US EPA ARCHIVE DOCUMENT

ICI AMERICAS INC.
Agricultural Chemicals Division
Wilmington, Delaware 19897

401413-22

COPY 1

VOLUME 22

Study Title

The Determination of Residues of Tefluthrin in Crops and Soil - A Gas-Liquid Chromatographic Method

Data Requirement

Guideline Ref. 171-4

Author

A. Sapiets

Study Completed On

November 28, 1986

Performing Laboratory

ICI Plant Protection Division
Jealott's Hill Research Station

Laboratory Project ID

Residue Analytical Method No. 85/1

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

[X] No claim of confidentiality under FIFRA Section 10(d)(1)(A), (B), or (C)

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C).

Company: ICI AMERICAS INC.

Company Agent: James M. Wagner Date: 3/6/87

Manager, EPA Registration
Title

James M Wagner
Signature

GOOD LABORATORY PRACTICE STATEMENT

[X] This document is not subject to the requirements of 40 CFR Part 160.

Submitter ICI AMERICAS INC.

Sponsor ICI AMERICAS INC.

Jealott's Hill Research Station
Bracknell Berkshire
RG12 6EY

Telephone Bracknell (0344) 424701
Telex 847556
Patents Telex 848495
Cables & Telegrams Plantector Bracknell Telex

Imperial
Chemical
Industries
PLC

Plant
Protection
Division

PLANT PROTECTION DIVISION RESIDUE ANALYTICAL METHOD NO. 85/1

THE DETERMINATION OF RESIDUES OF TEFLUTHRIN IN CROPS AND SOIL -

a gas-liquid chromatographic method

Author : A Sapiets

Study Director : A Sapiets

Date of Issue : 28 November 86

CONTENTS

1	SCOPE	1
2	SUMMARY	2
3	PROCEDURE	2
3.1	Sample Preparation	2
3.2	Extraction	2
3.3	Liquid-liquid partition Chromatography	3
3.4	Adsorption Column Chromatography	4
4	GAS LIQUID CHROMATOGRAPHY (GLC)	4
4.1	Packed Column Chromatography	4
4.2	Capillary Column Chromatography	5
4.3	Calculation of PP993 Residue Results	5
5	CONTROL AND RECOVERY EXPERIMENTS	6
6	LIMIT OF DETERMINATION	6
7	ESTIMATION OF THE PRECISION OF THE METHOD	6
8	METHOD VALIDATION STUDIES	7
9	EXAMPLES OF TYPICAL GAS CHROMATOGRAMS - see Appendix 6	7
10	CONFIRMATION OF RESIDUES OF PP993	7
11	REFERENCES	8

APPENDICES

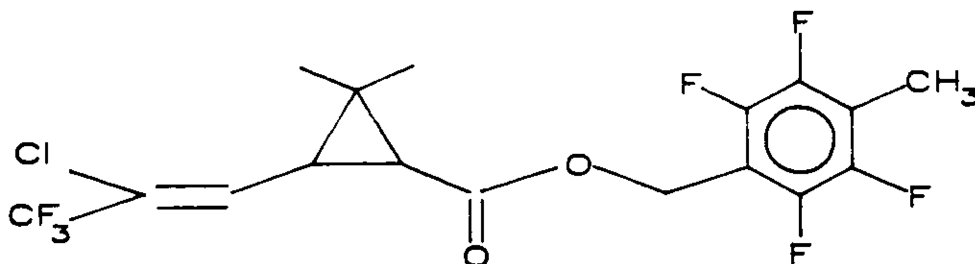
1. Apparatus
2. Reagents
3. Hazards
4. Preparation of analytical standards
5. Preparation of columns for gas liquid chromatography
6. Typical gas chromatograms for PP993 residue determination in crops
7. Typical calibration curve for PP993 residue determination in crops and soil

1 SCOPE

The analytical procedures described are suitable for the determination of residues of the insecticide tefluthrin (PP993 (I)) in crops and soil.

Although this method uses an internal standard it is equally valid to use external standardisation and fortified untreated samples to measure the efficiency of the analytical procedure. Examples of the typical range for external standard recoveries are given in Table 1.

To date, in these laboratories, the method has been applied to maize (plant, cobs, kernels), millet, oil seed rape, potatoes, soya, sugar beet, tobacco, wheat and soil samples. The limit of determination of the method is 0.005-0.01 mg kg^{-1}.



I. 2,3,5,6-tetrafluoro-4-methylbenzyl(1RS)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate.

2 SUMMARY

Samples which have been accurately fortified with an internal standard are extracted by homogenisation with acetone: hexane (crops) or reflux with acetonitrile (soil). The extracts are subjected to adsorption-chromatography on Florisil to remove interfering co-extractives. Final quantitative determination is by gas-liquid chromatography using electron capture detection.

