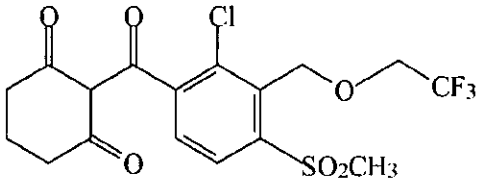
2. TEST AND REFERENCE SUBSTANCES

The following compounds were used as test and reference substances, and were supplied by Bayer CropScience. Neat standards were stored frozen. Solutions of standards were stored under refrigeration at approximately 4°C.

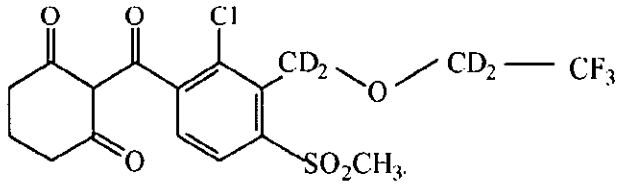
The structures for AE 0172747 and its metabolites AE 0456148 and AE 1392936 are shown below along with the structures for the deuterated internal standards.

Code Name: **AE 0172747**
(Active Ingredient, Parent Molecule)



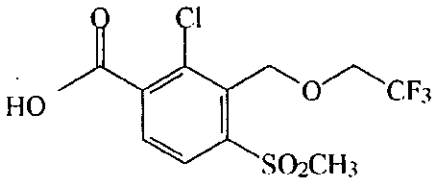
CAS Name:	2-[2-chloro-4-mesyl-3-((2,2,2-trifluroethoxy)methyl)benzoyl]cyclohexane-1,3-dione
CAS Number:	335104-84-2
Molecular Formula:	$C_{17}H_{16}O_6ClF_3S$
Molecular Weight:	440.82 g/mol

Code Name: **AE 0172747-*trifluoroethoxymethyl*-d_4**
(Parent Molecule, Deuterated Internal Standard)



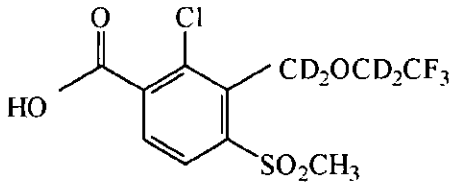
CAS Name:	2-[2-chloro-4-mesyl-3-((2,2,2-trifluroethoxy)methyl)benzoyl]cyclohexane-1,3-dione-d_4
Molecular Formula:	$C_{17}H_{12}D_4O_6ClF_3S$
Molecular Weight:	444.90 g/mol

Code Name: **AE 0456148**
(Metabolite)



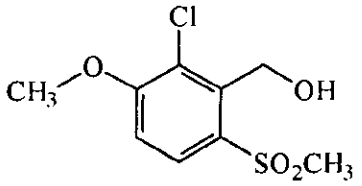
CAS Name:	2-[2-chloro-4-mesyl-3-[(2,2,2-trifluroethoxy)methyl]benzoic acid
CAS Number:	120100-77-8
Molecular Formula:	$C_{11}H_{10}O_5ClF_3S$
Molecular Weight:	346.71 g/mol

Code Name: **AE 0456148-*trifluoroethoxymethyl*-d_4**
(Metabolite, Deuterated Internal Standard)



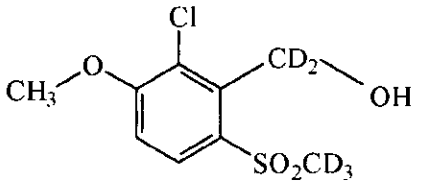
CAS Name:	2-[2-chloro-4-mesyl-3-((2,2,2-trifluroethoxy)methyl)benzoic acid-d_4
Molecular Formula:	$C_{11}H_6D_4O_5ClF_3S$
Molecular Weight:	350.70 g/mol

Code Name: **AE 1392936**
(Soil Metabolite)



CAS Name:	2-chloro-3-hydroxymethyl-4-mesylbenzoic acid
CAS Number:	120100-47-2
Molecular Formula:	$C_9H_9O_5ClS$
Molecular Weight:	264.69 g/mol

Code Name: **AE 1392936-*benzyl-methylsulfonyl*-d_5**
(Soil Metabolite, Deuterated Internal Standard)



CAS Name: 2-chloro-3-(hydroxymethyl)-4-mesylbenzoic acid-d_5
Molecular Formula: $C_9H_4D_5O_5ClS$
Molecular Weight: 269.70 g/mol

3. TEST SYSTEM – WATER SAMPLES

The method was validated using one surface water, one ground water and one tap water. The surface control water used in this study was collected for Bayer CropScience Study Number 00M19458, Ethoprophos Surface Water Monitoring. The ground control water used in this study was collected for Bayer CropScience Study Number MEGUY003 , Survey of Azinphos-Methyl (GUTHION) Residues in Well Water taken From Agricultural Areas of Virginia , Pennsylvania, and New York. The treated tap control water was collected from a treated drinking water tap in a laboratory building at Bayer Research Park, Stilwell, Kansas.

