1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM002.09A is suitable for the determination of Tefluthrin (Figure 1) in surface water. The limit of quantitation (LOQ) of the method has been established at 0.10 µg/L (0.10 ppb).

This method satisfies US EPA guidelines EPA OCSPP 650.6100.

1.2 Method Summary

Surface water samples (100 mL) are partitioned into toluene and analyzed directly by GC/MSD. A SPE procedure is available for optional cleanup.

The limit of quantitation of the method is 0.10 µg/L (0.10 ppb).

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.

2.3.1 Stock Solutions

Prepare a 100 µg/mL stock solution for Tefluthrin by one of the following methods:

Weigh out accurately, using a five figure balance, sufficient Tefluthrin analytical standard into an amber "Class A" volumetric flask (100-mL). Dilute to the mark with acetone and mix well to give a 100 µg/mL stock solutions of Tefluthrin. Standards should be prepared in amber bottles and stored under refrigeration.

Alternatively, the appropriate volume of acetone 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 acetone required
W	=	Weight, in mg, of the solid analytical standard
C	=	Desired concentration of the final solution, (µg/mL)
1000	=	Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions containing Tefluthrin should be prepared by serial dilution in acetone from the stock solution. It is recommended that the following solutions are prepared: 1.0 µg/mL, 0.10 µg/mL and 0.01 µg/mL for fortification purposes.

2.3.3 Preparation of Calibration Standards for GC-MSD

Calibration standards are prepared in toluene. An aliquot from the stock solution or fortification solution can be evaporated and reconstituted using toluene for serial dilution in preparation of calibration standards. Alternatively, a stock solution can be prepared as discussed in section 2.3.1 using toluene as the dilution solvent. Using the instrumentation found in Section 4.0, the following concentration range of standards were prepared and used for calibration: 0.25 ug/L -25 ug/L.

A calibration curve should be generated to quantify Tefluthrin residues. Standards over an appropriate concentration range should be prepared with a minimum of five levels.

Any observed matrix effects may be compensated for by use of matrix matched standards at the discretion of the study director, or by dilution of the final sample with toluene should instrument sensitivity permit.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in amber bottles and refrigerated 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 for Tefluthrin 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 1).

Solvent and Reagent hazards

	Toluene	Methanol	Acetone
Harmful Vapor	✓	✓	✓
Highly Flammable	✓	✓	✓
Harmful by Skin Absorption	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓
Causes severe burns	✖	✖	✖
Syngenta Hazard Category (SHC)	B, S	SHC-C, S	B
OES Short Term (mg/m^3)	560	310	3560
OES Long Term (mg/m^3)	188	260	1780

N/A not known

Syngenta Hazard Classification for Tefluthrin is SHC-D,S. The Syngenta Hazard Category scale rates highly toxic chemicals as category SHC-E and non-toxic chemicals as category SHC-A. An additional hazard category of S indicates the compound is a severe skin and eye irritant.

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

3.0 ANALYTICAL PROCEDURE

A summary of the method is included in flow-chart form as shown in Appendix 4. In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included in each sample set. At least one untreated control and two control samples fortified with known amounts of Tefluthrin should be analyzed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

3.1 Sample Preparation

a) If water samples are received frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity prior to subsequent aliquot for further treatment or analysis.

b) Transfer 100 mL of the water sample to be analyzed into a 250 mL nalgene bottle. Sample fortification is carried out at this time using fortification standards in acetone, if required.

c) To each sample add 20 mL of toluene. Shake for 15 minutes and centrifuge samples at 3500 rpm for 5 minutes.

d) Transfer 1.0 mL (top layer-toluene) to a suitable autosampler vial for determination by GC/MS. Final concentration is 0.5 ug/L. Further dilution can be performed using toluene if needed.

3.2 Alternate Solid Phase Extraction Procedure

a) Take one Oasis HLB cartridge (6 cc, 150 mg)) for each sample to be analyzed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add toluene (5 mL) and allow to percolate through each cartridge under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluate. Do not allow the cartridges to become dry. Add methanol (5 mL) and allow to percolate through each cartridge under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluate. Do not allow the cartridges to become dry. Add ultra-pure water (5 mL) to the top of each cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

b) Load water samples from Section 3.1 (b) onto the SPE cartridges (a suitable column reservoir may be used if desired) and allow to percolate through at a rate of approximately 10 mL/min, to the level of the top frit. Do not allow cartridges to become dry. Tefluthrin is retained on the SPE cartridge.

c) On completion of loading, wash the empty sample tubes with ultra-pure water (5 mL) and add the rinse to the column reservoir. Allow to percolate through at the same rate, again discarding the column eluate.

d) Remove the column reservoir and column connector from the SPE cartridge if used. Apply a high vacuum for approximately 15 minutes to remove all water from the cartridge.

e) Place suitable collection tubes (e.g. 10 mL glass test tubes or 15 mL falcon tubes) under each port, as required, in the manifold rack. Add 1 g of anhydrous sodium sulfate to each bottle to remove any remaining water. Add 5 mL of toluene to each bottle and swirl to rinse the bottle walls. Load the toluene rinsate into the cartridges to elute with toluene, under low vacuum at a rate of approximately 1 - 2 mL/min to the level of the top frit collecting the column eluate. Add an additional 5 mL of toluene to each cartridge to continue elution. Apply high vacuum for approximately 5 seconds to collect the excess solvent from the SPE cartridges. Tefluthrin is eluted in this step.

f) Dilute to a final volume of 10 mL with toluene.

g) Transfer 0.5 mL to a suitable autosampler vial and add 0.5 mL of toluene (1:1 dilution) for determination by GC/MS. Final concentration is 0.5 ug/L. Further dilution can be performed using toluene if needed.

