

## PREFACE

This report is final documentation of the 1992 environmental contaminants evaluation of largemouth bass from Great Bay National Wildlife Refuge, in Portsmouth, New Hampshire (PACF Catalog Number 5030016, Regional ID Number 5F01). Study design, implementation, data analysis, and reporting were completed by Environmental Contaminants personnel in the New England Field Offices, and Fisheries Assistance personnel in the Laconia F.A. Office, U.S. Fish and Wildlife Service, Department of the Interior. Funding for the project was provided by the Division of Refuges and Wildlife.

Questions, comments, and suggestions related to this report are encouraged. Written enquiries should refer to Report Number RY93-NEFO-2-EC and be directed to:

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# INTRODUCTION

Great Bay National Wildlife Refuge was formed from lands that were previously part of Pease Air Force Base. The former Pease AFB has numerous hazardous waste sites that are in various stages of remediation. The objective of this study was to conduct a gross screening level survey of fish from three impoundments on Peverly Brook to determine if contaminants from any of the hazardous waste sites are contaminating fish in parts of Great Bay NWR.

## METHODS

Largemouth bass (*Micropterus salmoides*) were collected from Upper Peverly Pond (21 September), Stubbs Pond (22 September) and Lower Peverly Pond (22 October), 1992 using boat-mounted electrofishing gear (Fig. 1). A minimum of three and a maximum of five fish were used for residue analysis. Organochlorines, pesticides, PCB's, arsenic, and elementary metals residue analyses were conducted by Hazelton Laboratories using procedures described in appendix 1.

## RESULTS AND DISCUSSION

### Organics

With the exception of DDD and DDE compounds, all other residue levels were below detection limits (0.1 for total PCB's and toxaphene, 0.01 for the remainder of the analytes). The concentrations of DDD, DDE, and total DDT are listed in table 1.

Table 1. DDD and DDE residue levels (PPM wet weight) in largemouth bass collected from Great Bay NWR, 1992.

Site	DDD	DDE	TOTAL DDT
Stubbs Pond	0.364	0.420	0.784
Lower Peverly Pond	<0.01	0.042	0.042
Upper Peverly Pond	0.021	0.076	0.097

Total DDT levels in largemouth bass collected from Stubbs Pond appear elevated when compared to other predatory fish in New England. Largemouth bass from the Hudson River (0.26 ppm); smallmouth bass (*Micropterus dolomieu*) from the Merrimack (0.15 ppm) and Penobscot (0.01 ppm) rivers; yellow perch (*Perca flavescens*) from the Connecticut (0.31 ppm), Androscoggen (0.09 ppm), and Kennebec (0.03 ppm) rivers; and northern pike (*Esox lucius*) from Lake Champlain (0.14 ppm) had lower total DDT levels (Schmitt et al. 1990). There is no evidence, however, that this level has any adverse effect on largemouth bass.

Levels of DDT and its metabolites have been correlated with eggshell thinning and reduced reproduction in various raptors including bald eagles (*Haliaeetus leucocephalus*) (Wiemeyer et al. 1984). Barn owls (*Tyto alba*), one of the most sensitive of the raptors tested, exhibited significant eggshell thinning and embryo mortality when fed 3.0 ppm (wet weight) DDE (Mendenhall et al. 1983). The largemouth bass tested were among the largest bass in the three ponds, and are, therefore, expected to exhibit the greatest degree of bioaccumulation of contaminants due to their ages and position in the food chain. Because the samples likely represent a worst-case bioaccumulation scenario in aquatic biota, and the dietary effect level for predatory birds are significantly greater than the highest sampled DDT levels in potential prey, there does not appear to be a significant risk to piscivorous birds from eating fish from any of the three ponds.

The FDA Action Level for DDT for the edible portion of the fish is 5.0 ppm. Since the levels in the fish from Great Bay NWR were lower than 5.0 ppm for the whole body of the fish, human health risks appear to be low as well.

## **Metals**

The results of the metal analyses from fish collected from the three ponds are presented in table 2. For ease of comparison, data from the National Contaminant Biomonitoring Program (NCBP) (Schmitt and Brumbaugh 1990) are also listed in table 2. The NCBP collected whole fish from 109 stations nationwide, and analyzed them for select metals.