3 PROCEDURE

3.1 Sample Preparation

Soil samples should be allowed to thaw overnight in the cold room (0°C) so they can be thoroughly mixed prior to sub sampling. All the stones and plant material should be removed and the total weight noted before and after preparation. Normally, the moisture content of the soil should be assessed. Crops should be homogenised in a suitable mincer until a representative sub sample can be taken. Frozen samples should be allowed to thaw for the minimum time before analysis to prevent partition of the endogenous water content.

3.2 Extraction

3.2.1 Soil

a) Thoroughly mix the prepared sample and weigh a representative aliquot (50g) into a round bottomed flask.

b) Fortify all samples with a suitable known amount of internal standard (R152379 - see Appendix 2 (i)).

c) Reflux for one hour with acetonitrile (100 cm^3) then allow to settle.

d) Pipette an aliquot (10 cm^3 = 5g soil) into a separating funnel and add glass distilled water (30cm^3). Partition into hexane (3 x 10cm^3) and combine the hexane extracts.

e) Dry the hexane by shaking with anhydrous sodium sulphate, transfer to a round bottomed flask and concentrate on a rotary evaporator keeping the temperature of the sample below 25°C to avoid losses due to volatility.

3.2.2 Crops

a) Thoroughly mix the prepared sample and weigh a representative aliquot (20g) into a centrifuge bottle.

b) Fortify all samples with a suitable known amount of internal standard (R152379 - see Appendix 2 (i)).

c) Homogenise for five minutes in 1:1 acetone:hexane (60 cm^3) and allow to settle.

d) Pipette an aliquot (9 cm^3 equivalent to 3g crop) into a separating funnel and shake with glass distilled water (2x10cm^3) to remove acetone. Discard the lower aqueous phase after each partition.

e) Dry the hexane layer by shaking with anhydrous sodium sulphate and transfer an aliquot equivalent to 1g crop (ie 1.5 cm^3) to a tube.

Crops with a high lipid content or those containing interfering plant co-extractives (eg tobacco) should be subjected to partition chromatography prior to adsorption chromatography. For all other samples continue to section 3.4.

3.3 Liquid - Liquid Partition Chromatography

Note - Throughout this section <u>hexane</u> refers to hexane equilibrated with acetonitrile and <u>acetonitrile</u> refers to acetonitrile equilibrated with hexane.

a) Prepare a liquid-liquid partition column by adding Florisil (7g) to a chromatography column (300 mm x 18 mm ID) containing **acetonitrile**.

b) Allow the **acetonitrile** to run through the column until level with the packing bed then add **hexane** (10 cm^3) and allow it to pass through the column. Do not let the column dry out at this stage.

c) Pipette an aliquot of the sample (equivalent to 1g crop) onto the column and run the solvent down to the level of the packing bed.

d) Wash the column with **hexane** (10 cm^3) and discard the washings.

e) Elute PP993 and the internal standard with 25% diethyl ether/hexane (15 cm^3) and collect the eluate in a round bottomed flask.

f) Evaporate to dryness on a rotary evaporator.

Do not place the flask in a water bath heated above 25°C as this may result in loss of PP993 through volatility.

g) Dissolve the residue in the flask in hexane (2 cm^3) and transfer to a Florisil adsorption column. (Section 3.4).

3.4 Adsorption Chromatography

a) Place a small glass wool plug in the bottom of a 10 mm diameter chromatography column and add hexane. Slowly, with gentle tapping, add Florisil (1g). Allow the hexane to percolate onto the column.

Note - Prior to use, each fresh batch of Florisil must be calibrated as follows:- Fortify a control sample extract with a mixed PP993/internal standard solution in hexane such that the concentration of each is 1 µg cm^{-3}. Load the sample onto the top of the column and elute with 10% diethyl ether:hexane and collect six fractions (5 cm^3 each) of the eluate. Analyse the fractions by GLC to determine determine the elution pattern.

b) Transfer the extract (from 3.2 or 3.3 above) and allow it to percolate onto the column. Elute the compounds of interest using the procedure determined from the column calibration. Collect the required diethyl ether:hexane fraction and evaporate to a small volume on a rotary evaporator (do not place the flask in a water bath heated above 25°C as this may result in loss of PP993 through volatility).

c) Transfer to a graduated centrifuge tube, rinsing the flask with further hexane, and combine the rinsings.

d) Reduce the volume of the collected fraction to 1 cm^3 using a stream of clean, dry air (or nitrogen). **Keep the sample temperature under 10°C.**

4 GAS-LIQUID CHROMATOGRAPHY (GLC)

The conditions for the analysis by GLC will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe and optimum use. The following conditions have been found to be satisfactory using a Hewlett Packard 5710A series instrument with an electron capture detector.

4.1 Packed Column Chromatography

Column	: 2% SP 2330 on Chromosorb W-HP (100-120 mesh). 1.2m x 2mm i.d glass.
Oven temperature	: 160°C.
Injector temperature	: 200°C.
Detector temperature	: 300°C.
Carrier gas	: Argon:Methane (95:5) at 50 cm^3 min^{-1}.

