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New England District
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Technical Support Services
General Electric (GE) Housatonic River Project
Pittsfield, Massachusetts

Contract No. DACW33-94-D-0009

FINAL

SUPPLEMENTAL INVESTIGATION WORK PLAN FOR THE LOWER HOUSATONIC RIVER

VOLUME II - APPENDICES

Task Order No. 0032

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FINAL

SUPPLEMENTAL INVESTIGATION WORK PLAN FOR THE LOWER HOUSATONIC RIVER

GENERAL ELECTRIC (GE) HOUSATONIC RIVER PROJECT PITTSFIELD, MASSACHUSETTS

Appendices (SIWP Addendum)

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APPENDIX A.1

HYDRODYNAMIC MODELING STUDY OF PCB CONTAMINATION

1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is in the process of conducting a hydrodynamic and water quality and fate and effect modeling study of the Housatonic River. The model study area will encompass the region of the Housatonic River between Dalton and Woods Pond Dam. The principal focus will be on those areas subjected to historical PCB contamination and where a determination will be made as to the extent and nature of remediation and restoration activities in affected areas. The scope of this modeling study will focus on both the main stem of the Housatonic River and adjacent floodplain and wetland regions, as appropriate.

2. OBJECTIVES

The objectives of this modeling study are as follows:

1. Quantify future spatial and temporal distribution of PCBs (both dissolved and particulate forms) within the water column and bed sediment.

2. Quantify the historical and relative contributions of various sources of PCBs on ambient water quality and bed sediment.

3. Quantify the historical and relative contribution of various PCB sources to bioaccumulation in targeted species.

4. Estimate the time required for PCB-laden sediment to be effectively sequestered by the deposition of “clean” sediment (i.e., natural recovery).

5. Estimate the time required for PCB concentrations in fish tissue to be reduced to levels that no longer pose either a human health or ecological risk based on various remediation and restoration scenarios, including natural recovery.

6. Quantify the relative risk(s) of extreme storm event(s) contributing to the resuspension of sequestered sediment or the redistribution of PCB-laden sediment within the area of study.

The objectives of this study require a modeling analysis that incorporates the relevant physical, chemical, and biological processes occurring within the area of study. Given the inherent complexity of these processes, the modeling study will evaluate the degree of uncertainty associated with answering each of the study objectives. For example, an estimate will be made of the expected uncertainty range over which recovery (e.g., fish tissue concentration or PCB bed

sediment concentrations) is likely to occur (i.e., +/- years) for a given alternative. In addition, the modeling analysis will document and rank the sensitivity of hydrodynamic, water quality, and biological input parameters specified in the model. The sensitivity of model parameters must be understood and expressed clearly in terms of their effect on model outputs.

While traditional water quality modeling investigations often have simulation periods on the order of minutes, hours, days, or months, this modeling study is expected to require a simulation period on the order of decades. This long-term, continuous simulation is necessary in order to adequately represent historical conditions and to predict the future response of the system to various remediation or restoration actions that are or may be implemented.

3. CRITICAL MODEL OUTPUTS

Outputs from the modeling study will be numerous and are intended to incorporate the effects of all critical physical, chemical, and biological processes. Since no single model adequately represents all these processes, several calibrated and validated models will be developed. Once calibrated and validated, these models will provide long-term predictions of flow, sediment concentration (both fine and coarse grain fractions), nutrient (nitrogen and phosphorus) concentrations, PCB concentrations (Aroclors and total PCBs), periphyton and phytoplankton populations (estimated from chlorophyll-a concentrations), and PCB fish tissue concentrations. The following identifies the various models that will be developed:

1. A hydrologic model of all contributing tributary areas within the study area including flow, suspended solids, biochemical oxygen demand (BOD), dissolved oxygen (DO), total organic carbon (TOC), and nutrients.

2. A hydrodynamic model of flow, sediment (fine grain and coarse grain) transport, and abiotic total PCB fate and transport.

3. A PCB fate and effects model for a specific fish species (large-mouth bass).

Major tasks elements required to perform this modeling study include the development of: 1) a model study design, 2) development of a quality assurance project plan (QAPP) and 3) model development and application. The purpose and extent of these tasks are discussed below.