The table below gives the sample ID, water type and sampling location for each of the waters. The raw data regarding the water sample identity and characterization are archived with the original studies listed in the table below (See Section 8, References.)

Sample ID	Original Study	Water Type	Source Location
19458-CA0097	00M19458	surface water	Lodi, CA
MK-2-9/10/04-A	MEGUY003	ground water	Biglerville, PA
BRP002	RAAEX021	treated tap water	Stilwell, KS

Storage
The untreated ground water samples were stored frozen at about minus 10°C and the surface waters in a refrigerator at approximately 4°C. The treated tap water was taken fresh for processing and was not stored before or during the study.

4. REAGENTS AND EQUIPMENT

4.1 Reagents and General Equipment

The reagents and equipment used in this study are listed in Section 2 of the attached method of analysis. Solution preparation using these reagents is described in the method in the same section.

General laboratory safety precautions were taken.

4.2 Liquid Chromatographic/Mass Spectrometer Detection System

Residues of AE 0172747 and its metabolites AE 0456148 and AE 1392936 in water were determined using an Applied Bio / Sciex API-4000 LC/MS/MS system with Sciex TurbolonSpray Electrospray Interface; Shimadzu LC-10AD VP HPLC pumps (2) with a high pressure mixer and SCL-10A VP Pump Controller; and a Gilson 215 autosampler. The Applied Bio / Sciex instrument software applications used was Analyst 1.4.1. The operating parameters that were used are outlined in Section 5 of the attached method. There were no modifications made and the method was run as written.

Method of Analysis for the Determination of Residues of AE 0172747 and its Metabolites AE 0456148 and AE 1392936 in Water Using LC/MS/MS – Revision W05-02

1 INTRODUCTION

1.1 Scope

This method sets forth the procedure for determining the residues of AE 0172747 and its metabolites AE 0456148 and AE 1392936 in water.

1.2 Principle

An aliquot of water is acidified with formic acid and injected onto the LC/MS/MS. Quantification is based on the use of deuterated internal standards and comparison of peak areas with those of known standards.

1.3 Method Limits

The target limit of quantitation for this method is 50 ppt (0.05ng/mL) for AE 0172747 and its metabolites AE 0456148 and AE 1392936 in water.

1.4 Purpose of Revision

The purpose of this revision is to add a procedure for determination of the analytes in waters that may contain free chlorine such as some finished drinking waters (tap waters).

2 MATERIALS

Unless otherwise noted, equivalent brands and/or suppliers can be used.

2.1 Reagents/Solvents

Acetic acid Guaranteed Reagent (GR) (EM Science Cat. No. AX0073)

Acetonitrile Omni-Solv, (EM Science, Cat. No. AX0142)

Water Omni-Solv, HPLC Grade (EM Science, Cat. No. WX0004)

Formic Acid, 88% Certified ACS (Fisher Scientific, A118-4)

2.2 Equipment and Supplies

Balance for analytical standards:
Accuracy ± 0.1 mg, Mettler AT 201 or equivalent

Disposable pipettes

Micropipetter, Eppendorf brand, and pipette tips

Graduated cylinders

Pipette bulb

Volumetric flasks

Volumetric pipettes

20 glass or plastic bottle or vial.

1 liter glass containers for HPLC solvent delivery.

2 autosampler vials

2.3 Solutions

Solution of 10:90 Acetonitrile: 0.1% Acetic Acid in Water.

Transfer about 200 mL of HPLC grade water into a 1000 mL mixing graduated cylinder. Add 900 µl of acetic acid and fill to the 900 mL mark with water. Fill to the 1000 mL mark with acetonitrile, stopper, and mix.

Solution of 1.5% acetic acid in HPLC grade water for use as a mobile phase component:

Add about 200 mL of HPLC grade water into a 1000 mL graduated cylinder or graduated mobile phase reservoir or container.

