Title: Determination of Chlorothalonil in Ground and Surface Water

1. Scope:

This section method (SM) provides stepwise procedure for chlorothalonil analysis in ground and surface water. It is followed by all authorized EA personnel.

2. Principle:

The chlorothalonil is extracted from the acidified ground water and surface water samples with methylene chloride. The extract is passed through sodium sulfate to remove residual water. The anhydrous extract is evaporated on a rotary evaporator and then a solvent exchange is performed with methanol. The extract is concentrated to a final volume of 1 mL and then vialed into 2 autosampler vials for analysis on an Ultra Performance Liquid Chromatography (UPLC) coupled to a negative atmosphere pressure chemical ionization triple quadrupole mass spectrometry (APCI-LC/MS/MS).

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.

3.2 Methylene chloride is a regulated and controlled carcinogenic hazardous substance. It must be stored and handled in accordance with California Code of Regulations, Title 8, Subchapter 7, Group 16, Article 110, Section 5202.

4. Interferences:

There were no matrix interferences for chlorothalonil at the time of method development.

5. Apparatus and Equipment:

5.1 Rotary Evaporator (Buchi/Brinkman or equivalent)
5.2 Nitrogen Evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
5.3 Balance (Mettler PC 4400 or equivalent)
5.4 Vortex-vibrating mixer
5.5 UPLC equipped with a triple quadrupole mass spectrometry and APCI ion source.

6. **Reagents and Supplies**:

6.1 Chlorothalonil CAS#1897-45-6
6.2 Methylene Chloride, nanograde or equivalent pesticide grade
6.3 Sulfuric Acid, Conc. ACS Grade
6.4 Water, MS grade, Burdick & Jackson or equivalent
6.5 Methanol, MS grade, Burdick & Jackson or equivalent
6.6 Separatory funnel, 2 L
6.7 Boiling flask, 500 mL
6.8 Sodium Sulfate, ACS grade
6.9 Funnels, long stem, 60°, 10 mm diameter
6.10 Graduated conical tubes with glass stopper, 15 mL
6.11 Glass wool, Pyrex® fiber glass slivers 8 microns
6.12 Disposable Pasteur pipettes, and other laboratory ware as needed
6.13 Recommended analytical column:
Waters Acquity BEH 1.7µm, 2.1 x 50 mm

7. **Standards Preparation:**

7.1 An individual stock standard of 1.0 mg/mL was obtained from the CDFA/CAC Standards Repository. The standard was diluted to 10 µg/mL with methanol for identification purposes.

The following concentrations: 1, 0.5, 0.25, 0.1, 0.05, 0.025, µg/mL were prepared in methanol for LC instrument calibration.

7.2 Keep all standards in the designated refrigerator for storage.

7.3 The expiration date of each standard is six months from the preparation date.

8. **Sample Preservation and Storage**:

Store all samples waiting for extraction in a separate refrigerator (4±3°C).

9. **Test Sample Preparation**:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the ground water and surface water for background to be used in method validation and QC.

9.2 Preparation of blank and spike

Matrix blank: Weigh out 1000 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 1000 g of background water. Spike a client requested amount of fungicide into the background water and let it stand for 1 minute. Follow the test sample extraction procedure.

9.3 Test Sample Extraction

9.3.1 Record the weight of water samples to 0.1 g by subtracting the weight of the sample container before and after water has been transferred into a funnel.

9.3.2 Add 2.5 mL of sulfuric acid to each separatory funnel and mix well.

9.3.3 Shake with 100 ± 5 mL of methylene chloride for 2 minutes. Vent frequently to relieve pressure.

9.3.4 After phases have separated, drain the lower methylene chloride layer through 25 ± 4 g of anhydrous sodium sulfate and glass wool into a 500 mL boiling flask.

9.3.5 Repeat steps 9.3.3 & 9.3.4 two more times using 80 ± 5 mL of methylene chloride for 1 minute each time. Combine the extracts in the same boiling flask.

9.3.6 After draining the final extraction, rinse the sodium sulfate with 25 ± 5 mL of methylene chloride.

9.3.7 Evaporate the sample extract to 2 - 4 mL on a rotary evaporator using a water bath at 35 ± 2 °C and 15 – 20 inch Hg vacuum. Add 2-4 mL of methanol and rotoevaporate to 1-2 mL. Transfer the extract to a calibrated 15 mL graduated test tube.