The fish collected from Lower Peverly Pond had considerably higher concentrations of copper (19.50 ppm) than fish collected from Upper Peverly Pond (0.49 ppm) or Stubbs Pond (0.48 ppm), and concentrations were also notably higher than the mean concentration found nationwide (0.65 ppm). Arsenic and aluminum levels were also found to be slightly higher in fish from Lower Peverly Pond, 0.71 ppm and 1.20 ppm, respectively, than the other two ponds (0.37 ppm and <0.99 ppm for Upper Peverly Pond, and 0.46 ppm and <0.99 ppm for Stubbs Pond). Nickel concentrations were also much higher in Lower Peverly Pond (1.08 ppm) than in Upper Peverly Pond (<0.12 ppm) or Stubbs Pond (<0.12 ppm). Zinc concentrations were much higher in fish from Lower Peverly Pond (24.7 ppm) than the other two ponds, (13.20 ppm for Upper Peverly Pond and 14.60 ppm for Stubbs Pond), but only slightly higher than the national mean (21.70 ppm).

Magnesium concentrations were found to be slightly higher in fish collected from Stubbs Pond (429.00 ppm) than from Lower Peverly Pond (397.00 ppm) or Upper Peverly Pond (396.00 ppm). Selenium was also found to be slightly higher in Stubbs Pond (0.89 ppm) than the other two ponds (0.17 ppm for Lower Peverly Pond and 0.47 for Upper Peverly Pond), and the national mean (0.42 ppm). Arsenic and mercury levels were found to be higher in fish collected from all three ponds on the refuge than the mean concentration found by the nationwide study. Arsenic levels for Stubbs Pond, Lower Peverly Pond, and Upper Peverly Pond were, respectively: 0.46 ppm, 0.71 ppm, and 0.37 ppm. The geometric mean found by the national study was 0.14 ppm. The concentrations of mercury found in the three ponds were: 0.33 ppm in Stubbs Pond, 0.18 ppm in Lower Peverly Pond, and 0.27 ppm in Upper Peverly Pond. The NCBP mean for mercury in fish was 0.10 ppm.

Table 2. Elementary metals residue levels in largemouth bass collected from Great Bay NWR, 1992, and levels of metals found in fish collected by the National Contaminant Biomonitoring Program (NCBP) (Schmitt and Brumbaugh 1990). Concentrations are presented in PPM (wet weight).

Analyte	Stubbs Pond	Lower Peverly Pond	Upper Peverly Pond	NCBP
Aluminum	<0.99	1.20	<0.99	-
Arsenic	0.46	0.71	0.37	0.14
Barium	0.24	0.26	0.25	-
Beryllium	<0.02	<0.02	<0.02	-
Boron	<0.40	<0.40	<0.40	-
Cadmium	<0.06	<0.06	<0.06	0.03
Chromium	0.50	0.52	0.46	-
Copper	0.48	19.50	0.49	0.65
Iron	12.30	11.90	15.00	-
Lead	<0.50	<0.50	<0.50	0.11
Magnesium	429.00	397.00	396.00	-
Manganese	4.69	5.01	4.94	-
Mercury	0.33	0.18	0.27	0.10
Molybdenum	<0.40	<0.40	<0.40	-
Nickel	<0.12	1.08	<0.12	-
Selenium	0.89	0.17	0.47	0.42
Strontium	26.10	22.10	19.40	-
Vanadium	<0.06	<0.06	<0.06	-
Zinc	14.60	24.70	13.20	21.70

- No data

Fish from Lower Peverly Pond appear to carry slightly higher metal burdens than fish in the other two ponds. However, with the exceptions of arsenic and mercury, all of the metals found to differ significantly in concentration between the ponds are present in all the ponds at levels well below reported effect levels.

