Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Cyfluthrin

MRID #: 415113-01

Matrix: Sediment

Analysis: GC/ECD

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Appendum 5 **Method Validation for the Analysis of Sediment for Cyfluthrin Residue**

I. Introduction and Background

Samples of Hydrosoil sediment were spiked with different amounts of cyfluthrin and aged for two time periods, after which they were extracted. The extract was analyzed to determine the percent recovery of cyfluthrin from the sediment. Standard operating procedure 4.13 was followed for the extraction and analysis of sediment samples. The validation for the procedure is given in sections II-IV below.

II. Validation Method

50 g samples of sediment were spiked with acetone solutions of cyfluthrin at concentrations between 10 ppb and 200 ppb. Samples were allowed to age in the freezer for two time periods, after which, they were extracted. The extracts were subjected to gas chromatographic analysis to determine the amount of cyfluthrin extracted from the sediment samples.

III. Validation Results

Samples Aged for:	Cyfluthrin Spike level	Average % Recovery	Std. Dev.
60 minutes	10 ppb	107%	7
	20 ppb	96%	2
	50 ppb	99%	12
4 days	100 ppb	85%	7
	200 ppb	88%	8

[Refer to Analytical report 76 for method validation results given above. Appendum number 11 describes the results of sediment spiked at 5 ppb and analyzed using the same procedure (SOP 4.13). 5.0 ppb is the quantitation limit for all sediment analysis conducted during study number MMP1.]

IV. Conclusion

Cyfluthrin can be extracted and detected from pond hydrosoil sediment, by the above method, with reasonable precision.

V. Standard Operating Procedure 4.13

SOP : 4.13 Analysis of Sediment for Cyfluthrin Residue

1. Principle:

Cyfluthrin is extracted from sediment samples with acetone in a 225ml amber glass jar by stirring and filtration. Acetonitrile is added to remove the water from the acetone fraction and the extract is evaporated to dryness by rotary evaporation. 10ml of hexane or hexanes is added and the flask

is sonicated for 2-3 minutes. The hexane extract is analyzed by electron capture gas chromatography using cool on-column injection and an uncoated deactivated retention gap.

2. Compound Information:

Cyfluthrin: Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2-2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate. Company BAY FCR 1272. Testing formulation: BAY FCR 1272 200 EC. Analytical grade standard of cyfluthrin as supplied by the study sponsor.

3. Equipment and Reagents

a. Equipment:

1. Balances - Mettler model PM460 top loading (0.001 g) and an AE240 analytical (0.00001 g), or equivalent.

2. Rotary flash evaporator - Buchi model 111B with temperature controlled water bath and refrigerated cooling fluid, or equivalent.

3. Amber glass wide-mouth jar - 225ml glass with teflon lined screw caps.

4. Stir bars - 3.8cm teflon coated octagonal stir bars.

5. Sonic water bath cleaner.

6. Pasteur capillary pipets, borosilicate glass, 23cm length.

7. Diatomaceous earth filtering aid-a 5-10mm bed of Celite 545.

8. Filter paper- Whatman #41, 7.0cm.

9. Buchner funnel- 8.0cm.

10. Evaporating flasks - 250ml glass with 24/40 ground glass joint, or equivalent.

11. Syringes - Hamilton brand gastight, or equivalent.

12. Gas chromatograph - Hewlett-Packard model 5890A with dedicated cool on-column capillary inlet and Ni^{63} electron capture detector or equivalent.

13. Gas chromatographic column - 5 meter x 0.53 mm ID fused silica megabore column with a 2.65 μm film of

methylsilicone stationary phase (HP-1 or equivalent).

14. Data Handling - Hewlett-Packard model 9000 series 300 computer and Hewlett-Packard GC Chemstation software, or IBM AT compatible computer with Nelson Analytical Turbochrom Chromatography software, or equivalent.

15. Stainless Steel or Teflon spatulas

16. Hack saw or band saw

17. Aluminum foil and aluminum weigh boats.

18. Drying oven.

b. Reagents:

1. Hexanes (ACS) - suitable for pesticide residue analysis (Mallinckrodt nanograde brand or equivalent).

2. Acetone (ACS) - suitable for pesticide residue analysis (Mallinckrodt nanograde brand or equivalent).

3. Acetonitrile (ACS) - suitable for pesticide residue analysis (Mallinckrodt ChromAR HPLC grade or equivalent).

4. Hydrogen gas - Liquid Carbonics zero grade or equivalent.

5. 90% argon: 10% methane gas - Liquid Carbonics zero grade or equivalent.

4. Procedures and Methods:

a. Preparation of Standard and Spike Solutions:

1. Standard Solutions.

All standards for injection into the gas chromatograph are to be in hexane or hexanes.