Under the above conditions PP993 gives a single peak, retention time 2.2 minutes, and the internal standard give a single peak, retention time 3.9 minutes. Sensitivity is such that 500 x 10^{-12}g PP993 injected on-column, with electrometer attenuation at x 32 and potentiometric recorder range on 1mV, gives approximately 70% full scale deflection.

4.2 **Capillary Column Chromatography**

a) OV 101 fused silica bonded phase capillary column 25 meters x 0.32 mm internal diameter.

b) Grob type splitless injector, 40 seconds purge delay.

c) Oven temperatures 47°C (hold for 2 minutes) program at 10°C min^{-1} to 165°C (hold for 12 minutes).

d) Injector 200°C; detector 300°C carrier gas : helium at 2 cm^3 min^{-1}.

Under the above conditions PP993 gives a single peak at retention time 15.4 minutes. R152379 has a retention time of 18.9 minutes. Sensitivity is such that, 10×10^{-12}g PP993 injected on column, with electrometer attenuation at x16 and recorder range on 1 mv, gives approximately 40% full scale deflection.

4.3 **Calculation of PP993 Residue Results**

Note - the internal standardisation procedure determines the concentration of the PP993 residue in the final extract relative to that of a known concentration of R152379 which is added by accurate fortification of the sample prior to extraction. Correction for percentage recovery throughout the procedure is thereby inherent for each individual sample; in addition, any small volume errors, particularly those associated with the final GLC injected solution are similarly corrected.

The calculation used for the determination of PP993 residues by internal standardisation using R152379 may be performed using a 'single point ratio calibration' (Ref. 1). It should be noted that such calibrations are only feasible when the internal standard chosen meets certain criteria (see Ref. 1 and Section 8).

a) Make repeated injections of 2-5µl of a standard solution containing a mixture of PP993 and R152379 at 0.1µg cm^{-3} and 0.2 µg cm^{-3} respectively into the GLC operated under conditions described in Section 4. When a consistent response is obtained measure the peak heights/areas obtained for PP993 and R152379 and calculate the PP993/R152379 peak ratio.

b) Make an injection of each sample solution and measure the peak heights/areas of the peaks corresponding to PP993 and R152379 and similarly calculate the peak ratios.

c) Re-inject the standard solution after a maximum of six injections of sample solutions.

d) Calculate the PP993 residue in the sample, expressed as mg kg^{-1}, by proportionation of the PP993/R152379 peak height or peak area ratio measured for the sample against that for the analytical standard solution.

* 0.2µg cm^{-3} internal standard solutions are used when the samples have been initially fortified at 0.2 mg kg^{-1}, and the final substrate to solvent ratio is 1.0g cm^{-3}.

eg,

$$\text{PP993 residue (mg kg}^{-1}) = \frac{\text{peak height ratio in sample}}{\text{peak height ratio in standard}} \times \frac{\text{concentration of PP993 in standard}}{\text{concentration of R152379 in standard}} \times \text{internal standard fortification level}$$

the units are commonly

$$\frac{\text{mm x mm}^{-1}}{\text{mm x mm}^{-1}} \times \frac{\mu\text{g cm}^{-3}}{\mu\text{g cm}^{-3}} \times \mu\text{g g}^{-1} = \mu\text{g g}^{-1} = \text{mg kg}^{-1}$$

Note - in the case where laboratory data systems/computing integrators are used the computer alogarithm may adopt a slightly different method for calculation of results. For example, a laboratory data system may use the relative detector response factor calculated from an analytical standard solution as the basis for calculation of results. The final calculated result is, of course, the same as the above manual calculation.

5 CONTROL AND RECOVERY EXPERIMENTS

At least one untreated sample must be analysed alongside any set of samples, using exactly the same method. This ensures that no contamination of the samples occurred prior to, or during, the analysis.

The amount of internal standard to be added should be decided by the residue levels expected in the sample. When low residues are expected, then the amount should not exceed 0.1 mg kg^{-1}.

For quantitation using external recoveries at least two control samples, accurately fortified with a suitable known amount of tefluthrin, should be analysed alongside every batch of treated samples. The percentage recovery of tefluthrin should be used to correct the residue found in the treated samples for the analytical efficiency.

e.g For a 80% recovery

$$\text{Corrected residue} = \text{Measured residue} \times \frac{100}{80}$$

Residues should not be corrected for recoveries greater than 100%

6 LIMIT OF DETERMINATION

The limit of determination of residues of PP993 can be assessed by carrying out recovery experiments at low levels of fortification (0.005-0.02mg kg^{-1}). The true limit of determination will give a final chromatographic response of at least 4 x the background noise at the retention time of PP993 and precision of reproducibility of better than ± 5%. In these laboratories the limit of determination has been set at 0.005-0.01 mg kg^{-1} depending upon the substate analysed.

