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TITLE

Independent Laboratory Validation of the
Analytical Method VP-38290 Tolclofos-methyl:
Determination of Tolclofos-methyl in Drinking Water

TEST GUIDELINES

850.6100

Abbreviation	Definition
GC/MS	gas chromatography with mass spectrometry
GLPs	Good Laboratory Practices
HPLC	high performance liquid chromatography
i.d.	inside diameter
ID	identification
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	liter
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantitation
m	meter
m/z	mass to charge ratio
µg	microgram
µL	microliter
MDL	method detection limit
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mmol	millimole
mol	mole
MS	mass spectrometry
MW	molecular weight
NA	not applicable
NCL	North Coast Laboratories, Ltd.
ND or nd	nondetect (below limit of detection)
ng	nanogram
No.	number
ppb	parts per billion or nanograms per gram or micrograms per kilogram
ppm	parts per million or microgram per gram or milligrams per kilogram
psi	pounds per square inch
QAU	quality assurance unit
q.s.	add a sufficient quantity to bring to the final volume from Latin quantum sufficiat or satis, as much as suffices;
r	correlation coefficient
R^2 (or r^2)	coefficient of determination; square of correlation coefficient
RSD	relative standard deviation
RT	retention time

Abbreviation	Definition
s	standard deviation
USDA	United States Department of Agriculture
UV	ultraviolet
v	volume
vol.	volume
wt	weight

This study is designed to fulfill the requirements of EPA's Ecological Effects Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation [3].

3.0 MATERIALS AND METHODS

3.1 Test / Reference Substance

The test/reference substance was shipped from Valent U.S.A. Corporation, Dublin, CA to NCL. The tolclofos-methyl was received on August 1, 2012. The test/reference substance that was used for the validation are described as follows:

Common name:	tolclofos-methyl
Chemical name:	O-2,6-dichloro-p-tolyl O,O-dimethylphosphorothioate equivalent to *O*-2,6-dichloro-4-methylphenyl *O,O*-dimethylphosphorothioate
CAS numbers:	57018-04-9
Batch identification:	KC-1374-01a
Stated purity:	99.3%
Expiration date:	February 29, 2014
Storage conditions:	frozen < - 10 ºC

Valent U.S.A. Corporation, Dublin, CA maintains the characterization and stability data for the test/reference substance.

Stock standard solutions were prepared from the neat test/reference substances for use in the preparation of fortification solutions and instrument calibration solutions. All standard solutions were prepared as per the method. The 1000 ppm stock standard was stored frozen and the remaining stock standard solutions were stored refrigerated when not in use. Section 3.5.4 describes of the preparation of the stock solutions, and section 4.7.2 provides example calculations.

3.2 Equipment and Reagents

3.2.1 Solvents and Reagents

Acetone, Omnisolve High Purity
Methanol, Sigma Chromasolv, HPLC grad
Distilled water, prepared by North Coast Laboratories
Dichloromethane, Omnisolve High Purity
Toluene, Omnisolve High Purity
Furnaced sodium sulfate, Macron Chemicals 10-60 mesh granular
Sodium thiosulfate (80 mg/L), JT Baker p/n 3954-01
Hach Pocket Colorimeter™ kit
DPD total chlorine powder pillows, p/n 21056-28

3.2.2 Apparatus

A list of apparatus used in the method validation trial is shown below. Similar equipment from other suppliers may also be used.