Arsenic was found to be higher in all of the ponds than the levels found by the NCBP. We have found background levels of arsenic in New Hampshire to be relatively high compared to national surveys (Shacklette and Boerngen 1984; U.S. Fish and Wildlife Service and NH Division of Public Health Services 1989), which may account for the relatively elevated levels found at the Great Bay NWR. Diminished growth and survival has been reported in immature bluegills (*Lepomis macrochirus*) when tissue concentrations of arsenic exceeded 1.3 ppm, or exceeded 5.0 ppm in adults (Eisler 1988). In birds, arsenic residue levels between 2.0 and 10.0 ppm in liver or kidney are considered elevated (Eisler 1988). Bioconcentration factors (BCF) for arsenic, experimentally determined, have been found to be relatively low, and arsenic is not known to biomagnify in the food chain. Therefore, it appears that the levels of arsenic in fish at the Great Bay NWR are not a threat to the aquatic communities, or to piscivorous predators.

The presence of mercury at detectable levels is always of concern because of its high

toxicity and its ability to biomagnify in the food chain. Reduced growth of rainbow trout (*Salmo gairdneri*) has been reported at water concentrations of mercury as low as 0.04 ug/l (Eisler 1987). Brook trout (*Salvelinus fontinalis*) have been reported to show signs of toxicity at whole body concentrations of 5.0 to 7.0 mg/kg (Eisler 1987). Mercury residues of 0.79 to 2.0 mg/kg in eggs, and 5.0 to 40.0 mg/kg have been reported to impair reproduction in a number of bird species (Eisler 1987). The FDA Action Level for mercury in the edible portion is 1.0 ppm. Given that the fish chosen for analyses at Great Bay NWR were some of the largest fish in the ponds, the concentrations reported provide the worst case scenario in terms of bioaccumulation. It, therefore, appears that there is no immediate hazard of mercury toxicity for fish and wildlife, or humans, at the refuge. However, periodic monitoring for mercury is warranted.

## CONCLUSIONS

No serious contamination was found in any of the three ponds sampled at the refuge. However, as long as contaminated sites are known to exist adjacent to the refuge, ongoing monitoring of the refuge is warranted. This ongoing monitoring is presently being conducted as part of the Pease AFB closure.

## LITERATURE CITED

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Appendix 1. Organic Analytical Results, QA/QC, and Methods.

ECDMS ANALYTICAL REPORT (6)

25-Jan-93

Catalog: 5030016

Regional Study Id: 5F01

Purchase Order: 85830-2-3997

User Id: R5NEFO

Submitter: Ken Carr - Concord, NH

Lab Name: Hazleton Environmental Services, Inc. (HAZL)

Report Includes the Following Sections:

- Weight, % Moisture, % Lipid, Total Suspended Solids
- Soil / Sediment Parameters
- Contaminant Concentrations
- Procedural Blanks
- Duplicates
- Reference Materials
- Spike Recoveries
- Comments (Result Modifiers and QA/QC Comments)
- Analytical Methods

## WEIGHT, % MOISTURE, % LIPID, TOTAL SUSPENDED SOLIDS

Sample Number	Sample Matrix	Sample Weight (g)	Percent Moisture	Percent Lipid	Total Suspended Solids ( % )
BassPond	Whole Body	1000	73.77	3.08	
LPeverly	Whole Body	1000	71.15	1.71	
UPeverly	Whole Body	1000	71.96	5.11	

SOIL / SEDIMENT PARAMETERS

Sample Number	Percent TVS	Percent TOC	----- Particle Size -----		
-----	-----	-----	%Sand	%Silt	%Clay
-----	-----	-----	-----	-----	-----

- NO DATA EXISTS FOR THIS SECTION.

## CONTAMINANT CONCENTRATIONS

Analyte	Sample Number	Sample Matrix	Result (ppm Dry Wt.)	Detection Limit (ppm Dry Wt.)	Result (ppm Wet Wt.)	Detection Limit (ppm Wet Wt.)
HCB	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
PCB-TOTAL	BassPond	Whole Body	< .381	.381	< .1	.1
	LPeverly	Whole Body	< .347	.347	< .1	.1
	UPeverly	Whole Body	< .357	.357	< .1	.1
alpha BHC	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
alpha chlordane	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
beta BHC	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
dieldrin	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
endrin	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
gamma BHC	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
gamma chlordane	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01

