APPENDIX IV, PART 9

Determination of PP321 Residues in Pond Hydrosoil by Gas-Liquid Chromatography (taken from PPRAM 124)

IV.9/1 SCOPE

The analytical procedures described are suitable for the determination of residues of the synthetic pyrethroid insecticides PP321 and PP563 in hydrosoil. The isomeric compositions of these compounds are described in Section 2.4.1 of the main text. PP321 is the enantiomer pair B and PP563 consists of enantiomer pairs A and B.

IV.9/2 SUMMARY

Residues are extracted from hydrosoil by refluxing in acetonitrile.

A two step clean-up then follows, involving sorbent extraction of an aliquot of the extract, in order to facilitate removal of co-extractives and to concentrate the analyte.

All samples are accurately fortified with internal standard before analysis. Final quantitative determination is by on-column capillary gas liquid chromatography using internal standardisation. The limit of determination for the method is 0.2 µg/kg dry wt hydrosoil (for each isomer).

IV.9/3 PROCEDURE

IV.9/3.1 Sample Preparation

a) When sufficient thawing of the frozen sample allows the soil to be removed from the core as a whole unit, the profile of soil required should be removed.

b) The water present in the hydrosoil on thawing should be separated by filtering in a Buchner funnel. The filtrate should be collected into a Buchner flask containing acetonitrile (50 cm^3) to prevent any adsorption of pyrethroids onto glassware.

c) A vacuum can be applied to aid filtration as the hydrosoil thaws.

IV.9/3.2 Extraction

a) Reflux the soil 'cake' with acetonitrite 100-200 cm^3 for 1 hour. Rinse condenser with further acetonitrile when cool.

b) Filter the soil (under vacuum) and collect the filtrate (acetonitrile extract).

c) Make the extract to a known volume with acetonitrile.

IV.9/3.3 Sorbent Clean-up

a) Prepare matrix cartridges (1g of C_8 octyl sepralyte sorbent) in a reservoir (75 cm^3).

b) Condition cartridges with methanol (25 cm^3) followed by water (5 cm^3) allowing each to percolate under gravity.

(N.B. Cartridges must be conditioned within half an hour of use and must not be allowed to dry out before use).

c) Take an aliquot of the acetonitrile extract equivalent to 1/100th of the total soil weight.

Add 1/100th of the total volume of aqueous phase (separated initially from the soil).

d) Dilute the solution with water to achieve 3:1 water: acetonitrile ratio, and mix thoroughly.

(N.B. The acetonitrile present in the aqueous phase must be taken into consideration here).

e) Apply the solution onto the C_8 cartridge. The elution rate should be approximately 1cm^3/min. Discard the eluate.

f) Wash cartridge with water (5 cm^3) followed by acetonitrile : water 50:50 (2 x 2.5 cm^3). Discard the eluates.

g) Take cartridges to dryness under vacuum (approximately 1hr).

h) Elute cartridges with hexane (5 x 2 cm^3) and collect the eluate.

IV.9/3.4 Silica Sorbent Clean-up

Note. In these laboratories the silica cartridges chosen were the 'Sep-pak' cartridges. These are fitted to the luer tip of a glass (20 cm^3) syringe. A plunger is then used to control percolation of solution/solvent through the sorbent bed.

a) Condition the cartridges with 10% ether/hexane (V/V) solution (10 cm^3) followed by hexane (20 cm^3).

b) Apply the hexane solution (h) to the cartridge. Leave to stand for 1 minute and then slowly push through the solvent. Discard the eluate.

c) Wash the cartridge with 10% ether/hexane (V/V))solution (1 cm^3). Discard the eluate.

d) Taking a clean syringe apply 10% ether/hexane (V/V) solution (4 cm^3) and collect the eluate.

e) Make to an appropriate volume for analysis by gas liquid chromatography.

Note:- i) At no time during the silica clean-up should the cartridges be allowed to dry out.

ii) All solutions/solvent should be allowed to stand for 1 minute and then pushed through the matrix at a rate no greater than 1 cm^3/min.

iii) The plunger should never be retracted with a cartridge still attached to the syringe.

IV.9/4 GAS LIQUID CHROMATOGRAPHY

The conditions for the analysis by GLC will depend upon the equipment available. A highly sensitive detector is required for analysis at low levels, and the following conditions have been found to be satisfactory.

IV.9/4.1 Instrumentation

Varian 3500 capillary gas chromatograph with automated on-column injector and fitted with a Ni^{63} (8 mCi) electron capture detector.

