

## 1.0 INTRODUCTION

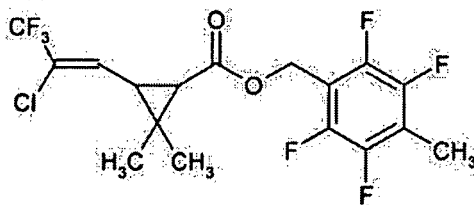
### Scope and chemical structures

Analytical method GRM002.04A is suitable for the determination of tefluthrin (Figure 1) in soil using an external standardisation procedure. The limit of quantitation (LOQ) of the method is 0.01 mg kg<sup>-1</sup>.

This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guideline OPPTS 850.7100.

#### Figure 1

<b>R Number</b>	: R151993
<b>Compound Code Number</b>	: ICI993
<b>Common Name</b>	: Tefluthrin
<b>CAS Number</b>	: 79538-32-2
<b>IUPAC Name</b>	: 2,3,5,6-tetrafluoro-4-methylbenzyl (1R,3R;1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate
<b>Molecular Formula</b>	: C <sub>17</sub> H <sub>14</sub> ClF <sub>7</sub> O <sub>2</sub>
<b>Molecular Mass</b>	: 418.7



### 1.2 Method summary

20 g soil samples are extracted by reflux with acetonitrile. Soil extracts are analysed directly by gas-liquid chromatography using mass selective detection (GC-MSD) in the selected ion monitoring mode. The limit of quantification is 0.01 mg kg<sup>-1</sup>.

## 2.0 MATERIALS AND APPARATUS

### 2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

## 2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass-distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

## 2.3 Preparation of analytical standard solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Prepare a  $200 \mu\text{g mL}^{-1}$  stock solution for tefluthrin by one of the following methods:

Weigh out accurately, using a five figure balance, sufficient tefluthrin analytical standard and carefully transfer into separate "Class A" volumetric flask (50 mL). Dilute to the mark with acetonitrile to give a  $200 \mu\text{g mL}^{-1}$  stock solution of tefluthrin.

Alternatively, the appropriate volume of acetonitrile to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P = Standard purity in decimal form (P%/100)

V = Volume of acetonitrile required

W = Weight, in mg, of the solid analytical standard

C = Desired concentration of the final solution, ( $\mu\text{g mL}^{-1}$ )

1000 = Unit conversion factor

The standard material is weighed into a "Class A" volumetric flask.

Sample fortification solutions should be prepared in acetonitrile from the primary stock solution in "Class A" volumetric flasks. It is recommended that, as a minimum, the following solutions are prepared by serial dilution with acetonitrile:  $10 \mu\text{g mL}^{-1}$ ,  $1.0 \mu\text{g mL}^{-1}$ ,  $0.1 \mu\text{g mL}^{-1}$  and  $0.01 \mu\text{g mL}^{-1}$ .

All stock solutions should be stored in a refrigerator or freezer when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of six months is recommended unless additional data are generated to support a longer expiration date.

## 2.4 Safety precautions and 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 any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 3).

### Reagent hazards

	Acetonitrile
Harmful Vapour	✓
Highly Flammable	✓
Harmful by Skin Absorption	✓
Irritant to eyes and respiratory tract, causes burns	×
Syngenta Hazard Category	SHC D, S
OES Short Term (mg m <sup>-3</sup> )	105
OES Long Term (mg m <sup>-3</sup> )	70

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

Tefluthrin has been assigned to Syngenta Hazard Category (SHC) D, S. The toxicity classification scale rates highly toxic chemicals as class E and non toxic chemicals as class A. An additional hazard category of S indicates the compound is a severe skin and eye irritant.

### 3.0 ANALYTICAL PROCEDURE

The method is summarized in flow chart form in Appendix 7.

#### 3.1 Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

#### 3.2 Extraction of Soil

- a) Weigh representative amounts of soil (20 g) into round bottomed flasks (250 mL size). At least one untreated control and two control samples fortified with known amounts of tefluthrin in acetonitrile should be analysed with each batch of samples to enable verification of the method and recovery corrections to be made.
- b) Add acetonitrile (100 mL) to the samples and reflux for 1 hour. Allow to cool to room temperature and the flask contents to settle. 1 mL aliquots of extract are now equivalent to 0.2 g soil. Transfer approximately 1 mL aliquots to GC vials and analyse extract directly by GC-MSD (Section 4).

