1.0 Introduction

1.1 Purpose of the Study

The purpose of the study was to demonstrate that BASF Analytical Method D1002, "Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS", could be performed successfully at an outside facility with no prior experience with the method.

1.2 Summary of the Results

The independent laboratory validation of the BASF method was successfully completed in the first trial without need for clarification of the method. Detailed results for cyflumetofen (BAS 9210 I), A-2, B-3, B-1, and AB-1 dimer are presented in Tables 1–5, respectively (Appendix A). Recommendations for BASF Analytical Method D1002 are presented in Appendix B. Representative chromatograms are presented in the Figures section (Appendix C). The detailed reports representing the analytical data are presented in Appendix D.

2.0 Reference Substance and Sample History

2.1 Reference Materials

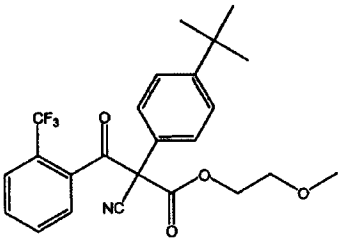
The reference standards shown below were used in this study. The certificates of analysis for the reference substances are provided in Appendix E.

The reference substances were maintained at the proper temperature for each compound until they were utilized in this study. Reference substance solutions were refrigerated during the course of this study. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical method.

Reference substances for BAS 9210 I, A-2, B-3, B-1, and AB-1 dimer were used for fortifications and LC-MS/MS calibration. Examples standard solution preparations are presented in Table 6 (Appendix A).

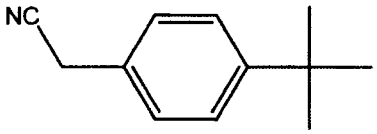
BASF Code Name:	BAS 9210 I
Common Name:	Cyflumetofen
Source:	Otsuka Chemical Co., Ltd.
BASF Registry Number:	5465430
CAS Number:	400882-07-7
IUPAC Name:	2-methoxyethyl-(*R*,*S*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl) propionate
Molecular Formula:	$C_{24}H_{24}F_3NO_4$
Molecular Weight:	447.5 g/mol
Lot Number:	006005
Purity:	99.5%
Date Assayed:	August 04, 2009
Expiration Date:	August 03, 2012

Chemical Structure:



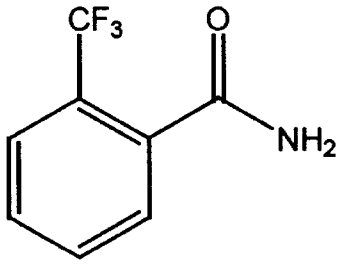
BASF Code Name: A-2
Source: BASF, Limburgerhof, Germany
BASF Registry Number: 133276
CAS Number: 3288-99-1
IUPAC Name: (4-*tert*-butylphenyl) acetonitrile
Molecular Formula: $C_{12}H_{15}N$
Molecular Weight: 173.3 g/mol
Lot Number: L84-64
Purity: 98.5%
Date Assayed: December 14, 2010
Expiration Date: December 1, 2012

Chemical Structure:



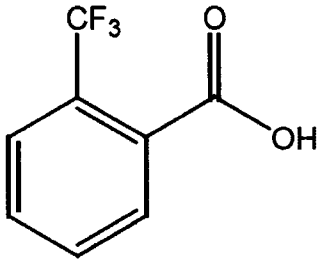
BASF Code Name: B-3
Source: Sigma–Aldrich
BASF Registry Number: 4288294
CAS Number: 360-64-5
IUPAC Name: 2-(trifluoromethyl) benzamide
Molecular Formula: $C_8H_6F_3NO$
Molecular Weight: 189.13 g/mol
Lot Number: 08721EB
Purity: 99.9%
Date Assayed: N/A
Expiration Date: January 7, 2012

Chemical Structure:



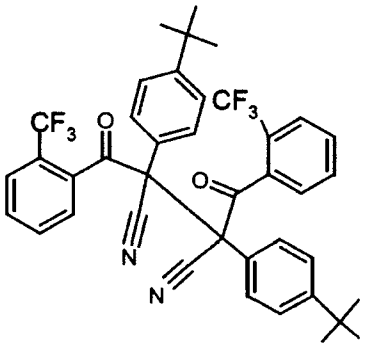
BASF Code Name: B-1
Common Name: trifluoro-*o*-toluic acid
Source: Sigma–Aldrich
BASF Registry Number: 104415
CAS Number: 433-97-6
IUPAC Name: 2-(trifluoromethyl) benzoic acid
Molecular Formula: $C_8H_5F_3O_2$
Molecular Weight: 190.12 g/mol
Lot Number: MKBB8135
Purity: 99.4%
Date Assayed: December 2008
Expiration Date: December 7, 2011

Chemical Structure:



BASF Code Name:	AB-1 Dimer
Source:	BASF, Limburgerhof, Germany
BASF Registry Number:	5756389
CAS Number:	N/A
Molecular Formula:	$C_{40}H_{34}F_6N_2O_2$
Molecular Weight:	688.7 g/mol
Lot Number:	L84-54
Purity:	92.9%
Date Assayed:	December 13, 2010
Expiration Date:	December 1, 2012

Chemical Structure:



The performance of the instrument was evaluated during each injection set. Standard solutions prepared for this study were stored under refrigerated conditions in refrigerator E-51, which had a temperature range of 2–4 °C during the course of the study.