IV.9/4.1 GLC Conditions

a) SE54 bonded phase fused silica open tubular capillary column 25m x 0.32mm ID.

b) Temperature programs:-
Column temperature:- 89°C (hold 1 minute) program at 10°C/min to 240°C (hold 21 minutes) program at 10°C/min to 250°C (hold 10 minutes).

Injector temperature:- 40°C program at 150°C/min to 250° (hold 35 minutes).

c) Detector temperature:- 300°C.

d) Carrier gas, helium at 2 cm^3/min. Make-up gas Nitrogen at 28 cm^3/min.

Under these conditions PP563 is resolved into its two isomers, isomer A with retention time 24.1 mins and isomer B (PP321) with retention time 24.85 minutes. The internal standard used - R171554 (see Section IV.9/13) has a retention time of 27.1 minutes. Sensitivity is such that 2.4 pg PP321 injected on column with electrometer attenuation at 10 x 8 and recorder on 1mV gives approximately 40% full scale deflection.

IV.9/5 CALCULATION OF PP563 RESIDUES

Residues of isomers A or B in the final extract are calculated using the internal standardisation procedure.

Prior to extraction each sample is accurately fortified with a known amount of internal standard. Each sample residue can thus be individually corrected by measuring the percentage recovery of internal standard through the analytical method.

The calculation for the determination of PP563 residues may be performed using a 'single point ratio calibration' (Caroline and Palermo, 1980). It should be noted that such calibrations are feasible only when the chosen internal standard meets certain criteria (Caroline and Palermo, 1980 and Section IV.9/9). The calculations below are shown for PP321 (isomer B) but are equally valid for isomer A.

a) Using the GLC operated under conditions described in Section IV 9/4.1 above, make repeated injections of 2 µl of a standard solution containing a mixture of R171554 and PP563 each at 2.5 ng/cm^3 until a consistent response is obtained.

b) Measure the peak heights or area obtained and calculate the detector 'Response Factor ratio'.

$$\text{i.e. Rf ratio} = \frac{\text{Peak ht PP321 (mm)}}{\text{Conc'n PP321 (ng/cm}^3\text{)}} \div \frac{\text{Peak ht internal standard (mm)}}{\text{Conc'n internal standard (ng/cm}^3\text{)}}$$

c) Make an injection of the sample solution (SA) and measure the peak heights or area obtained for PP32 1 and internal standard (R171554).

d) Since the response factor ratio will be constant for a detector with a linear response, the PP32 1 residue in sample can thus be calculated:-

$$\text{Rf ratio (Std)} = \frac{\text{Peak ht PP32 1}_{SA}}{\text{Conc'n PP32 1}_{SA}} \div \frac{\text{Peak ht R171554}_{SA}}{\text{Conc'n R171554}_{SA}}$$

$$\text{Therefore PP32 1 conc'n} = \frac{\text{Peak ht PP32 1}_{SA} \times \text{Conc'n R171554}_{SA}}{\text{Rf} \times \text{Peak ht R171554}}$$

$$\text{Units (ng/cm}^3\text{)} = \frac{\text{mm} \times \text{ng/cm}^3}{\text{mm}}$$

e) This concentration must then be corrected in order to express the residue in terms of the concentration of analyte in a known amount of matrix.

$$\text{Residue in ng/g} = \frac{\text{PP32 1 conc'n}}{\text{(Sample/matrix) conc'n in final solution}}$$

$$\text{Units (ng/g)} = \frac{\text{ng/cm}^3}{\text{g/cm}^3}$$

Note:- Injections of sample solutions (3 maximum) should be bracketed between standards.

IV.9/6 CONTROL AND RECOVERY EXPERIMENTS

a) To ensure that no observed contamination of the samples occurs during analysis at least one untreated sample must be analysed alongside any set of samples using exactly the same procedure.

b) Before extraction each sample should be accurately fortified with a concentration of internal standard within the range of the residue levels expected.

c) At least one untreated control sample must be fortified with suitable concentrations of both PP32 1 or PP563 and R171554 at/around expected residue to check the agreement between analyte and internal standard during analysis.

IV.9/7 LIMIT OF DETERMINATION

The limit of determination will give a final chromatographic response of at least 4 x the background noise at the retention time of PP321 (isomer B) or isomer A.

In these laboratories the LOD for isomers A and B has been set at 0.2 µg/kg.

