VALENT®

DATA SUBMISSION VOLUME 17

DATA REQUIREMENT:

200900102

96/46/EC (July 1996)
EU SANCO/825/00 rev.6
EU SANCO/3029/99 rev.4
(In Support of OPPTS 835.0000)

STUDY TITLE:

Validation of a Multi-Residue Method for the Determination of Tolclofos-methyl in Drinking Water

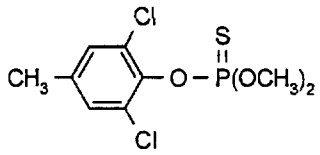
November 12, 2001

2 MATERIAL AND METHODS

2.1 TEST ITEM

All information concerning the test item were provided by the sponsor or were taken from the e-Pesticide Manual (12th Ed.) Ver. 2.0

2.1.1 TOLCLOFOS-METHYL

Common Name:	Tolclofos-methyl
Chemical Name (IUPAC):	*O*-2,6-dichloro-*p*-tolyl *O*,*O*-dimethyl phosphorothioate equivalent to *O*-2,6-dichloro-4-methylphenyl *O*,*O*-dimethyl phosphorothioate
Code No.:	S-3349
CAS No.:	57018-04-9
Structural Formula:	
Molecular Formula:	$C_9H_{11}Cl_2O_3PS$
Molecular Mass:	301.1 g/mol
Lot:	00404SG
Purity:	99.8%
Storage:	Store in a cool, dry and well-ventilated place
Effective Until:	April 01, 2003
Safety Precautions:	routine hygienic procedures will be sufficient to assure personnel health and safety.

2.2 TEST SYSTEM

The drinking water (regular tab water) was collected from the village Itingen, Switzerland. It was specified as follows.

Type	Drinking Water
Source	RCC Ltd Zelgliweg 1 CH-4452 Itingen/Switzerland
Date of sampling	October 09, 2001
Volume	About 25 L
Dissolved organic carbon (DOC) [mg C/L]	6.9
pH	7.00
Residue of evaporation [mg/L]	690
Hardness [°dH]	25

During the laboratory phase the untreated control samples were stored at room temperature at RCC Ltd.

2.3 REAGENTS AND APPARATUS

All reagents were of analytical, residue analytical or HPLC grade.

REAGENTS & APPARATUS	SUPPLIER	ARTICLE NO.
Acetone	Baker	9254
Methanol	Baker	8402
Water (distilled)	in-house prepared	
Dichloromethane	Baker	9264
Toluene	Baker	9336
Sodium sulphate (anhydrous)	Baker	0313
Mega bond Elut, C18 (2g/12mL)	Varian	12256015
Measuring flasks	various sizes	
Volumetric pipettes	various sizes	
Measuring cylinders	various sizes	
Laboratory bottles	1000 mL	
Funnels	various sizes	
Round bottom flasks	50 mL	
Vacuum Manifold	Supelco	
Hamilton syringes	various sizes	
Rotary evaporator	Büchi	011
Ultra sonic bath	Bender and Hobein	220
Analytical balance	Mettler	UMT-2

2.4 STANDARD SOLUTIONS

For measurement: 15.3599 mg of tolclofos-methyl (99.8 %) were dissolved in 15.33 mL acetone to obtain a stock solution of 1000 µg tolclofos-methyl/mL (100 %) using an ultra sonic bath for about 5 min. Defined volumes of this stock solution were diluted using acetone to obtain further stock solutions having a concentration of 100, 10 and 1 µg of tolclofos-methyl/mL. Defined volumes of the stock solution 100 µg of tolclofos-methyl/mL were diluted using toluene to obtain standard solutions in the range of 0.01 to 10.0 µg of tolclofos-methyl/mL.

The first stock solution was kept in the dark at about –20 °C (in a freezer), the other stock solutions and standard solutions were kept in the dark at about 4 °C (in a refrigerator).

Quantification was performed using Flame Photometric Detector (FPD; P-mode).

Tolclofos-methyl calibration standards were injected concurrently with the tolclofos-methyl sample injections for the determination of the retention time and for preparing of the standard calibration curve. Each analytical run was started and ended with a tolclofos-methyl calibration standard injection. A maximum of two sample injections were made between tolclofos-methyl calibration standard injections.

2.5 FORTIFICATION

To demonstrate the validity of the method used untreated samples were fortified with different volumes of tolclofos-methyl fortification solutions.

Fortification levels:

0.1 µg/L: 100 µL of the fortification solution (1.0 µg/mL*) were added to 1000 mL of the untreated water sample.

1.0 µg/L: 100 µL of the fortification solution (10 µg/mL*) were added to 1000 mL of the untreated water sample.

