INTRODUCTION

This document describes a procedure involving Gas Chromatography with Mass Spectrometry detection, capable of measuring Tolclofos-methyl residues in soil. Tolclofos-methyl is quantifiable at concentrations exceeding 0.01 mg/Kg using this procedure.

This procedure uses methodology from the multi-residue method for soil as described in the EU guidance document SANCO/825/000 and is based on the DFG S19 method.

Summary of the procedure
The method of analysis comprises of the following stages:

- Extraction with acetone /water.
- Partition with dichloromethane to remove the water.
- Clean up by Gel Permeation Chromatography.
- Quantification by GC/MS detection.

SAFETY PRECAUTIONS

The test article is an organo-phosphate, exposure to certain levels of these compounds can decrease levels of cholinesterase, and you should contact the Occupational Health Centre, to see if health surveillance is required. Operators should take the normal precaution of wearing gloves, laboratory coats and safety glasses when handling the test compound.

Safety assessments (Control of Substances Hazardous to Health, COSHH) have been made of those procedural steps involving preparation of solutions, reagents and extraction from soil samples. Appropriate safety codes have been included in the text and are defined in the section titled General Handling Control Categories.

The hazards and risks of the substances hazardous to health used in this method have been considered. Provided that the method is accurately followed and the control measures specified in the method are correctly used, there should be no foreseeable hazards to health.

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 Bűlher
• 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	Ratburns, 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°C) and are assumed to be stable for at least 1 month. All standard solutions must be stored in glass at or below 10°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 µg/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 µg/mL) with toluene to produce a 100 µg/mL solution. This should be further diluted to produce appropriate calibration standards in the concentration range 0.01 to 0.50 µg/mL.

Fortification solutions [1a/b, 4b]
Serially dilute the primary stock solution (1000 µg/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 µg/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^{-1}
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^{-1}
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 µm 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 µL
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 (µg/mL) = (Area – intercept)/slope

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

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

V_{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_{R1}	=	portion of volume V_{Ex} used for partition
V_{R2}	=	volume of solution of evaporation residue prepared for GPC
V_{R3}	=	portion of volume V_{R2} injected for GPC
V_{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 ≤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) ≥0.995 or a coefficient of determination (r^2) of ≥0.99.
- All test samples will be within the range of the calibration standards.

GENERAL HANDLING CONTROL CATEGORIES

CATEGORY Main	Division	CONTROL Name and Specification
1		GLOVES
	a	Disposable latex
	b	Disposable nitrile
	c	Rubber gloves
	d	Specific type for job (see assessment giving details)
2		PROTECTIVE CLOTHING
	a	Laboratory coat or equivalent
	b	Disposable overalls
	c	Oversleeves
	d	Overshoes
	e	Plastic apron
3		EYE/FACE PROTECTION
	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		ENGINEERING CONTROLS
	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		RESPIRATORY PROTECTIVE EQUIPMENT
	a	Disposable filtering facemask (HSE approved), i - organic vapour ii - dust iii – combination organic vapour/dust MUST SPECIFY TYPE
	b	Powered respirators/helmets with safety visor to BS 2092/2 C or better (HSE approved)
	c	Respirator with specified canister (HSE approved)
6		SPECIFIC IMMUNISATION REQUIRED (GIVE DETAILS)
7		ALLERGIC PERSONS PROHIBITED (SPECIFY ALLERGY)
8		REFER TO MATERIAL SAFETY DATA SHEET
9		KNOWN OR SUSPECTED REPRODUCTIVE HAZARD TO EITHER SEX (must specify details)
10		POISON – ensure antidote is available and is within its expiry date (must specify details)