saltwater algal nutrient medium were collected and transferred to 15 mL Culture tubes on 26 April 2010. Three low validation spike samples (0.485 µg/L) were fortified with 0.080 mL of a 0.0303 µg a.i./mL stock solution in 1% formic acid in methanol. Three high validation spike samples (18.2 µg/L) were fortified with 0.090 mL of a 1.01 µg a.i./mL stock solution in 1% formic acid in methanol. Three replicates were prepared for the control (matrix blank).

The samples were diluted with 5 mL of 0.1% formic acid in methanol, then centrifuged at 3,200 rpm for five minutes to pellet any precipitated salt. A 5 mL volume of the supernatant was diluted with 0.1% formic acid in methanol to produce an analyte concentration that was within the range of the standard curve. The samples were transferred to a glass

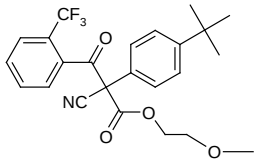
autosampler, capped and analyzed using high performance liquid chromatographic/mass spectrometry (HPLC/MS/MS).

Method:
Instrument/Detector: HPLC-MS/MS
Details on Method:
Instrument: HPLC
Detector: AB Sciex API 4000 mass spectrometer

LC conditions for the analysis of BAS 9210 I

Column:	Phenomenex Luna C18 HST, 50 mm × 3 mm, 2.5 µm		
Column temperature:	30°C		
Injection volume:	10 µL		
Mobile phase:	A: 0.1% formic acid in water		
	B: Methanol		
	Gradient:		
	Time (min)	%A	%B
	0.00	20	80
	3.00	20	80
	Flow rate: 0.2 mL/min		
Retention time:	Cyflumetofen	2.0 min	

Test and Reference Items

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name						
	BAS 9210 I	400882-07-7	$C_{24}H_{24}F_3NO_4$	447.45 g/mol	01H1	97.08%	16 April 2013
	BAS 9210 I	400882-07-7	$C_{24}H_{24}F_3NO_4$	447.45 g/mol	5J01	98.6%	21 January 2013
	2-methoxyethyl(RS)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-[2-(trifluoromethyl)phenyl] propionate						

Results:

Method Validation Results in Freshwater
Analysis of three replicate fortifications in freshwater at a concentration of 0.485 µg a.i./L resulted in recoveries ranging from 84 to 107% of the nominal concentration. Recoveries from three replicate fortifications at a concentration of 18.2 µg a.i./L ranged from 93 to 98% of the nominal concentration. No residues of cyflumetofen were detected in the controls above the minimum quantifiable limit (MQL) of 0.202 µg/L. The detailed results are given in the table shown below. These results indicate the method is acceptable for the recovery of cyflumetofen from laboratory freshwater.

Measured Concentrations of Cyflumetofen During the Method Validation in Laboratory Freshwater

Sample	Nominal Concentration (µg/L)	Calculated Concentration (µg/L)	Recovery (%)
Control A	0	<MQL	NA
Control B	0	<MQL	NA
Control C	0	<MQL	NA
Low Spike A	0.485	0.520	107
Low Spike B	0.485	0.408	84
Low Spike C	0.485	0.418	86
High Spike A	18.2	16.9	93
High Spike B	18.2	17.9	98
High Spike C	18.2	17.5	96

MQL = 0.202 µg/L

Method Validation Results in Saltwater Algal Nutrient Medium (SWAM)
Analysis of three replicate fortifications in SWAM at a concentration of 0.485 µg a.i./L resulted in recoveries ranging from 80 to 108% of the nominal concentration. Recoveries from three replicate fortifications at a concentration of 18.2 µg a.i./L ranged from 91 to 99% of the nominal concentration. No residues of cyflumetofen were detected in the controls above the minimum quantifiable limit (MQL) of 0.202 µg/L. The detailed results are given in the table shown below. These results indicate the method is acceptable for the recovery of cyflumetofen from SWAM.