3.1 MODEL STUDY DESIGN

The breadth and complexity of the modeling study objectives requires the development of a comprehensive and detailed model study design. The study design will document a scientific and technically defensible approach to meeting the study objectives. The study design will have, at a minimum, sections that discuss: 1) the purpose and objectives of the modeling study; 2) scope of the modeling study including study area boundaries, modeled constituents and processes, PCB sources, and simulation periods; 3) critical data requirements and their uses; 4) data availability; 5) recommendations on additional data collection needs, if any; 6) description of the modeling techniques that will be used, including model setup and physical domain of the system, specific

models to be used, approaches to be used for calibration and validation of each model component (i.e., hydrologic, hydrodynamic, sediment, toxic, nutrients and fate and effects); 7) uncertainty and sensitivity analyses; and 8) model outputs.

The modeling study and design document will also discuss the technical and scientific basis for the methods and techniques to be used in this study. It will document the purpose and means by which available data are to be used to conduct the study. It will also serve as a vehicle by which public and expert input can be incorporated into the final study design. In developing the model study, EPA is relying on recent and ongoing PCB modeling studies in the Lower Fox River and Hudson River as representative examples. In both instances, these studies documented the nature and scope of the modeling analyses that are necessary in order to satisfy objectives similar to those identified above.

A large part of the model study design will entail conducting an inventory of available data. Data of particular interest for this study are:

- Meteorological data (e.g., rainfall, snowfall, temperature, wind speed and direction, solar radiation, evaporation, evapotranspiration).
- Historical flow measurements.
- Ambient water quality data.
- Bed sediment chemistry including isotopic data.
- PCB concentration in sediment cores.
- Sediment grain-size distribution.
- Bed sediment erosion potential.
- River geomorphology including cross-sectional geometry.
- Floodplain profiles.
- Sediment PCB concentrations in floodplain, wetland, and backwater regions.
- Land cover distribution within all tributary areas.
- Digital elevation model (DEM) data.
- Soil properties.
- Wetlands inventory data.
- Point source inputs including publicly owned treatment works (POTW).
- Source PCB characterization data, e.g., historical PCB sources.

- Fish tissue data.
- Periphyton and phytoplankton PCB uptake.
- Bioturbation contributing to re-release of PCBs caused by the reworking of bed sediment.
- Configuration of urban stormwater conveyances.

In the model study design, a discussion will be provided of how outputs from one model will be used as inputs to other modeling tools, e.g., feeding hydrologic model outputs as a boundary condition to the hydrodynamic/water quality model.

3.2 QUALITY ASSURANCE PROJECT PLAN (QAPP)

The quality assurance project plan (QAPP) is being developed for the modeling study to ensure that the quality of the data used to perform the modeling study are sufficient to justify the conclusions of the study. The QAPP will address such aspects as the means by which the quality of secondary data sources, i.e., historical data, will be evaluated and treated in the study. The development of the QAPP will proceed in accordance with existing Agency guidelines and policies on the development of QAPP. A key element of the QAPP will involve the development of data quality objectives (DQOs). DQOs are specific, integrated statements and goals that are developed for each data type or information collection activity to ensure that the data, once collected, are of the required type, quality, and quantity necessary to meet the intended purposes of the study.

The following identifies typical elements of a QAPP that will be considered:

1. Problem statement.
2. Identify the decisions that will be made using the environmental data.
3. Identify the metrics that are required to support a specific decision.
4. Specify the boundaries (area and time period) by which a decision is applicable.
5. Specify how data are to be summarized and used to arrive at a decision.
6. Specify acceptable error rates and the consequences of making an incorrect decision.
7. Specify the most resource-efficient study design that will satisfy all the DQOs.
8. Specify how the management of model outputs will be used as inputs to other models including transformation, if necessary, of model outputs into formats appropriate for other models to be used (e.g., hydrologic model to hydrodynamic model to water quality and fate and effect model).