## CONTAMINANT CONCENTRATIONS (Cont.)

Analyte	Sample Number	Sample Matrix	Result (ppm Dry Wt.)	Detection Limit (ppm Dry Wt.)	Result (ppm Wet Wt.)	Detection Limit (ppm Wet Wt.)
gamma chlordane	UPeverly	Whole Body	< .036	.036	< .01	.01
heptachlor epoxide	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
mirex	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
o,p'-DDD	BassPond	Whole Body	.091	.038	.024	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
o,p'-DDE	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
o,p'-DDT	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
oxychlordane	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	< .036	.036	< .01	.01
p,p'-DDD	BassPond	Whole Body	1.296	.038	.34	.01
	LPeverly	Whole Body	< .035	.035	< .01	.01
	UPeverly	Whole Body	.075	.036	.021	.01
p,p'-DDE	BassPond	Whole Body	1.601	.038	.42	.01
	LPeverly	Whole Body	.146	.035	.042	.01
	UPeverly	Whole Body	.271	.036	.076	.01
p,p'-DDT	BassPond	Whole Body	< .038	.038	< .01	.01

## CONTAMINANT CONCENTRATIONS (Cont.)

Analyte	Sample Number	Sample Matrix	Result (ppm Dry Wt.)	Detection Limit (ppm Dry Wt.)	Result (ppm Wet Wt.)	Detection Limit (ppm Wet Wt.)
p,p'-DDT	LPeveryly	Whole Body	< .035	.035	< .01	.01
	UPevelyly	Whole Body	< .036	.036	< .01	.01
toxaphene	BassPond	Whole Body	< .381	.381	< .1	.1
	LPeveryly	Whole Body	< .347	.347	< .1	.1
	UPevelyly	Whole Body	< .357	.357	< .1	.1
trans-nonachlor	BassPond	Whole Body	< .038	.038	< .01	.01
	LPeveryly	Whole Body	< .035	.035	< .01	.01
	UPevelyly	Whole Body	< .036	.036	< .01	.01

## PROCEDURAL BLANKS

Analyte	Lab Sample Number	Result Total UG
-----	-----	-----
HCB	21201295	0
PCB-TOTAL	21201295	0
alpha BHC	21201295	0
alpha chlordane	21201295	0
beta BHC	21201295	0
dieldrin	21201295	0
endrin	21201295	0
gamma BHC	21201295	0
gamma chlordane	21201295	0
heptachlor epoxide	21201295	0
mirex	21201295	0
o,p'-DDD	21201295	0
o,p'-DDE	21201295	0
o,p'-DDT	21201295	0
oxychlordane	21201295	0
p,p'-DDD	21201295	0
p,p'-DDE	21201295	0
p,p'-DDT	21201295	0

## PROCEDURAL BLANKS (Cont.)

Analyte	Lab Sample Number	Result Total UG
-----	-----	-----
toxaphene	21201295	0
trans-nonachlor	21201295	0

## DUPLICATES

Analyte	Sample Number	Sample Matrix	Initial Result (ppm / %)	Duplicate Result (ppm / %)	Average	Relative % Difference
% Lipid	BassPond	Whole Body	3.08 %	3.37 %	3.225	8.99
% Moisture	BassPond	Whole Body	73.77 %	73.77 %	73.77	0
HCB	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
PCB-TOTAL	BassPond	Whole Body	< .1 Wet	< .1 Wet	0.05	0
alpha BHC	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
alpha chlordane	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
beta BHC	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
dieldrin	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
endrin	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
gamma BHC	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
gamma chlordane	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
heptachlor epoxide	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
mirex	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
o,p'-DDD	BassPond	Whole Body	.024 Wet	.023 Wet	0.0235	4.26
o,p'-DDE	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
o,p'-DDT	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
oxychlordane	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0

## DUPLICATES (Cont.)

Analyte	Sample Number	Sample Matrix	Initial Result (ppm / %)	Duplicate Result (ppm / %)	Average	Relative % Difference
p,p'-DDD	BassPond	Whole Body	.34 Wet	.34 Wet	0.34	0
p,p'-DDE	BassPond	Whole Body	.42 Wet	.44 Wet	0.43	4.65
p,p'-DDT	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0
toxaphene	BassPond	Whole Body	< .1 Wet	< .1 Wet	0.05	0
trans-nonachlor	BassPond	Whole Body	< .01 Wet	< .01 Wet	0.005	0

## REFERENCE MATERIALS

Analyte	Lab Sample Number	S.R.M. ID	S.R.M. Name	* Certified Reference Value (ppm / %)	95% Confidence Interval	Result (ppm / %)	Percent Recovery
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- NO DATA EXISTS FOR THIS SECTION.

