1.0 ABSTRACT

The control soil samples were obtained from Dow AgroSciences LLC. They were not characterized separately for this study. Residues of DE-537 acid, FHPBA, DE-537 amide, and DE-537 diacid were extracted from fortified control soil with acidified acetone. A hydrolysis step followed (converting cyhalofop-butyl to cyhalofop-acid). After acidification the residues were extracted from the aqueous solution with a mixture of 40% methyl-t-butyl ether (MTBE) and 60% 1-chlorobutane. A silica gel solid phase extraction (SPE) procedure was then utilized to further prepare the extracts. Determination of the analyte concentration was achieved by high performance liquid chromatography with mass selective detection (HPLC/MSD). Results were calculated using linear and quadratic regression from external standard responses normalized by an internal standard.

In Trial 1 for DE-537 metabolites analysis, five samples of control soil were fortified with a mixture of all analytes at each of two levels. Samples were fortified at 10 ppb and 100 ppb levels of all analytes. Two control soil samples and a reagent blank were also analyzed. Recoveries were satisfactory at both the 10 ppb and 100 ppb fortification levels for the diacid metabolite. Recoveries for FHPBA were less than 70% at both levels. An interfering peak at the retention time of FHPBA was present in the control extracts. The acid and amide metabolites produced acceptable recoveries only at the 100 ppb level, both having low recoveries at the 10 ppb level. Recovery values are shown in Table 1 on page 23.

In Trial 2 the fortification and sample preparation parts of the investigation were conducted in the same manner as the first trial; the only difference was that a new lot of control soil was used. The MSD analysis portion of investigation utilized parameters that more closely adhered to the settings listed in the provided method. The mean recoveries were satisfactory at both the 10 ppb and 100 ppb fortification levels for the diacid, acid, and amide metabolites. Recoveries for FHPBA were less than 70% at both levels. The sample extracts were processed through the method for FHPBA a second time. Recoveries for FHPBA were again less than 70% at both levels. No FHPBA interference peak was seen in the control extracts. Recovery values are shown in Tables 3 and 5-8 on pages 25 and 27-30.

2.0 INTRODUCTION

The purpose of this study was to evaluate the test method GRM 99.08 for the determination of DE-537 metabolites in a representative sample of soil. The method was evaluated at ABC Laboratories, Inc., an independent laboratory, unassociated with the sponsor, and initially unfamiliar with the methods.

3.0 MATERIALS

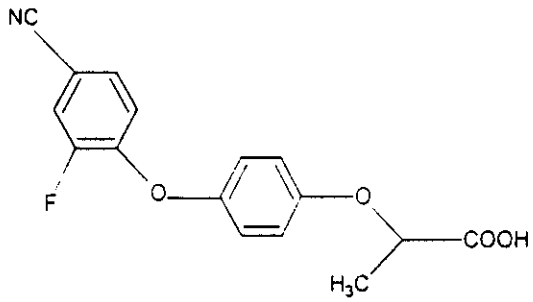
A. Test Materials

The test materials were received and stored as indicated below:

Test Material	Date Received	Storage Conditions
DE-537-Acid (ACID)	13 July 1999	Ambient
DE-537-Amide (AMIDE)	13 July 1999	Ambient
DE-537-Diacid (DIACID)	09 April 1999	Ambient
FHPBA	13 July 1999	Ambient
X460511 (Internal Standard)	13 July 1999	Ambient

A.1 DE-537-Acid (Acid):

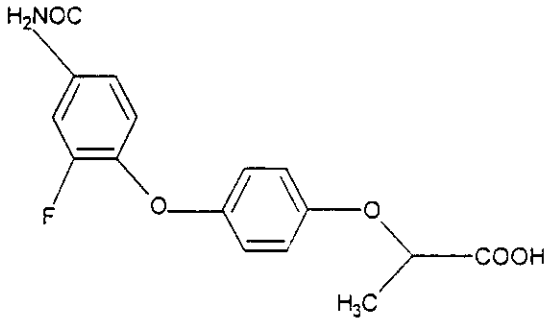
Alternate Names:	(R)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy] propanoic acid
Lot No:	ACPR 243-53
Purity:	99.4%, R+S
Expiration Date:	22 APR 2000
Source:	Dow AgroSciences LLC
Molecular Weight:	301.1
Molecular Formula:	$C_{16}H_{12}O_4FN$



DE-537-acid

A.2 DE-537-Amide (Amide):