*: 10 and 1.0 µg/mL fortification solution dissolved in acetone

2.6 ANALYTICAL METHOD

The used analytical method was the multi-residue method F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung), quoted in the guidance document SANCO/825/00.

A SPE C18 column was conditioned by passing 10 mL methanol and then 10 mL water (distilled) under suction, using a vacuum manifold. 1000 mL drinking water sample were transferred into a 1000-mL laboratory flask. Samples were fortified at this stage. The sample was sucked through the SPE C18 column at a flow rate of 5-10 mL/minute. The SPE C18 column was dried by suction of air through the column for 1-2 minutes. Tolclofos-methyl was eluted with 10 mL dichloromethane in 1-2 minutes. This step was repeated with additional 10 mL dichloromethane. The dichloromethane (20 mL) was collected and filtered over a funnel containing about 5-10 g anhydrous sodium sulphate into a 50 mL round bottom flask. The sodium sulphate was washed with 10-20 mL of dichloromethane. The dichloromethane was evaporated to about 1-2 mL under vacuum at about 30-40 °C using a rotary evaporator. After removing of the remaining solvent using a gentle steam of nitrogen, the residue was dissolved in 1.0 mL toluene using an ultrasonic bath.

All the samples were injected in duplicate into a GC.
The concentrations of tolclofos-methyl were determined after GC separation using an Flame Photometric Detector (FPD; P-mode). Peak verification was performed by GC/MS.

2.7 GC CONDITIONS

2.7.1 PRIMARY METHOD

Gas chromatograph:	Hewlett-Packard 5890
Auto sampler:	Hewlett-Packard 7683A
Software:	EZ Chrom Chromatography Data System Ver. 6.6
Column:	30 m x 0.25 mm DB-1 (0.25 µm) pre-column: deactivated fused silica (1.5 m x 0.53 mm)
Carrier Gas:	Helium, flow: 110 kPa (Total Flow: about 30 mL/min)
Temperatures:	Injector: oven track on Oven: 105 °C (initial time: 2 min rate: 10 °C/min to 230 °C for 6 min) Detector: 250 °C
Injection:	2 µL; on column
Detector:	Flame Photometric Detector (FPD; P-mode)
Retention Time:	13.1 min

2.7.2 CONFIRMATORY METHOD

Gas chromatograph:	Hewlett-Packard 6890
Auto sampler:	Hewlett-Packard 7683
Column:	30 m x 0.25 mm Rtx-1 (0.25 µm)
Carrier Gas:	Helium, flow: 1.4 mL/min (constant flow)
Temperatures:	Injector: 250 °C Oven: 105 °C (initial time: 2 min rate: 10 °C/min to 230 °C for 6 min)
Injection:	1 µL; splitless
Detector:	MS 5973; ChemStation Ver. B.01.00, Hewlett Packard Ionization Mode: EI, negative Electron Energy: 70 eV EMV: about 1700 V Scan Mode: Fullscan (m/z 10-550) & SIM (m/z: 265 - quantifier & 267, 250 & 125 qualifier) Scan time: 3.77 s Ion Source Temperature: 230 °C Transfer Line Temperature: 300 °C Quadrupole Temperature: 150 °C
Retention Time:	11.2 min

2.8 EVALUATION OF RESULTS

Injected samples were quantified by peak area with reference to the calibration curve. The latter was obtained by correlation of the peak area (mean value in counts from duplicate injection) of the analytical standards with their corresponding concentration in µg/mL.
The correlation is performed using a least squares fit of a non linear function (equation 1).
A non-linear function was chosen for calculation of the calibration curve as the relative deviation between $counts_{measured}$ and $counts_{calculated}$ was found to be higher than 20 % for a linear function due to the large range (LOD up to 100 times LOD) of the calibration curve.

$$Y = a \cdot x^b \quad \text{or} \quad x = (Y/a)^{1/b} \qquad (1)$$

where

Y = Peak area of injected sample (mean value in counts from duplicate injection)

x = Tolclofos-methyl in injected sample [µg/mL]

a = Constant factor

b = Exponent

The residue of tolclofos-methyl in the samples is calculated according to equation 2.

$$R = \frac{X \cdot V_F}{W} \qquad (2)$$

where

R = Residue of tolclofos-methyl in sample material [µg/L]

X = Concentration of injected sample [µg/mL] calculated from equation 1

V_F = Final volume [mL]

W = Sample volume [L]

The recovery of tolclofos-methyl in the samples is calculated according to equation 3.

$$Rec = \frac{R \cdot 100}{F} \qquad (3)$$

where

Rec = Recovery of tolclofos-methyl [%]

R = Residue of tolclofos-methyl in sample material [µg/L]

F = Fortification level [µg/L]

3.3 SPECIFICITY

The method allows the determination of tolclofos-methyl in drinking water. There was no interference with other substances observed at the retention times of tolclofos-methyl

3.4 LIMIT OF DETECTION (LOD)

The limit of detection was found to be about 0.01 µg/L. The LOD was estimated from the lowest calibration standard concentration used (0.01 µg/mL).

