Title: Analysis of Chlorpyrifos in Sediment

1. Scope:

   This section method (SM) documents chlorpyrifos analysis in sediment and is followed by all authorized EMON personnel.

2. Principle:

   The SM describes the method for determination of chlorpyrifos in sediment.
   The samples are homogenized and extracted with 1:1 acetone/hexane by shaking on an obitial shaker. The extracts are cleaned with florisil before being analyzed with a gas chromatography equipped with a triple stage quadropole detector (MS/MS).

3. Safety:

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

   3.2 Acetone and hexanes are flammable and toxic solvents; they should be handled with care in a ventilated area.

4. Interferences:

   There were no matrix interferences that caused quantitative problems during method development and validation.

5. Apparatus and Equipment:

   5.1 Shaker, (Lab-Line Force Orbital Air Shaker or equivalent)
   5.2 Rotary Evaporator (Buchi/Brinkman or equivalent)
   5.3 Nitrogen evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
   5.4 Balance, (Mettler PC 4400 or equivalent)
   5.5 Vortex-vibrating mixer
   5.6 Gas Chromatograph equipped with a triple stage quadruple detector (MS/MS)

6. Reagents and Supplies:

   6.1 Chlorpyrifos CAS# 2921-88-2

6.12 Acetone, nanograde or equivalent pesticide grade
6.13 Hexanes, nanograde or equivalent pesticide grade
6.14 Diethylether, nanograde or equivalent pesticide grade
6.15 Mason jars, pint size with lids
6.16 Magnesium sulfate, anhydrous
6.17 Whatman filter paper, #4, 15 cm
6.18 Funnels, short stem, 60°, 8 cm diameter
6.19 Copper powder, purified
6.20 Florisil SPE cartridge, 2 grams with 20 mL reservoir
6.21 Pipette, 1-mL
6.22 Test tube, 50 mL
6.23 Graduated conical tubes with glass stopper, 15-mL
6.24 Disposable Pasteur pipettes, and other laboratory ware as needed
6.25 Analytical columns:
Varian Factor Four VF-5ms 30Mx 0.25mm x 0.025µm or equivalent.

7. Standards Preparation:

7.1 The chlorpyrifos stock standard of 1.0 mg/mL was obtained from the CDFA/CAC Standards Repository. The standard was diluted to 10 ng/µL with hexanes for identification purposes.

The standard was also used to dilute the following concentrations: 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, 0.5 ng/µL in hexanes 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 freezer. If samples are to be extracted the next day, they may be stored in the refrigerator. Sample extracts shall be stored in the refrigerator (32-40 °F).

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the sediment for background to be used in method validation. The background sediment was provided in a 5 gal bucket. Excess water was decanted off before the sediment was mixed. The sediment was mixed well with a paddle attached to a drill and then passed through a Tyler equivalent #9 mesh sieve to remove debris. The sieved background was placed in quart size mason jars and stored in the refrigerator.

9.1.1 Blank

Remove background sample from refrigerator and allow it to come to room temperature. Mix background sample well before weighing out 20 g. Proceed to step 9.2.2 of section 9.2.

9.1.2 Spike

Remove background sample from refrigerator and allow it to come to room temperature. Mix background sample well before weighing out 20 g. Fortify at the level requested by client and mix well to ensure that the pesticide are well distributed. The spiked background was allowed to sit for 30 minutes before proceeding to step 9.2.2 of section 9.2.

9.1.3 Moistures

9.1.3.1 Thaw sediment sample and then decant any excess water from the sample. Homogenized the sediment thoroughly.

9.1.3.2 Weigh out a 15 – 20 g sub-sample into a pre-weighed aluminum weighing pan.

9.1.3.3 Dry the pan with sediment for at least 6 hours in a ~ 105°C oven.

9.1.3.4 Reweigh sediment after cooling in a dessicator.

9.1.3.5 Report the wet and dry weights on Chain of Custody sample sheets.

9.2 Test Sample Extraction

9.2.1 Thaw sediment sample and then decant any excess water from the sample. Thoroughly homogenized the sediment and remove any debris (e.g., gravel, sticks). Weigh out a 20 ± 0.5 g sub-sample into a pint size mason jar.

9.2.2 Add 5 g of copper powder to each sample and mix well.

9.2.3 Place the Mason jar containing the sample on ice and add ~ 2 spatulas of anhydrous $MgSO_4$ and mix well. Keep adding $MgSO_4$ to the sample until it is dried (sandy condition).

9.2.4 Add 75 mL of 1:1 mixture of acetone/hexane to the mason jar, cover with foil and cap. Place on shaker and shake for 15 min at 185 rpm.

9.2.5 Decant the extract and filter through a piece of Whatman # 4 filter paper containing approximately 2 g anhydrous $MgSO_4$ into a 250 mL boiling flask. Repeat step 9.2.4 & 9.2.5 again, but this time transfer solvent and soil to the funnel and rinse with 1:1 acetone/hexane. The filtered extracts are combined.

