1 Summary

An independent laboratory validation (ILV) of the "Method AE002-S04-02 for the Determination of Residues of AE 0172747 and its Metabolites AE 0456148, AE 0968400, AE 1392936, AE 0941989 by HPLC-MS/MS and of AE 1124336 by GC/MSD in Soil" was conducted using soils Höfchen (silt loam, Germany) and Laacher Hof (sandy loam) Germany).

The independent laboratory validation (ILV) was conducted using two untreated control soils from Germany (Höfchen and Laacher Hof) chosen as representative matrices. The analyses included a solvent blank with no internal standards added (GC-MSD) and with internal standards added (LC-MS/MS), and a set of duplicate untreated controls, five control soil samples fortified at the limit of quantitation (LOQ) and five samples fortified at 10× LOQ for both soils. The LOQ for all analytes was 0.01 mg/kg (ppm).

In the first trial the method was successfully validated for AE 0172747 and its metabolites AE 0456148, AE 0968400, AE 1392936 and AE 0941989 (HPLC-MS/MS measurements). However, for AE 1124336 (GC-MSD measurements) some recoveries were less than 70% and it was found that the losses were caused by the evaporator which was used (TurboVap LV). A second trial was performed using a vacuum rotary evaporator. On the second trial, the method gave acceptable recoveries for AE 1124336 in both test soils.

2 Objective

The objective of this study was to demonstrate that method AE002-S04-02 ("AE 0172747: Analytical Method for the Determination of AE 0172747 and its Metabolites AE 0456148, AE 0968400, AE 1392936, AE 0941989 by HPLC-MS/MS and for the Determination of AE 1124336 by GC/MSD") can be performed with acceptable recoveries for determination of the compounds AE 0172747, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 at an independent laboratory having no prior experience with the method. The method was developed by Bayer CropScience LP, Stilwell, USA, and validated in that laboratory with results reported as Method AE002-S04-02, by D.J. Netzband & J.M. Wade, in report dated July 6, 2004. Soil Höfchen (Germany) and soil Laacher Hof (Germany) were chosen as representative matrices for validation within the present study.

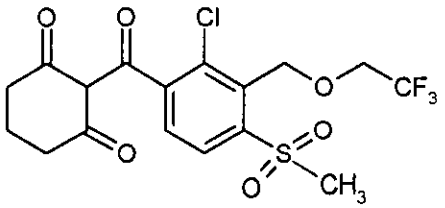
This study was performed in accordance with EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 of March 17, 2004, Commission Directive 96/46/EC amending Council Directive 91/414/EEC of July 16, 1996, and BBA Guideline on Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998 and Ecological Effects Test Guidelines, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods, EPA 712-C-96-348, April 1996.

3 Materials

3.1 Test and Reference Items

AE 0172747:

Structural formula:



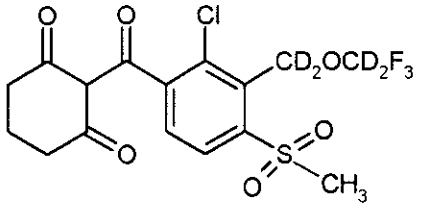
Chemical code:	AE 0172747
Chemical name (CAS):	2-{2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy)methyl]-benzoyl}cyclohexane-1,3-dione
Empirical formula:	$C_{17}H_{16}ClF_3O_6S$
Molecular weight:	440.82 g/mol

Reference standard:

Certificate of analysis:	AZ 10961
Purity:	98.9 %
Expiry date:	August 2006
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

AE 0172747-trifluoroethoxymethyl-d4 (used as internal standard for the active ingredient AE 0172747):

Structural formula:



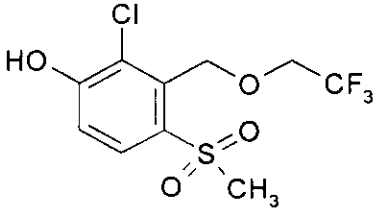
Code name:	AE 0172747-trifluoroethoxymethyl-d4
Chemical name (CAS):	2-{2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy-1,1-d_2)-methyl-d_2]-benzoyl}-1,3-cyclohexanedione
Empirical formula:	$C_{17}H_{12}ClD_4F_3O_6S$
Molecular weight:	444.84 g/mol

