

*MAN***DATA SUBMISSION VOLUME 16****DATA REQUIREMENT:**

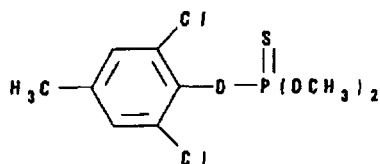
91/414/EEC (July 1991)  
Amended by 96/46/EC (July 1991)  
EU SANCO/825/00 rev.6  
EU SANCO/3029/99 rev.4  
(In Support of OPPTS 835.0000)

**200900101****STUDY TITLE:**

Tolclofos-methyl: Validation of an Analytical Method for the  
Determination of Residues in Soil

## INTRODUCTION

Tolclofos-methyl is a protectant organophosphorus fungicide for use against soil-borne diseases in a variety of crops. Tolclofos-methyl has the following structure:



Chemical name: *O*-2,6-dichloro-*p*-tolyl *O*, *O*-dimethyl phosphorothioate equivalent to *O*-2,6-dichloro-4-methylphenyl *O*,*O*-dimethyl phosphorothioate

Molecular formula: C<sub>9</sub>H<sub>11</sub>Cl<sub>2</sub>O<sub>3</sub>PS

Molecular weight: 301.1

The objective of this study was to validate an analytical method for the determination of tolclofos-methyl in soil, in compliance with the Council Directive 91/414/EEC of 15 July 1991 as amended by Commission Directive 96/46/EC of 16 July 1996 and the EU Guidance document on residue analytical methods SANCO/825/00 rev.6 (20/06/00) for post registration and EU guidance document on residue analytical methods SANCO/3029/99, rev.4 (11/07/00) for pre registration.

## PROCEDURES

### Protocol adherence

The study was performed in accordance with the protocol and amendment with the following deviation.

### Analytical standard

Tolclofos-methyl AS analytical standard (batch 00404SG) was supplied by the sponsor and was received at Covance Laboratories on the 1 February 2001. The purity of the standard was 99.8%, and the expiry date was 1 April 2003. The standard was stored refrigerated (1 to 10°C). The certificate of analysis is presented in Appendix 6.

### Control sample

Control soil was obtained via Covance Laboratories Ltd Environmental Fate Department on 8 March 2001 and was given a unique Covance (CLE) number according to Residue Chemistry Standard Operating Procedures (SOP). Full characterisation of the soil is reported in Appendix 5 and the soil was classified as a sandy loam. The sample had been passed through a 2mm sieve and was stored frozen in the Residue Chemistry Department (nominally -20°C) until required for analysis.

### Analytical method

The analytical method CLE 333/153-04R 'Tolclofos-methyl: Analytical Method of Determination of Residues in Soil' is based on the DFG S19 multi-residue method. The analytical method was originally issued as CLE 333/153-01R and was used to perform the validation with some minor deviation. The method was re-issued as CLE 333/153-02R to incorporate the deviations and correct some typographical errors. The method was re-issued again as CLE 333/153-03R to remove the option of using calibration standards prepared in matrix, as this was found not to be necessary. A final

version of the method CLE 333/153-04R was issued to incorporate some minor format changes suggested by the client.

The analytical procedure is presented in Appendix 1.

The analytical method involved extraction of the soil with acetone/water. Partition of the extraction mixture with dichloromethane. Clean-up of the extract with Gel Permeation Chromatography and quantification of the final extracts by Gas Chromatography with Mass Selective detection (GC/MS).

#### **Validation procedure**

The analytical method was validated by fortifying sub-samples of untreated control soil with known amounts of tolclofos-methyl. The fortified samples were analysed using the analytical method and the recovery of tolclofos-methyl for each sample was determined.

Validation included consideration to the criteria in the following sections.

#### **Linearity**

In order to establish the linearity of response of the analytical chromatographic system to tolclofos-methyl six standards solutions of increasing concentration were prepared over the range 0.01 to 0.50 µg/mL. The lowest concentration was equivalent to a sample at less than 50%, of the limit of quantification. The solutions were injected onto the chromatograph in random order, and a concentration/response curve prepared.

#### **Specificity**

The ability of the method to distinguish between tolclofos-methyl and other substance present in the control samples was demonstrated. Components present in control samples were considered to interfere with the analysis if they were present at levels greater than 30% of the limit of quantification.

