Determination of Thiram in Surface Water

1. **Scope**:

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

2. **Principle**:

Thiram is extracted from the surface water sample with methylene chloride. The extract is passed through a very small amount of sodium sulfate to remove residual water. The anhydrous extract is evaporated on a rotary evaporator and then a solvent exchange to methanol is performed. The extract is concentrated to a final volume of 2 mL and then vialed for analysis on high performance liquid chromatography equipped with a C18 column and UV detector at 272 nm.

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 thiram analysis 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 HPLC: Waters Liquid Chromatography model 2695 with Empower 2 software, C18 column and UV detector

6. **Reagents and Supplies**:

6.1 Thiram CAS#137-26-8
6.2 Methylene Chloride, nanograde or equivalent pesticide grade
6.3 Water, grade suitable for pesticide residue analysis, Burdick & Jackson or equivalent
6.4 Methanol, MS grade, Burdick & Jackson or equivalent
6.5 Acetonitrile, nanograde or equivalent pesticide grade
6.6 Separatory funnel, 2 L
6.7 Boiling flask, 500 mL
6.8 Anhydrous sodium sulfate, ACS grade
6.9 Funnels, long stem, 60°, 50 mm I.D.
6.10 Graduated conical tubes with glass stopper, 15 mL
6.11 Disposable Pasteur pipettes, and other laboratory ware as needed
6.12 Recommended analytical column:

Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 150 mm column

Guard column: Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 20 mm cartridge
Guard column holder: Waters Sentry guard holder universal.

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 to10 µg/mL with methanol for identification purposes.

The 10 µg/mL standard was also used to dilute the following concentrations: 0.1 0.2, 0.5, 0.75 and 1.0 µg/mL in methanol for 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 Regulations (DPR) provides the background water for matrix blank and spikes.

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 Wash all glassware with 1:3 hydrochloric acid and then rinse well with water, distilled water and acetone.

9.3.2 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 separatory funnel.

9.3.3 Add 100 ± 5 mL of methylene chloride to the water sample and shake gently for 2 minutes. Vent frequently to relieve pressure.

9.3.4 After phases have completely separated, drain the lower methylene chloride layer except ~ 1 inch of solvent through 1 g of anhydrous sodium sulfate without glass wool into a 500 mL boiling flask. (Leaving the 1 inch of solvent should help keep the sodium sulfate from hardening and keep water out of the sample extract) Using more sodium sulfate leads to low recovery of thiram.

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. When draining the solvent make sure to leave the 1 inch of solvent each time.

9.3.6 Evaporate the sample extract to ~ 10 mL on a rotary evaporator using a water bath at 35 ± 2 °C and 15 -16 inch Hg vacuum. As the sample extract concentrates reduce the speed of flask rotation. Transfer the extract to a calibrated 15 mL graduated test tube with methylene chloride.

9.3.7 Evaporate the sample extract to ~ 2 mL in a water bath at 35 - 40 °C under a gentle stream of nitrogen. Add ~ 4 mL methanol, mix well and continue to evaporate down to ~ 1.5 mL's Occasional mixing is necessary during evaporation, because the top layer will contain methanol and the bottom layer will contain methylene chloride. Then bring to a final volume of 2.0 mL with methanol, mix well and vial for analysis. (Care must be taken not to evaporate to dryness otherwise low or no recovery may occur)

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 LC instrument is obtained using quadratic fit.

11. **Analysis:**

11.1 HPLC

11.1.1 HPLC Instrument: Waters model 2695 HPLC and auto-sampler with column heater and equipped with UV detector.

Guard Column: Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 20 mm cartridge
Column: Waters Symmetry RP_{18} 5 µm, 3.9 x 150 mm column
Column Temperature: 35 °C
Mobile Phase: Gradient
Solvent 1: Water
Solvent 2: Acetonitrile

Gradient:

Time(min)	Flow rate mL/min	Solvent 1	Solvent 2
0	1.0	70.0	30.0
1.0	1.0	70.0	30.0
5.0	1.0	10.0	90.0
8.0	1.0	10.0	90.0
8.5	1.0	70.0	30.0
10.0	1.0	70.0	30.0

Injection Volume: 20 µL

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 surface water samples are spiked at 1.0 ppb 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 thiram is 0.5 ppb.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four 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 analyte are shown in Appendix 2.

12.4 Control Chart and Limits

A control chart was generated using the data from the method validation for the analyte. 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 LC software used a quadratic curve fit, with all levels weighted 1/x. Alternatively, at the chemist's discretion, sample results may be calculated using the response factor for the standard.

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

14. **Reporting Procedure:**

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

15. **Discussion and References:**

15.1 A storage stability study was done with this project. The storage stability study consisted of a 10.0 ppb spike level and 3 replicates over a 26 day period. Nine liters of background surface water were pH adjusted to ~ 3 and then spiked. The spiked water was transferred to one liter amber bottles. These spiked samples were stored in the refrigerator until analyzed on 5, 12 and 26 days. Along with the storage spikes a blank and method control spike were also extracted. This storage study showed no significant degradation. Results for the storage study are shown in Appendix 3.

15.2 Some of the samples were also analyzed by ESI+/LC/MS/MS (Finnigan Ion Trap MS Deca I). LC/MS acquisition was performed in SRM mode with the following transition: *m/z* 241→ 196 at normalized collision energy of 20%. Results were comparable to those obtained using LC/UV.

15.3 References:

Hefner, Karen; Lee, Paul; Lew, Robert; *Determination of Thiram in Water*, 1994, Environmental monitoring method, Center for Analytical Chemistry, CDFA

Appendix 1

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

Lab #	Spk\Analyte	Thiram (ppb)
2006-2206	Blank	nd
2006-2207	1.0 ppb spk 1	0.720
2006-2208	1.0 ppb spk 2	0.883
2006-2209	1.0 ppb spk 3	0.577
2006-2210	1.0 ppb spk 4	0.903
2006-2211	1.0 ppb spk 5	0.874
2006-2212	1.0 ppb spk 6	0.817
2006-2213	1.0 ppb spk 7	0.781
	SD	0.11508
Reported	**MDL**	**0.3614**
	RL	**0.50**

Appendix 2

Method Validation Data

	Spike	Recovery (%)						
Analyte	ppb	Set1	Set2	Set3	Set4	Set5		%
Thiram	1.0	34.4	88	83.8	67.4	81.5	Mean:	87.4
	2.0	87.0	90.5	87.9	88.3	93.0	SD:	14.5
	5.0	91.6	97.2	86	90.7	97.7	UCL:	131
	10.0	93.4	97.8	93.7	101	96.4	UWL:	116
							LWL:	58.4
							LCL:	43.9

Appendix 3

Storage Stability Study

Analyte \ Recovery %		Day 5	Day 12	Day 26
Thiram	Blank	ND	ND	ND
	QC spike	97.1%	87.2%	92.2%
	Spike 1	90.1%	80.5%	80.0%
	Spike 2	96.3%	80.8%	79.4%
	Spike 3	95.6%	79.2%	70.1%

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