Reference standard:

Standard no.:	K-1418
Reference no.:	2003BRP003-215
Origin:	Bayer CropScience, Stilwell, KS, USA

AE 0968400:

Structural formula:



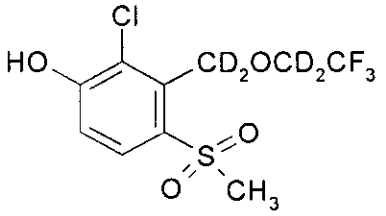
Chemical code:	AE 0968400
Chemical name (CAS):	2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy)methyl]phenol
Empirical formula:	$C_{10}H_{10}ClF_3O_4S$
Molecular weight:	318.70 g/mol

Reference standard:

Certificate of analysis:	AZ 10312
Purity:	97.6 %
Expiry date:	December 2004
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

AE 0968400-trifluoroethoxymethyl-d4 (used as internal standard for the test item AE 0968400):

Structural formula:



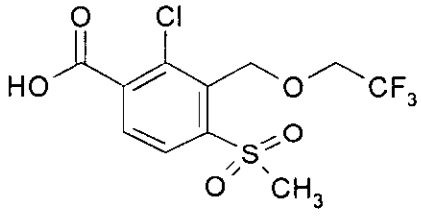
Code name:	AE 0968400-trifluoroethoxymethyl-d4, AE 0172747-P-1- trifluoroethoxymethyl-d4
Chemical name (CAS):	2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy-1,1-d_2)methyl-d_2]phenol
Empirical formula:	$C_{10}H_6ClD_4F_3O_4S$
Molecular weight:	322.72 g/mol

Reference standard:

Standard no.:	K-1272
Purity:	3.98% w/w solution in acetonitrile (test item purity 99.4%)
Expiry date:	June 2008
Origin:	Bayer CropScience, Stilwell, KS, USA

AE 0456148:

Structural formula:



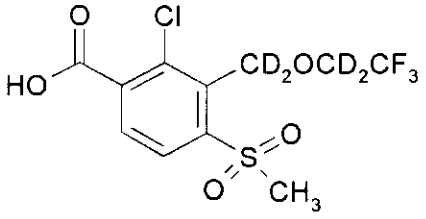
Chemical code:	AE 0456148
Chemical name (CAS):	2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy)methyl]-benzoic acid
Empirical formula:	$C_{11}H_{10}ClF_3O_5S$
Molecular weight:	346.71 g/mol

Reference standard:

Certificate of analysis:	AZ 10576
Purity:	93.8%
Expiry date:	April 2006
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

AE 0456148-trifluoroethoxymethyl-d4 (used as internal standard for the test item AE 0456148):

Structural formula:



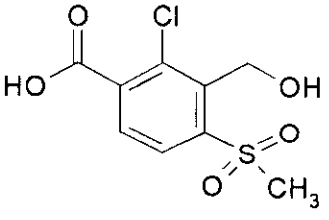
Code name:	Free acid-trifluoroethoxymethyl-d4, AE 0172747-acid–trifluoroethoxymethyl-d4
Chemical name (CAS):	2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy-1,1-d_2)methyl-d_2]benzoic acid
Empirical formula:	$C_{11}H_6ClD_4F_3O_5S$
Molecular weight:	350.73 g/mol

Reference standard:

Standard no.:	K-1229
Purity:	98.7%
Expiry date:	October 2008
Origin:	Bayer CropScience, Stilwell, KS, USA

AE 1392936:

Structural formula:



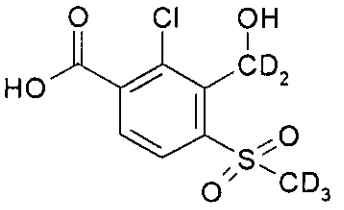
Chemical code:	AE 1392936
Chemical name (CAS):	2-chloro-3-hydroxymethyl-4-mesylbenzoic acid
Empirical formula:	$C_9H_9ClO_5S$
Molecular weight:	264.68 g/mol

Reference standard:

Certificate of analysis:	AZ 10910
Purity:	93%
Expiry date:	July 2005
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