## SPIKE RECOVERIES

Analyte	Sample Number	Sample Matrix	Spike Level (ppm / %)	Amount Recovered (ppm / %)	* Spike / Background	Percent Recovery
HCB 62	BassPond	Whole Body	.5 Wet	0.31 Wet	50	
alpha BHC 54	BassPond	Whole Body	.5 Wet	0.27 Wet	50	
alpha chlordane 58	BassPond	Whole Body	.5 Wet	0.29 Wet	50	
dieldrin 70	BassPond	Whole Body	.5 Wet	0.35 Wet	50	
endrin 64	BassPond	Whole Body	.5 Wet	0.32 Wet	50	
gamma BHC 52	BassPond	Whole Body	.5 Wet	0.26 Wet	50	
gamma chlordane 58	BassPond	Whole Body	.5 Wet	0.29 Wet	50	
heptachlor epoxide 60	BassPond	Whole Body	.5 Wet	0.3 Wet	50	
mirex 58	BassPond	Whole Body	.5 Wet	0.29 Wet	50	
o,p'-DDD 75.2	BassPond	Whole Body	.5 Wet	0.376 Wet	20.83	
o,p'-DDE 68	BassPond	Whole Body	.5 Wet	0.34 Wet	50	
o,p'-DDT 76	BassPond	Whole Body	.5 Wet	0.38 Wet	50	
oxychlordane	BassPond	Whole Body	.5 Wet	0.27 Wet	50	

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p,p'-DDD 70	BassPond	Whole Body	.5	Wet	0.35	Wet	1.47
p,p'-DDE 44	BassPond	Whole Body	.5	Wet	0.22	Wet	1.19
p,p'-DDT 80	BassPond	Whole Body	.5	Wet	0.4	Wet	50

\* For a spike to be a valid measure of method accuracy, this ratio must be higher than 1.0.

## SPIKE RECOVERIES (Cont.)

Analyte	Sample Number	Sample Matrix	Spike Level (ppm / %)	Amount Recovered (ppm / %)	* Spike / Background	Percent Recovery
trans-nonachlor 60	BassPond	Whole Body	.5 Wet	0.3 Wet	50	

\* For a spike to be a valid measure of method accuracy, this ratio must be higher than 1.0.

## COMMENTS (RESULT MODIFIERS AND QA/QC COMMENTS)

Analyte	Sample Number		Result Modifier
p,p'-DDD	BassPond	M	Compound identity was confirmed by GC/MS.
	BassPond	M	Compound identity was confirmed by GC/MS.
p,p'-DDE	BassPond	M	Compound identity was confirmed by GC/MS.
	BassPond	M	Compound identity was confirmed by GC/MS.

\* Result Modifier 'M' = Compound identity was confirmed by GC/MS.

## QA/QC Comments

-----  
APPROVED, CSH

## ANALYTICAL METHODS

Method  
Code

Method Description

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010      LABORATORY: Hazleton Laboratories America, Inc.

## Extraction by Soxhlet

## X.    SCOPE:

This method covers the extraction of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in biological tissues.

## SAMPLE PREPARATION:

Blend 20 grams of ground tissue with 40 grams of anhydrous sodium sulfate in a 250 ml beaker. If there is not 20 grams of sample available the remove at least one gram for percent moistures and weigh the remainder for extracting. For wet samples, more sodium sulfate may be required. If a sufficient amount has been added the sample will appear granular. Add 500 ul of the pesticide spiking solution to the matrix spike and the control spike. Add 100 ul of the 2,4,5,6-tetrachloro-m-xylene (TMX) surrogate spiking solution to all samples and QC samples. Allow the ground tissue/sodium sulfate to dry under a hood for a couple of hours, stirring it occasionally.