9.3.8 Rinse flask 3 more times with 2 - 4 mL of methanol and transfer each rinse to the same test tube.

9.3.9 Evaporate the sample extract to a volume slightly less than 1 mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Then bring to a final volume of 1.0 mL with methanol, mix well and transfer to 2 autosampler vials with inserts.

10. **Instrument Calibration**:

10.1 The calibration standard curve consists of a minimum of three levels. The lowest level must be at or below the corresponding reporting limits.

10.2 The calibration curve for the LCMS instrument was obtained using Linear fit.

11. **Analysis:**

11.1 UPLC-MS/MS

11.1.1 UPLC Instrument: Waters Acquity Ultra Performance LC
Column: Waters Acquity BEH 1.7µm, 2.1 x 50 mm
Column Temperature: 60 °C
Mobile Phase: Gradient
Solvent 1: Water
Solvent 2: Methanol
Gradient:

Time(min)	Flow rate	Solvent 1	Solvent 2
0	0.50	90.0	10.0
1.0	0.50	90.0	10.0
1.5	0.50	5.0	95.0
3.5	0.50	5.0	95.0
3.55	0.50	90.0	10.0
5.0	0.50	90.0	10.0

Injection Volume:2.0 µL

11.1.2 Mass Spectrometry and Operating Parameters
Model: Waters Xevo Triple Quadrupole
Ion ProbeType: Atmospheric Pressure Chemical Ionization (APCI)
Ion Mode: APCI-
APCI Probe Temp: 500 °C
Source Temp: 150 °C

Compound	Retention Time (min)	Precursor ion	Product Ion	Dwell (s)	Cone(V)	Collision Energy/-ev
Chlorothalonil	1.90	244.95	174.95	0.061	46.0	28.0
		244.95	**181.91**	0.061	46.0	30.0

Quantitation ions are in bold.

Note: The column conditions, temperature, mobile phase, etc. may slightly shift retention time.

12. **Quality Control**:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 well water samples and 7 surface water samples are spiked at 0.1ppb and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for each analyte using the following equation:

MDL = tS

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The RL is chosen in a range 1-5 times the MDL, as per client agreement. The reporting limit for Chlorothalonil in well water and surface water is 0.05 ppb.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included five levels of fortification and a method blank. All spikes and method blanks were processed through the entire analytical method. Spike levels and recoveries for the analytes are shown in Appendix 2.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the % recovery, respectively, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a spiked matrix sample.
12.5.2 The retention time should be within ± 2 per cent of that of the standards.
12.5.3 The recoveries of the matrix spikes shall be within the control limits.
12.5.4 The sample shall be diluted if results fall outside of the calibration curve.

13. Calculations:

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The LCMS software used a linear curve fit, with all levels weighted 1/x.

$$ppb = \frac{(\text{sample peak area or ht}) \times (\text{std conc}) \times (\text{std vol. Injected}) \times (\text{final vol of sample})(1000\ \mu L/mL)}{(\text{std.peak area or ht}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

14. Reporting Procedure:

Sample results are reported out according to the client's analytical laboratory specification sheets.

15. Discussion and References:

15.1 Upon infusion of chlorothalonil, we found the principal ion to be 245 ion rather than the anticipated molecular ion at 264. This is consistent with substitution of the chlorine by hydroxyl within the source.

15.2 Acid is not necessary for the extraction of chlorothalonil but was added with the intent of including its metabolites at a later date.

15.3 A storage stability study was done with this project for well water only. The storage stability study consisted of a 1.0 ppb spike level and 2 replicates over a 28 day period. Fourteen liters of background well water were spiked and then transferred to fourteen one liter amber bottles. These spiked samples were stored in the refrigerator until analyzed at 0, 2, 5, 7, 14, 21 and 28 days. Along with the storage spikes a blank and method control spike were also extracted. This storage study showed no degradation for the chlorothalonil within the 28 days. Results for the storage studies are shown in Appendix 3.

15.4 We have observed gradual losses in sensitivity and peak tailing caused by the sample matrix. We recommend cleaning the cones when this occurs.