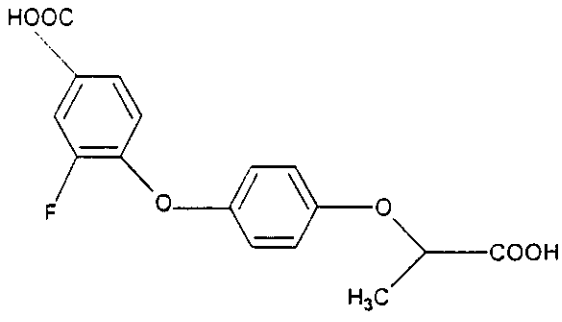
Alternate Names:	(R)-2-[4-(4-aminocarbonyl-2-fluorophenoxy) phenoxy]propanoic acid
Lot No:	E0432-44
Purity:	98% active ingredient
Expiration Date:	04 JUN 2000
Source:	Dow AgroSciences LLC
Molecular Weight:	319.1
Molecular Formula:	$C_{16}H_{14}O_5FN$



DE-537-amide

A.3 DE-537-Diacid (Diacid):

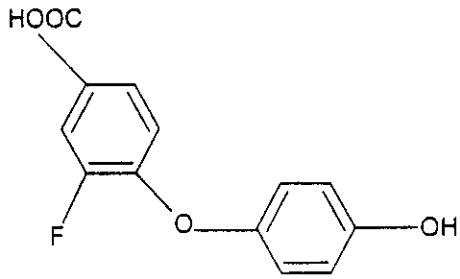
Alternate Names:	(R)-2-[4-(4-carboxy-2-fluorophenoxy)phenoxy] propanoic acid
Lot No:	ACPR 205-106
Purity:	98% active ingredient
Expiration Date:	03 APR 2000
Source:	Dow AgroSciences LLC
Molecular Weight:	320.1
Molecular Formula:	$C_{16}H_{13}O_6F$



DE-537-diacid

A.4 FHPBA:

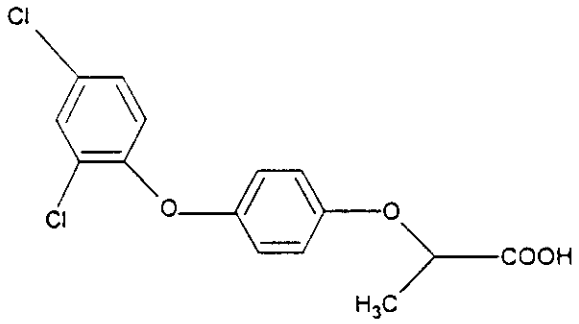
Alternate Names:	3-Fluoro-4-(4-hydroxyphenoxy)benzoic acid
Lot No:	F0449-35
Purity:	99% active ingredient
Expiration Date:	22 APR 2000
Source:	Dow AgroSciences LLC
Molecular Weight:	248.0
Molecular Formula:	$C_{13}H_9O_4F$



FHPBA

A.5 X460511 (Internal Standard):

Alternate Names:	None given
Lot No:	13404A1
Purity:	Assumed to be 100%
Expiration Date:	Not specified
Source:	Dow AgroSciences LLC
Molecular Weight:	326.1
Molecular Formula:	$C_{15}H_{12}C_2O_4$



ISTD. X460511

4.0 METHODS

A. Method

The analytical method and the protocol are provided in Appendix A. The method was performed as written without modification to extraction; however, chromatographic conditions were modified to optimize response and separation from interferences.

Sample calculations were performed in four ways, as a specific method of calculation was not presented in the analytical method. The sample concentrations were calculated from a linear standard curve ranging from 2.5 to 50 ng/mL, 2.5 to 500 ng/mL, and a quadratic standard curve ranging from 2.5 to 1000 ng/mL with and without weighting.

B. HPLC Chromatographic Conditions for Trial One

Instrument:	HP Series 1100
Auto Injector:	HP Series 1100, Material ID# 1625-980046G
Column Oven:	HP Series 1100, Material ID# 1625-980046F set at 30 °C
Pump:	HP Series 1100, Material ID# 1625-980046H
Column:	Zorbax C8 25 cm x 4.6 mm i.d. x 3.5 um P/N 866953-906, S/N USEB003730
Injection Volume:	50 µL
Detector:	HP Series 1100 MSD, Material ID# 1625-980046A
Mobile Phase:	A. 0.5% Acetic acid/water B. 0.5% Acetic acid/acetonitrile
Flow Rate:	0.5 mL/min.