3.3 Problems and Modifications

For low level residue analysis it is recommended to perform sample container rinses using the extraction solvent where applicable to increase procedural recoveries. It is also recommended to use disposable labware when possible to avoid cross-contamination.

The SPE procedure has been developed using cartridges from the stated manufacturer. Similar cartridges from other manufacturers may be used. In all cases however, it is strongly recommended that the elution profile of the chosen batch of cartridges is checked prior to commencing analysis to assess any variation in manufacturers' products and between batches.

3.4 Time Required for Analysis

The methodology is normally performed with a batch of 15 samples. One skilled analyst can complete the analysis of 1-2 sample sets in 1 day (8 hour working period).

3.5 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekends 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 method has been developed for use on an Hewlett Packard 6890. The system is controlled and data is processed by Chemstation™ Software. The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimization 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.

4.1 Instrument Description

GC/MS

GC System	:	Hewlett Packard 6890
Detector	:	Hewlett Packard 5973

4.2 Chromatography Conditions

Column : HP-5MS (30.0m x 0.25 mm i.d)

Injection Port : Splitless-carbofrit liner (4mm)

Carrier Gas : Helium at 1.0 mL/min

Injection Mode : Pulsed (pressure 30 psi)

Purge Time : 2 minutes

Injection Volume : 2 µL

Injector Temperature : 275°C

Transfer Line Temperature : 280°C

Ion Source Temperature : 230°C

Quadrupole Temperature : 150°C

Oven Temperature Gradient

Step	Rate (°C/min)	Temperature	Time (min)
1	-	150	1
1	20	300	2

Under these conditions the retention time for tefluthrin is approximately 5.1 minutes.

4.3 Mass Spectrometer Conditions

Ionization Mode	:	Chemical (SIM)
Polarity	:	Negative
Calibration	:	AutoTune
Analyte	:	Tefluthrin
Target Ion	:	241 *m/z*
Qualifier 1	:	243 *m/z*
Qualifier 2	:	205 *m/z*
Ion Ratio	:	100:33:13

4.4 Confirmatory Procedures for Tefluthrin

Final determination by GC/MS with two qualifier ions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi Point Calibration Procedure

Residues of Tefluthrin may be calculated in µg/L for each sample as follows:

a) 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 five levels).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to respective target ions. Quality Control standard solutions should be interspersed throughout the analysis to monitor any matrix effects.

c) Generate calibration curve parameters using an appropriate regression package.

d) 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 (slope) 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

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

e) Calculate residues of interest in a sample, expressed as µg/L, as follows:

$$Residue\ (\mu g/L\ or\ ppb) = \frac{Analyte\ Found\ (pg)}{Water\ Sample\ Injected\ (mg\ or\ \mu L)}$$

Where on-column *Analyte Found (pg)* is calculated from the standard calibration curve and on-column *Water Sample (matrix) Injected* is calculated as follows:

$$Water\ Sample\ Injected\ (mg\ or\ \mu L) = Sample\ Volume\ (mL) \times \frac{Injection\ Volume\ (\mu L)}{Sample\ Final\ Volume\ (mL)}$$

f) Determine the recovery by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery as a percentage (%) by the equation:

$$Recovery\ (\%) = \frac{(Residue\ in\ Recovery\ Sample) - (Residue\ in\ Control)}{Amount\ Fortified} \times 100\%$$

g) If residues need to be corrected for average percentage recovery, *e.g.* for storage stability studies, then the equation below should be used.

$$Corrected\ Residue\ (\mu g/L\ or\ ppb) = \frac{Residue\ (\mu g/L\ or\ ppb)}{Average\ Precent\ Recovery}$$

5.2 Single Point Calibration Procedure

Tefluthrin residues may be calculated in µg/L (ppb) for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) 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.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to Tefluthrin.

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

d) Calculate the Tefluthrin residues in the sample, expressed as µg/L (ppb) using a mean standard response from each of the injections bracketing the sample as follows:

$$Residue\ (\mu g/L\ or\ ppb) = \frac{PK\ area\ (SA)}{PK\ area\ (STD)} \times \frac{Standard\ Conc.}{Sample\ Conc.}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (µg/mL)
Sample Conc. = Sample concentration (L/mL)

e) If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$Corrected\ Residue\ (\mu g/L\ or\ ppb) = \frac{Residue\ (\mu g/L\ or\ ppb)}{Average\ Precent\ Recovery}$$

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analyzed with each batch of samples. Control samples from the same matrix are recommended to monitor any instrumental matrix effects present.