Standard solutions will be prepared using analytical grade Cyfluthrin as received from the study sponsor. A stock standard of Cyfluthrin is prepared by dissolving analytical grade Cyfluthrin in hexane in a 50 or 100 ml volumetric flask. Intermediate standards are prepared by dilution of these solutions. Working standards are brought to volume with hexane. Preparation of standards is recorded on standard

preparation data sheets.

All standards are labeled as described in SOP 15.6. Control charts, the use of which are outlined is SOP 4.18, are maintained to document the stability and freshness of standard solutions. Use of an analytical standard is documented on the "Analytical Standard Log" form as described in SOP 4.8.

2. Spike Solutions

Spiking solutions are prepared in acetone in the same manner as are standard solutions.

b. Application of Spikes:

Field and lab spikes are performed for each days samples. The application of the spiking solutions is outlined in SOP 4.16.

Record the following information on the spiking data sheet.

A. Sample ID spiked.
B. Level spiked.
C. Solution number and Volume of spike.
D. Time and date of spike.
E. Initials of person spiking.

c. Sample Preparation:

1. Sediment Core Samples

a. Three cores are collected from each pond on a sediment core sampling day (excluding lab and field spike cores collected from control ponds) and frozen in the lab until sample analysis.
b. Perform the following steps to remove a one cm section from each of the three frozen cores.
   1. Lay the core on clean aluminum foil.
   2. Measure one cm down from the lowest top point of the sediment. Mark the 1 cm location on the plastic core.
   3. Cut the plastic core with a hexane-rinsed band saw along the 1 cm mark.
   4. Separate the top layer.
   5. Place core sections in aluminum weigh boats until cores defrost enough to be cut into smaller pieces with a stainless steel or teflon spatula.
   6. Transfer the core sections to a 225 ml amber glass jar using the stainless steel or teflon spatula.

c. Using the spatula, mix the three sections in a teflon beaker. Remove a subsample and determine the percent dry sediment as described in step 5.f. Also, weigh approximately a 50 g(wet weight) subsample in a tared 225 ml amber glass jar. Proceed with step 4.d. (SOP 4.5).

[Note: A dry weight subsample should not be taken from the sediment core field spike sample.]

2. Sediment Core Lab Spike Samples

a. One lab spiking core is collected from the control pond on each sediment core sampling day and frozen in the lab until sample analysis.
b. In the lab, remove a one cm section as described in step 4.c.1.b.
c. Using a stainless steel or a teflon spatula, mix the hydrosoil placed in a 225 ml amber glass jar. Remove approximately a 10g subsample and determine the percent dry sediment as described in step 5.f. Next, weigh approximately a 50 g (wet weight) subsample in a tared 225 ml amber glass jar. Spike the hydrosoil in the flask as described in step B.3 (SOP 4.13). Proceed with step 4.d (SOP 4.13).

3. Sediment Trap Samples

a. Sediment trap samples are collected in tared 225 ml amber glass jars as described in step 3 of SOP 4.1 (Use of Sediment Traps for Collection Residue Samples).
b. In the field, sediment trap field spike samples are spiked as described in step A.2.b of SOP 4.16.
c. All samples are returned to the lab and frozen until residue analysis.
d. To begin analysis in the lab, remove a subsample and determine the percent dry sediment as described in step 5.f. [Note: A dry weight subsample should not be taken from the sediment trap field spike sample.]
e. Record a weight for the remaining sediment trap sample (approximately 50 g) in each tared flask. Sediment trap lab spike samples are spiked at this time as described in step B.2 (SOP 4.16). Proceed with step 4.d.

4. Run-Off Tank Mix Samples

a. Run-off tank mix samples are collected in tared 225 ml amber glass jars as described in step 2.D.3. of SOP 17.4 (Application of Baythroid as a Run-off Simulation).
b. In the field, run-off tank mix field spike samples are spiked according to step A.2b of SOP 4.16.
c. All samples are returned to the lab and frozen until residue analysis.
d. To begin analysis, remove a subsample and determine the percent dry sediment as described in step 5.f [Note: A dry weight subsample should not be taken from the sediment core field spike sample.].
e. Record a weight for the remaining run-off tank mix sample -approximately 50g(wet weight)- in each tared flask. Next, run-off tank lab spike samples are spiked as described in step B.2 (SOP 4.16). Proceed to step 4.d.

d. Liquid-Solid Extraction

1. Before extraction, the sample is homogenized with a teflon or stainless steel spatula and a sample for dry weight analysis is removed from all samples except field spikes (a subsample for dry weight analysis is removed from the field spike sample prior to spiking). The determination of the dry weight of the sample is described in section 5.f. of this SOP.

2. A wet weight is recorded for each sample in an amber glass jar. The goal weight of each sample is 50 ± 10g wet weight for each sample, so excess sample may be removed and discarded, except for field and lab spike samples.

3. 50ml of acetone is added to the sample in the jar and a stir bar is added. The sample is tightly capped and stirred at moderate speed for 15 minutes.