Measured Concentrations of Cyflumetofen During the Method Validation in SWAM

Sample	Nominal Concentration (µg/L)	Calculated Concentration (µg/L)	Recovery (%)
Control A	0	<MQL	NA
Control B	0	<MQL	NA
Control C	0	<MQL	NA
Low Spike A	0.485	0.524	108
Low Spike B	0.485	0.392	81
Low Spike C	0.485	0.386	80
High Spike A	18.2	16.6	91
High Spike B	18.2	17.5	96
High Spike C	18.2	18.0	99

MQL = 0.202 µg/L

MDL and PQL Determination:
Seven replicates of the 0.101 µg/L standard were injected and measured concentrations ranged from 0.16277 to 0.18921 µg/L. Results are shown in the table below. The standard deviation of the seven replicate samples was 0.010. The Method Detection Limit (MDL) was calculated as 3.14 times the standard deviation of the replicate samples, or 0.0314 µg/L and the Practical Quantitation Limit (PQL) was calculated as five times the MDL, or 0.157 µg/L.

Determination of the Method Detection Limit (MDL) and Practical Quantitation Limit (PQL)

Sample ID	Nominal Concentration (µg/L)	Measured Concentration (µg/L)
Replicate – A	0.101	0.16467
Replicate – B	0.101	0.16582
Replicate – C	0.101	0.16277
Replicate – D	0.101	0.18047
Replicate – E	0.101	0.17506
Replicate – F	0.101	0.18154
Replicate - G	0.101	0.18921
	Mean	0.17422
	STDEV	0.010
MDL=	3.14×STDEV	0.0314
PQL=	5×MDL	0.157

Conclusion:
The analytical method for recovering cyflumetofen from laboratory freshwater and saltwater algal nutrient medium was determined to be acceptable. The Method Detection Limit (MDL) was determined to be 0.0314 µg/L and the Practical Quantitation Limit (PQL) was determined to be 0.157 µg/L. The method was assessed to be acceptable as a post registration monitoring method.

B.5.3.2 Sediment (Annex IIA 4.6, Annex IIIA 5.6)

An analytical method has been developed for the determination of metabolite AB-1 in sediment.

Report:	II A 4.6/01
Reference Type:	Study Report
Author(s):	Oudhoff K.A. 2009(c)
Year:	2009
Title:	Development and validation of an analytical method for the analysis of AB-1 in sediment
Testing Laboratory:	Notox B.V.
Report Number:	490354
Owner Company:	BASF
Company Study Number:	BASF Reg. Doc. # 2009/1126662
MRID:	48542657
Report Date:	24 April 2009
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes

PMRA # 2146760

Executive Summary

The aim of the study was to develop and to validate an analytical method for the quantitative analysis of the test substance in sediment. For the quantitative analysis of metabolite AB-1 in sediment, a HPLC method coupled to tandem mass spectrometric detection (HPLC-MS/MS) was developed and validated in sediment for pre-registration purposes.

This study was conducted in compliance with EEC 91/414 Annex II (Part A Section 4); EEC 91/414 Annex III (Part A Section 5); SANCO/3029/99 rev. 4 (11 July 2000).

The limit of quantification for metabolite AB-1 was set at 100 mg/kg sediment. At this level all criteria of SANCO/825/00, i.e. the lowest concentration of the test substance with accuracy in the range of 70-110% and a repeatability of <20%, were met in sediment samples.

A brief description of the methodology follows:

Sediment (60 gram based on dry weight) was spiked with the test substance at a target concentration of 100 or 1000 mg/kg. The accuracy samples were extracted at 200 rpm with 100 mL of 50/50 (v/v) acetonitrile/water containing 1% (v/v) ammonium solution. The shaking time was 30 minutes. The samples were centrifuged at 16421 g and 20°C for 5 minutes, and thereafter the samples were filtered through a 0.2 µm Spartan FP 30/0.2 CA-S filter (Whatman, Dassel, Germany) and further diluted with 50/50 acetonitrile/water to obtain concentrations within the calibration range.

The blank accuracy sample was prepared and treated similar to the accuracy samples.

The samples were analyzed for metabolite AB-1 by LC/MS/MS in the negative ion mode monitoring ion transitions m/z 344.2 → m/z 304.0.

Based on the above findings, the described HPLC-MS/MS method is sufficiently validated and suitable for determination of the metabolite AB-1, in sediment samples at a LOQ of 100 mg/kg.