Mettler AB204-2 Analytical Balance
HACH Pocket Colorimeter™ kit, p/n 46700-00
1-L graduated cylinders
1-L amber glass bottles, ESS precleaned 34-oz, p/n 1000-0150-PC
Büchner funnels, 7 cm or 9 cm
50 mL round bottom flasks
SPE reservoir adaptors
Organomation Associates N-EVAP™112 nitrogen evaporator
Pipettors, Automatic - capable of accurately dispensing volumes of 1.0 µL to 50 mL
Pipettes, Graduated or Volumetric suitable of accurately delivering 0.5 to 10 mL
Pipettes, Pasteur, disposable
Brinkmann Buchi 121 Rotary Vacuum Evaporators
Branson 1510 Sonicator
Supelco Visiprep 24™ DL SPE Vacuum Manifold
SPE cartridges, Agilent C18 12 cc 2 gm, Catalog No. 12256015
Screw Thread Amber 15-mL glass vials with Teflon-lined screw-caps
CRS 1.8-mL clear screw top standard mouth glass autosampler vials with caps
Whatman 41 ashless 110 mm filter papers, p/n 1441 110

3.2.3 GC/MS Instrumentation

Analysis was performed using a gas chromatograph with a mass specific detector (GC/MS). The following equipment was used:

HP 5890 Series II Gas Chromatograph with a HP 5972 Mass Specific Detector
HP 7673 Autosampler and Injector
Restek Column Rxi™ -XLB, 30 m, 0.25 mm ID, 0.25 µm df

3.3 Safety and Health

This method was performed by trained personnel who acted in accord with the analyte material safety data sheet (Appendix 2) that documents the hazards associated with the use of this chemical.

3.4 Test System and Sample Storage

The matrix, bulk control water, used for the validation was collected from a private well. The sample was collected in four 4-L amber glass bottles. The water sample received a unique North Coast Laboratories, Ltd. (NCL) sample number. The sample was not stored after collection as the extraction was performed on the day the sample was collected.

The control water sample used in this ILV was collected from a private drinking water well in McKinleyville, CA for Valent Study VP-38291 (labeled as “laundry room tap (control)” with Sample Numeric ID of 1209235-01A).

3.5 Analytical Method and Method Establishment

3.5.1 Principle of the Method

The water samples were prepared by passing them through a conditioned SPE C18 cartridge. The analytes were eluted from the cartridges using dichloromethane which was then dried and concentrated using a rotary evaporator. The samples were blown to dryness and dissolved in toluene. The extracts were analyzed by GC/MS.

3.5.2 Limits of Quantitation

The limit of quantitation (LOQ) for tolclofos-methyl was 0.10 µg/L (ppb). The LOD was estimated to be approximately half of the LOQ (0.05 µg/L).

3.5.3 Validation Sample Set

The validation set consisted of the following samples:

Instrument calibration working standards
One reagent blank
Two unfortified control samples
Five samples fortified with tolclofos-methyl at 0.10 µg/L (ppb; 1xLOQ)
Five samples fortified with tolclofos-methyl at 1.0 µg/L (ppb; 10xLOQ)

3.5.4 Preparation of 1000 PPM Tolclofos-Methyl Standard Solutions

Section 4.7.1 provides an example calculation describing the preparation of the 1000-ppm stock standard solution.

An aliquot of 0.0152 g of analyte was weighed out into an amber glass vial. The appropriate amount of acetone was added to the vial to yield a 1000 µg/mL standard solution. The 1000 ppm tolclofos-methyl standard was stored frozen and the other concentrations of the tolclofos-methyl standard solutions were stored refrigerated at 2 to 6 ºC.

3.5.5 Preparation of Tolclofos-methyl Fortification and Calibration Standard Solutions

A 100 µg/mL standard solution was prepared by combining 1.0 mL of the 1000 µg/mL standard into a 10-mL volumetric flask and bringing the solution up to the 10-mL final volume with acetone. From this 100 µg/mL standard, a 10 µg/mL standard solution was

prepared by combining 1.0 mL of the 100 µg/mL standard into a 10-mL volumetric flask and bringing the solution up to the 10-mL final volume with acetone. From this 10 µg/mL standard, a 1.0 µg/mL standard solution was prepared by combining 1.0 mL of the 10 µg/mL standard into a 10-mL volumetric flask and bringing the solution up to the 10-mL final volume with acetone.

