48809102

201300025

TITLE

Independent Laboratory Validation of Valent Method
VP-38287 Tolclofos-methyl:
Determination of Tolclofos-methyl in Soil

TEST GUIDELINES

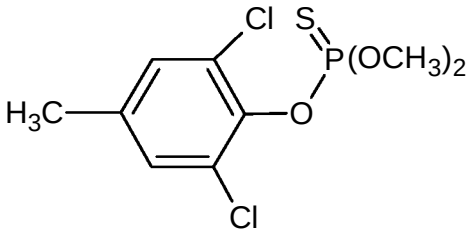
850.6100

STUDY COMPLETION DATE

2012-10-05

Analytical Reference Standard

Standard Name:	Tolclofos-methyl
Lot:	AS 2218d
IUPAC Name:	O-2,6-dichloro-p-tolyl O,O-dimethyl phosphorothioate
CAS Number:	57018-04-9
Sample Archive No.:	V-Arc-2150
Manufacturer's ID:	Valent Reference VTC-1269-22
Purity:	99.3%
Molecular Formula:	$C_9H_{11}Cl_2O_3PS$
Average Mass:	301.1
Molecular Structure:	



3.0 MATERIALS AND METHODS

3.1 Test Substance

Standard name: Tolclofos-methyl
Lot: AS 2218d
IUPAC name: O-2,6-dichloro-p-tolyl O,O-dimethyl phosphorothioate
CAS number: 57018-04-9
Sample Archive no.: V-Arc-2150
Manufacturer's ID: Valent Reference VTC-1269-22
Purity: 99.3%
Date of analysis: 29 February 2012
Expiration date: 29 February 2014
Storage conditions: Frozen

3.2 Test System

The test system used for the validation was a Penn series soil sample obtained from Baptistown, New Jersey, by Valent U.S.A. Corporation. The sample was stored at room temperature until needed for analysis.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in Valent Method VP-38287 for Trial 1 (Appendix 5, Appendix 1: Apparatus, Materials, Reagents and Solutions). Identical or equivalent apparatus and materials were used.

3.3.1 Equipment and Apparatus

Agilent 1100® HPLC System (Agilent Technologies)
Agilent 1260® Infinity Manual Injector (Agilent Technologies)
Agilent Manual Syringe, 10 mL, PTFE, Luer-Lok (Agilent Technologies)
Analytical Balance (Mettler Toledo)
GPC Column 500 mm × 40 mm, 25 mm id with 50 g of BioBeads S-X3 (LC Tech)
Laboratory Oven (Quincy LAB-Model 20 GC Lab Oven)
Manual Micro Pipettor 200 µL (VWR International)
Manual Micro Pipettor 1000 µL (VWR International)
Manual Micro Pipettor 5000 µL (VWR International)
N-EVAP (Organomation)
Recirculating Water Chiller (Polystat K8-S3)
Reciprocating Shaker 12 × 18 in. (Eberbach Corporation)
Refrigerator/Freezer (Nor-lake® Scientific)
Restek Rtx®-5MS 30 m × 0.25 mm id, 0.25 µm df
Rotavapor® R-124 (Büchi)
Thermo Scientific® AS3000 (Autosampler)

Thermo Scientific® DSQ II Mass Spectrometer
Thermo Scientific® Trace GC Ultra
Top-loading Balance (Mettler Toledo)
Ultrasonic Cleaner 5210 (Branson)
Ultrasonic Cleaner 5510 (Branson)
Whatman® No. 1 Filter Paper 90 mm ∅ × 100 circles

3.3.2 Reagents

Acetone (Pharmco-AAPER)
Cyclohexane (J.T. Baker®)
Dichloromethane (EMD)
Ethyl Acetate (EMD)
HPLC-grade Water (EMD)
Sodium Chloride (J.T. Baker®)
Sodium Sulfate (EMD)
Toluene (Pharmco-AAPER)

3.4 Experimental Design

3.4.1 Establishment of the Method

Prior to performing the ILV, the analyte retention times, instrument detection limits, and linearity of instrument responses to a range of analyte concentrations were determined, and the test system was verified as free of interferences at appropriate retention times.

3.4.2 Sample Validation Sets, Fortification, and Extraction Procedure

Sample Validation Sets
Each analytical set consisted of 13 samples: one reagent blank, two untreated controls, five untreated controls fortified with tolclofos-methyl at the Limit of Quantitation (LOQ; 0.010 ppm), and five untreated controls fortified with tolclofos-methyl at 10×LOQ (0.100 ppm).