#### **Recovery**

The recovery of tolclofos-methyl from control soil fortified at 0.01 mg/kg (LOQ) and 10 mg/kg was determined in quintuplet. The tolclofos-methyl was added using 100 µL of a 5 µg/mL acetone solution for the 0.01 mg/kg level and 1 mL of a 500 µg/mL acetone solution for the 10 mg/kg level. In addition, control samples were extracted and analysed in duplicate. The equivalent concentration of any response to test article from the control commodity (the 0.00 mg/kg recovery) was

subtracted from each of the validation levels, before calculation of the recovery values. Quantification was performed using the most abundant ion (265) and the two confirmation ions (267 and 250). The mean recovery values for each level were considered acceptable if they fell within the range 70 to 110%.

**Precision**

The repeatability of the method was demonstrated by analysing each validation level in quintuplet. The relative standard deviation (RSD) was determined at each validation level and was considered acceptable if  $\leq 20\%$ .

**Limit of quantification (LOQ)**

The limit of quantification, defined as the lowest fortification level where an acceptable mean recovery was obtained (70 to 110%), and a relative standard deviation of  $\leq 20\%$  was achieved, was also demonstrated. The documented limit of quantification for the analytical method is 0.01 mg/kg.

**Method confirmation**

No separate confirmation analysis was carried out as final detection was by GC/MS monitoring three ions. Quantification was performed using the two confirmation ions to assess the method confirmation technique. The same criteria for acceptability of recovery values determined from the primary quantification ion was used.

**Limit of detection (LOD)**

The LOD was assessed from the GC/MS response to the lowest concentration standard that was injected.

**Appendix 1**  
**Analytical Procedure CLE 333/153-04R**  
**Tolclofos-methyl: Analytical Method of Determination of Residues in Soil**

## APPARATUS, MATERIALS, REAGENTS AND SOLUTIONS

### Apparatus and glassware

• 500 mL Glass Jars	Fisher Scientific
• Buchner Funnel	Fisher Scientific
• 500 mL Separating Funnels	Fisher Scientific
• Round Bottom Flasks	Fisher Scientific
• Beakers	Fisher Scientific
• Volumetric flasks	Fisher Scientific
• Various pipettes	Fisher Scientific
• Short form pipettes	Fisher Scientific
• Bio-Beads S-X3 (200-400 mesh)	Bio Rad
• Filter Papers (QL100)	Fisher Scientific
• Analytical Balance (MT5)	Mettler Toledo
• Sample Balance (BD601)	Mettler Toledo
• Mechanical shaker (SM 25)	Edmund Bülher
• GPC Column (Superformance)	Merck
• HPLC pump (880 PU)	Jasco
• HPLC autosampler (ASPEC XL)	Gilson
• Fraction Collector (Medel 202)	Gilson
• GC/MS instrument (MD 800)	Fisons

Equivalent equipment may be used.

### Materials

The specification and supplier of the materials are as follows:

• Ultra pure water	Elgastat deionised
• Acetone	Rathburns, Glass Distilled
• Dichloromethane	Rathburns, HPLC grade
• Ethyl acetate	Rathburns, HPLC grade
• Cyclohexane	Rathburns, HPLC grade
• Toluene	Rathburns, Glass Distilled
• Sodium Chloride	AnalaR
• Sodium Sulphate	AnalaR

Equivalent or higher grade reagents/solvents may be used.

**Reagents and solutions [1a/b, 4b]**

**Cyclohexane: Ethyl acetate (1:1, v/v)**

Add 2500 mL of cyclohexane to 2500 mL ethyl acetate. Mix by shaking and degas.

**Preparation of Standard Solutions [1a/b, 4b]**

Each stock standard solution is prepared in acetone and stored frozen (nominally  $-20^{\circ}\text{C}$ ) and are assumed to be stable for at least 1 month. All standard solutions must be stored in glass at or below  $10^{\circ}\text{C}$  when not in use. Solutions should be allowed to warm to room temperature prior to use.

**Preparation of stock solutions [1a/b, 4b]**

In duplicate accurately weigh *ca* 10 mg (corrected for purity) of Tolclofos-methyl into a 10 mL volumetric flask and dilute to the mark using acetone to give standards of concentration 1000  $\mu\text{g/mL}$ .

Note - Duplicate solutions are prepared to check both the accuracy of the weighings and the solubility of the test articles. Confirmation is achieved by GC/MS quantification of appropriately diluted solutions. Only one stock standard is used for the preparation of both fortification and calibration solutions.

