ANALYSIS OF ORGANOPHOSPHATE PESTICIDES IN SEDIMENT

1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of organophosphorous pesticides in soil and sediment by high resolution gas chromatography using electron capture (ECD), nitrogen phosphorous (TSD), or flame photometric (FPD) detectors or gas chromatography-mass spectrometry (GC-MS). Table 1 lists the target pesticide compounds currently analyzed with their method detection limits and reporting limits for sediment.

1.2 These procedures are applicable when low part per billion analyses are required to monitor differences between sediments from relatively uncontaminated reference areas and contaminated areas.

2.0 Summary of Method

2.1 Sets of 12-16 sediment samples are scheduled for extraction by the project lead chemist. Extraction methods employed were developed and validated by the Water Pollution Control Laboratory. Extract cleanup and partitioning methods are modifications of the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1

Wet or dry sediment samples are removed from the freezer and allowed to thaw. A separate extraction bench sheet is initiated for each project, and analysis type.

2.2 Water that has separated from wet samples is decanted off and discarded. The sample is mixed thoroughly and a l-5 g sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 hours to determine moisture content. A 10 g sample is weighed

into a pre-cleaned 250 mL jar and is mixed with

Table 1. Organophosphorous Pesticides Analyzed and Their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Sediment[1].

	MDL ng/g ppb Dry wt.	RL ng/g ppb Dry wt.
Biphenthrin	5.0	10
Chlorpyrifos	5.0	10
Diazinon	5.0	10

1 Ten grams of sample extracted (assumes 50% moisture)

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approximately 7 g of pre-extracted Hydromatrix® until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE ZOO) extractor cell and packed by tamping the mixture. A solution containing an appropriate pesticide surrogate is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted with a 50/50 mixture of acetone/dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in 60 mL VOA vials.

2.3 The extracts are dried using sodium sulfate, evaporated to approximately 0.5 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 5 mL with DCM. The extracts are then filtered through a 0.45 µm syringe filter into Autoprep 2000 Gel Permeation Chromatograph (GPC) autosampler tubes.

2.4 The GPC autosampler tubes are then placed on the GPC autosampler for sulfur removal.

2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroluem ether. The extracts are then fractionated using a standard 4 inch x 22 mm Florisil® column. The Florisil® columns are eluted with petroleum ether (PE) (Fraction 1- discarded), 6% diethyl ether/PE (Fraction 2) and 15% diethyl ether/PE (Fraction 3). The fractions are concentrated to an appropriate volume using K-D/micro

K-D apparatus prior to analysis by dual column high resolution gas chromatography. A mixture of organophosphorous pesticide standards is eluted through the Florisil® column to determine the recovery and separation characteristics of the column. The distribution of synthetic organic compounds in the four fractions is listed in Table 2.

Table 2. Distribution of Organophosphorous Pesticides Among the Three Fractions of a Standard Florisil® Column.

(0%) Fraction 1/	**(6%) Fraction 2/**	**(15%) Fraction 3/**
(Discarded)	Biphenthrin Chlorpyrifos	Diazinon

1/ 0% ethyl ether in petroleum ether.
2/ 6% ethyl ether in petroleum ether.
3/ 15% ethyl ether in petroleum ether.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination.

3.3 Interferences co-extracted from tissue samples limit the method detection and quantitation limits. For this reason, sample extract cleanup is necessary to yield reproducible and reliable analyses of low level contaminants in the tissue sample.

4.0 Apparatus and Materials

4.1 Beakers, borosilicate glass, 250 mL

4.2 Chromatographic Column - 300 cm x 22 cm borosilicate glass chromatography column with Teflon stopcock.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 mL graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22, joint, is used to prevent evaporation of extracts.

4.4.2 Evaporation flask - 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 Boiling chips, Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.5 Water bath, Blue M or Organomation 5-position, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.

4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.6.1 Extraction Cells, 33 mL Dionex P/N 049562

4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.6.3 VOA Vials, 60 mL pre-cleaned and certified.

4.7 Sample vials - glass, 2-5 mL with PTFE-lined screw cap.

4.8 Analytical balance - capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Balance - capable of 100 g to the nearest 0.01 g.

4.11 Disposable Pasteur Pipets - (rinsed with solvents before use).

4.12 Aluminum dishes for moisture determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL

4.16 Culture tubes, 16 x 100 mm, with PTFE lined cap.

4.17 Gas chromatograph, Hewlett-Packard HP 6890 plus, equipped with two micro ECD detectors with EPC, split-splitless injector with EPC, and autosampler.