AE 1392936-benzyl-methylsulfonyl-d5 (used as internal standard for the test item AE 1392936):

Structural formula:



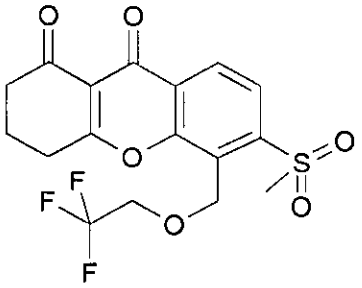
Code name:	AE 0172747 P-2-benzyl-methysulfonyl-d5
Chemical name (CAS):	2-chloro-3-(hydroxymethyl-d_2)-4-(methyl-d_3-sulfonyl)-benzoic acid
Empirical formula:	$C_9 H_4 Cl D_5 O_5 S$
Molecular weight:	269.71 g/mol

Reference standard:

Standard no.:	K-1239
Purity:	91.8%
Expiry date:	October 2008
Origin:	Bayer CropScience, Stilwell, KS, USA

AE 0941989:

Structural formula:



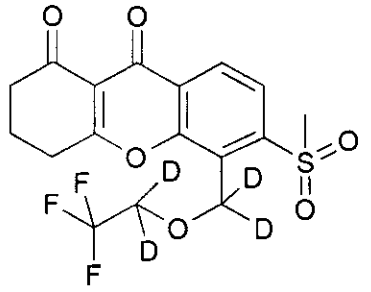
Chemical code:	AE 0941989
Chemical name:	6-mesyl-5-(2,2,2-trifluoro-ethoxymethyl)-3,4-dihydro-2H-xanthene-1,9-dione
Empirical formula:	$C_{17} H_{15} F_3 O_6 S$
Molecular weight:	404.36 g/mol

Reference standard:

Certificate of analysis:	AZ 11511
Purity:	99.7%
Expiry date:	April 2007
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

AE 0172747-xanthenedione-trifluoroethoxymethyl-d4 (used as internal standard for the test item AE 0941989):

Structural formula:



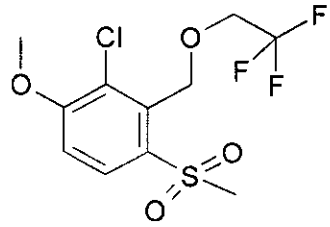
Code name:	AE 0172747 xanthedione-trifluoroethoxymethyl-d4
Chemical name (CAS):	3,4-Dihydro-6-(methylsulfonyl)-5-[(2,2,2-trifluoroethoxy-1,1-d2)methyl-d2]-1H-xanthene-1,9-(2H)-dione
Empirical formula:	$C_{17}H_{11}D_4F_3O_6S$
Molecular weight:	408.38 g/mol

Reference standard:

Standard no.:	K-1341
Purity:	96.1%
Expiry date:	December 2008
Origin:	Bayer CropScience, Stilwell, KS, USA

AE 1124336:

Structural formula:



Chemical code:	AE 1124336
Chemical name:	2-chloro-4-mesyl-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene
Empirical formula:	$C_{11}H_{12}ClF_3O_4S$
Molecular weight:	332.72 g/mol

Reference standard:

Certificate of analysis:	AZ 10323 & AZ 12129
Purity:	97.7%
Expiry date:	December 2004 & November 2007
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

3.2 Test System

The method was validated using two German soils *Höfchen* and *Laacher Hof*. Two different soils were used in order to assess a possible influence of different soil characteristics. The soil samples were classified according to DIN and/or USDA specifications. Soil characteristics of the used soils are summarised in Table 1.
Complete soil parameterisation is reported in Table 11 and Table 12.