## PROCEDURE:

Load the prepared sample into the soxhlet extractor between two plugs of pre-extracted glass wool. Place 250 ml of methylene chloride into a pre-rinsed 500 ml erlenmeyer flask containing three to five teflon boiling chips. Attach the flask to the extractor. Add 100 ml of methylene chloride to the mixing beaker, swirl, and add the solvent to the extractor prior to attaching the condenser. Adjust the temperature of the heating mantle so that the extractors cycle at a rate of 12 to 15 cycles per hour. All the system to cycle for 16 to 20 hours.

## ANALYTICAL METHODS (Cont.)

Method Code	Method Description
010	<p>Allow the extract to cool after the extraction is complete. Rinse the condenser with extraction solvent and drain the soxhlet apparatus into the bottom collection flask.</p> <p>Pour the extract through a Whatman #4 filter into a 500 ml K-D flask fitted with a 10 ml concentrator tube. Attach a three-ball snyder column to the K-D flask and concentrate the extract on a hot water bath, adjusting the temperature so that the concentration is completed within 15 to 20 minutes.</p> <p>When the apparent volume reaches approximately 5.0 ml, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Bring up to a volume of 10 ml with methylene chloride.</p> <p>REFERENCES:</p> <ol style="list-style-type: none"><li>1. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste - Physical/Chemical Methods - EPA Publication No. SW-846," Method 3540, Office of Solid Waste and Emergency Response, Washington, D.C. (September 1986)</li><li>2. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Biological Tissues." Method MP-FWST-MA, Hazleton Laboratories America, Inc., Madison, Wisconsin.</li></ol>
011	<p>LABORATORY: Hazleton Laboratories America, Inc.</p> <p>Determination of Percent Lipids</p> <p>XI. SCOPE:</p> <p>This method covers the gravimetric determination of percent lipids in biological tissue samples.</p> <p>PROCEDURE:</p>

## ANALYTICAL METHODS (Cont.)

Method  
Code

Method Description

011

One milliliter of the 10 ml extract is placed into a preweighed aluminum weighing pan. The pan is allowed to sit lightly covered in a hood overnight to allow the solvent to evaporate. The pan is then weighed again. The following equation is then used to calculate the percent lipid:

$$\frac{((\text{weight(g) of pan + lipid}) - \text{weight(g) of pan}) \times 10 \text{ ml} \times 100}{\text{grams extracted}} = \% \text{lipid}$$

## REFERENCES:

1. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Biological Tissues." Method MP-FWST-MA, Hazleton Laboratories America, Inc., Madison, Wisconsin.

012 LABORATORY: Hazleton Laboratories America, Inc.

## Determination of Percent Moisture

## XII. SCOPE:

This method covers the gravimetric determination of percent moisture in soil, sediment and biological tissue samples.

## PROCEDURE:

One to 10 g of the sample is placed into a preweighed aluminum weighing pan. The pan is weighed again with the sample in it. The pan and sample are then placed into an oven at 105 C for 16 hours. The sample is allowed to cool in a desiccator and then weighed again. The following equation is used to calculate the percent moisture:

$$\frac{(\text{mass(g) pan + wet sample}) - \text{mass(g) pan + dry sample}}{\text{grams of sample}} \times 100 = \% \text{ moisture}$$

## ANALYTICAL METHODS (Cont.)

Method  
Code

Method Description

012

If samples are to be calculated based on dry weight, the percent moisture is converted to a correction factor (M). The calculation of the factor is:

$$100 / (100 - \% \text{ moisture}) = M$$

## REFERENCES:

1. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste - Physical/Chemical Methods - EPA Publication No. SW-846," Method 3550, Office of Solid Waste and Emergency Response, Washington, D.C. (September 1986)
2. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Soils and Sediments." Method MP-FWSS-MA, Hazleton Laboratories America, Inc., Madison, Wisconsin.

013      LABORATORY: Hazleton Laboratories America, Inc.

## Gel-Permeation Chromatography Cleanup

## XIII. SCOPE:

This method covers the cleanup of soil, sediment and biological samples by gel-permeation chromatography (GPC).