7 ESTIMATION OF THE PRECISION OF THE METHOD

The use of internal standardisation ensures that small inter sample differences in percentage recovery of PP993 throughout the method are corrected. Hence the expected precision is significantly better than that expected for external standard methodology. Recovery experiments carried out on a range of crops gave a mean recovery of 102% of theoretical with standard deviation of 13% when calculated against an external standard (see table 1). Calculation of the same results by internal standardisation gave a standard mean recovery of 101% of theoretical with a standard deviation of 7%.

TABLE 1 : PP993 External Recovery Data from Various Crops.

Crop	PP993 Fortification Level (mg/kg)								
	0.01	0.025	0.05	0.07	0.1	0.2	0.3	0.4	0.5
Root Vegetables					93,108,124 115,94,103				
Leafy Vegetables					96,103				
Cereal grains			107		113,82,73,95 108,87,100,105 91,93,112	116			
Fodders and straws	127 111		84 101		127,105,102 125,92,99,104 102,90,108,110 103,108,117	85,105 115			
Oilseed rape					75				
Soil		113 101	112 98	94 111	126,81 85	76,101	98 106	95 94	88 90
Mean	119	107	100	103	102	100	102	95	89
Standard Deviation	8	6	10	9	14	15	4	1	1

8 METHOD VALIDATION STUDIES

In these laboratories to date the method has been applied to the analysis of the following substrates: soils, maize (whole plant, cobs and kernels), millet, oil seed rape, potatoes, soya, sugar beet, tobacco and wheat. No endogenous materials from these crops have been observed to interfere with either PP993 or the internal standard during the final chromatographic determination step.

The validity of the internal standardisation procedure has been demonstrated by plotting calibration graphs for PP993/internal standard peak ratios against the residue concentration of PP993 in accurately fortified samples. the resultant plot is always a straight line (correlation coefficient, $r>0.99$) with the intercept at zero, see Appendix 7. The percentage recovery of PP993 and the internal standard throughout The procedure has been shown to be essentially identical and therefore the slope of the extracted calibration line is found to be virtually identical to that obtained for reference standard mixtures of PP993 and internal standard. Hence R152379 is shown to be a 'true' internal standard for the measurement of PP993 and consequently, 'single point ratio' calculations may be used instead of graphical interpolation for day to day assays.

9 EXAMPLES OF CHROMATOGRAPHIC TRACES - see Appendix 6

10 CONFIRMATION OF RESIDUES OF PP993

Combined gas chromatography-mass spectrometry (GCMS) operated in the selected ion monitoring (SIM) mode may be used for the qualitative and quantitative confirmation of PP993 residues down to levels at the limit of determination ie, 0.01 mg kg^{-1}. Samples obtained from the residue analytical method are examined by SIM i.e. three or more of the most abundant m/z values present in the mass spectrum are continuously monitored throughout the gas chromatographic run and recorded using a multi-channel pen recorder. Qualitative confirmation of residues is given by the appearance of a peak at the correct gas chromatographic retention time for all the specific m/z values monitored. In addition, the ratios between the peak height responses given for each m/z value should be identical to that given by a standard solution of PP993.

Quantitative confirmation of PP993 residues is carried out by comparison of the peak height measured for the most abundant m/z value recorded, against that for an external standard.

The selectivity of the technique is such that high sample to solvent ratios eg 20 g cm^{-3} may be injected into the instrument.

11 REFERENCES

1 Cardone M J and Palermo P J : Potential Error in single point ratio calculations based on linear curves with a significant intercept. Anal. Chem., 52, pp 1187-1191, 1980.

APPENDIX

1 **APPARATUS**

a) Equipment for the initial preparation of samples, eg, Hobart food chopper.

b) High speed homogeniser, eg, Silverson Homogeniser; available from Silverson Machines Limited, Chesham, Bucks.

c) Glass columns, 300 mm x 10 mm internal diameter and 300 mm x 18 mm ID for column chromatography.

d) Graduated glass centrifuge tubes of 10cm^3 capacity calibrated down to 1.0cm^3 in 0.1cm^3 units, with an accuracy of at least ± 1% measured at 10 cm^3.

e) Gas-liquid chromatograph or capillary gas chromatograph fitted with an electron capture detector.

f) Syringes for gas-liquid chromatography.

Note: the use of an autosampler apparatus with GLC equipment is satisfactory provided (a) suitably precise injections are achieved, ie, reproducibility better than ± 5%, (b) no cross-contamination from consecutive injections is observed, and (c) that no contamination arises in the final sample due to the autosampler vials or vial caps.

g) Potentiometric pen recorder (1mV), eg, Perkin Elmer 56 or equivalent instrument.

Note - An electronic integrator for measurement of peak areas, can be used (in addition to the chromatographic trace of of the pen recorder) provided that the analyst is satisfied that the area response given is both accurate and precise.