Calibration standard solutions (0.020 to 0.500 µg/mL) and blanks were also included in each sample set.

The method detection limit (MDL) is 0.0200 ± 0.0040 µg/mL (measured) or 0.0060 ±0.0012 ppm (sample equivalent).

Fortification

The LOQ and 10×LOQ samples were fortified with 0.100 mL of the appropriate fortification standard solutions of tolclofos-methyl. The fortification standard solutions had a concentration of 5.00 µg/mL for the LOQ and a concentration of 50.0 µg/mL for the 10×LOQ.

Extraction and Workup

The following extraction steps were followed for each sample.

1. Using a top-loading balance, weighed 50 g of control sample into tared 1-L glass jars.
2. Added the appropriate amount of fortification solution to the sample.
    a. For the reagent blank and the untreated controls, added 0 mL.
    b. Added 0.100 mL of 5.00 µg/mL tolclofos-methyl fortification solution for the LOQ samples.
    c. Added 0.100 mL of 50.0 µg/mL tolclofos-methyl fortification solution for the 10×LOQ samples.
3. Added 80 mL of HPLC-grade water to each sample, and allowed to stand for 10 minutes. For the reagent blank, added 100 mL of HPLC-grade water to a clean 1-L glass jar.
4. Added 200 mL of acetone to each sample.
5. Using a reciprocating shaker, shook samples for 10 minutes.
6. For each sample, filtered through No.1 filter paper (90 mm ∅ ×100 circles) in a Buchner funnel, collecting the filtrate with a 500-mL glass filtering flask.
7. Decanted 200 mL of the filtrate into a 500-mL separatory funnel, added 20 g of sodium chloride, and shook vigorously. Added 100 mL of dichloromethane (DCM), and shook again.
8. Discarded the lower aqueous layer, and collected the organic layer in a 500-mL round bottom flask by filtering through sodium sulphate.
9. Rinsed the separatory funnel and filter cake with a further 20 mL of ethyl acetate in duplicate.
10. Rotary evaporated the extract to dryness at 30°C, removing the last traces of solvent with a gentle stream of nitrogen.
11. Reconstituted in 10 mL of (50:50) cyclohexane/ethyl acetate, added a small amount of sodium sulphate, mixed, and decanted the solution into clean tubes.
12. Stored samples in freezer (-15 ± 10°C) overnight.
13. Removed samples from storage, and equilibrated to room temperature on benchtop.
14. Equilibrated the GPC column before usage by eliminating all air bubbles.
15. For each sample, manually injected 5 mL onto the GPC column and collected an 8 minute fraction starting at 23 minutes and stopping at 31 minutes using a clean tube.
16. Evaporated samples under nitrogen using an N-EVAP with water bath at 30°C, and reconstituted in 5 mL of toluene.
17. Transferred a 1-mL aliquot of sample to autosampler vials to be analyzed by GC-MS.

3.4.3 Sample Processing and Analysis

The samples were processed and analyzed as described by Valent Method VP-38287 [1] for Trial 1.

3.4.4 Fortification and Calibration Standard Solutions Preparation

Trial 1
The primary stock solution for the reference standard was prepared by weighing approximately 10.0 mg of tolclofos-methyl into a tared 20-mL glass scintillation vial and diluting with 10 mL of acetone using a Class A glass 10-mL pipette. After addition of acetone, sonication was performed on the primary stock for 5 minutes. A fortification solution was prepared at a concentration of 50.0 µg/mL by adding an appropriate amount of the primary stock solution to a 10-mL volumetric flask and diluting to volume with acetone. A second fortification solution was prepared at a concentration of 5.00 µg/mL by measuring 1 mL of the initial fortification solution into a 10-mL volumetric flask and diluting to volume with acetone.

A secondary stock solution was prepared at a concentration of 100 µg/mL by adding an appropriate amount of primary stock solution to a 10-mL volumetric flask and diluting to volume with toluene. A second secondary stock solution was prepared at a concentration of 10.0 µg/mL measuring 1 mL of the initial secondary stock solution into a 10-mL volumetric flask and diluting to volume with toluene.

The calibration standard solutions were prepared at concentrations ranging from 0.020 to 0.500 µg/mL by adding an appropriate amount of secondary stock solution to a 10-mL volumetric flask and diluting to volume with toluene.

All solutions were stored in a freezer (-15 ± 10°C) when not in use.