#### 3.3 Preparation of GC-MSD Calibration Standard

No significant suppression or enhancement of the instrument response for tefluthrin has been observed in the soil types tested using the above procedure in this laboratory, hence non-matrix standards should be used for calibration. Calibration standards should be prepared as described below:

To prepare a  $0.002 \mu\text{g mL}^{-1}$  calibration standard, transfer 2 mL of a  $0.01 \mu\text{g mL}^{-1}$  tefluthrin standard in acetonitrile to a 10 mL volumetric flask. Adjust to the mark with acetonitrile. Stopper securely and shake to mix thoroughly. Transfer an approximately 1 mL aliquot of the standard in acetonitrile to a suitable autosampler vial. The standard is ready for final determination by GC-MSD.

A calibration curve may also be generated to quantify tefluthrin residues. Standards over the concentration range  $0.001 - 1.0 \mu\text{g mL}^{-1}$  (or appropriate to the residue expected in the samples) should be prepared as described above, using appropriate amounts of tefluthrin standard in acetonitrile.

#### 3.4 Time required for analysis

The methodology is normally performed with a batch of 12 or more samples. One person can complete the analysis of 20 samples in 1 day (8 hour working period).

### 3.5 Method stopping points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

### 4.0 FINAL DETERMINATION

The following instrument and conditions have been found to be suitable for this analysis in this laboratory. Other instruments can be equally used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

This method has been developed for use on an Agilent 5973 mass selective detector instrument, with an Agilent 6890 series GC system.

#### Instrument description

##### Chromatography conditions

Column	: Agilent HP5MS (30.0 m x 0.25 mm i.d., df = 0.25 µm)
Injection Port	: Splitless with silanised glass wool plug
Carrier gas and head pressure	: Helium at 1.0 mL min <sup>-1</sup> constant flow
Injection mode	: Pulsed (pulse pressure 30.0 psi) for 1 minute
Injection volume	: 2 µL
Injector temperature	: 275°C
Detector temperature	: 300°C
Transfer line temperature	: 280°C
Ion source temperature	: 230°C
Quadrupole temperature	: 150°C
Temperature programme	: 100°C (hold for 1 minute), 30°C/min to 300°C (hold for 1 minute).

##### MSD Conditions

Electron energy	: Maximum 70 eV (set by autotune)
System Calibration	: Autotune

## Acquisition Parameters

Compound Name	Low Mass Resolution	SIM	MODE
Tefluthrin	Yes	Target Ion Qualifier 1 Qualifier 2 Retention Time	177 <i>m/z</i> 197 <i>m/z</i> 199 <i>m/z</i> 6.2 mins

Final determination is by GC-MSD monitoring 3 ions with  $m/z \geq 100$ . This is considered to be highly specific; hence no confirmatory conditions are included. Quantification may be carried out on any of the above ions. Typical chromatograms are shown in Appendix 4. The full scan spectrum showing the fragmentation of tefluthrin is included in Appendix 6.

## 5.0 CALCULATION OF RESULTS

Residues may be calculated using an external standardisation procedure. Tefluthrin residues may be calculated in  $\text{mg kg}^{-1}$  for each sample using a mean standard response from each of the injections bracketing the sample as follows:

### 5.1 Single point calibration procedure

- Make repeated injections of a standard containing tefluthrin at an appropriate concentration into the GC-MSD operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for tefluthrin.
- Make an injection of each sample solution and measure the peak areas of the peaks corresponding tefluthrin.
- Re-inject the standard solution after a maximum of four injections of sample solutions.
- Calculate the tefluthrin residues in the sample, expressed as  $\text{mg kg}^{-1}$  using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue concentration} = \frac{\text{PK area(SA)}}{\text{PK area(STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample

PK area (STD) = Average peak response for bracketing standards

Standard Conc. = Concentration of standard ( $\mu\text{g mL}^{-1}$ )

Sample Conc. = Sample concentration ( $\text{g mL}^{-1}$ )

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue concentration} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg kg}^{-1}\text{)}$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

## 5.2 Multi point calibration procedure

Tefluthrin residues may be calculated in mg kg<sup>-1</sup> for each sample as follows.

- Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four).
- Make an injection of each sample solution and measure the areas of the peaks corresponding to tefluthrin. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.
- Generate calibration curve parameters using an appropriate regression package.
- The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where  $y$  is the instrument response value,  $x$  is the standard concentration,  $m$  is the gradient of the line of best fit ("X-variable 1" in MS Excel) and  $c$  is the intercept value. An example of this equation generated using the experimental values of  $m$  and  $c$  should be included in the raw data, as should the "R-Squared" value for the regression. Re-arrangement for  $x$  gives

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

- Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where  $y$  is the instrument response value,  $x$  is the standard concentration and  $a$ ,  $b$ ,  $c$  are constants.

- Calculate the tefluthrin residues in the sample, expressed as mg kg<sup>-1</sup>, as follows

$$\text{Residue concentration (mg kg}^{-1}\text{)} = \frac{\text{Analyte found (}\mu\text{g mL}^{-1}\text{)}}{\text{Sample conc. (g mL}^{-1}\text{)}}$$

Where analyte found ( $\mu\text{g mL}^{-1}$ ) is calculated from the standard calibration curve and sample conc. is the final sample concentration in g mL<sup>-1</sup>.