4.18 Gas chromatograph, Varian 3600, equipped with two TSD detectors, direct and SPI injectors and 8200 autosampler.

4.19 Capillary columns, 60 meter DB5 and 60 meter DB17 (J&W Scientific) (0.25 mm I.D. and 0.25 μm film thickness) connected to a single injection port using a "Y" press fit connector.

4.20 Capillary columns, 30 meter DB5 and 30 meter DB17 (J&W Scientific) (0.32 mm I.D. and 0.25 μm film thickness) connected to a single injection port using a "Y" press fit connector.

4.21 Data System, Hewlett-Packard, to collect and record GC data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

4.22 Gel Permeation (size exclusion) Chromatograph, automated, 01 Analytical (ABC) Model 2000, equipped with 50 cm Optima column (100% DCM).

5.0 Reagents

5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Acetone. (Same as above).

5.3 Iso-octane. (Same as above).

5.4 Diethyl ether preserved with 2% ethanol. (Same as above).

5.5 Dichloromethane (DCM). (Same as above).

5.6 Chem Elut-Hydromatrix', Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.

5.7 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.8 Florisil®, 60/100 mesh, PR grade, U.S. Silica Corp.

5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE, GPC and solvent evaporation).

5.10 Nitrogen, ultra-pure (99.99999%) for GC makeup.

5.11 Helium, ultra-pure (99.99999%) for GC carrier gas.

5.12 Air, compressed, breathing quality, for ASE pneumatics.

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 **Sample Collection, Preparation, and Storage**

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. The samples should be collected using pre-cleaned polycarbonate containers and frozen as soon as possible after collection.

6.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned. (e.g., stainless

steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed with a high-purity acetone, and finally rinsed with Type II water.

7.0 **Sample Extraction**

7.1 Remove sediment samples from freezer and allow to thaw. Prior to extraction, decant off any water that has separated from the sediment sample then mix well (stirred) by hand using a clean Teflon spatula. Sample sets of 12-16 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes, and laboratory control spikes.

7.2 A separate extraction bench sheet is started for each project, sample matrix type, and analysis type. Several bench sheets may be used for an extraction set.

7.3 Prepare a 250 mL beaker and glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle. If a pre-cleaned and certified jar is substituted for the beaker it is not necessary to rinse it with solvent.

7.4 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap, if they are placed outside of this area they will interfere with the ASE optical sensor. Use two VOA vials for each sample to be extracted. Label with Project Number (eg. L#) or Project Name and the sample identifier with the second VOA vial for each sample additionally labeled "RE-EXTRACT". Label and weigh aluminum planchets for moisture determinations (samples ID can be made on the bottom of planchets using a ball point pen.

7.5 Tare beaker or pre-cleaned jar. Using a clean glass rod, stir the sediment and make sure that water has not separated from the sediment. Weigh 10 g of sediment into the beaker, record the weight on the bench sheet, and add the Hydromatrix from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free. flowing.

7.6 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 48 hours and re-weigh dry weight.

7.7 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter *(the filter is the one that was used to pre-extract the Hydromatrix).*

7.8 Pour the sediment/Hydromatrix mixture through the powder funnel back into the extraction cell that the Hydromatrix was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the method blank and laboratory control spike and its duplicate *(if used)* will contain only Hydromatrix.

7.9 All of the extraction cells are spiked with the pesticide surrogate standard. Spike each cell with exactly 1.0 mL of the pesticide surrogate solution (200 ng/mL). Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCD) and its duplicate (LCSD) (if used) are spiked with exactly 1.0 mL of the pesticide matrix spike solution (200 ng/mL). Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.11 The extraction cells are capped *(firmly tightened)* and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carrousel with the position numbers corresponding to the numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.

7.12 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat 0 min.

Heat	5 min.
Static	5 min.
Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100°C
Sol A	Other 100%

7.13 After the initial extraction is complete, remove full VOA vials and place in a Nalgene rack and replace collection VOA vials with the vials labeled RE-EXTRACT. Check each of the extraction cells to make sure that the caps are finger tight as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.14 When extraction is completed place VOA vials in a Nalgene rack with the RE-EXTRACT vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography (GPC)

IMPORTANT: All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.

8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps.

8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Add a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of sodium sulfate to the funnel.