Table 1: Soil Types

Soil	Texture of Soil	Organic Matter [%]
Höfchen	silt loam (USDA)	1.58
Laacher Hof	sandy loam (USDA)	2.06

4 Experimental

4.1 Analytical Method

The recovery data for the study were generated using the following method, which gives full details of preparing the analytical sample extracts and the conditions for high performance liquid chromatography (HPLC):

Number of the method:	AE002-S04-02
Title of the method:	AE 0172747: Analytical Method for the Determination of AE 0172747 and its Metabolites AE 0456148, AE 0968400, AE 1392936, AE 0941989 by HPLC-MS/MS and for the Determination of AE 1124336 by GC/MSD.
Author of the method:	Derek J. Netzband Jami M. Wade Bayer CropScience LP 17745 S Metcalf Avenue Stilwell, Kansas 66085
Reference:	Method AE002-S04-02
Limit of quantitation:	0.01 mg/kg

The following sample sets were analysed:

Table 2: Level and Number of Recoveries per Fortification Level

Soil	Control sample	Level 0.01 mg/kg	Level 0.10 mg/kg
Höfchen	2	5	5
Laacher Hof	2	5	5

Additionally, a solvent blank with no internal standards added (GC-MSD) and a solvent blank with internal standards added (LC-MS/MS) were analysed.

4.1.1 Outline of the Method

AE 0172747 and its associated metabolites were extracted from soils using microwave extraction with acetonitrile / 0.1% acetic acid in water (50:50 v/v). Following extraction, the extracts were quantitatively transferred to a 250-mL graduated cylinder and diluted to volume with acetonitrile.
An aliquot of this extract was transferred to a 200-mL Zymark® Evaporation Flask, a deuterated internal standard containing AE 0172747-d4, AE 045614-d4, AE 0968400-d4, AE 0941989-d4 and AE 1392936-d5 was added to the extraction solution and diluted to volume with 0.1% (v/v) acetic acid in deionised water and analyzed by LC/MS/MS for AE 0172747, AE 045614, AE 0968400, AE 0941989 and AE 1392936 by internal standard.
An additional aliquot was transferred from the original 250-mL measuring cylinder to a round bottom flask with ground joint, evaporated to dryness using a rotary evaporator and reconstituted in ethyl acetate and analyzed by GC/MSD for AE 1124336 by external standard.

4.1.2 Instruments

Microwave Extractor:	MLS-Ethos MWS Vertriebs GmbH 88299 Leutkirch, Germany
Balances:	PC 4400, PM 4800 and AT 261 Mettler Instruments GmbH 35387 Giessen, Germany
Ultrasonic Bath:	Transsonic 890/H Heinrich Faust 51145 Cologne, Germany
Liquid Chromatograph:	HP 1100 Column Compartment G1316A HP 1100 Binary Pump G1312A HP 1100 Isocratic Pump G1310A HP 1100 Degasser G1322A Agilent 40880 Ratingen, Germany
Autosampler:	HTC PAL System CTC Analytics AG 4222 Zwingen, Switzerland
Mass Spectrometer:	IONICS EP 10+ with turbo-ionspray interface mass selective detector (MS/MS) performance-enhanced Sciex API-365 Ionics Concord, ON, Canada

Note: Some mass spectrometric conditions are instrument specific. The spectrometric conditions were optimised by a competent operator prior to analysis.

Gas Chromatograph: 6890 Plus gas chromatograph G1530N
5973N mass selective detector G2589A
7683 autosampler G2613A
Agilent
40880 Ratingen, Germany

Vacuum rotary evaporator: with round bottom flask
Büchi Labortechnik GmbH
Postfach 100351
45003 Essen, Germany

4.1.3 Reagents and Equipment

Column (HPLC): SymmetryShield RP8 5 µm, length 150 mm, i.d. 3 mm
Order No. WAT094243
Waters GmbH
65760 Eschborn, Germany

Column (GC): HP Ultra 1 Methyl Siloxane 12 m x 0.2 mm,
0.33 µm film thickness
Order No. 19091A-101
Agilent
40880 Ratingen, Germany

Magnetic stirring bar: plain (large, e.g. 35 x 8 mm [length x i.d.]) or "dumb-bell" type (e.g. 35 x 8 mm [length x i.d.], diameter of end disk is 20 mm, from COWIE Technology, parts no. 1.1335)

Porcelain Büchner funnel

Filter paper: Whatman GF/A 90 mm glass microfibre filter
Cat no. 1820 090
Whatman

Syringe filter: Acrodisc® CR 13 mm syringe filter with 0.45 µm PTFE membrane, parts no. 514-4008
VWR International GmbH
64301 Darmstadt, Germany

Acetonitrile: for HPLC, super gradient grade
Riedel de Haen, No. 34998
30926 Seelze, Germany

Ethyl acetate:	for residue analysis Promochem 46485 Wesel, Germany
Acetic acid (100%):	p.a. Merck, No.1.00063.1011 64271 Darmstadt, Germany
Water:	purified in a Milli-Q unit Milli-Pore GmbH 65731 Eschborn, Germany

Volumetric flasks, pipettes and other equipment commonly used in the laboratory.