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue concentration} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg kg}^{-1}\text{)}$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

## 6.0 CONTROL AND RECOVERY SAMPLES

Analysis of control soil samples should be completed as detailed in Sections 3.1-3.2 for each set of samples analysed to verify that the soil is free from tefluthrin contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (untreated soil samples accurately fortified with a known amount of tefluthrin prior to extraction) should also be completed alongside each batch of samples. Provided the recovery values are acceptable they may be used to correct any tefluthrin residues found. The recovery levels should be appropriate to the residue levels expected.

Recovery data is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of  $\leq 20\%$ .

## 7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

### 7.1 Matrix interference

GC-MSD monitoring of three ions with  $m/z > 100$  is a highly specific detection technique. Interference arising from the soil types tested has not been observed.

### Reagent and solvent interference

Using high purity solvents and reagents no interference has been found.

### 7.3 Labware interference

This method uses disposable labware as far as possible. Glassware should be detergent washed and rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.



## 11.0 REFERENCES

1. Hargreaves S L and Sapiets A (2000). RAM085/05: Determination of Tefluthrin in Crops and Soil.
2. Hargreaves S L (2000). RAM349/01: Determination of Tefluthrin in Soil.
3. Luxon S G (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge, CB4 4WF, UK. ISBN 0-85186-229-2.
4. Hargreaves S L, (2000). Provision of Additional Validation Data to Supplement Residue Analytical Method RAM 085/04: The Determination of Residues of Tefluthrin in Crops and Soil. Technical Letter 47463/01.
5. Cardone M J, Palermo P J and Sybrand L B: Potential error in single point ratio calculations based on linear calibration curves with a significant intercept. Anal Chem., 52 pp 1187-1191, 1980.
6. Hill I R, Bewick D W, Bartlett D W, Weissler M S, Hendley P, Newell S A and Wolfe R (1987). Tefluthrin: Fate of Radiolabelled Material in Soil Under Field Conditions. Report No. RJ0546B.
7. Ussary J P and Fitzpatrick R D (1987). Tefluthrin: Dissipation in U.S. Soils. Report No. TMU3086B.

## APPENDIX 1 APPARATUS

### UK suppliers

General laboratory glassware, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

Crimp cap auto sampler vials and caps available from Agilent Technologies UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire, SK8 3GR, UK.

Agilent 5973 MSD system equipped with an Agilent 6890 GC system and Agilent 7683 autosampler injector system, available from Agilent Technologies UK Ltd., Lakeside, Cheadle Royal Business Park, Stockport, Cheshire, SK8 3GR, UK.

GC column, Agilent HP5MS (5% phenyl 95% dimethylpolysiloxane), available from Agilent 7683 autosampler injector system, available from Agilent Technologies UK Ltd., Lakeside, Cheadle Royal Business Park, Stockport, Cheshire, SK8 3GR, UK.

Double gooseneck injection liner 4 mm i.d. for HP splitless injectors, available from Thames chromatography, Fairacres Industrial Centre, Dedworth Road, Windsor, Berkshire, SL4 4LE, UK.

Deactivated glass wool, available from Thames chromatography, Fairacres Industrial Centre, Dedworth Road, Windsor, Berkshire, SL4 4LE, UK.

### US suppliers

General laboratory glassware, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Crimp cap auto sampler vials and caps available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304, USA.

Agilent 5973 MSD system equipped with an Agilent 6890 GC system and Agilent 7683 autosampler injector system, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304, USA.

GC column, Agilent HP5MS (5% phenyl 95% dimethylpolysiloxane), available from Agilent 7683 autosampler injector system, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304, USA.

Double gooseneck injection liner 4 mm i.d. for HP splitless injectors, available from Restek Corporation, 110 Benner Circle, Bellafonte, PA 168230, USA.

Deactivated glass wool, available from Restek Corporation, 110 Benner Circle, Bellafonte, PA 168230, USA.

## APPENDIX 2 REAGENTS

### UK suppliers

Solvents: Acetonitrile, available from Rathburn Chemicals Ltd., Walkerburn, EH43 6AU, UK.

Tefluthrin analytical standard, available from GLP Testing Facility EZA, Syngenta, CH-4333, Munchweilen, Switzerland.

### US Suppliers

Solvents: Acetonitrile, available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA.

Tefluthrin analytical standard, available from Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300, USA.

**APPENDIX 7      METHOD FLOWCHART**

Reflux soil (20 g) samples in MeCN (100 mL) for 1 hour



Allow to cool then analyse aliquot directly by GC-MSD