8.3 Pour sample extracts from the VOA vials through sodium

sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial cap and shake and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (-10-20 mL). *Use a small clean glass beaker to transfer DCM for rinses, use Teflon wash bottle for rinsing glassware only.... never for dispensing DCM.*

8.4 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 80-82°C. Evaporate the solvent until the reflux line falls below the top of the Snyder column. At this point there should be between l-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column.

8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath repeat steps l-5 for the remaining samples extracted with this set.

8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate . If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath. Evaporate the solvent until only 0.5 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

8.7 When the solvent has been evaporated to 0.5 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 5.0 mL.

8.8 Draw the sample up into a clean 10 mL syringe with a 0.45 µm filter attached. Filter the sample into a GPC' autosampler tube. Attach the GPC autosampler tube to the autosampler of the Autoprep 2000 GPC.

8.9 Gel permation chromatography system : (01 Analytical (ABC) AS2000),an automated system equipped with:

- Column: optima teflon column(50cm X 0.75" id) packed with 39.4g Envirobead select S-X3 resin
- 2.5 ml injector loop

- Ultraviolet detector,254nm
- strip-chart recorder set at 20cm/hr
- solvent - 100% dichloromethane

8.9 Samples to be analyzed for pesticide in sediment use

GPC Method 4:

Dump time:	9.0
Collect time:	17.58
Wash time:	7.0

Start the GPC using the following procedure:

8.9.1 Turn on the pump and nitrogen gas, set the column switch to *Inline* and allow solvent to pump through the system for about 30 minutes to allow column to stabilize and to rinse out any contaminants remaining in the column and/or detector. Make sure the solvent rinse and eluant reservoirs are full of DCM. Check to make sure that no bubbles of air are entering the detector from the column. Set up the GPC for calibration by entering the following:

Dump Time:	45 minutes
Collect Time:	None

8.9.2 Measure and adjust the flow rate to 5 mL/min. Load calibration solution using a 10 mL syringe and 5 mL of pre-filtered GPC calibration standard solution in a dry GPC sample vial. Check UV detector parameters (0.2 AUFS, 0.3 sec Rise Time) and system pressure (10-15 psi) and make sure that the recorder is turned on

with the following settings (50 mV and 20 cm/hr). Run the calibration solution and obtain a UV chromatogram showing a discrete peak for each calibration component.

8.10 The GPC eluate is collected in 125 mL pre-cleaned Boston round bottles if small GPC column is used or 250 mL Erlenmeyer flask if the large column is used. Pour GPC eluate into K-D flask and add 0.5 mL of iso-octane (2,4,5-trimethyl pentane) to each flask as a "keeper". Add a micro boiling chip, attach a Snyder column to the flask and evaporate solvent on the hot water bath. When the apparent volume of solvent in the concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. Repeat a third time. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Remove the concentrator tube from the K-D apparatus.

8.12 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon faced cap. Place extracts in a refrigerator for storage until the final Florisil® column cleanup is done.

9.0 Florisil® Column Fractionation

IMPORTANT: All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure. Florisil® must be activated in an oven at 130°C for at least 24 hours prior to use.

9.1 This procedure is performed after the GPC cleanup procedure for all sediment samples analyzed for Chlorpyrifos, Diazinon and Bifenthrin. **No** more than 30 minutes prior to performing the Florisil® cleanup, add a small amount (~1 g) of sodium sulfate to the culture tubes to remove residual water from the extract. If the sodium sulfate becomes a solid plug in the bottom of the tube, add more until some of the sodium sulfate is free flowing when the tube is shaken. *If extracts are allowed to remain in contact with sodium sulfate for longer that 30 minutes, target analyte loss may result.*

9.2 Prepare the reagents to be used for Florisil® cleanup: 6% ethyl ether in petroleum ether, 15% ethyl ether in PE. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 200 mL per sample for the 6% (F2) and 15%(F3), fractions. Fill the 250 mL separatory funnels located above the Florisil® columns with 200 mL of petroleum ether (0% or F1 fraction). These funnels will be used for eluant reservoirs.

9.3 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Measure 4 inches from the top of sodium sulfate and mark column outside of the column with a permanent marker. Fill the column with Florisil® to about 3/4 inch beyond the mark and tap column with rubber "mallet" to firmly settle the Florisil®. Add more Florisil® as necessary so that it is even with the mark after settling. Top column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water. Tie a Kimwipe around the column to catch any condensation or accidental overflow which could roll down the outside of the column and contaminate the sample.

9.4 Place a 600 mL beaker under the column. Fill the solvent reservoir above the column with 200 mL of petroleum ether (F1). Pre-wet the column with about 60 mL of petroleum ether. Filling the column to 1 inch above the "Kimax" label is usually sufficient.