Gradient Description:

Time (min.)	%A	%B
0.00	75	25
1.00	75	25
9.00	25	75
10.00	15	85
15.00	15	85
15.10	75	25
22.00	75	25

C. MSD Conditions for Trial One

Ionization Mode:	API-ES
Polarity:	Negative
SIM Ions:	203.0 and 247.0 for FHPBA, 228.0 and 300.0 for DE-537 Acid, 246.0 and 318.0 for DE-537 Amide, 247.0 and 319.0 for DE-537 Diacid, 325.0 for Internal Standard (X460511)
Gain:	3.0 EMV
Fragmentor:	80V
Gas Temperature:	350 °C
Drying Gas:	7.0 l/min.
Nebulizer Pressure:	25 psig
Vcap:	4250V

D. HPLC Chromatographic Conditions for Trial Two

Instrument:	HP Series 1100
Auto Injector:	HP Series 1100, Material ID# 1625-980046G
Column Oven:	HP Series 1100, Material ID# 1625-980046F set at 30 °C
Pump:	HP Series 1100, Material ID# 1625-980046H
Column:	Du Pont Zorbax C8 25 cm x 4.6 mm i.d. x 3.5 um P/N 866953-906, S/N USEB003730
Injection Volume:	50 µL
Detector:	HP Series 1100 MSD, Material ID# 1625-980046A
Mobile Phase:	A. 0.5% Acetic acid/water B. 0.5% Acetic acid/acetonitrile
Flow Rate:	1.0 mL/min.

Gradient Description:

Time (min.)	%A	%B
0.00	75	25
1.00	75	25
9.00	25	75
10.00	15	85
10.01	75	25
15.00	75	25

E. MSD Conditions for Trial Two:

Ionization Mode:	API-ES
Polarity:	Negative
SIM Ions:	203.0 and 247.0 for FHPBA, 228.0 and 300.0 for DE-537 Acid, 246.0 and 318.0 for DE-537 Amide, 247.0 and 319.0 for DE-537 Diacid, 325.0 for Internal Standard (X460511)
Gain:	10.0 EMV
Fragmentor:	80V
Gas Temperature:	350 °C
Drying Gas:	12.0 l/min.
Nebulizer Pressure:	50 psig
Vcap:	5700V

5.0 CALCULATIONS

A. Residue Calculation using Quadratic Regression

The formula to calculate residues of DE-537 metabolites listed below is based on the solution to the quadratic regression of the calibration curve. For the quadratic curve formula:

$$y = ax^2 + bx + c$$

where y = peak area ratio of analyte to internal standard,
x = concentration in ng/mL, and
a, b, and c are the coefficients from the quadratic regression of the calibration curve

Rearranging to solve for x:

$$x = \frac{-b + \sqrt{b^2 - 4a(c - y)}}{2a}$$

Then the concentration in ppb is as follows:

$$ppb = x\ ng/mL \times \frac{1.0\ mL\ final\ volume}{5.00\ g\ initial\ weight} \times \frac{40.0\ mL\ sample\ extract}{8.0\ mL\ aliquot} \times 50.5$$

where:

50.5 = the concentration of the internal standard. This factor was employed in the construction of the calibration curve, and thus also is required to calculate sample residues.

An example calculation for DE-537 acid analyte in sample 45518-038 follows:

$$995\ ppb = \frac{-b + \sqrt{b^2 - 4 \times a \times \left(c - \frac{478399}{234647}\right)}}{2 \times a} \times \frac{1.0\ mL}{5.00\ g} \times \frac{40.0\ mL}{8.0\ mL} \times 50.5$$

where a = -0.00664994
b = 1.04678
c = 0.00227077

B. <u>Residue Calculation using Linear Regression</u>

The formula to calculate residues of DE-537 metabolites listed below is based on the solution to the linear regression of the calibration curve.

if $y = mx + b$

where y = peak area ratio of analyte to internal standard, and
m = slope
x = concentration in µg/mL
b = y intercept

$$x = \frac{y - b}{m}$$

Then the concentration in ppb is as follows:

$$ppb = x\ ng/mL \times \frac{1.0\ mL\ final\ volume}{5.00\ g\ initial\ weight} \times \frac{40.0\ mL\ sample\ extract}{8.0\ mL\ aliquot}$$

An example calculation for FHPBA analyte in sample 45518-038 follows:

$$2.6\ ppb = \frac{\left(\frac{177708}{234647}\right) - 0.09930}{0.02525} \times \frac{1.0\ mL}{5.00\ g} \times \frac{40.0\ mL}{8.0\ mL}$$

7.0 COMMUNICATIONS

Prior to the first trials initiation several questions were asked, via telephone, about method specifics for Dow AgroSciences LLC Method GRM 99.08, "Determination of DE-537 Metabolites in Soil.". These questions were permissible under the relevant US guidelines (PR96-1), which allow discussions of the method prior to running the first trial. The items discussed dealt with the type of vial listed in the method and HPLC/MSD optimization. It was also decided that the protocol would be written to specify a 10 ppb LOQ. Also an e-mail was sent by the sponsor specifying the method number (GRM 99.08) and providing a fuller title "Determination of Residues of Cyhalofop-butyl and Metabolites in Sediment and Soil by Liquid Chromatography with Mass Spectrometry Detection".