**GC/MS Calibration standards [1a/b, 4b]**

Serially dilute the primary stock solution (1000  $\mu\text{g/mL}$ ) with toluene to produce a 100  $\mu\text{g/mL}$  solution. This should be further diluted to produce appropriate calibration standards in the concentration range 0.01 to 0.50  $\mu\text{g/mL}$ .

**Fortification solutions [1a/b, 4b]**

Serially dilute the primary stock solution (1000  $\mu\text{g/mL}$ ) with acetone to produce solutions suitable for fortifying samples at the required level. For fortification of a control sample at the limit of quantification a 5.0  $\mu\text{g/mL}$  solution should be prepared.



## PROCEDURES

All work should be carried out under the minimum control categories listed under the safety procedures section. Additional controls are listed with the individual steps of the procedure.

### **Packing of GPC Column**

Allow approximately 50 g of Bio-Beads S-X3 (200-400 mesh) to swell overnight in 500 mL cyclohexane:ethyl acetate (1:1 v/v). Pour all the suspension into the column (capacity of <200 mL). As soon as the gel bed has settled free from air bubbles insert the plunger, lower it to the bed level and screw it into place. If the gel bed shrinks after prolonged use, the plunger must be adjusted accordingly.

### **Calibration of the GPC Column**

Elute as described below and determine the fraction to be collected to recover the analyte.

### GPC Conditions

Flow rate :	5 mL min <sup>-1</sup>
Eluent :	Cyclohexane : ethyl acetate (1:1 v/v)
Injection Volume :	5.0 mL
Program :	Collect 200 mL in 2 minute fractions

### **Determination of procedural recovery**

Procedural recovery will be determined by directly fortifying 50 g of soil with appropriate standard solutions of tolclofos-methyl and subjecting them to the analytical procedure. Fortification at the LOQ (0.01 mg/kg) of the method can be achieved by the addition of 0.5 mL of a 5 µg/mL solution. The amount of tolclofos-methyl recovered should be compared with the amount fortified onto the soil to calculate the procedural recovery.

### Analysis of the Soil

1. Determine the moisture content of the soil.
2. Weigh 50 g of soil into a 500 mL glass jar, add the appropriate amount of water (100 mL minus the water content of the samples), and allow to stand for 10 minutes.

$$\text{Volume of water to be added} = 100 - (W \times M/100) \text{ mL}$$

W = Weight of soil    M = Moisture content of soil in percent

3. Add 200 mL acetone and shake for 10 minutes.
4. Filter through a No.1 filter paper in a Buchner funnel.
5. Decant 200 mL of this solution into a 500 mL separating funnel, add 20 g Sodium chloride and shake vigorously. Add 100 mL DCM and shake again.
6. Discard the lower aqueous phase and collect the organic phase in a 500 mL round bottom flask, filter through sodium sulphate, and rinse the separating funnel and filter cake with a further 20 mL of ethyl acetate in duplicate.
7. Rotary evaporate the extract to dryness at 30°C, removing the last traces of solvent with a gentle stream of nitrogen.
8. Reconstitute in 10 mL cyclohexane: ethyl acetate (1:1 v/v), add a small amount of sodium sulphate, mix, allow to settle and decant the solution into a clean tube. 5 mL of this solution is required for injection onto the GPC column (Conditions for the GPC separation are shown below).
9. Rotary evaporate the fraction collected from the GPC to dryness at 30°C, and reconstitute in 5 mL toluene, for analysis by GC/MS.

### Gel Permeation Chromatography conditions

The following HPLC conditions are suitable for the GPC clean up of Tolclofos-methyl.

Column:                    50 g of Bio-Beads S-X3  
Eluent:                    Cyclohexane: Ethyl acetate (1/1, v/v)  
Flow rate:                5.0 mL min<sup>-1</sup>  
Injection volume:        5.0 mL

**Gas Chromatography/ Mass spectrometry conditions**

Analysis of samples should be carried out against at least 6 calibration standards. Extracts containing concentrations greater than the top calibration point should be diluted so that they fall within the calibration range. Each sample should be injected singly and interspersed with the calibration standards.