3.5 LC-MS/MS Instrumentation

Instrumentation
Agilent 1100® HPLC System (Agilent Technologies)
Agilent 1260® Infinity Manual Injector (Agilent Technologies)
GPC Column 500 mm × 40 mm, 25 mm id with 50 g of BioBeads S-X3 (LC Tech)
Thermo Scientific® AS3000 (Autosampler)
Thermo Scientific® DSQ II Mass Spectrometer
Thermo Scientific® Trace GC Ultra

3.6 Data Acquisition and Reporting

Peak integration was performed by Xcalibur® software version 2.1.0.
The MS detector responses (peak area) for various injected standard concentrations were

used to generate an external calibration curve for the analyte of interest. The overall purpose for the external calibration curve was to display acceptable linearity ($r^2 \geq 0.99$) of the assigned calibration range. The recoveries of the analyte from the fortified samples were calculated by multi-point calibration.

Recovery results were computed for each sample. The equations used for quantification are presented in Appendix 2. A statistical treatment of the data includes the calculation of means, standard deviations (SD), RSDs as percentages (%), and the 95% confidence intervals. All statistics were calculated using Microsoft® Office Excel 2003.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment

The tolclofos-methyl ions m/z 265.00 (quantification), 267.00 (confirmation 1), and 250.00 (confirmation 2) were used to quantitate and confirm the analyte.

Prior to performing the ILV, the analyte retention times, instrument detection limits, and linearity of instrument responses to a range of analyte concentrations were determined, and the test system was verified as free of interferences at appropriate retention times.

4.2 Independent Laboratory Validation Trial Results

Tolclofos-methyl eluted as well-resolved chromatographic peak in toluene with a retention time of approximately 9.66 minutes.

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Trial 1 met all validation requirements, and thus can be considered successful.

4.3 Potential Interferences

No significant interference from the test system was observed.

4.4 Time Required for Analysis

It took one person approximately 16.0 hours to complete the extraction of one set of 13 samples (one reagent blank, two unfortified matrix control samples, and 10 fortified samples). Time of analysis was approximately 7.3 hours. To complete one set, including extraction and analysis, took approximately 2.9 days.

TABLE 7 GEL PERMEATION CHROMATOGRAPHY PARAMETERS FOR VALENT METHOD VP-38287 (TRIAL 1)

HPLC System:	Agilent 1100® HPLC System (Agilent Technologies)
GPC Column:	LC Tech GPC Column 500 mm × 40 mm, 25 mm id with 50 g of BioBeads S-X3
Injection Volume:	5.00 mL
Eluent:	(50:50) cyclohexane/ethyl acetate
Flow rate:	5.00 mL/minute

TABLE 8 GC-MS OPERATING PARAMETERS FOR VALENT METHOD VP-38287 (TRIAL 1)

GC-MS System:	Thermo Scientific® AS3000 Thermo Scientific® Trace GC Ultra Thermo Scientific® DSQ II Mass Spectrometer
Software:	Xcalibur® 2.1.0
GC Column:	Restek Rtx®-5MS 30 m × 0.25 mm id, 0.25 μm df

The following parameters were used for operation of the GC-MS system:

Autosampler method

Sampling

Sample volume:	2.00 μL
Plunger strokes:	10
Viscous sample:	No
Sampling depth in vial:	Bottom

Injection

Injection depth:	Standard
Pre-inj. dwell time:	4 seconds
Post-inj. dwell time:	0 seconds

Washes

Pre-inj. solvent:	A (toluene)
Pre-inj. solvent cycles:	3
Sample rinses:	2
Post-inj. solvent:	A (toluene)
Post-inj. solvent cycles:	5

MS method

Aquisition time:	GC Run Time
Cal gas:	Off
Reagent gas:	Off
Acquire profile:	No
Acq threshold:	0
Source temperature:	200°C

Segment 1

Start time:	7.00 minutes
Detector gain:	100000
Chrom filter width:	Off
Reagent gas flow:	Off

Scan Event 1

Mass defect:	0.00
Polarity:	POS

Use tune file emission current

Scan mode:	SIM
Mass: 267.00 (quantification):	Width: 1.00, Dwell Time: 50.0
Mass: 265.00 (confirmation 1):	Width: 1.00, Dwell Time: 50.0
Mass: 250.00 (confirmation 2):	Width: 1.00, Dwell Time: 50.0