## PROCEDURE:

After extraction, the sample extracts are concentrated in Kuderna-Danish (K-D) apparatus and the volume is adjusted to 10 ml with methylene chloride. Five milliliters of this extract is then injected on an ABC Laboratories Model 1002B GPC system using a column packed with 70 g of S-X3 Bio-beads and methylene chloride as the carrier solvent. A dump, collect, and rinse cycle is then run which is

## ANALYTICAL METHODS (Cont.)

Method  
Code

Method Description

013

consistent with exhibit D, section 7.1 of reference 1 below.

The collected fraction is then quantitatively transferred to a 500 ml K-D apparatus fitted with a 10 ml concentrator tube. A three ball snyder column is attached and the extract is concentrated on a hot water bath, adjusting the temperature such that the concentration is completed within 15-20 minutes.

When the apparent volume reaches approximately 5.0 ml, the K-D apparatus is removed from the water bath and allowed to cool for at least 10 minutes. 50 ml of hexane is added to the flask and it is returned to the hot water bath and the extract is concentrated to 5.0 ml.

## REFERENCES:

1. USEPA Contract Laboratory Program, "Statement of Work for Organic Analysis, multi-media, multi-concentration", Document number OLM01.0 (March 1990) including revisions OLM01.1 (December 1990 and OLM01.2 (January 1991)
2. "Determination of Organochlorine Pesticides and Polychlorinated Biphenyls (PCBs) in Biological Tissues." Method MP-FWST-MA, Hazleton Laboratories America, Inc., Madison, Wisconsin.
3. Instrument Operating Procedure for Gel-Permeation Chromatograph, Method OP-6004-36, Hazleton Laboratories America, Inc., Madison, Wisconsin.

017 LABORATORY: Hazleton Laboratories America, Inc.

Silica Gel Cleanup and Separation

XVII. SCOPE:

## ANALYTICAL METHODS (Cont.)

Method Code	Method Description
017	<p>This method is applicable to any sample extract in hexane which requires additional cleanup and the separation of polychlorinated biphenyls (PCBs) from many of the organochlorine pesticides.</p> <p>SAMPLE PREPARATION:</p> <p>The sample extract should be at a volume of 5.0 ml in hexane.</p> <p>PROCEDURE:</p> <p>The silica gel (100/200 mesh) is prepared by swirling it in a slurry of 40% acetonitrile and 60% methylene chloride, vacuum filtering, and then rinsing it successively with methylene chloride and hexane. It is then dyed at 140 C overnight and deactivated with 0.5% (w/v) distilled water.</p> <p>Fifteen grams of this silica gel is then slurried in petroleum ether, poured into a chromatography column, and topped with anhydrous sodium sulfate. The sample extracts are then drawn into the top of the column. The first fraction is eluted with 140 ml petroleum ether. The second fraction is eluted with 250 ml of a mixture of 1% acetonitrile, 19% hexane, and 80% methylene chloride (v/v).</p> <p>The first fraction should include all PCBs, p,p'-DDE, hexachlorobenzene, and mirex. It may also include some portion of p,p'-DDT, o,p'-DDE, o,p'-DDT, and trans-nonachlor. The remaining portion of these 4 pesticides, along with all other organochlorine pesticides, will be found in the second fraction.</p> <p>Both fractions are then quantitatively transferred to a 500 ml K-D apparatus fitted with a 10 ml concentrator tube. A three ball snyder column is attached and the extract is concentrated on a hot water bath, adjusting the temperature such that the concentration is completed within 15-20 minutes.</p> <p>When the apparent volume reaches approximately 5.0 ml, the K-D</p>

## ANALYTICAL METHODS (Cont.)

Method  
Code

Method Description

-----  
017

apparatus is removed from the water bath and allowed to cool for at least 10 minutes. 50 ml of hexane is added to the flask and it is returned to the hot water bath. If the extract was cleaned by gel-permeation chromatography (GPC), it is concentrated to 5.0 ml. If it did not undergo GPC cleanup then it is concentrated to 10.0 ml.

## REFERENCES:

1. Technical Operating Procedure, "Silica Gel Cleanup and Separation of Organochlorine Pesticides and PCBs", Method OP-6004-45, Hazleton Laboratories America Inc., Environmental Chemistry, Madison, Wisconsin.