2 REAGENTS

a) Solvents: Redistilled acetone, acetonitrile, diethyl ether and hexane. Particular care must be taken to avoid contact with materials, eg, plastics, which may contaminate the solvents.

b) Granular anhydrous sodium sulphate - Analar grade, BDH Chemicals Ltd., Poole, UK. Heated in an oven at 140°C for 24 hours to remove volatile contaminants.

c) Glass wool - Contaminants are removed by treatment of the glass wool in a Soxhlett apparatus with refluxing n-hexane (redistilled) for 2 hours.

d) Presilanised glass wool (for GLC Columns) - obtainable from chromatography suppliers.

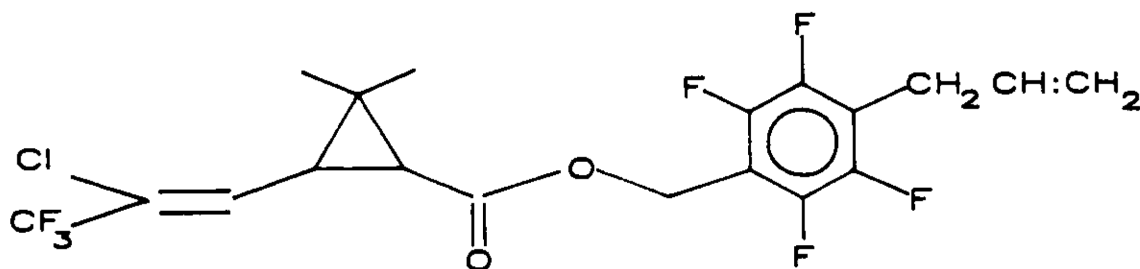
e) Florisil (100-120 US mesh) for chromatographic use available from BDH Ltd., Poole, UK.

f) Stationary phases for gas-liquid chromatography, a semi-polar cyanopropylmethyl silicone oil, SP2330 available from chromatography suppliers.

g) Gases for gas liquid chromatography - helium and argon:methane (95:5), dried by passing through molecular sieve.

h) A sample of PP993 of known purity.

i) A sample of R152379 of known purity for use as internal standard.



R152379 4-allyl-2,3,5,6-tetrafluorobenzyl-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate.

Note - while all the reagents and apparatus may be individually checked for purity, it is necessary to analyse reagent blank samples, where the complete procedure has been carried out in the absence of crops. This will enable the analyst to verify whether the system produces a GLC trace which is free of interference at the retention times of PP993 and the internal standard.

3 HAZARDS

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in doubt, consult the appropriate safety manual (eg, ICI Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and a monograph such as 'Hazards in the Chemical Laboratory', Ed G D Muir, The Chemical Society, London.

(a) Solvent Hazards

	Acetone	Acetonitrile	Dichloromethane	Diethyl ether	Hexane
Harmful vapour	✓	✓	✓	✓	✓
Highly flammable	✓	✓	-	✓	✓
Harmful by skin absorption	-	✓		-	-
TLV mg m^{-3}	2400	70	360	1200	180

In all cases avoid breathing vapour. Avoid contact with eyes/skin.

(b) PP993 and R152379 are synthetic pyrethroid insecticides with a mammalian toxicity (acute oral LD_{50}) in the rat in the order of 25-50 mg kg^{-1} (PP993).

4 PREPARATION OF ANALYTICAL STANDARDS

Weigh out accurately, using a five figure balance, sufficient PP993 or R152379 to allow dilution in acetonitrile to give a 1000 µg cm^{-3} stock solution in a volumetric flask. Make serial dilutions of this stock to give 100 µg cm^{-3}, 10 µg cm^{-3} and 1.0 µg cm^{-3} standard solutions in acetonitrile (used for fortification of crop samples). Prepare serial dilutions of mixed standards of PP993 and R152379 in hexane (for use as GLC reference standards) down to 0.1 µg cm^{-3} or less as required.

When not in use, always store the standard solutions in a refrigerator at <4°C to prevent decomposition/evaporation/concentration. Analytical standards should be replaced with freshly prepared standards after 3 months of use.

5 PREPARATION OF COLUMNS FOR GAS-LIQUID CHROMATOGRAPHY

Stationary phases may be obtained from most chromatography suppliers precoated onto the support phase at the required loading. However, preparation of the required column packing may be performed in the laboratory by the following method: Dissolve 0.2g SP2330 in acetone (100 cm^3) and add to 9.8g Chromosorb W-HP (100-120 mesh). Gently stir the mixture with a glass rod to ensure thorough mixing.

Remove the solvent under a vacuum on a rotary evaporator until a dry, free-flowing powder is obtained then place on a petri-dish in an oven (100°C) for 2 hours. Columns may be packed under vacuum or under pressure from a gas cylinder, gentle vibration of the column during this process will ensure uniform packing. Condition the packed column by heating at 250°C for 24 hours with the carrier gas flow rate at 30 cm^3 min^{-1} leaving the detector end of the column disconnected to avoid contamination of the detector.