15.5 References:

15.51 Wakefield, Mike (Principal MS Applications Specialist); UPLC-MS/MS conditions for Chlorothalonil, Waters Corporation

15.52 Hsu, J. and White, J.; *Determination of Azoxystrobin, Azoxystrobin Acid, Azoxystrobin Z-metabolite,Dicloran, Iprodione,Isoiprodione,Vinclozalin and 3,5-Dichloroaniline in Well Water*, 2010, Environmental Analysis Section Method, Center for Analytical Chemistry, CDFA

Appendix 1

The Determination of Method Detection Limit (MDL) and Reporting Limit (RL)

Results:	Well Water	Surface Water
Spk\Analyte	Chlorothalonil	Chlorothalonil
	Spike Level: 0.1 ppb	Spike Level: 0.1 ppb
Spike 1	0.0919	0.0898
Spike 2	0.127	0.0959
Spike 3	0.0958	0.102
Spike 4	0.106	0.0797
Spike 5	0.104	0.115
Spike 6	0.107	0.101
Spike 7	0.106	0.0928
SD	0.0112	0.0111
MDL	0.0351	0.0348
RL	0.05	0.05

Appendix 2

Results: Well Water

Analyte	Spike ppb	Recovery (%) Set 1	(%) set 2	(%) set 3	(%) set 4	(%) set 5	%	%
Chlorothalonil	0.1	113	100	95.8	111	85.4	Mean:	93.2
	0.2	98.3	110	79.7	115	84.0	SD:	12.4
	0.5	73.2	116	78.3	91.8	79.1	UCL:	130.4
	1.0	93.3	91.6	87.7	89.0	82.9	UWL:	118.0
	2.0	83.0	101	85.8	99.5	86.2	LWL:	68.5
							LCL:	56.1

Results:Surface Water

Analyte	Spike ppb	Recovery (%) Set 1	(%) set 2	(%) set 3	(%) set 4	(%) set 5	%	%
Chlorothalonil	0.1	95.2	79.7	76.2	94.3	100	Mean:	93.3
	0.2	103	89.8	81.8	102	110	SD:	9.9
	0.5	101	92.4	74.9	102	97.6	UCL:	123.0
	1.0	90.8	92.7	81.8	104	90.7	UWL:	113.1
	2.0	106	91.8	79.3	104	90.5	LWL:	73.4
							LCL:	63.5

Appendix 3 Storage Stability Study

Spike Level: 1.0ppb

Chlorothalonil Results:

Storage Day	EMON Lab#	Sample	1st injection result ppb	2nd injection result ppb	Avgerage ppb	% Recovery
Day 0	2010-1618	Blank	ND	ND	ND	N/A
	2010-1619	SPK 1	0.785	0.676	0.731	73.1%
	2010-1620	Spk 2	0.765	0.721	0.743	74.3%
Day 2	2010-1621	Blank	ND	ND	ND	N/A
	2010-1622	QC spk	0.887	0.844	0.866	86.6%
	2010-1623	SPK 1	1.04	1.07	1.055	106%
	2010-1624	Spk 2	1.02	0.931	0.976	97.6%
Day 5	2010-1625	Blank	ND	ND	ND	N/A
	2010-1626	QC spk	0.783	0.718	0.751	75.1%
	2010-1627	SPK 1	0.810	0.764	0.787	78.7%
	2010-1628	Spk 2	0.882	0.718	0.800	80.0%
Day 7	2010-1629	Blank	ND	ND	ND	N/A
	2010-1630	QC spk	0.902	0.907	0.905	90.5%
	2010-1631	SPK 1	0.889	0.842	0.866	86.6%
	2010-1632	Spk 2	0.903	0.848	0.876	87.6%
Day 14	2010-1633	Blank	ND	ND	ND	N/A
	2010-1634	QC spk	1.21	1.23	1.22	122%
	2010-1635	SPK 1	0.984	0.849	0.917	91.7%
	2010-1636	Spk 2	0.968	0.878	0.923	92.3%

Appendix 3 Storage Stability Study continued:

Day 21	2010-1637	Blank	ND	ND	ND	N/A
	2010-1638	QC spk	0.977	0.959	0.968	96.8%
	2010-1639	SPK 1	0.766	0.739	0.753	75.3%
	2010-1640	Spk 2	0.834	0.787	0.811	81.1%
Day 28	2010-1641	Blank	ND	ND	ND	N/A
	2010-1642	QC spk	0.953	0.899	0.926	92.6%
	2010-1643	SPK 1	0.887	0.875	0.881	88.1%
	2010-1644	Spk 2	0.994	0.914	0.954	95.4%

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