3.5.6 Preparation of Tolclofos-Methyl Instrument Calibration Working Standard Solutions

Seven levels of instrument calibration working standards were prepared (0.5x, 1x, 2x, 5x, 10x, 20x and 50xLOQ) and named with respect to the concentration in the fortified samples (see the table below and the example calculations presented in section 4.7.3). The standards described in the table below were brought up to a final 1.0-mL volume with toluene.

Tolclofos-methyl Instrument Calibration Working Standard Solutions				
Tolclofos-methyl Concentration Relative to the Sample	Concentration Tolclofos-methyl Stock Solution (ng/µL)	Volume of Tolclofos-methyl Stock Solution (µL)	Final Volume (mL)	In-solution Tolclofos-methyl Concentration (ng/mL)
0.5xLOQ = 0.05 µg/L	10	5	1.0	50
1xLOQ = 0.10 µg/L	10	10	1.0	100
2xLOQ = 0.20 µg/L	10	20	1.0	200
5xLOQ = 0.50 µg/L	10	50	1.0	500
10xLOQ = 1.0 µg/L	10	100	1.0	1000
20xLOQ = 2.0 µg/L	10	200	1.0	2000
50xLOQ = 5.0 µg/L	10	500	1.0	5000

3.5.7 Preparation of Samples

The control water sample used in this ILV was collected by North Coast Laboratories from a private drinking water well in McKinleyville, CA.

3.5.8 Preparation of Fortification Samples

A 100-µL aliquot of the 1.0 ng/µL, (ppm) standard solution was applied to each replicate LOQ fortification. A 100-µL aliquot of the 10 ng/µL, (ppm) standard solution was applied to each replicate 10xLOQ fortification. The fortified samples were allowed to sit for at least 5 minutes before proceeding with the extraction. Section 4.7.2 presents the calculations used to prepare the fortified samples.

3.5.9 Extraction Procedure

The extraction procedure was performed as written in the method Valent Method VP-38290. The method is incorporated into the Study Protocol which is presented in

Appendix 3 page 19 Section 2.6 of the Protocol. The extraction procedure as followed at NCL is described below:

Tolclofos-methyl residues were extracted from the aqueous samples using solid phase extraction. The SPE C18 cartridges were equipped with reservoir adaptors and were placed in two connected vacuum manifold boxes to accommodate thirteen cartridges with attached reservoir adaptors. The cartridges were conditioned by passing 10 mL of methanol and then 10 mL of distilled water under vacuum through each cartridge. Each sample was measured into a 1-L graduated cylinder and then poured through funnels into labeled amber glass small-mouth bottles. Samples were fortified at this stage with appropriate fortification solution and inverted five times to mix. About 6-7 mL of sample were added to the appropriate cartridge. The adaptor and reservoir were then fitted on to the cartridge. The reservoirs were filled with appropriate sample, and drawn through the SPE C18 cartridge at a flow rate of 5-10 mL/minute. The SPE C18 cartridges were dried by vacuum through the cartridges for 1-2 minutes. The analyte was eluted from the SPE with 10 mL of dichloromethane in approximately 1-2 minutes. The eluate was received into an intermediate vessel (15-mL glass vial) that fit into the manifold box. The eluate was poured through filter paper/funnels containing furnaced sodium sulfate into labeled round-bottom flasks. This step was repeated with an additional 10 mL of dichloromethane. The sodium sulfate was rinsed with 15 mL of dichloromethane. At this point, insufficient time prevented completion of the extraction. The dried extracts were stored frozen overnight in the capped round-bottom flasks. The following day, after bringing the extracts to room temperature, the dichloromethane was evaporated to about 1-2 mL under vacuum on the rotary evaporator at 30-40ºC at approximately 15-20 mm in. Hg vacuum. After removing the remaining solvent using a gentle stream of nitrogen using a blowdown manifold, the residues were dissolved in 1.0 mL of toluene with sonication for approximately 30-50 sec. The samples were then transferred to autosampler vials using Pasteur pipettes.