Transfer 15.0 mL of acetic acid to that cylinder or container, then make up to the 1000 mL mark with HPLC grade water.

If necessary, transfer the solution to a clean, dry mobile phase reservoir.

Swirl to mix thoroughly, but do not shake, to prevent dissolving more air into the solution.

Place the container or reservoir in a sonicator bath and apply vacuum while sonicating for about 10 minutes or until air bubble formation or cavitation subsides to a minimum or use an in-line degasser.

Mobile phase is produced by high pressure mixing of the above with pure acetonitrile to produce the mobile phase gradient as outlined in the instrument conditions below. It has not been found necessary to sonicate the acetonitrile.

Solution of 10 ppm sodium thiosulfate:

Weigh approximately 100 mg of sodium thiosulfate into a 100 mL volumetric flask. Dissolve the amount in approximately 50 mL of HPLC grade water and make up the volume to the 100 mL mark. Mix thoroughly by inverting the flask several times. This solution is 1mg/mL or 1000ppm. Transferring 1 mL of this solution to a 100 mL water sample will produce 10 ppm concentration of sodium thiosulfate in that sample. Transfer the sodium thiosulfate solution in to 100 mL amber bottle and store refrigerated at approximately 6°C (±5°C).

Solution of HPLC grade water chlorinated with sodium hypochlorite (NaOCl):

Pipet 128uL of NaOCl (13% chlorine, density 1.209g/mL) into a 100mL volumetric flask. Fill to volume with deionized, HPLC grade water. The resulting free chlorine concentration is 200ug/mL. To simulate a chlorinated finished drinking water add an appropriate amount of this solution to a water sample. For example, add 100uL of the 200ug/mL free chlorine solution to a 10mL HPLC grade water sample. The resulting level of free chlorine is 2ug/mL (ppm). Chlorine is volatile, so this solution should be stored tightly sealed, in the dark under refrigeration at approximately 6°C (±5°C) and should be remade if more than three weeks old.

3 FORTIFICATION AND CALIBRATION SOLUTIONS

3.1 Preparation

Use class "A" volumetric pipettes to make standards. The following is an example of a procedure to follow in preparing standard solutions. Alternate or additional standards of appropriate concentration and volume may be prepared as needed. The "~" symbol indicates approximately.

All the standard solutions must be stored in amber glass bottles. Standard solutions will be stored in a refrigerator at approximately 6°C ± 5°C when not in use. Solutions should be allowed to warm to room temperature prior to use.

Note: All reusable glassware should be baked in a muffle oven at ~ 400°C for at least 2 hours to remove possible contamination before use.

3.2 Native, Non- Isotopically Labeled, Stock Standard Solutions

1. Transfer ~0.0100 g (corrected for purity) each of AE 0172747, AE0456148, and AE 1392936 into separate 100-mL volumetric flasks and dilute to volume with acetonitrile. Cap and mix by inversion. The concentration of these stock standards is ~100 µg/mL.

2. Transfer a 5 mL aliquot of each of the 100 µg/ AE 0172747, AE 0456148, and AE 1392936 stock solutions into a 100 mL volumetric flask. Dilute to 100 mL with acetonitrile. The concentration of this solution is 5 µg/mL.

3. Pipet 1 mL of the 5 µg/mL solution into a 100 mL volumetric flask. Fill to volume with acetonitrile. The concentration of this solution is 50 ng/mL.

4. Pipet 0.5 mL of the 5 µg/mL solution into a 100 mL volumetric flask and fill to volume with acetonitrile. The concentration of this solution is 25 ng/mL.

5. Pipet 10 mL of the 50 ng/mL solution into a 50 mL volumetric flask and fill to volume with acetonitrile. The concentration of this solution is 10 ng/mL .

6. Pipet 10 mL of the 50 ng/mL solution into a 100 mL volumetric flask and fill to volume with acetonitrile. The concentration of this solution is 5 ng/mL.

7. Pipet 1 mL of the 50 ng/mL solution into a 50 mL volumetric flask and fill to volume with acetonitrile. The concentration of this solution is 1 ng/mL.

3.3 Fortification Solutions

1. Use the 5.0 ng/mL mixed native solution prepared in Step 3.2.6 to spike water at the target LOQ of 0.05 ng/mL. A 10 mL sample is fortified to 0.05 ng/mL by the addition of 100 µl of the 5.0 ng/mL solution.