Ref : AS/CL
Disc No : 256
PPRAM No : 85_1
Date : July 1986

APPENDIX 6

Typical gas chromatograms for PP993 residue determination in crops and soil

1. Packed column chromatography

Figure 1 : 0.01 ug cm^{-3} PP993 and 0.02 ug cm^{-3} internal standard

Figure 2 : Untreated sample at 0.1 g cm^{-3}

Figure 3 : Treated soil sample at 0.1 g cm^{-3}
Residue = 0.03 mg kg^{-1}

2. Capillary column chromatography

Figure 1 : 0.05 ug cm^{-3} PP993 and 0.1 ug cm^{-3} internal standard

Figure 2 : Untreated sample at 1 g cm^{-3}

Figure 3 : Treated maize sample at 1 g cm^{-3}
Residue = 0.01 mg kg^{-1}

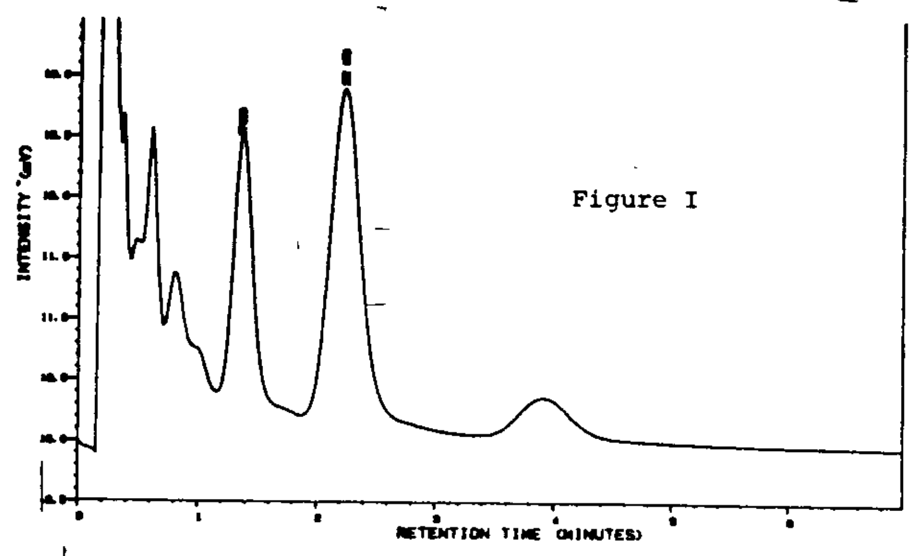


Figure I
INTENSITY (mV)
RETENTION TIME (MINUTES)

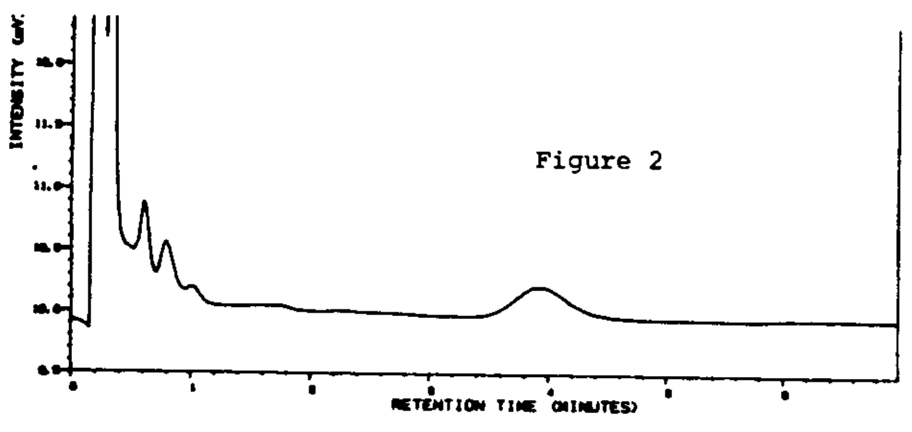


Figure 2
INTENSITY (mV)
RETENTION TIME (MINUTES)