3.3 MODEL DEVELOPMENT

Model development will largely begin following the development of the model study design and QAPP. Model development entails data development and organizing data in appropriate formats, e.g., model input data, model setup, model calibration and validation, sensitivity and uncertainty analyses, and presentation of modeling results. It is envisioned that an iterative, phased approach will be necessary to satisfy the study objectives.

In order to carry out the modeling, EPA intends to use three models as the backbone of the modeling analysis. They include the Hydrologic Simulation Program-Fortran (HSPF) model, Environmental Fluid Dynamics Computer Code (EFDC), and AQUATOX. Each of these models is described briefly in terms of their intended use in this study.

HSPF is a lumped-parameter, watershed-scale hydrologic model that allows for simulation of both water quantity and quality in simple to complex watersheds. It includes numerous facilities to handle a diversity of water quality constituents, the inclusion of hydraulic structures and complex operational scenarios, and the ability to simulate the effects of constant and variable water withdrawals. This model will be used to simulate the hydrology within the study area and will set the external boundary conditions to the hydrodynamic/water quality model EFDC. A continuous output from HSPF will be fed to EFDC such as flow, suspended solids, nutrients, BOD, and DO.

EFDC is a sophisticated hydrodynamic/water quality model that is capable of operating in 1-, 2- and/or 3-Dimensional modes. EFDC is written in a generic form such that rewriting of the internal code is not necessary for a given environmental setting. The model can be used in a wide diversity of environmental settings including rivers, lakes, reservoirs, and estuaries. In addition to a robust numerical solution scheme to predict the system's hydrodynamic behavior, it also has facilities to simulate cohesive and non-cohesive sediment transport, fate and transport of conventional pollutants and toxics, eutrophication, and thermal loadings. It also has the ability to simulate bed sediment dynamics (erosion and deposition) and diagenesis, and the effects of bioturbation. EFDC is capable of operating over an indefinite period of time and has been used to simulate hydrodynamics and water quality in complex environmental settings.

AQUATOX is a time-variable environmental fate and effects model that has the ability to model the fate and effects of organic toxicants and other environmental stressors in aquatic ecosystems. It simulates the fate and transfer of pollutants to the water, sediment, and biotic components and their accumulation through a food web. Key ecological processes simulated include primary and secondary productivity, trophic structure and dynamics, seasonally varying growth rates, reproduction and mortality, direct and indirect toxic effects, predator/prey interactions, and nutrient dynamics. AQUATOX will be used to simulate the dominant PCB fate and effect pathways. Time-variable inputs will be supplied to AQUATOX via outputs from the EFDC model.

Outputs from each of these models will be subjected to calibration and validation techniques, and sensitivity and uncertainty analyses. Key input parameters in each model will also be ranked in terms of their sensitivity and their significance to modeling results.

4. RESULTS

Results from the modeling study will be presented in an iterative and phased fashion for each of the major model components. Internal Peer Review Group input will be used to further refine the modeling results as necessary. Initial results will focus on how well the various models describe historical conditions. Independent and separate data set will be used to calibrate and validate the models to historical conditions. Following calibration and validation of the various models, results will then be developed to characterize the expected response of the system to various remedial alternatives. Internal Peer Review Group input will be used to refine the modeling results, as necessary. All results will be represented in terms of satisfying the modeling objectives.

APPENDIX A.2

STANDARD OPERATING PROCEDURE FOR PORE WATER SAMPLING

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APPENDIX A.2

STANDARD OPERATING PROCEDURE FOR PORE WATER SAMPLING

1. SCOPE AND APPLICATION

The objective of this standard operating procedure (SOP) is to provide guidance and general reference information on sampling of sediment pore water. Pore water, or "interstitial water," is defined as the water contained within the interstitial spaces between sediment particles. These procedures are designed to be used in conjunction with analyses for the most common types of pore water contaminants (e.g., volatile and semivolatile organic compounds, pesticides/PCBs, metals) and/or toxicity studies, specifically toxicity identification evaluation (TIE).

Several pore water sampling techniques exist. Since the pore water sampling technique used will vary with site-specific characteristics and ultimate project analytical objectives, two pore water sampling procedures are provided. Subsection 2 is intended as a general guideline; Subsection 3 provides a specific protocol for collection of pore water samples for TIE and analytical chemistry. In all instances, any deviation from this SOP will be documented in the final report.