2. Use the 25.0 ng/mL mixed native solution prepared in Step 3.2.4 to spike water at 5X the target LOQ (0.25 ng/mL.) A 10 mL sample is fortified to 0.25 ng/mL by the addition of 100 µl of the 25.0 ng/ solution.

3.4 Labeled Internal Standards

1. Weigh ~0.01000 g each of AE 0172747-d_4, AE0456148-d_4, and AE 1392936-d_5 into separate 100 mL volumetric flasks and dilute to the marks with acetonitrile. Cap and mix by inversion. The concentration of these stock labeled standards is ~100 µg/mL.

2. Transfer 5 mL each of the ~100 µg/mL solutions to one 100 mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by

inversion. The concentration of this mixed labeled standard is ~5 µg/mL AE 0172747-d_4, AE0456148-d_4, and AE 1392936-d_5.

3. Transfer 1 mL of the ~5 µg/mL deuterated mixed standard to a 100 mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed labeled standard is ~.05 µg/mL AE 0172747-d_4, AE0456148-d_4, and AE 1392936-d_5.

3.5 Calibration Standards

1. Transfer 2 mL of the ~1.0 ng/mL *native* mixed standard solution (from Step 3.2.7) and 2 mL of the ~.05 µg/mL deuterated mixed standard solution (from Step 3.4.3) to a 100 mL volumetric flask. Dilute to volume with 10:90 acetonitrile: 0.1% acetic acid in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.02 ng/mL native mixed standard and ~1 ng/mL deuterated internal standard.

2. Transfer 5 mL of the ~1.0 ng/mL *native* mixed standard solution (from Step 3.2.7) and 2 mL of the ~.05 µg/mL deuterated mixed standard solution (from Step 3.4.3) to a 100 mL volumetric flask. Dilute to volume with 10:90 acetonitrile: 0.1% acetic acid in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.05 ng/mL native mixed standard and ~1 ng/ mL deuterated internal standard.

3. Transfer 1 mL of the ~10 ng/mL *native* mixed standard solution (from Step 3.2.5) and 2 mL of the ~.05 µg/ mL deuterated mixed standard solution (from Step 3.4.3) to a 100 mL volumetric flask. Dilute to volume with 10:90 acetonitrile: 0.1% acetic acid in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.10 ng/mL native mixed standard and ~1 ng/mL deuterated internal standard.

4. Transfer 2 mL of the ~10 ng/mL *native* mixed standard solution (from Step 3.2.5) and 2 mL of the ~.05 µg/mL deuterated mixed standard solution (from Step 3.4.3) to a 100 mL volumetric flask. Dilute to volume with 10:90 acetonitrile: 0.1% acetic acid in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.20 ng/mL native mixed standard and ~1 ng/mL deuterated internal standard.

5. Transfer 5 mL of the ~10 ng/mL *native* mixed standard solution (from Step 3.2.5) and 2 mL of the ~.05 µg/mL deuterated mixed standard solution (from Step 3.4.3) to a 100 mL volumetric flask. Dilute to volume with 10:90 acetonitrile: 0.1% acetic acid in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.50 ng/mL native mixed standard and ~1 ng/mL deuterated internal standard.

3.6 Stability of the Calibration Standard Solutions

The stock concentrate solutions in acetonitrile when stored in the dark in a freezer at ≤ −18 degrees centigrade should be stable for at least three months.

Fortification and calibration standard solutions are stable for a minimum of 3 months when stored in the dark at approximately 6°C or less.

4. METHOD PROCEDURE

Analysis of Water Samples by LC/MS/MS

1. Samples are brought to room temperature. Mix the sample completely before removing a sub-sample for analysis. Use a large volume auto-pipette and disposable pipette tip to remove and transfer a 10 mL sub-sample to a suitable container such as a disposable glass vial or plastic bottle.

2. Add 250 µL of formic acid to the 10 mL sample aliquot.

3. Add an appropriate volume of fortification solution to the LOQ and 5X LOQ control samples. For example, add 100 µL of a 5 ng/ mL fortification solution (from Step 3.2.6) to a 10 mL sample for LOQ and 100 µL of a 25 ng/mL fortification solution (from Step 3.2.4) to a 10 mL sample for 5X LOQ to give approximately 50 ppt and 250 ppt analyte concentrations respectively.