3.5.10 GC/MS Operating Parameters

3.5.10.1 Conditions

Column:	Restek Rxi™ -XLB, 30 m, 0.25 mm ID, 0.25 µm df
Injection volume:	1 µL
Temperature program:	105ºC, hold 2 min, to 240ºC at 15 ºC/min, hold 5 min.
Expected Retention time:	tolclofos-methyl 10.6 min
Target Fragment m/z:	265.0
Qualifier Fragments m/z:	Q1= 267.0, Q2 = 125.0 and Q3 = 250.0

Copies of example chromatograms are included in the Figures Section and the operating parameters for the GC/MS are included in Appendix 4.

3.5.10.2 Calibration Procedures

Instrument calibration working standard solutions were prepared as described in section 3.5.6. Seven instrument calibration working standards were positioned within the

analytical batch sequence, bracketing no more than two samples between standards. The standard concentrations were 0.5x, 1x, 2x, 5x, 10x, 20x, and 50xLOQ for each analyte (0.05, 0.10, 0.20, 0.50, 1.0, 2.0, and 5.0 µg/mL, ppb, respectively, relative to the sample). The ChemStation software generated a linear non-weighted calibration curve and the associated coefficient of determination (r^2) for tolclofos-methyl by plotting the analyte peak area count versus analyte concentration. The correlation coefficient (r^2 for each analyte was required to be greater than or equal to 0.995. The equation generated by ChemStation was verified using Microsoft® Excel.

3.5.11 Data Acquisition and Reporting

The analysis of samples by GC/MS generated electronic data via the ChemStation Software interface. The hardware, security, and report configurations were set through the software modules. These modules also enabled instrument tuning, provided a mechanism for setting the acquisition methods and batches, processed the data, and quantified the data.

The ChemService Software generated raw data files from which the data were tabulated, and the chromatograms and the standard curves were generated. The data and the resulting descriptive statistics are summarized in Table 1 (Tables section) and in Appendix 5. Representative chromatograms are presented in the Figures section.

3.5.12 Qualifier Ions

Qualifier ions were used to test the quantifier ion (m/z 265) using qualifier ion ratio tests. Data were collected for three qualifier ions (m/z 267.0, 125.0, and 250.0). Qualifier ions were set for 20% tolerance of the expected ratio of qualifier to quantifier ion response.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment

4.1.1 Specificity

The analysis of tolclofos-methyl by GC/MS was highly specific and provided signals that effectively identified the analyte in the water. There were no significant interfering peaks in the range of the quantifier ion.

4.1.2 Calibration/Linearity

The calibration curves were generated from the seven standards at concentrations of 0.5x, 1x, 2x, 5x, 10x, 20x and 50xLOQ (0.05, 010, 0.20, 0.50, 1.0, 2.0 and 5.0 µg/mL, ppb, respectively, relative to the sample).

4.1.3 Accuracy/Precision

The descriptive statistics indicate the accuracy of the method and are presented in as the mean, the standard deviation, the range of values, and the 95% confidence interval for the percent recoveries of five replicate fortifications at each of two fortification levels and overall [5]. Individual recoveries were each within the acceptance interval of 70 to 120% across all analytes and fortification levels.

The precision of the method is indicated by the relative standard deviation (RSD) over the five replicate fortifications at each of the two fortification levels and overall. The RSD of replicate measurements was well below the target level of less than or equal to 20%.

4.2 Independent Laboratory Validation Trial Results

The individual water sample recovery results for the set which met the method acceptance criteria and were analyzed utilizing extra conditioning shots are shown in

4.3 Potential Interferences

No peak within the window of the expected retention time of tolclofos methyl (10.6 min ± 25 sec) was noted in either control #1 and control #2 water subsamples. A peak was seen in the reagent blank for tolclofos methyl. The area counts of the peak observed were below the limit that is considered negligible [3], *i.e.* < 50% of the area count of the 0.050 µg/L calibration standard.

4.4 Time Required for Extractions and Analyses

North Coast Laboratories, Ltd. laboratory personnel required approximately 10 hours to complete a set of 13 samples.