2. GENERAL METHOD SUMMARY

2.1 OVERVIEW

To obtain a representative sample of pore water, it is important that the water collected have minimal mixing with the overlying surface water. There are two general pore water sampling approaches for ensuring that mixing does not occur. The first approach is ex situ and consists of collecting a sediment sample and extracting pore water via centrifugation or other pressurization device. The second approach consists of collecting pore water in situ via placement of "peepers" or similar containers in the sediment, allowing pore water to diffuse into the sampling container over time, or else suctioning pore water directly into a container.

A variety of pore water methods exist, each with its own advantages and disadvantages (Table 1). Of these methods, squeezing and suction filtration are not recommended (especially for metals), since they may underestimate the concentrations of contaminants in the interstitial water (Bufflap and Allen, 1995). Because both of these methods require a specialized sampling apparatus, they may also be expensive. The remaining, recommended methods consist of centrifugation, seepage meters, and dialysis ("peeper") methods.

The following variables should be considered before choosing a pore water method:

1. Water body characteristics (lake, pond, river, stream).

Table 1
Comparison of Sediment Pore Water Sampling Methods

Method	Description of Approach	In Situ	Sufficient Volume for Tox Testing	Advantages	Disadvantages
Centrifugation	Collect sediment core in airtight container, extract sample under inert conditions, centrifuge sample, and analyze.	No	Yes	Simple to collect; sufficient volume for chemistry and toxicity analyses; most widespread method.	Collection tube must be airtight; centrifuge oxidizes sample unless done in a nitrogen chamber; must spin sample twice or filter it to avoid suspending fine particles.
Squeezing	Collect sediment core in sampling apparatus that forces pore water out by applying pressure.	No	Yes	Simple to collect; sufficient volume for chemistry and analysis.	Samplers are expensive and difficult to make; oxidation or temperature artifacts may occur through sample handling; pore water concentrations may be altered by forcing water through sediment.
Seepage Meters	Install vented cylinder into sediment and collect water that flows through it.	Yes	Yes	Simple to install/collect samples, sufficient volume for all analyses, provides flux data also.	Can use only in areas of groundwater discharge and water depth <2 ft. Must wait to equilibrate before sampling.
Suction Filtration	Insert device into sediment that sucks pore water into holding vessel.	Yes	No	Avoids sample handling artifacts associated with ex situ handling.	Complex and expensive to produce.
Dialysis	Allow deionized, distilled water to come into equilibrium with the pore water in order to determine concentrations.	Yes	No	“Peepers” may be quickly and cheaply constructed, and are easy to install; most ex situ artifacts are avoided.	Some oxidation of sediments may still occur via water supplied.

2. Sediment particle size (gravel, sand, silt, clay).
3. Analytical objectives (parameters, detection limits, data quality objectives).
4. Analytical volume required (toxicity testing, chemistry, etc.)
5. Project duration and time available.
6. Groundwater/surface water interrelationships (gaining/losing stream).
7. Cost.

Sample processing methods vary with the technique used, but generally samples should be sealed and labeled, then placed immediately in coolers with ice to maintain a temperature of 4 ºC. Samples are subsequently shipped to a subcontracted analytical or toxicity testing laboratory.

2.2 RECOMMENDED SAMPLING PROCEDURES

Procedures to be followed are summarized below for each of the three recommended sampling procedures.

2.2.1 Centrifugation

1. Collect sediment core using a standard sediment coring device such as the K-B corer (Wildco, Inc.) or polycarbonate coring device. There should be no head space within the core.

2. Seal the core with polyethlyene (or equivalent) caps and tape, then label the top end of each core as "top." Subsequently label the top end of the core with the sample location number. Cut the core into sections if it is necessary to sample at multiple depths. Cap and label each section with the sampling location, depth, and date.

3. Place the cores in a cooler at 4 °C and ship to the laboratory or processing area.

4. If metals are to be analyzed, sample handling and centrifugation should be undertaken within a nitrogen chamber. Otherwise certain metals may oxidize, affecting analytical results of the metals as well as phosphates.