Matrix/Medium:	Sediment
Test Guideline: According to:	EEC 91/414 Annex II (Part A Section 4); EEC 91/414 Annex III (Part A Section 5); SANCO/3029/99 rev. 4 (11 July 2000)
Deviations from Guideline:	No
GLP Compliance:	Yes

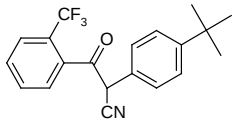
Method:

Instrument/Detector:	UPLC-MS/MS
Details on Method:	
Instrument:	Acquity UPLC
Detector:	AB Sciex API 5000 mass spectrometer

<u>LC conditions for the analysis of AB-1</u>

Column:	Acquity UPLC BEH Shield RP-18, 50 mm × 2.1 mm, 1.7 µm
Column temperature:	40°C
Injection volume:	10 µL
Mobile phase:	A: 5/95 (v/v) acetonitrile/water containing 2.5 mM ammonium formate and 0.1% formic acid B: 95/5 (v/v) acetonitrile/water containing 2.5 mM ammonium formate and 0.1% formic acid Isocratic: 40/60 (v/v) A/B Flow rate: 0.8 mL/min
Retention time:	AB-1 0.75 min

Test and Reference Items

Structural Formula	Codes	CAS No.	Chem. formula	Mol. weight	Lot No.	Purity	Expiry date
	Chemical Name						
	AB1	NA	C20H18F3NO	345.4 g/mol	0863NT2	99.8%	16 June 2009
	(RS)-2-(4-tert-butylphenyl)-3-oxo-3-[2-(trifluoromethyl)phenyl] propanenitrile						

Results:

Linearity:
Linearity was determined over a range of 0.100-10.0 µg/L (in solution) and showed an acceptable linear relationship with a correlation coefficient (r) of > 0.99. Two data points at 7 µg/L were excluded because the back calculated accuracy was > 10% of the nominal concentration.

Specificity:
The chromatogram of the test substance showed one test substance peak, the blank sediment sample showed a small peak at the test substance retention time. Since this response was maximum 12% of the LOQ level, the specificity requirements (<30% at LOQ level) were met and comply with SANCO/3029/99. Therefore the method is considered specific for the test substance.

Accuracy:
Accuracy samples were analyzed by single injection into the analytical system. The analytical method was considered applicable for the determination of the test substance if the mean accuracy was in the range 70-110% and the coefficient of variation was ≤20%. The SANCO/3029/99 criterion (70%-110%) was met for sediment samples with mean accuracy of 98% (individual values: 100, 100, 103, 96 and 92%) and 88% (individual values: 92, 86, 90, 83 and 87%) at the 0.1 and 1 g/kg level respectively. The coefficients of variation at the 0.1 and 1 g/kg levels were 4.4 and 4.1 % respectively.

Precision/repeatability:
The criterion for precision, as laid out in SANCO/3029/99, was met, since the coefficients of variation were well below 20% (see Accuracy).

Accuracy of the analysis of AB-1 in sediment

Fortification level (mg/kg)	Individual values (%)	Mean accuracy (%)	Coefficient of variation (%)
100	100, 100, 103, 95.5, 92.1	98	4.4
1000	91.9, 85.6, 90.3, 82.9, 86.6	88	4.1

Reproducibility:
Each fortification level was sufficiently replicated and as the mean accuracy at each concentration level was within 70-110% and the coefficient of variation was < 20%, the

analytical method was accepted for the analysis of the test substance in sediment in the target concentration range of 100 - 1000 mg/kg (0.1 - 1 g/kg).

Limit of Quantification/Detection:
The limit of quantification for metabolite AB-1 was set at 100 mg/kg sediment. At this level all criteria of SANCO/825/00, i.e. the lowest concentration of the test substance with accuracy in the range of 70-110% and a repeatability of <20%, were met in sediment samples.

Conclusion:
Based on the above findings, the described HPLC-MS/MS method is sufficiently validated and suitable for determination of the metabolite AB-1 in sediment samples at a LOQ of 100 mg/kg.

	Result
Specificity	specific
Linearity	r = 0.997
Precision	4.1 and 4.4%
Accuracy	88 and 98%
Limit of quantification	100 mg/kg
Stability of the chromatographic system and end solution	Stable

PRIMARY REVIEWER`S REMARKS (USEPA-EFED)

No deficiencies were noted. However, the LOQ of 100 mg/kg is quite high which limits the utility of this method.