4.5 Potential Problems, Hazards, or Precautions

The method did not provide sufficient detail about the setup and procedure used to collect dichloromethane eluant from the SPE cartridges. Additional information regarding the size and size and type of receiving vessels inside the manifold box, the specific filter paper to be used, and whether or not the sodium sulfate should be pre-rinsed are examples of helpful information that could be included in the method description so that extractions are performed by different technicians or different laboratories in a consistent manner.

It would be helpful to include steps in the extraction method where the procedure could be stopped overnight if necessary. Since the sample preparation took longer than 8 hours to complete, the extraction was stopped after the methylene chloride was dried with sodium sulfate, and the extracts were stored in the capped round-bottom flasks overnight in a freezer. The recoveries were acceptable, indicating this was a reasonable stopping point.

Residual chlorine in the water sample affected the recovery of the tolclofos-methyl. The method was initially tested for interferences using tap water. This test yielded very low recoveries of the tolclofos-methyl. A second test was performed on dechlorinated tap water. The water was dechlorinated with 320 mg of sodium thiosulfate. Residual chlorine was not detected using the Hach Pocket Colorimeter™ kit.

The procedure for dealing with chlorinated tap water should be addressed in the method as drinking water matrices are often treated with chlorine.

The tolclofos-methyl response was affected by the amount of “conditioning” that occurred prior to the start of the analytical sequence. Prior to starting the analytical sequence, several standards and treated control samples were analyzed followed by a 0.50 µg/L continuing calibration verification standard (CCV). The first five analytical sets showed varying recoveries for this CCV. The sixth analysis was “conditioned” with additional injections of standards and fortified samples. This variability was most likely due to active sites on our analytical system inlet. It may help to change from a splitless injection to a direct column injection using an inert or deactivated liner. This would help reduce the exposure of the tolclofos-methyl to active sites during the analysis.

Dechlorination of Water:

- It appears residual chlorine causes a loss of tolclofos-methyl.
- Initially the tap water was dechlorinated with sodium thiosulfate but the recoveries were still low. This may have been due to low level residual chlorine remaining.
- The sponsor approved using untreated well water as an alternative to dechlorinated tap water. A protocol deviation was written for this change.

4.7 Calculations

4.7.1 Calculation of the Amount of Solvent Needed to Bring a Weighed Amount of Standard to the Required Concentration

$$\text{Volume of solvent} = \frac{(\text{weight of standard}) \times (\text{percent purity})}{\text{desired concentration}}$$

Example calculation
Preparation of the 1000 µg/mL (ng/µL, ppm) analyte standard stock solution:

Weight of analyte: 0.0152 g
Percent purity: 99.3%

Weight of analyte corrected for percent purity = (0.0152 g) x (993000 µg/g)
= 15094 µg

Volume of solvent needed = (1509.4 µg) / 1000 µg/mL
= 15.094 mL

4.7.2 Calculations used in the Preparation of Fortification Samples

LOQ Fortification

An LOQ fortification on a 1-L water sample was prepared by fortifying the sample with 100-µL aliquot of the 1.0 ng/µL, (ppm) fortification standard solution:

LOQ fortification = (100 µL)x(1.0 ng/µL)x(1/1000 mL) = 0.10 ng/mL (ppb)

10xLOQ Fortification

A 10xLOQ fortification on a 1-L water sample was prepared by fortifying the sample with 100-µL aliquot of the 10 ng/µL, (ppm) fortification standard solution:

LOQ fortification = (100 µL) x (10 ng/µL) x (1/1000 mL) = 1.0 ng/mL (ppb)

Analyte concentration in a final sample volume of 1.0 mL:

From a 1xLOQ (0.10 ng/mL) fortification on a 1-L water sample, applied to an SPE cartridge and brought to a 1.0-mL final sample volume, at 100% recovery:

Total Analyte = (1000 mL water) x (0.10 ng analyte/mL water) / (1.0 mL final sample vol)
= 100 ng/mL