At least two recovery samples (control samples accurately fortified with known amounts of analyte), including one at the method LOQ and one at the expected residue level, should also be analyzed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found in the sample. The fortification levels should be appropriate to the residue levels expected in the sample.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of $\leq$ 20%.

When the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

7.0 SPECIFICITY

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

7.1 Matrix

GC/MS is a highly specific detection technique. Interferences arising from the matrices tested have not been observed.

7.2 Reagent and Solvent Interference

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

7.3 Labware Interference

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

7.4 Limit of Quantitation (LOQ)

The limit of quantitation of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-120% with a relative standard deviation of $\leq 20\%$ has been obtained. Generally, for accurate quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantitation of the method has been established at 0.10 µg/L (0.10 ppb).

7.5 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. Note that the LOD may vary between runs and from instrument to instrument. The LOD was determined as 0.25 pg injected on column, equivalent to 0.125µg/L when using a 2 µL injection volume.

7.6 Matrix Effects

No significant matrix effects were observed in the water types tested during method validation and non-matrix standards should generally be used for quantitation.

7.7 Detector Linearity

For accurate quantitation of residue concentrations, analyses should be carried out within the linear range of the detector. For multi-point calibration, detector range and linearity will be demonstrated within each sample set.

The linearity of the GC/MS detector response for Tefluthrin was tested in the range from 0.50 pg to 50 pg injected on column (equivalent to 0.25 µg/L to 25 µg/L standards when using a 2 µL injection volume) and was found to be linear.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested linear range prior to quantitation.

At least five different standard concentration levels were injected and the response plotted against the amount injected, using Chemstation™ software for target and confirmatory ions for Tefluthrin.

Representative plots of the detector responses versus the analyte concentration for all calibration points are presented in the Figures Section.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of Tefluthrin residues of environmental surface water. Only commercially available laboratory equipment and reagents are required. The analysis of 30 water samples can be completed by one skilled analyst in 1 day (8 working hour period). Untreated and fortified samples should be analyzed with each set of samples to demonstrate absence of any interference and adequate recoveries, if possible. The limit of quantitation of the method is 0.10 µg/L (0.10 ppb). This method satisfies US EPA OCSPP 850.6100.

11.0 REFERENCES

1. 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.

2. 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

3 Underwood T (2014): "Independent Laboratory Validation of Analytical Method (GRM002.09A) for the Determination of Tefluthrin in Surface Water by GC/MSD." TK0225184.

FIGURE 1 Chemical Structure

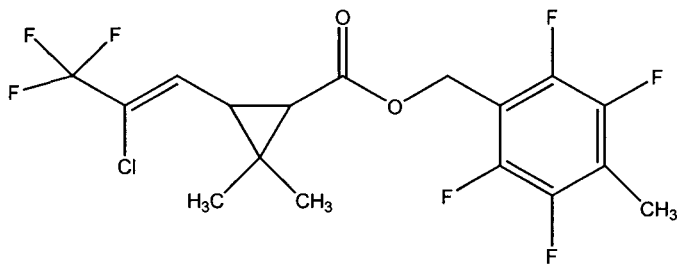
Common Name : Tefluthrin

Code Name : ICI993

CA Index Name : Cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-, (2,3,5,6-Tetrafluoro-4-methylphenyl)methyl ester, [1.alpha.,3.alpha.(Z)]-(.+-.)-

Molecular Formula : $C_{17}H_{14}ClF_7O_2$

Molecular Weight : 418.7



APPENDIX 1 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General lab glassware	General lab glassware	www.thermoscientific.com
General lab plastic-ware	General lab plastic-ware	www.thermoscientific.com
Autosampler vials	2 mL size	www.thermoscientific.com
GC Column	HP5-MS, 30m x0.25 m	www.agilent.com
SPE	OASIS HLB, 6cc/150mg	www.thermoscientific.com

APPENDIX 2 Reagents/Chemicals

Recommended Suppliers

Reagent	Description	Supplier
Toluene	Optima/HPLC grade	www.thermoscientific.com
Methanol	Optima/HPLC grade	www.thermoscientific.com
Acetone	Optima/HPLC grade	www.thermoscientific.com
Water	Optima/HPLC grade	www.thermoscientific.com
Sodium Sulfate	ACS grade	www.thermoscientific.com
Tefluthrin Standard	GLP certified	Syngenta Crop Protection, LLC

APPENDIX 3 Method Flow Chart for GC/MSD

Transfer sample (100 mL) to a 250 mL nalgene bottle

↓

Fortify recovery sample, if needed

↓

Add 20 mL toluene and place on shaker (15 minutes)

↓

Centrifuge 3500 rpm for 5 minutes

↓

Vial for GC/MSD determination

Optional SPE Procedure

Transfer sample (100 mL) to a 250 mL nalgene bottle

↓

Fortify recovery sample, if needed

↓

Load into Pre-Conditioned Oasis HLB SPE Cartridge (6 cc, 150 mg)

↓

Elute with 10 mL toluene

↓

Vial for GC/MSD determination