Instrumentation	Fisons MD800
Column:	DB 5MS (30 m x 0.25 mm, 0.25 $\mu$ m film thickness)
Column oven:	100°C for 1 minute. Ramp at 15°C/minute to 250°C and hold for 3 minute.
Injector:	250°C (Splitless)
Interface temperature	250°C
Detector temperature:	200°C EI positive
Carrier gas	Helium 10 psi
Injection Volume :	1 $\mu$ L
Ions monitored (SIR):	250, 265 and 267 Da Quantification on 265
Ionisation mode:	EI positive
Retention Times	Approximately 10 minutes

## CALCULATION OF RESULTS

The presence of tolclofos-methyl in a sample is confirmed if the resulting peak arising from the test sample has the same chromatographic retention time as a standard.

Residues of tolclofos-methyl are determined by following the interpolation of the sum of the resulting peak areas of the components of tolclofos-methyl, from the standard curve linear regression equation as follows:

Concentration of extract ( $\mu\text{g/mL}$ ) = (Area - intercept)/slope

The residue tolclofos-methyl in the test samples is calculated as follows:

$$\text{Residue (mg/Kg)} = \frac{\text{extract concentration } (\mu\text{g/mL}) \times V_{\text{End}} (\text{mL}) \times V_{\text{Ex}} (\text{mL}) \times V_{\text{R2}}}{V_{\text{R1}} (\text{mL}) \times V_{\text{R3}} \times \text{sample wt (g)}} \times D$$

- $V_{\text{Ex}}$  = volume of acetone and water added in extraction, plus water contained in sample in mL, less an empirical volume shrinkage of 5 mL.  
 $V_{\text{R1}}$  = portion of volume  $V_{\text{Ex}}$  used for partition  
 $V_{\text{R2}}$  = volume of solution of evaporation residue prepared for GPC  
 $V_{\text{R3}}$  = portion of volume  $V_{\text{R2}}$  injected for GPC  
 $V_{\text{End}}$  = final volume.  
 $D$  = dilution factor

Recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery (\%)} = \frac{A - C}{S} \times 100$$

Where:-

A = amount of tolclofos-methyl found in fortified soil (mg/Kg)

C = amount of tolclofos-methyl (or interference) found in control soil (mg/Kg)

S = amount of tolclofos-methyl added to fortified soil (mg/Kg)

### **METHOD CRITERIA**

The analysis will be considered successful only if the following criteria are met.

- A procedural recovery of 70 to 110% will be obtained for each batch of analysis.
- Control sample contains a concentration  $\leq 30\%$  the limit of quantification.
- At least 6 calibration standards will be used in the determine linearity of the calibration line.
- The calibration line will have a correlation coefficient ( $r$ )  $\geq 0.995$  or a coefficient of determination ( $r^2$ ) of  $\geq 0.99$ .
- All test samples will be within the range of the calibration standards.

**GENERAL HANDLING CONTROL CATEGORIES**

CATEGORY		CONTROL
Main	Division	Name and Specification
1		<b>GLOVES</b>
	a	Disposable latex
	b	Disposable nitrile
	c	Rubber gloves
	d	Specific type for job (see assessment giving details)
2		<b>PROTECTIVE CLOTHING</b>
	a	Laboratory coat or equivalent
	b	Disposable overalls
	c	Oversleeves
	d	Overshoes
	e	Plastic apron
3		<b>EYE/FACE PROTECTION</b>
	a	Safety glasses to BS 2092/2 or better
	b	Face shield to BS 2092/2 C or better
	c	Safety goggles to BS 2092/2 C or better
4		<b>ENGINEERING CONTROLS</b>
	a	Open bench in ventilated area
	b	Fume cupboard to BS 7258
	c	Laminar flow cabinet to BS 5295 Class 1
	d	Re-circulating fume chamber
	e	Radioisotope lab
	f	Biohazard lab
	g	Glove box
5		<b>RESPIRATORY PROTECTIVE EQUIPMENT</b>
	a	Disposable filtering facemask (HSE approved), i - organic vapour ii - dust iii - combination organic vapour/dust <b>MUST SPECIFY TYPE</b>
	b	Powered respirators/helmets with safety visor to BS 2092/2 C or better (HSE approved)
	c	Respirator with specified canister (HSE approved)
6		<b>SPECIFIC IMMUNISATION REQUIRED (GIVE DETAILS)</b>
7		<b>ALLERGIC PERSONS PROHIBITED (SPECIFY ALLERGY)</b>
8		<b>REFER TO MATERIAL SAFETY DATA SHEET</b>
9		<b>KNOWN OR SUSPECTED REPRODUCTIVE HAZARD TO EITHER SEX (must specify details)</b>
10		<b>POISON - ensure antidote is available and is within its expiry date (must specify details)</b>