4.1.4 Chromatographic Conditions and Mass Spectrometric Parameters

Liquid as well as gas chromatographic conditions were identical to those described in Appendix 1 of the original method report AE002-S04-02.
MS/MS parameter settings were optimized for the instrument being used and therefore not identical with those reported in method AE002-S04-02.

4.1.5 Calculation

Calculations were performed using the computer software MS-EXCEL. In general, the program uses nine decimal places for calculations. The results given are rounded values. Thus, rounding "errors" may occur if recalculations are made using the listed figures.

4.1.5.1 Calculation of AE 0172747, AE 045614, AE 0968400, AE 0941989, AE 1392936 and AE 1124336 Residues (LC-MS/MS & GC-MSD)

NOTE: Evaluation is performed according to the external bracketing standard procedure.

1. Calculate the mean peak area (GC-MSD) and area ratio (LC-MS/MS), respectively, of the bracketing standards for each sample. This value will be used as F_{ST}.
2. Determine the peak area / area ratio for the analysed sample. This value will be used as F_A.
3. Calculate the residue level in mg/kg (ppm) as follows:

$$R = \frac{F_A \times V_{End} \times W_{St}}{F_{St} \times V_{inj} \times G}$$

R: Determined amount of residue in mg/kg
F_A: Mean peak area / area ratio in the sample solution obtained from V_{inj}
F_{St}: Mean peak area / area ratio in the bracketing standards obtained from W_{St}

V_{End}: Final volume of the sample solution in mL (may be adjusted according to the expected residue level)
W_{St}: Amount of analyte in V_{inj} of the standard solution in ng
V_{inj}: Injection volume in mL
G: Sample weight of analytical samples in g

4.1.5.2 Calculation of Recoveries

1. Calculate the mean residue in the recovery sample according to 4.1.4.1.
2. Calculate the percent recovery as follows:

$$\text{Recovery} = \frac{\text{Mean Residue} \times 100}{\text{Fortification Level}}$$

Recovery: Recovered amount of analyte in % found in the fortified sample
Mean residue: Mean residue in the fortified sample in mg/kg determined according to 4.1.4.1

Fortification level: Fortified concentration of analyte in mg/kg

4.1.6 Deviations from the Method

Within the analytical procedure for determination of AE 1124336 by GC-MSD the solvent has to be evaporated after extraction. According to method AE002-S04-02 a Zymark **TurboVap II** has been used. Within the ILV procedure a Zymark **TurboVap LV** was used, since the other model was not available within the test facility. However, it was found that recoveries were not acceptable (<70%) when using the **TurboVap LV** but were acceptable when using a **vacuum rotary evaporator**. The unexpected losses obtained with **TurboVap LV** (compared to **TurboVap II**) can be explained by differences in technology implemented in the two different models. The ILV was performed using the **vacuum rotary evaporator** instead of a **TurboVap LV**. Acceptable recoveries were obtained using the rotary evaporator.

4.2 Linearity of the Detector

The linearity of the detector response for AE 0172747 and its metabolites AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 were tested by injections of standard solutions. The following concentrations were measured:

Table 3: Standard Concentrations for the Determination of Detector Linearity

	Concentration [µg/L]						
AE 0172747	0.05	0.1	0.25	0.5	1	2	5
AE 0456148	0.05	0.1	0.25	0.5	1	2	5
AE 0968400	0.05	0.1	0.25	0.5	1	2	5
AE 1392936	0.05	0.1	0.25	0.5	1	2	5
AE 0941989	0.05	0.1	0.25	0.5	1	2	5
AE 1124336	0.5	1	2	3	4	5	-