4. Add 200 µl of the 50 ng/mL deuterated internal standard (from step 3.4.3) to each sample. Cap the vial or bottle and shake to mix.

   Note: If the sample extract is too concentrated in any analyte for the calibration curves used, the extract will have to be diluted further.

5. Transfer approximately 1 to 1.5 mL of sample to an autosampler vial and cap. Sample is now ready for analysis by LC/MS/MS.

Analysis of Finished Drinking Waters (Tap Waters) Containing Free Chlorine

AE 0172747 degrades to its metabolite AE 0456148 in water containing free chlorine. This takes place over a fairly short period depending on the level of chlorine present in the water. (See Reference 2 in section 8 below) In order to

accurately detect AE 0172747 residues present in a chlorine treated water, these residues would have to be stabilized at the time of sampling the water. Stabilization of AE 0172747 residues can be achieved by adding sodium thiosulfate to the finished water sample at the time of collection. 10 ppm of sodium thiosulfate added to the water sample is sufficient to remove 2ppm of chlorine and stabilize AE 0172747 residues. For example, sample bottles that are used for collecting 100mL samples of treated water should contain 1 mL of a 1000 ppm solution of sodium thiosulfate. Addition of the sodium thiosulfate to the sample bottles may be done in the lab prior to transport to the water collection sites to prevent any potential contamination of the bottles in the field. The samples so treated are then analyzed as per the method for non-free chlorine containing waters as described above.

Tap water or HPLC water chlorinated in the lab may be used for a finished drinking water method recovery sample. Appropriate amounts, for example, 100µl of the 1000 ppm solution of sodium thiosulfate and 10mL of water, should be used, with the thiosufate being added *before* the water is spiked with a known amount of a fortification solution to give the desired level of fortification. See Solutions, Section 2.3 above for making free chlorine and thiosulfate solutions.

5 CHROMATOGRAPHIC SYSTEM

Acquisition Parameters

Instrument Used: Perkin Elmer Sciex API 4000 LC/MS/MS System with Valco Divert Valve
Interface: PE Sciex Turbo Ion Spray Electrospray

Scan Type:	MRM
Polarity:	Negative
Resolution Q1:	Low
Resolution Q3:	Low

Scan Type:	MRM
Polarity:	Negative
Resolution Q1:	Low
Resolution Q3:	Low

Analyte (4.58 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
AE 0172747	439.00	403.00	500	DP	-45.0	-45.0
				EP	-13.00	-13.00
				CE	-15.00	-15.00
				CXP	-15.00	-15.00

LC/MS/MS Parameters (con't)

Analyte (4.53 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
AE 0172747-d4	443.00	407.00	500	DP	-45.00	-45.00
				EP	-8.00	-8.00
				CE	-15.00	-15.00
				CXP	-16.00	-16.00

Analyte (5.44 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
AE 0456148	345.00	217.00	500	DP	-55.00	-55.00
				EP	-10.00	-10.00
				CE	-18.00	-18.00
				CXP	-13.00	-13.00

Analyte (5.43 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
AE 0456148-d_4	349.00	221.00	500	DP	-55.00	-55.00
				EP	-10.00	-10.00
				CE	-22.00	-22.00
				CXP	-9.00	-9.00

Analyte (4.63 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
AE 1392936	263.00	189.00	600	DP	-35.00	-35.00
				EP	-4.00	-4.00
				CE	-18.00	-18.00
				CXP	-11.00	-11.00

Analyte (4.63 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
AE 1392936-d_5	268.00	192.00	250	DP	-35.00	-35.00
				EP	-4.00	-4.00
				CE	-18.00	-18.00
				CXP	-11.00	-11.00

Parameter Table

NEB:	7.00 /min.
CUR:	10.00 /min.
IS:	-4200.0 volts
TEM:	500.00° C
CAD:	8.00 /min.