PRIMARY REVIEWER`S CONCLUSION (USEPA-EFED)

This study is classified as **Acceptable**.

B.5.3.3 Air (Annex IIA 4.7, Annex IIIA 5.7)

Not required.

B.5.4 Analytical methods for the determination of residues in body fluids and tissues (Annex IIA 4.8; Annex IIIA 5.8)

This section not reviewed by the PMRA chemistry reviewer.

B.5.5 Evaluation and assessment

A validated analytical method was provided for the determination of the active, and was assessed to be selective, accurate and precise for this purpose.

Analytical methods for the determination of the active and its major metabolites in soil, water and sediment have been provided. The analytical methods have been validated and assessed to be acceptable for use as post-registration monitoring methods.

An analytical method was provided for the determination of cyflumetofen in Nealta Miticide/Sultan Miticide (BAS 9210 2 I formulation). The method was assessed to be linear, selective, precise and accurate for use as an enforcement analytical method.

B.5.6 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
IIA 4.2.1	Saka, M.	2004	OK-5101 Technical: Determination of Purity and Stability The Institute of Environmental Toxicology, Ibaraki, Japan, Study No. IET 03-5047 BASF Reg. Doc. No. OTSA-0021(EN)-FR(2) GLP	Y	BASF
IIA 4.2.1	Saka, M.	2004	Composition of Components in OK-5101 Technical (Include Validation of Analytical Methods) The Institute of Environmental Toxicology, Ibaraki, Japan, Study No. IET 03-5047 BASF Reg. Doc. No. OTSA-0167(EN)-FR(2) GLP	Y	BASF
IIA 4.4	Carter, M.L. et al.	2011	Validation of Analytical Method Number D1002: Validation of BASF Analytical Method D1002: Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS ADPEN Laboratories, Inc., Jacksonville, Florida, Study No. 416947 BASF Reg. Doc. No. 2011/7005073 GLP	Y	BASF
IIA 4.4	Perez, S. et al.	2011	Independent Laboratory Validation of BASF Analytical Method D1002: Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS ADPEN Laboratories, Inc., Jacksonville, Florida, Study No. 379626 BASF Reg. Doc. No. 2011/7005075 GLP	Y	BASF
IIA 4.5	Baltussen, E.	2007	Development and Validation of an Analytical Method for the Determination of OK-5101 Residues in Water Notox B.V., DD's-Hertogenbosch, The Netherlands, Study No. 475741 BASF Reg. Doc. No. OTSA-0326-FR GLP	Y	BASF
IIA 4.5	Oudhoff, K.A.	2009	Development and Validation of an Analytical Method for the Analysis of Residues of B-1 in Water Notox B.V., DD's-Hertogenbosch, The Netherlands, Study No. 490990 BASF Reg. Doc. No. 2009/1126660 GLP	Y	BASF

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
IIA 4.5	Oudhoff, K.A.	2009	Development and Validation of an Analytical Method for the Analysis of Residues of B-3 in Water Notox B.V., DD's-Hertogenbosch, The Netherlands, Study No. 490989 BASF Reg. Doc. No. 2009/1126661 GLP	Y	BASF
IIA 4.6	Leak, T.	2012	Validation of Test Solution Preparations and Analytical Methods for Use in the Determination of BAS 9210-I in Various Media Used in Environmental Toxicity Studies (Including Final Report Amendment and Final Report Amendment 2) ABC Laboratories, Inc., Columbia, Missouri, Study Nos. 379967 and 65234 BASF Reg. Doc. No. 2012/7004298 GLP	Y	BASF
IIA 4.6	Oudhoff, K.A.	2009	Development and Validation of an Analytical Method for the Analysis of AB-1 in Sediment Notox B.V., DD's-Hertogenbosch, The Netherlands, Study No. 490354 BASF Reg. Doc. No. 2009/1126662 GLP	Y	BASF
Annex III Data and Information					
IIIA 5.2.1	Brill, J.H.	2011	Validation of Analytical Method AFR0088/01: Determination of BAS 9210 I in BAS 9210 I SC Formulation by Reverse-Phase HPLC using UV Detection BASF Corporation, Research Triangle Park, North Carolina, Study No. 409943 BASF Doc. ID 2011/7005142 GLP	Y	BASF