2.2 Test System

A homogenized control soil sample was provided by BASF from Study Number 350785, BASF sample code RSN R0905070016. This soil sample has been identified as silt loam and with high water content (>20% moisture) at 24–30 inches. The untreated soil sample was sent from BASF Crop Protection, Inc. on August 1, 2011 and received by ADPEN Laboratories, Inc. on August 2, 2011.

Upon receipt, samples were logged in and stored in freezer E-16, which had a temperature range of −20 to −6 °C during the course of this study. The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 110810001-001) and is cross-referenced on the detailed reports to the assigned unique sample number. Sample extracts awaiting LC-MS/MS analysis were stored in refrigerator E-20, which had a temperature range of 6–7 °C during the course of this study.

3.0 Procedure – Method Synopsis

BASF Analytical Method D1002, "Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS".

3.1 Summary of Analytical Procedure

Briefly, for the analysis of cyflumetofen (BAS 9210 I), B-1, B-3, A-2, and AB-1 Dimer, a 0.1 g soil sample aliquot is extracted by shaking with acetonitrile followed by mixture of acetonitrile and water (60:40, v/v). The combined extract is then diluted with 1:1 (v/v) with 0.1% formic acid in acetonitrile for the residue determination of BAS 9210 I, B-1, and B-3 using LC-MS/MS. 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. Instrument parameters are described in Table 7 (Appendix A). A flow diagram for

the method procedure is presented in Figure 1 (Appendix C) and the detailed technical procedure can be found in Appendix F.

The primary (quantitative) and secondary (confirmatory) transition ions monitored are found below:

Analyte	Injection	Transition (m/z)		Ionization Mode	Retention Time (min.)
		Primary	Secondary		
BAS 9210 I	#1	448.2 → 173.0	448.2 → 145.1	Positive (Switch at 2.50 min)	2.51
B-3		190.0 → 130.0	190.0 → 102.0	Positive (Switch at 1.99 min)	1.85
B-1		189.1 → 69.0	189.1 → 145.1	Negative (Switch at 1.99–2.50 min)	2.01
A-2	#2	174.1 → 147.1	174.1 → 117.1	Positive	1.74
AB-1 Dimer	#3	689.4 → 288.2	689.4 → 268.2	Positive	2.80
B-1	#4	189.1 → 69.0	189.1 → 145.1	Negative	2.01

4.0 Limits of Quantitation and Detection

The limit of quantitation (LOQ) for residues of BAS 9210 I and its metabolites in soil is 0.01 ppm for each analyte. The limit of detection (LOD) is 20% of the LOQ, equivalent to 0.002 ppm for each analyte.

5.0 Calibration, Calculations, and Statistics

Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substances at multiple concentrations.

A standard curve was prepared by injected standard solutions at appropriate concentrations for each analyte. Calibration standard concentrations for cyflumetofen (BAS 9210 I) and its metabolites ranged from 0.125–10.0 ng/mL. A calibration standard was typically injected every two to four sample injections. Analyst® 1.5.1 created the standard curve based on linear regression, typically using 1/x weighting. The regression functions were used to calculate the best-fit line by plotting the standard concentrations (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis. Typical calibration curves are presented in Figures 2–6 (Appendix C) for BAS 9210 I, A-2, B-3, B-1, and AB-1 dimer, respectively and representative chromatograms of calibration standards for BAS 9210 I, A-2, B-3, B-1, and AB-1 dimer are presented in Figures 7–42 (Appendix C), respectively. The performance of the instrument was evaluated during each injection set.

Peak integration and quantitation were performed within Analyst 1.5.1 software; using the calibration curve equation to determine sample concentrations of the analyte found during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples within Microsoft® Office Excel and reported in spreadsheet data reports, which are presented in Appendix D.

The equation used for quantitation is: $y = mx + b$
Where:

y = peak area
x = ng found for peak of interest
m = slope
b = y-intercept

a) Solving for x: $x = \frac{y - b}{m}$

The following equations are used for residue and recovery calculations within Microsoft® Office Excel:

b) Amount of sample injected (mg) = $\frac{\text{sample weight (mg)}}{\text{final volume (mL)}} \times \text{injection size}(\mu L) \times \frac{1\,mL}{1000\,\mu L}$

c) ppm = $\frac{\text{ng found}}{\text{mg injected}}$

d) Percent recovery = $\frac{(\text{ppm in the sample} - \text{ppm in the control})}{\text{ppm added}} \times 100$

As an example, calculations to obtain BAS 9210 I recovery results using BASF Sample Number 11081001-001C from work order WO-11090902 are shown below:

a) ng found = $\frac{11085 - 422}{2.47e+006} = 0.004317$ ng

b) mg of sample injected = $\frac{100\,mg}{1.6\,mL} \times 7.0\,\mu L \times \frac{1\,mL}{1000\,\mu L} = 0.4375$ mg

c) ppm = $\frac{0.004317\,ng}{0.4375\,mg} = 0.00985$ ppm

Average residue found in the control samples (ADPEN Lab Code: 110810001-001C and 110810001-001A) = 0.00000 ppm

d) Percent recovery = $\frac{(0.00985 - 0.00000)\,ppm}{0.01\,ppm} \times 100 = 98.5\%$

Statistical treatment of the data included calculation of means, standard deviations (SD) and percent relative standard deviations (%RSD). These calculations were performed using Excel. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

Appendix B. Recommendations/Suggestions for BASF Analytical Method D1002

Recommendations for BASF Analytical Method D1002: 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 BAS 9210 I 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 should be used for the analysis of B-1 for confirmatory purposes.

Figure 1. Flow Diagram of Analytical Method Number D1002 for BAS 9210 I, B-1, B-3, A-2, and AB-1 Dimer