Trace GC ultra method

Aquisition time

Use oven run time:	Yes

Oven method

Initial temperature:	100°C
Initial time:	1.00 minute
Number of ramps:	1
Rate #1:	15.0 deg/minutes
Final temperature #1:	250°C
Hold time #1:	3.00 minutes
Post run temperature:	Off
Enable cryogenics:	Off
Maximum temperature:	250°C
Prep run timeout (min.):	INF
Equilibration time:	0.50 minutes

Right SSL method

Base temperature:	On
Base temperature:	250°C
Mode:	Splitless
Split flow:	On
Split flow flow:	50 mL/minute
Splitless time:	1.00 minute
Surge pressure:	Off
Surge pressure:	0.44 psi
Surge duration:	0.00 minute
Constant purge:	On
Stop purge at:	0.00 minute

Right carrier method

Mode:	Constant flow
Initial value:	On
Initial value:	1.00 mL/minute
Initial time:	1.00 minute
Gas saver:	Off
Gas saver flow:	30 mL/minute
Gas saver time:	5.00
Vacuum compensation:	On

No left inlet

Right FID method

Base temperature:	Off
Base temperature:	50°C
Flame:	Off
Flameout retry:	Off
Ignition threshold:	0.5 mA
H_2:	Off
H_2 flow:	35 mL/minute
Air:	Off
Air flow:	350 mL/minute
Makeup gas:	Off
Makeup gas flow:	30 mL/minute

Right signal method

Offset:	Off
Offset value:	0
Autozero:	Off
Range:	1
Gain:	1
Negative polarity:	Off
Analog filter:	Off

No left detector

No aux detector

Aux zones

Aux 1 MS transfer line:	On
Aux 1 MS transfer line (C):	250
Aux 2:	Off
Aux 2 (C):	30

Run Table

External event #1 prep-run default:	Off
External event #2 prep-run default:	Off
External event #3 prep-run default:	Off
External event #4 prep-run default:	Off
External event #5 prep-run default:	Off

APPENDIX 2 CALCULATIONS

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Finnigan™, Xcalibur® software version 1.4 using linear regression. Further calculations were performed using the software Microsoft® Office Excel 2003.

The linear equation is expressed as:

$$y = MX + B$$

where

- y = Native peak area
- M = Calibration line slope
- X = Concentration of the reference standard in μg/mL
- B = Calibration line intercept

Trial 1

By means of the linear equation, the content of tolclofos-methyl in soil or recoveries can be calculated as follows:

$$\text{Extraction Concentration (μg/mL)} = \frac{y - B}{M}$$

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

$$\text{Residue (ppm)} = \frac{\text{Extraction Concentration (μg/mL)} \times V_{End}\,(\text{mL}) \times V_{Ex}\,(\text{mL}) \times V_{R2}\,(\text{mL}) \times D}{V_{R1}\,(\text{mL}) \times V_{R3}\,(\text{mL}) \times \text{Sample Wt (g)}}$$

where

- 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 = (300 mL)
- V_{R1} = Portion of volume V_{Ex} used for partition = (200 mL)
- V_{R2} = Volume of solution of evaporation residue prepared for GPC = (10 mL)
- V_{R3} = Portion of volume V_{R2} injected for GPC = (5 mL)
- V_{End} = Final volume = (5 mL)
- D = Dilution factor = (1)

Calculate recoveries using the following equation:

$$\text{Recovery (\%)} = \frac{(R)}{T} \times 100$$

where

R = ppm of target analyte found in fortified sample
T = Theoretical ppm in fortified sample

1. EPA REQUIREMENT

1.1. OCSPP Guideline 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation

2. INTRODUCTION

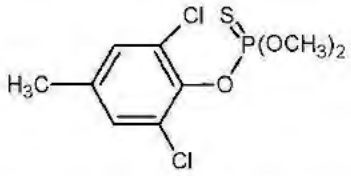
2.1. The objective of this study is to validate the Valent method VP -38287, "Tolclofos-methyl: Determination of Tolclofos-methyl in Soil." (See Appendix 1.)