IV.9/8 CONFIRMATION OF RESIDUES

Capillary gas chromatography-mass spectrometry (GCMS) operated in the selected ion monitoring mode (SIM) may be used for the qualitative and quantitative confirmation of residues. Samples obtained from the residue analytical method are examined by SIM ie. 2 or more of the most abundant m/z values present in the mass spectrum are continuously monitored throughout the gas chromatographic run and collected on a data capture system. 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 these laboratories GCMS was used to qualitatively and semi-quantitatively confirm the presence of residues of PP563 and also the epimer of the internal standard R171554, formed by inversion of the optically active α-CN position.

IV.9/9 METHOD VALIDATION

No endogenous material from the hydrosoil matrix has been observed to interfere with PP563 (cyhalothrin) or the internal standard during the final chromatographic analysis. The percentage recovery of PP563 and R171554 has been observed to be virtually identical for a range of external recoveries between 0.8 and 25 ppb.

IV.9/10 EXTRACTABILITY

The extraction of PP321 from soil using acetonitrile has been found to be >85% efficient: Berwick et al (1986).

IV.9/11 EXAMPLES OF CHROMATOGRAPHIC TRACES

These are shown in Figures 50 a-d.

IV.9/12 APPARATUS

a) Filtering equipment, Buchner flasks and funnels.

b) Equipment for reflux.

c) Vacuum manifold system.

d) Graduated tubes 10cm^3 calibrated down to 1.0cm^3 in 0.1cm^3 units.

e) Glass reservoir and plungers (10 or 20 cm^3).

f) Capillary gas chromatograph fitted with an electron capture detector which will meet the sensitivity requirements.

NB. An autosampler may be used with the gas chromatograph providing:

1) it is ensured that it is not a source of cross contamination between samples.

2) Suitably precise injections can be achieved with a reproductibilty better than ± 5%.

g) Reservoirs (polyethylene) 75ml capacity and porous polyethylene frits 20 µm pore size, (Jones chromatography).

IV.9/13 REAGENTS

a) Solvents: Redistilled methanol, acetonitrile, diethyl ether, n-hexane, and ultra-pure water.

b) C_8 octyl sepralyte sorbent (Supplier:- Jones Chromatography).

c) A sample of PP321/PP563 of known purity.

d) A sample of R171554 for use as an internal standard.

The internal standard used is the resolved cis isomer assumed by analogy to the chromatographic GLC characteristics of PP563 and other synthetic pyrethroids to be:-

(RS)-α-cyano-3-phenoxybenzyl-(1RS)-cis-3-(Z-2-bromo-3, 3, 3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate.

IV.9/14 Great care must be taken when working at low levels to minimise the risk of contamination.

a) To achieve the set limits of determination, whenever possible all analysis should be carried out in a designated 'low level' area.

b) All glassware must be segregated and used solely for the low level analysis. If possible a washing machine should be designated for such glassware.

c) All glassware should be thoroughly soaked and rinsed with appropriate solvents before use.

d) Plastic and glassware for the analysis should be minimised.

e) All new 'batches' of solvent and sorbent should be analysed (for interfering contaminants in ECD analysis) before use.

f) Solvents should be freshly dispensed into glass vessels for each analysis and any solution which is required, should be freshly prepared.

IV.9/15 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 monographs such as 'Hazards in the Chemical Laboratory', Ed G D Muir, The Chemical Society, London.

a) Solvent Hazards

	Acetone	Acetonitrile	Diethyl ether	Hexane	Methanol
Harmful vapour	Yes	Yes	Yes	Yes	Yes
Highly flammable	Yes	Yes	Yes	Yes	Yes
Harmful by skin absorption	-	Yes	-	-	Yes
TLV mg m^3	2400	70	1200	180	260

b) PP321 and PP563 and R171554 are synthetic pyrethroid insecticides with a mammalian toxicity (acute oral LD_{50}) in the rat in the order of 50-60 mg kg^{-1} (PP321).

IV.9/16 PREPARATION OF ANALYTICAL STANDARDS

Weigh out accurately, using a five figure balance, sufficient PP321, PP563 or R171554 to allow dilution in acetone to give a 1000 $\mu g/cm^3$ stock solution in a volumetric flask. Make serial dilutions of this stock to give 100 $\mu g/cm^3$, 10 $\mu g/cm^3$ and 1.0 $\mu g/cm^3$ standard solutions in hexane. Prepare serial dilutions of mixed standards of PP321 and internal standard in hexane (for use as GLC reference standards) down to 2.5 ng/cm^3.

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