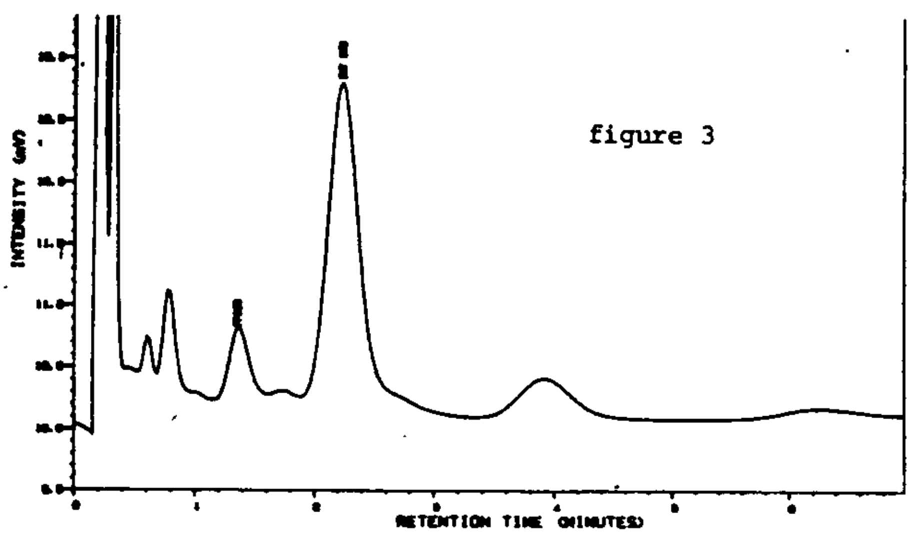


figure 3
INTENSITY (mV)
RETENTION TIME (MINUTES)

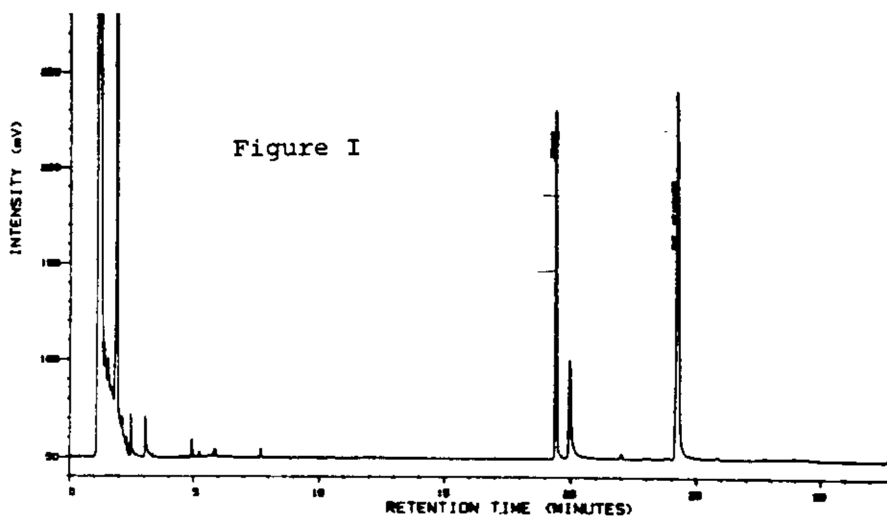


Figure I
INTENSITY (mV)
RETENTION TIME (MINUTES)

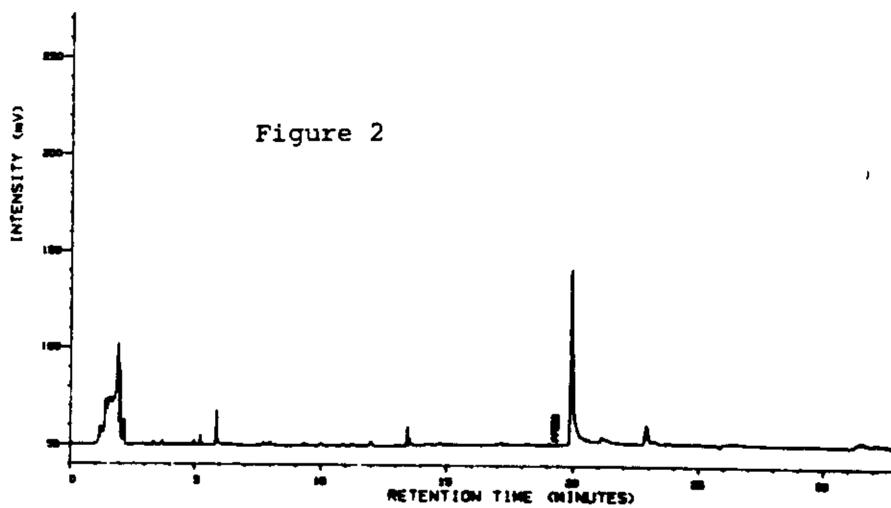


Figure 2
INTENSITY (mV)
RETENTION TIME (MINUTES)