2.2. The laboratory personnel, including the Study Director, have had no prior laboratory experience with the Valent method, "Tolclofos-methyl: Determination of Tolclofos-methyl in Soil". This study is designed to fulfill the requirements of the EPA's OCSPP Guideline 850.6100. In addition, this study will be conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

3. TEST MATERIALS

3.1. Analytical Reference Standard

Standard name:	Tolclofos-methyl
Lot:	AS 2218d
IUPAC name:	*O-2,6-dichloro-p-tolyl O,O-dimethyl phosphorothioate*
CAS number:	57018-04-9
Sample Archive no.:	V-Arc-2150
Manufacturer's ID:	Valent Reference VTC-1269-22
Purity:	99.3%
Date of analysis:	February 29, 2012
Expiration date:	February 29, 2014
Storage conditions:	Frozen
Molecular structure:	



The reference standard will be supplied by Valent Technical Center. Methods of synthesis, fabrication, and/or derivation of the reference standard are maintained by Valent Technical Center.

A record of reference standard storage conditions and records of weights and dilutions will be maintained and checked.

CPS Study ID Number: 12-CPS-006

Material Safety Data Sheets (MSDS) or other information necessary for proper and safe handling, shipping, and storage of the test materials will be provided by Valent Technical Center.

3.2. Reagents and Materials

All solvents shall be HPLC grade. Water shall be HPLC grade, or equivalent quality. Chemicals (grade and supplier) and materials will be documented in the raw data.

4. EXPERIMENTAL DESIGN

4.1. Establishment of the Method

Prior to performing the Independent Laboratory Validation (ILV), it will be necessary to establish the method, e.g., determine analyte retention times, instrument detection limits, linearity of instrument responses to a range of analyte concentrations, and verify that the test system is free of interferences at appropriate retention times. In general, Critical Path Services, LLC (CPS), will demonstrate that the method is under control before initiating the ILV.

Clarification of the method will be provided by the Valent Study Monitor or method developer if requested by the Study Director. All contacts made during the establishment of the method will be documented and presented in the final report.

4.2. Test System

The test system used for the validation will be an untreated Penn series soil sample obtained from Baptistown, New Jersey by Valent U.S.A Corporation. The soil characterization data will be included in the study records.

4.3. Sample Identification

Each weighed portion of sample used for validation will be assigned a unique code number/label within the analytical set during preparation and analysis. Minimally, documentation will also associate all samples with the study number and sample type.

4.4. Validation Sets

One set of samples is defined as one reagent blank, two untreated control samples, five untreated controls fortified with tolclofos-methyl at the Limit of Quantitation (LOQ; 0.01 ppm), and five untreated controls fortified with tolclofos-methyl at 10×LOQ (0.1 ppm). Fortifications will be made by addition of a diluted standard onto the control matrix via pipette or syringe.

raw data and in the final report. The validation will be considered acceptable if the mean recovery at each spiking level, at or above the LOQ, is between 70% and 120%. Data from matrix control samples will not be used to correct values from spiked matrix controls for recoveries. Interferences with peak areas that are <50% at the method detection limit (MDL) or limit of detection (LOD), are considered not significant. The control matrix should be essentially free of any interference at the retention time of the compound(s) of interest.

The recoveries will be expressed as a percentage of the analyte concentration(s) determined relative to the concentration(s) added. The mean and individual values for recoveries, the standard deviations and relative standard deviations (RSD) in fortified samples at each fortification level, and the 95% confidence interval for the recoveries at each fortification level will be reported. The RSD of replicate measurements of analyte concentrations should not exceed the target level of ≤ 20% for each fortification level at or above the LOQ.

Additional statistical specifications/suggestions are described in the method (e.g., linear regression with 1/X weighting). The regression may not be linear depending on the concentration range. The coefficient of determination (r^2) should be ≥ 0.99, respectively.

Results of the validation trial will be reviewed by the Study Director and the Study Monitor. If the results are determined to be acceptable, a final report will be written. If the validation trial is unsuccessful, the Study Director and the Study Monitor will consult with the method developer to clarify directions given in the method. This communication will be documented and presented in the final report. A second set of validation samples will then be analyzed.

If a second validation trial is conducted, the results will be reviewed by the same personnel. If the results are determined to be acceptable, a final report will be written. If the second validation trial is unsuccessful, the same process of consultation with the method developer will take place to further clarify directions given in the method. This communication will also be documented and presented in the final report. A third set of validation samples will then be analyzed.

If a third validation trial is conducted, the results will be reviewed by the same personnel. If the results are acceptable, a final report will be written. If the third validation trial is unsuccessful, method validation will be terminated, and a final report will be written.

APPENDIX 5 VALENT METHOD VP-38287

MRID: 48842102

201200167

TITLE

Tolclofos-methyl: Determination of Tolclofos-methyl in Soil

TEST GUIDELINE

860.1340

STUDY COMPLETION DATE

July 10, 2012

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:

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

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)