IMPORTANT: **From this point and through the elution process, the solvent level should <u>never</u> be allowed to go below the top of the sodium sulfate layer and the column stopcock should never be closed.**

9.5 When approximately 1 inch of PE remains above the surface of the column, adjust flow rate to about 5 mL/min (32 drop/l2 sec). When the meniscus of the rinse PE reaches the column bed surface, pour the sample extract onto the column. Immediately add 5 mL

of PE to the tube, shake vigorously, and set aside. When the collected volume reaches 10 mL, pre-wet the next column. If the columns are started in this sequential fashion, the lag time will be adequate to perform the necessary tasks for up to six columns.

9.6 When the sample extract reaches the sodium sulfate layer, add the PE rinse from the culture tube. Add another 5 mL to the culture tube, shake and immediately add this rinse to the top of the column. Repeat rinse a third time. When the final rinse reaches the sodium sulfate layer, fill the column one half full with PE from the reservoir. Adjust the drip rate from the separatory funnel to approximately equal that from the column tip. Try to keep the solvent level in the column constant to avoid variations in flow rate.

9.7 When all of the F1 solvent has been transferred to the column from the solvent reservoir, close the reservoir stopcock and fill the separatory funnel with 200 mL of the 6% diethyl ether/PE mixture. Just before the PE reaches the sodium sulfate layer, remove the waste PE beaker and place a K-D flask under the column. When the solvent reaches the sodium sulfate, add the 6% diethyl ether/PE to the column and elute as before.

9.8' Repeat the above using 200 mL of 15% diethyl ether/PE mixture. Add 0.5ml iso octane, a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 6% (F2) fraction and place vessel in the hot water bath with the temperature set at 80-82 °C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.9 When the vessels are cool, remove the concentrator tube from the K-D flask add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 85°C on a hot plate. Evaporate the solvent until only 0.5-l mL remains in the concentrator tube. Four or five tubes

can be evaporated at one time.

9.10 When the solvent has been evaporated to 0.5-l mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step. Transfer the extract to a clean labeled culture tube and cap.

9.11 Repeat for 15% (F3), extracts. The extracts are ready for analysis by GC or GC-MS.

10.0 Analytical Procedure

10.1 Bifenthrin analysis:

10.1.1 Hewlett-Packard 6890 plus gas chromatograph equipped with two ^{63}Ni micro-electron capture detectors with EPC and autosampler. Two 60 meter, 0.25 mm ID, 0.25 um (film thickness) fused silica columns (J&W) are used. A 5 meter length of DB-5 column is connected to a press fit "Y" union which splits the column effluent into two 60 m columns, a DB-5 and a DB-17. The injector is a split-splitless injector with EPC.

10.1.2 Chromatograph conditions:
The injector is operated isothermal at 240°C. The oven has an initial temperature of 70°C and is immediately temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 11 min. Helium is used as the carrier gas at a linear velocity of 35 cm/sec. Nitrogen is used for the detector makeup at 30 mL/min.

10.2 Chlorpyrifos and Diazinon analysis:

10.2.1 Gas chromatograph, Varian 3600, equipped with varian 8200 autosampler,Septum-equipped Progammable Injector (SPI) and Thermionic Specific Detector (TSD). Dual megabore columns, 15 meter DB17 and DB-5 (J&W Scientific) (0.53 mm I.D. and 1.5µm film thickness)

10.2.2 Chromatograph conditions:
The injector is operated isothermal at 190°C. The oven has an initial temperature of 190°C and held for 3, then temperature programmed to 250°C at a rate of 5°C/min and held for 10 min. Nitrogen is used as the carrier gas and detector makeup at 30 mL/min.

10.2.3 Data System, Hewlett-Packard, to collect and record both Varian and HP GC data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

11.0 References

Tetra Tech, 'Inc. 1986. Bio Accumulation monitoring Guidance: 4. Analytical Methods for U.S. Priority Pollutants and 301 (h) Pesticides in tissues from Estuarine and Marine Organisms. TC-3953-03. U.S. EPA Washington, DC.

U.S. Environmental Protection Agency. 1993. Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories, Volume I, Fish Sampling and Analysis. EPA 823-R-93-002. U.S. EPA, Office of Water, Washington D.C.

U.S. Food and Drug Administration. 1994. Pesticide Analytical Manual. Volume 1, Chapter 3, Multiclass Multiresidue Methods. U.S. Food and Drug Administration, Rockville, MD.