4.7.3 Calculations Used in the Preparation of a 1xLOQ, 1 µg/L (ppb), Instrument Calibration Working Standard

In-solution concentration (calibration standard):

In-solution concentration (calibration standard) = (volume of stock standard) x (concentration of stock standard) x (1/final volume)

In-solution concentration (calibration standard) = (10 µL) x (10 ng/µL) x (1/1.0 mL)
= 100 ng/mL (ppb)

This agrees with the analyte concentration in a final sample volume of 1.0 mLat 100% recovery (section 4.7.2). Instrument calibration working standards at other concentrations were calculated in a similar manner as listed in section 3.5.6.

4.7.4 Calculation of Analyte Concentrations

The ChemStation software generated regression equations with a quadratic fit and a 1/x weighting factor and linear fits from the concentration of each standard and its peak area count. The reported data in Table 1 employed a linear non-weighted fit. The regression equation was used to calculate the concentration of each sample by applying the respective

peak area count to the curve. The calculated concentration that is recorded on each chromatogram in the Figures section and reported in Table 1 is expressed in terms of µg/L (parts per billion). The equation generated by ChemStation for the linear curve was verified using Microsoft® Excel. The calibration curve and regression equation generated by ChemStation for tolclofos-methyl are shown in Figure 1.

The linear calibration equation is calculated as $Y = mX + b$
Rearranging:

$X = (Y - b) / m$

Y = analyte peak area count
X = analyte concentration (µg/L)
m = slope
b = y-intercept

Example proof of calculation (see Figure 1 for regression equation) for the LOQ analyte fortification #1 (Figure 11), where the coefficients were calculated by Microsoft® Excel to show increased significant figures where:

Y = 6998
m = 79088
b = 1000.4
X = unknown

$X = (Y - b) / m$

$X = (6998 - 1000.4) / 79088$

$X = 0.0758$ µg/L

NOTE: The regression coefficients used by the by the ChemStation software to calculate residues include more significant figures than the rounded numbers printed out in the data packages. Therefore, the software calculation cannot always be reproduced exactly beyond the third/fourth significant figure by Microsoft® Excel or a hand-held calculator.

4.7.5 Calculation of Method Fortification Percent Recovery

$$\text{Method fortification recovery (\%)} = \frac{\text{residue found (µg/L)}}{\text{target fortification concentration (µg/L)}} \times 100\%$$

The LOQ tolclofos-methyl fortification #1, 0.10 µg/L (ppb), (see Table 1, and Figure 11)

Method fortification recovery (%) = (0.0758 µg/L / 0.1 µg/L) x 100% = 75.8%

4.7.6 Calculation of Standard Deviation (s) and Relative Standard Deviation (RSD) for a Mean

Standard deviation

Standard deviations for the mean percent recoveries in Table 1 were calculated in a Microsoft® Excel spreadsheet and verified using a calculator. The standard deviation is designated as "s" and the percentage is expressed as an absolute number.

Relative standard deviation (RSD)

Relative standard deviation = (standard deviation / mean) x 100%

Example calculation
The five replicates of the LOQ analyte fortification presented in Table 1 resulted in an unrounded mean recovery of 80.68% and an unrounded standard deviation of 2.991154%. The RSD was calculated as:

RSD = (2.991154% / 80.68%) x 100%

= 3.707%

= 3.7%

4.7.7 Calculation of 95% Confidence Interval for a Mean

$$95\%\ \text{confidence interval} = \text{mean} \pm \frac{t \times \text{standard deviation}}{\sqrt{n}}$$

Where: n = number of measurements
t = student t variate for n-1 degrees of freedom at the 95% confidence interval (2.776 for 4 degrees of freedom) [5].