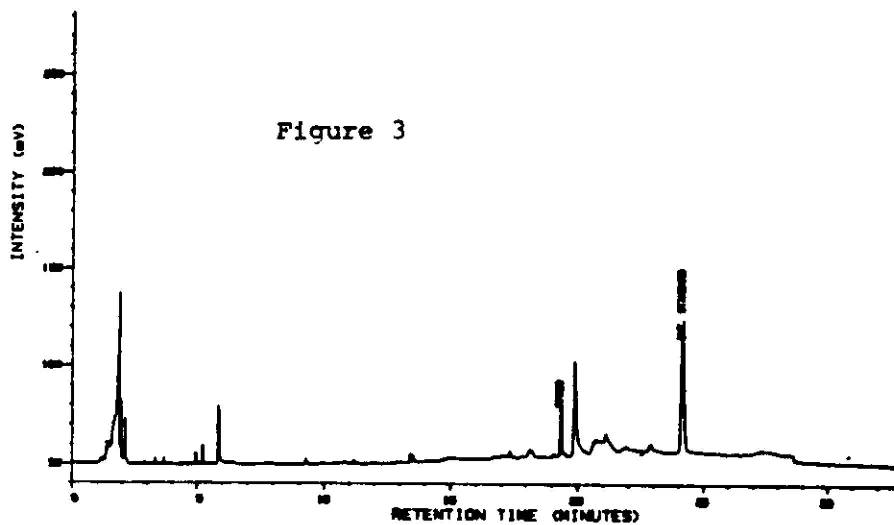
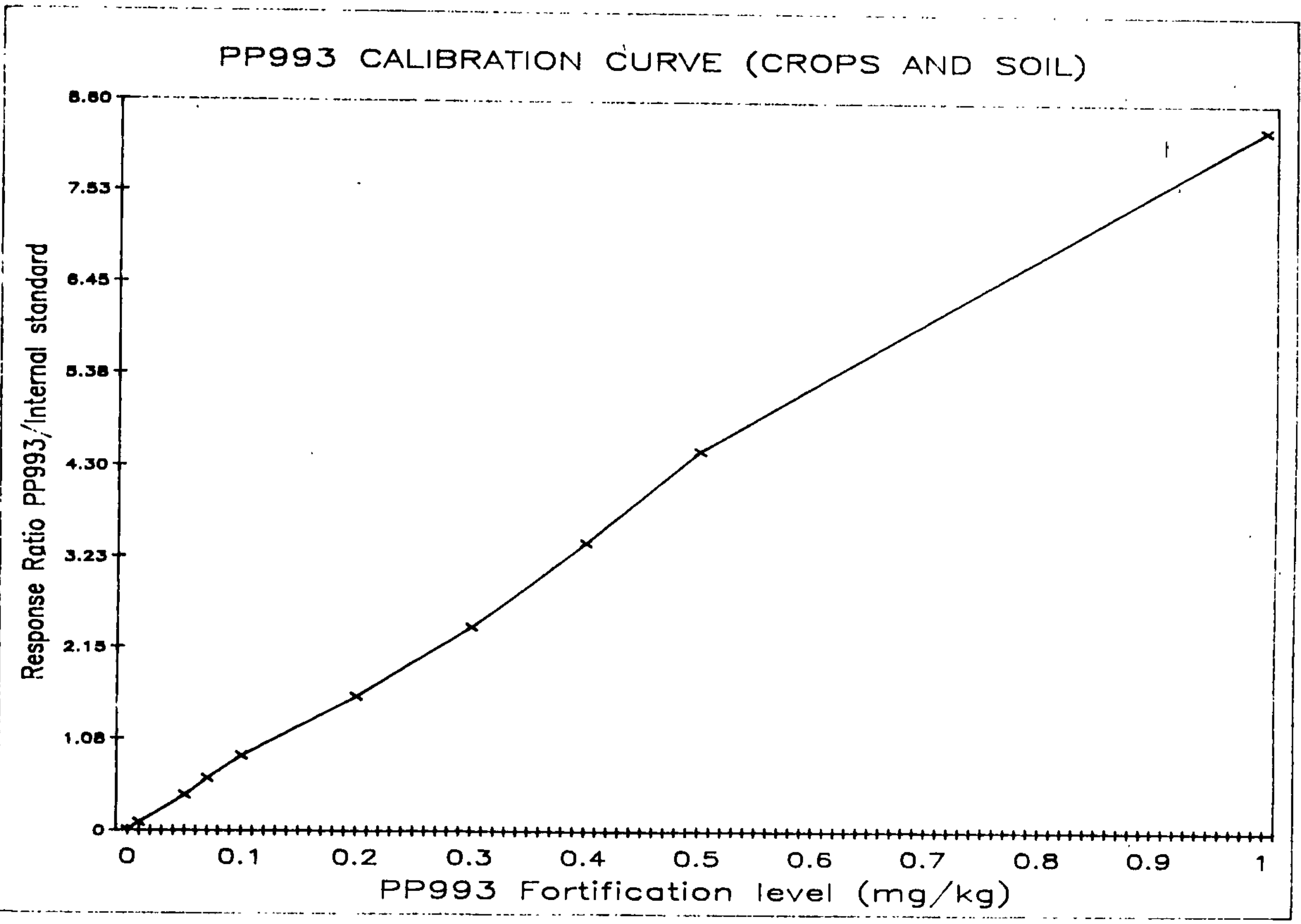


Figure 3
INTENSITY (mV)
RETENTION TIME (MINUTES)

APPENDIX 7

Typical calibration curve for PP993 residue determination in Crops and Soil


PP993 CALIBRATION CURVE (CROPS AND SOIL)
Response Ratio PP993/Internal standard
8.60
7.53
6.45
5.38
4.30
3.23
2.15
1.08
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
PP993 Fortification level (mg/kg)