On August 11, 1999, results of the first trial were discussed with the sponsor, Dow AgroSciences. Because of some unacceptably low recoveries, it was decided to re-profile the silica SPE cartridge to verify that 10 mL of 99% ethyl acetate/1% acetic acid was sufficient to elute all four analytes. Also, it was agreed that a different batch of control soil would be evaluated since a high FHPBA background was observed in the control for each ion monitored. SPE cartridges were shipped to Dow AgroSciences, where the profile verification and new control soil evaluation were performed. Dow AgroSciences verified acceptable silica SPE performance using the conditions specified in the method. They determined that the new control soil was acceptable for use and shipped it to ABC Laboratories for receipt on September 30, 1999.

During a conference call on October 1, 1999 (prior to Trial #2), the sponsor suggested that a standard and a control soil extract from Trial #1 be injected using the exact LC-MSD conditions provided in the method. ABC personnel had previously been hesitant to use the voltage and gain settings specified in the method because of uncertainty concerning the effect of what were perceived as excessively high settings on the MSD. However, the sponsor indicated that in their laboratory these high settings had been employed for 6 months with no deleterious effects observed on their MSD. The sponsor requested that a standard and control extract from Trial #1 be injected under the specific method conditions. This was done on October 11, 1999. In addition to the standard and control soil extract from Trial #1, a fortified sample from Trial #1 was injected as well. Under these conditions, the apparent response of FHPBA in the control was minimal compared to that seen under the alternate MSD conditions used by ABC Laboratories for Trial #1. Based upon comparison with the single standard injection made on October 11, recoveries seen in the single fortified sample injected on that date were 70% or greater for all analytes, with the exception of 26% recovery for FHPBA. These results were discussed with Ed Olberding of Dow AgroSciences on October 22, 1999. Mr. Olberding indicated that the low FHPBA result could be a result of degradation occurring in the stored fortified soil extract. It was proposed that a second trial be performed, using the method-specified MSD conditions for analysis. Mr. Olberding agreed with this proposal.

Following the second trial, Mr. Olberding was contacted by telephone on December 8, 1999. Results were generally acceptable, except for FHPBA. Recoveries for FHPBA averaged 25% at the 100 ppb fortification level and were even lower at the 10 ppb level. It was agreed that the 10 ppb results would be recalculated using a curve with a smaller range, since poor back-calculated values were being observed for the low-end standards using the full-range calibration curve. Upon discussion with Mr. Olberding, it was speculated that temporary storage of the soil extracts in acetone:formic acid solution could be the source of the low FHPBA recoveries. It was decided to reprocess a second aliquot of the retained extracts through the method and to analyze them for FHPBA only. Mr. Olberding suggested that a more appropriate stopping point in the method would be at the step that the extracts are in chlorobutane. Following this re-processing, the sponsor was contacted via a telephone message left on December 14, 1999 and an e-mail sent on December 16, 1999 to let him know that the re-processed samples gave recoveries that were even lower than the first aliquots that were stored in the acetone:formic acid. It was proposed to the sponsor that the fortification solutions could be assayed for purity to see if they had degraded in terms of FHPBA content and/or that fresh FHPBA fortification solutions could be made and a third trial initiated for FHPBA. Alternatively, the study could be terminated and the FHPBA results already generated could be reported, based upon the sponsor's previous indication that FHPBA is a relatively unimportant soil metabolite. During a telephone conversation on December 17, 1999, Mr. Olberding indicated that ABC should terminate the study.

Written logs of communications with the sponsor are provided in Appendix B.

To aid in the complete execution of the method for the determination of DE-537 metabolites in soil, the independent laboratory recommends adding specifications regarding what type of calibration (quadratic regression with linear amount weighting, i.e., 1/x-weighting, or others) is to be used, along with formulae to calculate residue values and recovery values. It is also recommended that the LC-MSD section of the method describe the expected retention times and ions to be acquired for each compound. An explanation of why a hydrolysis step (converting cyhalofop-butyl to cyhalofop-acid) was included in the method would also be useful.

10.0 REFERENCES

1. Analytical Method Analytical Method GRM 99.08, "Determination of Residues of Cyhalofop-butyl metabolites in Sediment and Soil by Liquid Chromatography with Mass Spectrometry Detection." The version provided to ABC Laboratories was Method GRM 99.08, "Determination of DE-537 Metabolites in Soil."