Example calculation
From the five recovery values of water samples fortified with analyte at LOQ level (Table 1), the unrounded mean recovery was 80.68% and the unrounded standard deviation was 2.991154%. The confidence interval was calculated as:

$$95\%\ \text{confidence interval} = 80.68\% \pm \frac{(2.776)\,(2.991154\%)}{\sqrt{5}}$$

= 80.68% ± 3.7134%

= 80.68% ± 3.7%

4.8 Statistics Statement

The mean percent recoveries were arithmetic means, and standard deviations for the mean percent recoveries were calculated and designated as "s" with the percentage expressed as an absolute number. ChemStation software calculated the regression coefficients and the coefficient of determination (r^2) associated with the linear standard calibration. ChemStation calculated the concentration of each sample by applying the peak area count of each sample to the calibration curve.

The individual recoveries of all fortifications were between 70 and 120%. The coefficient of determination (r^2) for each standard curve was greater than or equal to 0.995. Mean percent recoveries, standard deviations, relative standard deviations, and 95% confidence intervals were calculated in a Microsoft® Excel spreadsheet and verified with a calculator.

4.9 Protocol, Method, or SOP Deviations

Two Protocol Deviations were prepared and are at the end of Appendix 3. Protocol Deviation #1 changed the matrix for the ILV from tap water to well water due to low recoveries on chlorinated water. The second deviation discussed analyzing the extracts after the eight-day storage time listed in the method. The changes were approved by the Sponsor Representative via e-mail prior to implementation. Due to an oversight by NCL personnel, the Protocol Amendments were not prepared.

APPENDIX 3 Protocol, Amendments and Deviations

Tolclofos-methyl

Independent Laboratory Validation (ILV) of the Analytical Method VP-38290
Tolclofos-methyl: Determination of Tolclofos-methyl in Drinking Water

PROTOCOL

DATA REQUIREMENTS: OCSPP 850.6100

APPENDIX 3 Protocol, Amendments and Deviations (continued)

1. STUDY OBJECTIVE

The object of this study is to validate the residue method VP-38290 for determining residues of tolclofos-methyl in drinking water. This study is designed to fulfill the requirements of EPA's Ecological Effects Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation.

2. TEST SYSTEM

The test system will be water taken from the tap at North Coast Laboratories, Ltd. (NCL) in Arcata, California.

3. JUSTIFICATION OF TEST SYSTEM

EPA's Ecological Effects Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation, requires that analytical methods be independently validated.

4. STUDY CONDUCT

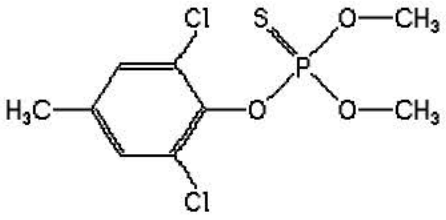
This study is intended to support the registration of tolclofos-methyl. The intent is to submit it to the EPA; therefore, the study shall conform to FIFRA Good Laboratory Practice (GLP) Standards specified in 40 CFR Part 160.

APPENDIX 3 Protocol, Amendments and Deviations (continued)

5. MATERIALS

The test/reference substance for this study will be supplied by Valent U.S.A. Corporation. Methods of synthesis, fabrication, and/or derivation of the test/reference substance are maintained by Valent. The Certificate of Analysis is presented in Appendix 1. The test substance is to be stored according to the CoA. Stock standards are to be stored per the method (Appendix 2).

5.1 Test Substance

Common name:	Tolclofos-methyl
Chemical name:	O-2,6-dichloro-p-tolyl O,O-dimethylphosphorothioate equivalent to *O*-2,6-dichloro-4-methylphenyl *O,O*-dimethylphosphorothioate
CAS number:	57018-04-9
Lot number:	AS 2218d
Stated purity:	99.3%
Expiration date:	February 29, 2014
Structure:	

APPENDIX 3 Protocol, Amendments and Deviations (continued)

6. METHOD SUMMARY

The method to be used in this study is Valent Method VP-38290 (Appendix 2). The Study Director and analyst have had no previous laboratory experience with any residue anlaytical methods involving the deterination of tolclofos-methyl.