4. The extract is vacuum filtered into a 250ml evaporating flask through a Buchner funnel lined with a disc of Whatman #41 filter paper and a 5-10mm bed of Celite 545 which has been previously rinsed with acetone. The sample jar is rinsed with an additional 50ml of acetone and this fraction is filtered and combined with the first acetone extract. The acetone is allowed to evaporate in the rotovapor until water remains.

5. 125ml of acetonitrile is added to the water fraction and the extracts are evaporated to dryness by gentle rotary evaporation. If the water was not totally

evaporated, an additional 30ml of acetonitrile is added to complete the evaporation of the water. 10ml of hexane is added to dissolve the residues and the flask is sonicated for 2-3 minutes. The final hexane extract is carefully transferred via a Pasteur pipet and pipet pump to an autosampler storage vial with a foil- or teflon-lined cap for storage at -15 ± 10°C.

e. Lab Tracking of Soil and Hydrosoil Residue Samples.

Lab tracking forms will be completed for all sediment residue samples processed. Record the following on tracking forms:

A. Sample condition upon arrival in laboratory.
B. Date arrived.
C. Sample lab ID number.
D. Sample storage location.
E. Extract storage location.
F. Date extracted.
G. Date gas chromatographic analysis completed.
H. Analytical laboratory supervisor sign-off when analysis is completed.

5. Gas Chromatography:

a. Pre-analysis instrument checks:

Prior to initiation of analysis using the gas chromatograph, a few critical checks of instrument fitness will be performed.

The HP5890 display should indicate 'ready' after run parameters are programmed.

The gas cylinder regulators should indicate enough gas supply to complete the run.

The column head pressure gauges should indicate carrier gas pressure. Also, the flow rate should be checked.

Other instrumental problems will be indicated by lack of performance during the analysis. Corrective action may be taken as indicated in SOP 16.2. [Use and Maintenance of Gas Chromatograph (Hewlett Packard 5890A)]. The operator of the gas chromatograph is responsible for recording routine and nonroutine maintenance events in the gas chromatograph maintenance log book as described in step 3.b of SOP 16.2.

b. Instrumental Setpoints

The chromatographic setpoint values may be changed only by the supervisor of the analytical lab and only if proper record of the change is made in the gas chromatograph run log. The critical setpoints are listed in the gas chromatograph run log.

c. Gases

The carrier gas used is Hydrogen. The Electron Capture make-up gas is 90% argon and 10% methane. Gas flow rates will be checked at least once a week or whenever a change in the flows is suspected. Flow checks will be recorded on the G.C. run log forms under the maintenance section. A molecular sieve moisture trap and an oxygen trap should be installed in line for each of the gasses used on the gas chromatograph.

d. Analysis of Extracts

The extracts and standards from section 4 are analyzed by high resolution gas chromatography using a 5 meter X 0.53mm fused silica column. Samples are grouped by dose level and each sample is bracketed by standards at a comparable level. The non-polar methylsilicone stationary phase is 2.65μm thick. On-Column injection is used with injection volumes of 0.5μl to 5μl. The injections are made using the auto-injector on the HP5890 gas chromatograph. Manual injections may be made up to 10μl. In all cases, the injection volume of standards and samples must be identical. The oven is temperature programmed to a temperature which allows the pyrethroid to migrate through the analytical column. The chromatograms are stored in computer readable form and on printouts generated at the end of each separation. An arrow is drawn on the printed chromatogram indicating the analyte peak location. The analyst will initial all printed chromatograms.

e. Correction to Dry Weight Basis:

Water content of sediment samples is determined by drying an aliquot in a tared aluminum drying pan to constant weight in a drying oven at 105°C ±5°C. The dry weight of the sample is determined from the following calculations:

Aliquot Wet Weight(g)=[Wet Sediment + Pan(g)] - Pan Wt.(g)

Aliquot Dry Weight(g)=[Dry Wt.(Pan + Sed.)(g)]-Pan Wt.(g)

$$\text{Percent Dry Sediment} = \frac{\text{Final Dry Aliquot Wt.(g)}}{\text{Initial Wet Aliquot Wt.(g)}} \times 100$$

Dry Sample Mass = Wet Sample Mass X Percent Dry Sediment(÷100)

f. Calculations:

Calculation of cyfluthrin concentration in the samples will be by direct comparison to the standards run on the GC that bracket the sample. This may be carried out by calculator or computer using peak areas or automatically by the data acquisition computer. After comparison of the peak areas for samples to the peak areas for standards, the analyte concentration in the original sample in ng/g (ppb) may be calculated as follows:

$$\text{Cyfluthrin concentration in dry sample (ng/g)} = \frac{\text{extract conc. (pg/}\mu\text{l) X extract vol. (}\mu\text{l)}}{\text{1000 (pg/ng) X dry sample mass (g)}}$$