3.5 ANALYTICAL SAMPLE STORAGE PERIOD

The maximum time between starting the extraction and measurement was 1 day (for GC/MS confirmatory: 8 days). Extracts were stored in a freezer before GC measurement.

APPENDIX II

COPY OF THE STUDY PLAN

2 MATERIALS AND METHODS

2.1 TEST ITEM

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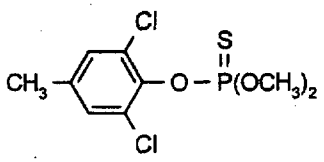
CAS No.:	57018-04-9
Structural Formula:	
Molecular Formula:	$C_9H_{11}Cl_2O_3PS$
Molecular Mass:	301.1 g/mol
Lot:	00404SG
Purity:	99.8%
Storage:	Store in a cool, dry and well-ventilated place
Effective Until:	April 01, 2003
Safety Precautions:	routine hygienic procedures will be sufficient to assure personnel health and safety.

2.2 TEST SYSTEM

The test system drinking water was selected by the sponsor in accordance with the quoted guidelines.
The origin, date of sampling, the pH-Value, the dissolved organic carbon content (DOC), hardness and the residue of evaporation will be determined and described in the final report to characterise the test system.

2.3 ANALYTICAL METHOD

The analytical method to be used during this study will be one of the multi-residue analytical methods and will be described in detail in the final report.

2.4 CALIBRATION PROCEDURE

The instrument calibration standards for this study will be prepared by making appropriate dilutions of a stock standard solution to produce analytical standards. The standard solutions should bracket the working range. The lowest standard of the working range should be at least half the concentration of the lowest sample concentration expected.

Calibration standards will be injected in duplicate concurrently with the sample injections for the determination of the retention time and of the standard calibration curve. Each analytical run will begin and end with a standard injection. A maximum of two injections will be made between standard injections.

For Quantification a e.g. GC/FPD will be used. Samples will be quantified after separation. Details will be given in the final report. Peak identity (specificity) will be determined using e.g. GC/MSD on recovery samples in comparison to analytical standard solutions.

2.5 EXPERIMENTAL DESIGN

The following procedure will be undertaken:
Known amounts of fortification solutions of tolclofos-methyl will be added to untreated drinking water to achieve the following fortification levels:

0	µg/L	Control
0.10	µg/L	Limit of Quantitation (LOQ)
1.00	µg/L	10 times of LOQ (upper working range)

At least two control samples and five samples of each fortification level for drinking water will be analyzed during this study.

Quantification will be performed using a e.g. GC/FPD.
Peak identification will be performed using a e.g. GC/MSD.
For the peak confirmatory the following procedure will be undertaken:
One untreated sample, three samples fortified at 0.10 µg/L and one sample fortified at 1.00 µg/L will be measured by e.g. GC/MSD.

The percent recovery of tolclofos-methyl will be determined as follows:

$$\%\,\text{Recovery} = \left[\frac{\text{Found}\,(\mu g/L)}{\text{Fortification Amount}\,(\mu g/L)}\right] * 100\,(\%)$$

The results will be interpreted as follows:

The mean recovery should be within 70 and 110% of the nominal concentration. Its relative standard deviation should be ≤ 20%.
Control values at the retention time of tolclofos-methyl should be less than 30 % of the LOQ.

If the matrix requires modification to the procedures, the changes will be reported to the Sponsors representative and will be detailed in the final report.

2.6 STATISTICAL METHODS

The mean recovery and relative standard deviation will be calculated for all samples as well as for each single fortification level and included in the final report.

2.7 EVALUATION OF RESULTS

The quantity of the residues in each sample shall be determined by comparison to a calibration curve consisting at least 5 points, one of these shall be at the quantification limit of the method. The calibration curves should bracket the working range. If the concentration of a sample is beyond the range of the calibration curve it will be diluted appropriately and rerun.

Calibration curves will be generated along with every sample series. Peak heights or peak areas are plotted versus µg/mL analytical standard. The concentration of tolclofos-methyl in drinking water samples is then determined from the standard curve.

All results will be calculated in accordance to the analytical method described above.

Repeatability (Precision and Accuracy), Specificity (including confirmatory method), Linearity, Limit of determination and Limit of detection (along with its definition, such as the theoretical concentration in water specimen related to the lowest concentration of calibration curve) will be described in the final report.

2.8 SAMPLE IDENTIFICATION

All samples and/or vessels containing sample aliquots will be labeled individually with RCC study number and further information regarding the test item and/or sample to assure an unmistakable identification.