Tolclofos-methyl residues are extracted from the aqueous samples using solid phase extraction. The SPE C18 column is conditioned by passing 10 mL of methanol and then 10 mL of distilled water under vacuum through the SPE column. A 1000-mL water sample is transferred into a 1000-mL laboratory flask. Samples are fortified at this stage. The sample is drawn through the SPE C18 column at a flow rate of 5-10 mL/minute. The SPE C18 column is dried by suction of air through the column for 1-2 minutes. The analyte is eluted from the SPE with 10 mL of dichloromethane in approximately 1-2 minutes. This step is repeated with an additional 10 mL of dichloromethane. The 20 mL of dichloromethane is then filtered though 5-10 g of anhydrous sodium sulfate and collected in a 50-mL boiling flask. The sodium sulfate is rinsed with 10-20 mL of dichloromethane. The dichloromethane is evaporated to about 1-2 mL under vacuum on the rotary evaporator at 30-40°C. After removing the remaining solvent using a gentle stream of nitrogen, the residue is dissolved in 1.0 mL of toluene with sonication.

The method will be validated as written and according to the requirements of EPA's Ecological Effects Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation.

The validation will involve the assay of a reagent blank (taken through the method), duplicate untreated control (UTC) water samples, five UTC water samples fortified at 0.10 µg/L (the LOQ of the method) and five UTC water samples fortified at 1.0 µg/L (ten times the LOQ of the method). Each sample will be injected twice. The mean area count for each duplicate set of calibration standards will be used to generate the regression equation. The mean results from each duplicate set of injected samples will be those reported.

The validation will be considered acceptable if all recoveries are between 70% and 120%. The relative standard deviation (RSD) of replicate measurements of recoveries should not exceed plus or minus 20%. The control matrix should be essentially free of any interference at the retention time of the compound of interest. Interferences with peak areas that are less than 50% at the LOD are considered insignificant.

7. SAMPLE IDENTIFICATION

The bulk untreated control tap water collected at NCL must have a unique identification number. The matrix controls and fortified subsamples during the extraction and analysis phases will be labeled appropriately. Minimally, documentation will also associate all samples with the study number and sample type.

APPENDIX 3 Protocol, Amendments and Deviations (continued)

8. SAMPLE HANDLING

Tap water sufficient to complete one analytical set will be collected and logged in as one sample according to NCL standard operating procedures (SOP). If any additional sets are required to complete a successful ILV, another sampling of tap water will be collected and labeled appropriately. The untreated control water is stored at ambient room temperature. The maximum time between starting the extraction and analysis is 8 days for the GC/MS analysis. Extracts should be stored in a freezer.

9. STATISTICAL ANALYSIS

Recoveries will be expressed as a percentage of the analyte concentration(s) determined relative to the concentration(s) added. Additional statistical methods (e.g.- linear regression of the instrument calibration data for a linear fit with a non-zero-intercept) are described in the method.

10. REPORTING OF DATA

A report will be issued after the completion of the study. Minimally, it will include all the applicable items specified in 40 CFR 160.185. It will also contain an outline of the contact with the method developer (if any) required for successful validation of the method. The final report will undergo a quality assurance audit as required by 40 CFR 160.35(b)(6).

13. PROTOCOL AMENDMENTS AND DEVIATIONS

Planned changes (changes before an event) to this protocol will require the approval of the Study Director and the Sponsor Representative. The Study Director will issue a written, signed protocol amendment that includes a description of the amendment, the reason for the amendment, and the impact, if any, on the study.

APPENDIX 3 Protocol, Amendments and Deviations (continued)

Changes to the procedures outlined in this protocol, to any protocol amendments, or to the method are deviations. All deviations must be recorded in writing as soon as possible after the occurrence, and the Study Director and Sponsor Representative must be notified. The written deviation must include a description of the deviation, the reason for the deviation, and the impact, if any, on the study. The written deviation will be placed in the study file.

14. STANDARD OPERATING PROCEDURES

If there is a conflict between existing standard operating procedures (SOPs) and this protocol, the protocol shall take precedence.