LC/MS/MS Parameters (con't)

Autosampler Used:	Gilson 215 Autosampler
Injection Volume:	90 µL
Pre-inject Flushes:	0
Post inject Flushes:	4
Air Cushion:	10 µL
Excess Volume:	5 µL
Sample Speed:	2.00 mL/min.
Inject Delay Time:	0.00 min.
Needle Z-Direction Speed	Very Fast
Inject Time Delay	0.0 min.
Needle Flush Volume:	250 µL
Flush Speed	5.00 mL/min.
Port Flush Volume	250 µL

Pump Used:	Shimadzu LC-10AVP (High Pressure Mixer)
Minimum Pressure:	0.0 psi
Maximum Pressure:	4300 psi
Column Temperature:	Ambient

Column: Use 2 columns in series

Manufacturer:	Waters
Type:	SymmetryShield
Phase:	RP8
Particle Size:	5 µ
Diameter:	2.1 mm
Length:	50 mm (total length is 100mm)
Pore Size:	100 Å
Mobile Phase A:	1.5% Acetic Acid in DI Water
Mobile Phase B:	Acetonitrile

Gradient Program:

Step	Total Time (min.)	Flow	A(%)	B(%)
0	0.00	200 µL/min.	90.0	10.0
1	0.50	200 µL/min.	90.0	10.0
2	2.50	200 µL/min.	5.0	95.0
3	5.00	200 µL/min.	5.0	95.0
4	5.01	200 µL/min.	90.0	10.0
5	7	Stop	------	-----

Divert Valve Program:

Step	Total Time (min.)	Divert Location
1	0.0 - 1.0	To Waste
2	1.0 - 7.0	To LC/MS
3	7.0 - End	To Waste

6 CALCULATIONS

Generate calibration curves for AE 0174747, AE 0456148, and AE 1392936. A minimum of four standards over a range of concentration levels should be included with a set of samples. To bracket samples with residues near the LOQ, the lowest standard run will be between the LOQ and LOD.

Standards should be interspersed with samples or bracket sample runs to compensate for any minor change in instrument response.

Linear regression coefficients should be calculated for the ratio of analyte to internal standard area or height plotted versus the ratio of analyte to internal standard concentration in the calibration standards. The data from the analytical standards should then be fit to the linear model,

$$y = A + Bx$$

$$x = \text{Conc. Ratio} = \frac{\text{conc.}}{\text{IS} \cdot \text{conc.}}$$ where IS = labeled internal standard

$$y = \text{response ratio} = \frac{\text{response} \cdot \langle\text{area}\rangle}{\text{IS} \cdot \text{response} \cdot \langle\text{area}\rangle}$$

The equation to be used to estimate the residues in the samples is:

$$E = \frac{(y - A)}{B} \times D \times f$$

where: E = concentration of analyte in sample in parts per billion (ppb or ng/mL)
y = ratio of analyte response (area or height) to internal standard response (area or height)
A = intercept from linear regression analysis
B = slope from linear regression analysis (area ratio per conc. ratio)

$$D = \text{ng/mL internal standard in the starting sample} = \frac{V \times c}{S}$$

V = volume in mL of internal standard solution added to sample
c = ng/ concentration of internal standard solution
S = volume of starting sample in mL.
f = dilution factor, if applicable

For a better estimation of any residues between the lowest standard and the limit of detection, the linear through zero regression may be used and high standards may be omitted from the regression, or only the lowest standard and zero may be used.

7 SAFETY

All available appropriate Material Safety Data Sheets should be available to the study personnel during the conduct of the method. General laboratory safety precautions should be taken.

8 REFERENCES

1. In House Laboratory Validation Of An Analytical Method for the Determination of Residues of AE 0172747 and its Metabolites AE 0456148, AE 0968400, AE 0941989, and AE 1392936 in Soil Using LC/MS/MS and GC/MSD, Bayer CropScience Study Number 03RAAEX022, D. Netzband, in press.

2. AE 0172747 Drinking Water Treatment Study, Bayer CropScience Study Number, MEAEX098, A. R. Dominic and E. L. Arthur, in press.

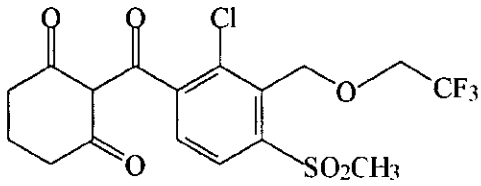
Appendix 1 Analytical Method Summary Parameters

Analyte(s)	AE 0172747, AE 0456148 and AE 1392936
Extraction solvent / Technique	Direct injection of water samples after addition of small amount of acetic acid to improve chromatographic separation. Thiosulfate may be added to tap waters to quench free chlorine if present.
Cleanup Strategies	None beyond HPLC separation with divert valve for early eluting matrix components.
Instrumentation and detection	Shimadzu LC-10AD VP HPLC pump with Gilson 215 Liquid handler and Gilson 819 Valve Actuator Applied Biosystems API 4000 MS/MS
Column	Waters SymmetryShield RP8, 2.1 x 100mm (two 50mm columns in series), 5µ
Standardization Method	Multi point calibration curve (Internal standard)
Stability of Standard Solutions	Stock standard solutions are stable for a minimum of 3 months when stored in the dark at ≤-18°C Fortification and calibration standard solutions are stable for a minimum of 3 months when stored in the dark at approximately 6°C or less.
Retention times	AE 0172747 (4.53 min), AE 0456148 (5.44 min) and AE 1392936(4.63 min)