9.2.6 Rotory evaporate to ~ 5 mL under vacuum at approximately 17-20 inch Hg in a water bath at 40° C.

9.2.7 Transfer the extract to a 15 mL graduated test tube. Rinse flask 3 times with approximately 2 mL of hexane and transfer each rinsate to the same test tube.

9.2.8 Place the test tube on nitrogen evaporator under a gentle stream of nitrogen with water bath set at 40° C and solvent-exchange with hexane. Bring to final volume of 2 mL.

Cleanup

9.2.8 Condition a 2 g florisil SPE cartridge with 10 mL of 15% diethylether followed by 20 mL hexane. Do not allow cartridges to go to dryness.

9.2.9 Carefully load the sample extract onto the conditioned florisil SPE cartridge. Rinse the tube that previously contained the extract twice with 2 mL hexane. Add rinses to florisil cartridge.

9.2.10 Elude the chlorpyrifos from the cartridge with 30 mL of 15% diethylether and collect in a 50 mL tube.

9.2.11 Evaporate the sample eluants to dryness under a gentle stream of nitrogen in a 40° C water bath.

9.2.12 Pipet 2mL of hexane into the test tube and vortex well. Vial contents of test tube in 2 autosampler vials. Submit one for analysis and refrigerate the other.

10. Instrument Calibration:

10.1 The calibration standard curve consists of a minimum of three levels. The recommended concentrations levels of standards are 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, or 0.5 ng/µL.

10.2 The Triple Quad used linear regression with a correlation coefficient (r) equal to or greater than 0.995.

11. Analysis:

11.1 Injection Scheme

The instrument may need to be conditioned with a matrix blank or old sample before running the following sequence of Standard Curve, Hexane, Matrix Blank, Matrix Spike, Test Samples (maximum of 10 – 12) and Standard Curve.

11.2 GC-Triple Quad Instrumentation

Model: Varian Triple Quad 320-MS
Column: Varian Factor Four VF-5ms 30M x 0.25mm x 0.025µm

Temperature Program: initial column temperature 80 °C, hold 1 min., ramp at 25 °C/min. to temperature of 280 °C and hold for 5 min.;

Injector temperature: 250˚ C
Injection volume: 1 µL
Transfer line heater 280 °C
Source temperature: 200 °C

Compound	Retention Time (min)	Precursor ion	Product Ion	Collison Energy/-ev
Chloropyrifos	8.0	197	169	15
		314	258	9.0

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 sediment 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 chlorpyrifos 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 reporting limit for chlorpyrifos is 1.0 ppb. This reporting limit was chosen after taking into account the matrix effect and various sample background that could be encountered.

12.3 Method Validation

The method validation consisted of 3 sample sets. Each set included four levels of fortification (1, 5, 20 and 400 ppb) and a method blank. All spikes and method blanks were processed through the entire analytical method. Recoveries for chlorpyrifos are tabulated 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 exceed the calibration curve.

13. Calculations:

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The Triple Quad uses linear regression fit, with all levels weighted 1/nx2 and forced origin. Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

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

14. Reporting Procedure:

Sample results are reported accordance with the client's analytical laboratory specification sheets.

15. Discussion:

15.1 The sample matrix may require that the liner be changed more frequently and the column trimmed to maintain sensitivity.

15.2 This method was adapted from the methods listed in the references below.

16. References:

16.1 J. You, D.P. Weston, M. J. Lydy, *A Sonication Extraction Method for the Analysis of Prethroid, Organophosphate, and Organchlorine Pesticides from Sediment by Gas Chromatography with Electron-Capture Detection,* Archives Environmental Contamination and Toxicology 47, 141-147 (2004)

16.2 J. You, M. J. Lydy, *Evaluation of Desulfuration Methods for Pyrethroid, Organophosphate, and Organochloride Pesticides in Sediment with High Sulfur Content,* Archives Environmental Contamination and Toxicology 47, 148 -153 (2004)

16.3 White, Jane, *Analysis of Pyrethroids in Sediment* , California Department of Food and Agriculture, Center for Analytical Chemistry, Environmental Analysis Section, EMON-SM-52-9 (2005)

Appendix 1

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

Chlorpyrifos	1.0 ppb Spike Level	
	ppb	%
blk sed	n/d	
spk 1	0.758	75.8
spk 2	0.923	92.3
spk 3	0.801	80.1
spk 4	0.798	79.8
spk 5	0.815	81.5
spk 6	0.810	81.0
spk 7	0.859	85.9
Std dev	0.0530	
MDL	0.16662	
RL	1.00ppb	

Appendix 2

Method Validation Data and Control Limits

Results:

Analyte	Spike ppb	Recovery (%) Set 1	Set 2	Set 3		
Chlorpyrifos	1.00	97.9	92.8	95.3	Mean:	90.892
	5.00	86.0	77.6	90.8	SD:	9.557
	20.0	88.5	81.5	92.5	UCL:	119.6
	400	114	81.0	92.8	UWL:	110.0
					LWL:	71.78
					LCL:	62.2

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