Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Cyfluthrin

MRID #: 415113-01

Matrix: Water

Analysis: GC/ECD

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Appendum 4 Method Validation for the Analysis of Cyfluthrin Residue in Pond Water

I. Introduction and Background

The compound cyfluthrin was applied to test ponds, at various concentrations, by either a spray method using cyfluthrin in water, or by a soil application using cyfluthrin in a soil mixture. The pond water was sampled at various times before and after application and at various depths. The water samples were extracted and the extracts were subjected to analysis to determine the amount of cyfluthrin in the sample. Standard operating procedure 4.12, described in section V, was followed for the extraction and analysis of pond water samples. The validation for the procedure is given in sections II-III below.

II. Validation Methods

One liter samples of pond water were spiked with various concentrations of cyfluthrin. Spike levels were within the range of 5.6 ppt to 1440 ppt. Both lab spikes and field spikes were performed. The samples were extracted and the extracts were subjected to analysis by gas chromatography to determine the recovery of cyfluthrin from the spiked water samples.

III. Validation Results

Lab Spikes	Average % Recovery	Std. Dev.
5.6 ppt	62%	28
11 ppt	91%	5
28 ppt	83%	9
90 ppt	87%	11
1440 ppt	85%	7

Average % Recovery of Replicates

Spike level	Lab spikes	Std. Dev.	Field spikes	Std. Dev.
10 ppt	134%	17	97%	39
50 ppt	104%	0	83%	16
100 ppt	101%	1	90%	13

(Refer to Analytical Reports 79 and 80)

IV. Conclusions

Cyfluthrin can be detected in pond water by the above method with reasonable precision. 10 ppt was selected as the quantitation limit for all water residue analysis conducted during study number MMP1.

V. Standard Operating Procedure 4.12

SOP : 4.12 Analysis of Pond or Reagent Water for Cyfluthrin Residue.

1. Principle

Cyfluthrin is extracted from pond water samples with hexane or hexanes in a one liter screw cap volumetric flask. When the hexane:water mix is stirred at moderate speed on a magnetic stir plate the hexane is drawn down into the water by vortex action and the two phases are thoroughly mixed by the action of the stir bar. The organic layer is allowed to separate and is recovered from the narrow neck of the volumetric flask. The extract is analyzed by electron capture gas chromatography using cool on-column injection and an uncoated 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. One liter volumetric flasks with teflon lined screw caps (Pyrex no. 5650, or equivalent).

3. Volumetric flasks with teflon lined screw caps.

4. Magnetic stir plates and 3.8 cm teflon coated octagonal magnetic stir bars.

5. Pasteur capillary pipet, 23 cm length.

6. Pipet pumps, 10 ml capacity.

7. Syringes - Hamilton brand gastight, or equivalent.

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

9. 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). With a 1 m x 0.53 mm ID uncoated gap attached with a glass connector.

10. 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.

b. Reagents:

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

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

3. 90% argon:10% methane gas - Liquid Carbonics zero grade or equivalent.

4. Procedures and Methods

a. Preparation of 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 approximately 25 mg analytical grade cyfluthrin in hexane in a 50 or 100 ml volumetric flask. Intermediate standards are prepared by dilution of these solutions. Standards will be prepared at concentrations which will bracket the sample concentrations. Preparation of all standards is recorded on standard preparation data sheets.

Standards are stored at -15±10°C and a control chart is used to monitor their storage stability. Fresh standards will be prepared when standard values fall outside acceptable ranges on the control chart. Only one injection is made from each autosampler vial of working standard. All standards are labeled as described in SOP 15.6. Use of an analytical standard is documented on the "Analytical Standard Log" form as described in SOP 4.8.

b. Preparation and Application of Spikes

One laboratory and one field spike for each set of samples is prepared along with each days sampling. Spiking solutions are prepared in acetone. All spikes will be done in flasks to receive samples from control ponds. One of these samples is spiked in the field and the other, collected in an unspiked volumetric flask, is returned to the lab and transferred to a spiked volumetric flask. Two samples, one field spike and one lab spike sample, will be taken from the control pond designated for that day. These will be taken at the same time and place as the normal mid-depth pre-dose sample. The cyfluthrin levels in the spikes will approximate the level of cyfluthrin expected in the samples.

Record the following information on the spiking data sheet.

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

c. Liquid Liquid Extraction

A one liter volumetric flask is brought to volume with water to be analyzed and covered tightly with aluminum foil. A 3.8 cm long teflon coated octagonal magnetic stir bar is placed in the flask and the flask is placed on a magnetic stir bench.

5.0 ml of pesticide grade hexane is added and the flask is tightly capped. The sample is stirred at moderate speed for 15 minutes, providing intimate contact between the hexane and the water. The phases are allowed to separate and the hexane layer is removed with a Pasteur pipet and pipet pump and transferred to a 20 ml scintillation vial. Another 5 ml of hexane is added to the volumetric flask and the sample is again stirred at moderate speed for 15 minutes. The hexane layer is removed and added to the previous hexane fraction. The scintillation vial containing the hexane fraction, part which may be in an emulsion, is covered with foil, tightly capped, and placed in a -15±10°C freezer until the water portion freezes. The vial is removed from the freezer and the contents are allowed to come to room temperature, during which time the emulsion breaks.

The hexane fraction is carefully removed with a Pasteur pipet and pipet pump such that none of the sample comes

in contact with the pipet pump. If this does accidentally occur, the sample and the bulb are discarded since interference peaks arise from the bulb. Part of the sample is transferred directly to an amber autosampler vial for gas chromatographic analysis and the remainder is transferred to a labeled 8 ml amber storage vial and stored at -15±10°C.

d. Lab Tracking of Water Residue Samples.

Lab tracking forms will be completed for all water 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 gas chromatograph 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 gases 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 rapidly 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. The analyst will initial all printed chromatograms.

e. Calculations:

Calculation of cyfluthrin concentration in the sample will be by direct comparison to the standards that bracket the sample. This may be carried out by calculator or computer using peak areas or automatically by the data acquisition computer. The analyte concentration in one ul of the original sample may be calculated as follows:

$$\frac{\text{Conc. of Standard(pg/ul)}}{\text{Peak Area}} = \frac{\text{Conc of Sample(pg/ul)[unknown]}}{\text{Peak Area}}$$

The analyte concentration in the original sample in ng/l(ppt) may be calculated as follows:

$$\text{Cyfluthrin Concentration in Sample(ng/l)} = \frac{\text{Extract Conc.(pg/ul) x Extract Vol(ul)}}{\text{1000pg/ng x Original Sample Volume(l)}}$$