Appendix 2 Structures of the Test Substances

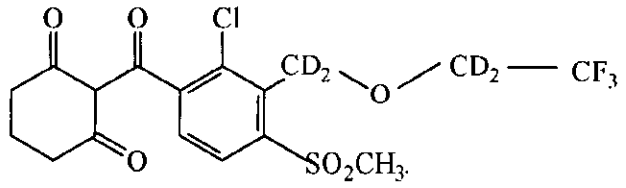
The structures for AE 0172747 and its metabolites AE 0456148 and AE 1392936 and their isotopically labeled analogs are presented below:

Code Name: **AE 0172747**
(Active Ingredient, Parent Molecule)



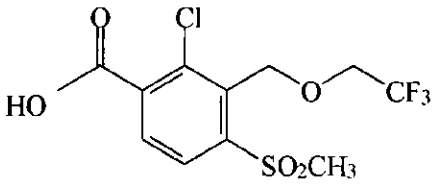
CAS Name:	2-[2-chloro-4-mesyl-3-((2,2,2-trifluroethoxy)methyl)benzoyl]cyclohexane-1,3-dione
Molecular Formula:	$C_{17}H_{16}O_6ClF_3S$
Molecular Weight:	440.82 g/mol
CAS Number:	335104-84-2

Code Name: **AE 0172747-*trifluoroethoxymethyl*-d_4**
(Parent Molecule, Deuterated Internal Standard)



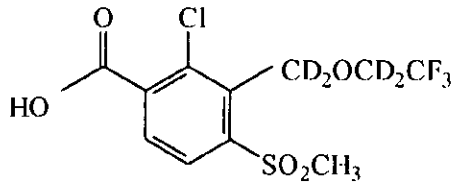
CAS Name:	2-[2-chloro-4-methylsulfonyl-3-[(2,2,2-trifluroethoxy-1,1-d_2)methyl-d_2]benzoyl]-1,3-cyclohexanedione
Molecular Formula:	$C_{17}H_{12}D_4O_6ClF_3S$
Molecular Weight:	444.90 g/mol

Code Name: **AE 0456148**
(Metabolite)



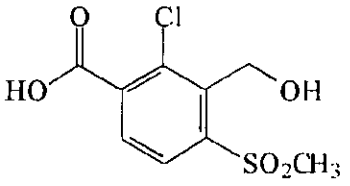
CAS Name:	2-[2-chloro-4-mesyl-3-[(2,2,2-trifluroethoxy)methyl]benzoic acid
Molecular Formula:	$C_{11}H_{10}O_5ClF_3S$
Molecular Weight:	346.71 g/mol
CAS Number:	120100-77-8

Code Name: **AE 0456148-*trifluoroethoxymethyl*-d_4**
(Metabolite, Deuterated Internal Standard)



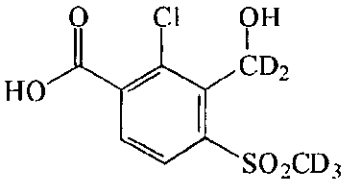
CAS Name:	2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluroethoxy-1,1-d_2)methyl- d_2]benzoic acid
Molecular Formula:	$C_{11}H_6D_4O_5ClF_3S$
Molecular Weight:	350.70 g/mol

Code Name: **AE 1392936**
(Metabolite)



CAS Name:	2-chloro-3-hydroxymethyl-4-mesylbenzoic acid
Molecular Formula:	$C_9H_9O_5ClS$
Molecular Weight:	264.69 g/mol
CAS Number:	120100-47-2

Code Name: **AE 1392936 -d_5**
(Metabolite, Deuterated Internal Standard)



CAS Name:	2-chloro-3-(hydroxymethyl-d_2)-4-(methyl-d_3-sulfonyl)benzoic acid
Molecular Formula:	$C_9H_4D_5O_5ClS$
Molecular Weight:	269.70 g/mol