5. Samples should be processed immediately upon receipt by the laboratory, since coagulation and precipitation of humic material in interstitial water was observed in samples stored for more than 1 week at 4 °C (Burton and Landrum, 1989, Landrum et al., 1987).

6. The laboratory should centrifuge each sample at 3,000 revolutions per minute (rpm) for 20 minutes; this should be done in a nitrogen chamber if necessary. The supernatant should be filtered through a 0.45-micrometer (µm) paper filter before analysis. Filtration with glass or plastic filters is inappropriate since nonionic organics may be removed (Burton and Landrum, 1989, Word et al., 1987).

7. The resultant filtered supernatant may be analyzed for chemistry and/or used for toxicity bioassays.

2.2.2 Seepage Meters

1. At least 1 day prior to the sampling event, install seepage meters into the sediment until its top is about 2 cm above the sediment surface. The vent hole on top of the seepage meter is fitted with a rubber stopper and tube. A deflated plastic bag is connected to the tube and left overnight to collect the sediment pore water sample.

2. On the day of sample collection, estimate the depth of water to the nearest 0.1 ft. Record these measurements in the logbook.

3. Measure/observe the physical characteristics of the water body, such as odor, color, temperature, pH, conductivity, oxidation-reduction potential (ORP), DO, presence of dead vegetation, and surface sheens. Use the polyethylene cups for collection of a sample for field monitoring. Record the measurements/observations in the logbook.

4. Collect the pore water sample by removing the plastic sample collection bag from the vent hole tube. The sample can be directly transferred into the laboratory sample bottles. Record a description of the sampling location.

5. Samples for volatile organic analysis will be collected first. Following the collection of a sample in a volatile organic analysis (VOA) vial, ensure that there are no air bubbles in the vial by turning the vial upside down and tapping it lightly. All other sample containers should be filled to at least ¾ full.

6. Immediately label, preserve (if necessary; see Subsection 2.3), refrigerate/ice, and log the samples in the field logbook.

7. Collect a final sample aliquot in a disposable container and immediately measure and record the pH, temperature, conductivity, ORP, and DO of the sample.

2.2.3 Dialysis

1. Construct “peepers” from a 6-mL polyethylene snap vial by boring out the center of the cap.

2. Fill each vial with deionized water, then place a 1-µm filter screen over the opening and tighten the cap. The screen acts as a membrane across which the pore water diffuses over time and enters the bottle.

3. Once in the field, mark the sampling location with a stake, flagging, or other means.

4. Submerge the vial in the sediment and leave it for 1 to 10 days to allow it to equilibrate with the pore water. In gravel or coarse sand environments it will equilibrate faster than in fine-grained silts.

5. Retrieve the sample and label it according to sample location.

6. Place the samples in a cooler at 4 °C and ship to the laboratory.

7. In the laboratory immediately extract the sample by inserting a pipette or syringe through the membrane.

8. Analyze the sample.

2.3 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

The type of analysis for which a sample is being collected determines the type of bottle, preservative, holding time, and filtering requirements. When dedicated sampling containers (e.g., peepers) are used, the sample will be retained within the container, sealed, and shipped. Otherwise, samples should be collected directly from the sampling device into appropriate laboratory-cleaned containers.

After the sample is collected, complete a field data sheet, a chain-of-custody form, and record all pertinent data in the site logbook. Samples shall be appropriately preserved, labeled, logged, and placed in a cooler to be maintained at 4 °C. Samples must be shipped well before the holding time expires and ideally should be shipped within 24 hours of sample collection. It is imperative that samples be shipped or delivered daily to the analytical laboratory in order to maximize the time available for the laboratory to perform the analyses. The samples should be shipped with adequate packing and cooling to ensure that they arrive intact and at the appropriate temperature.

2.4 INTERFERENCES AND POTENTIAL PROBLEMS

2.4.1 General

The primary goal in performing sediment pore water sampling is to obtain a representative sample of the water present within the interstitial spaces between sediment particles. To characterize sediment quality, pore water analyses should be conducted concurrently with analyses of water quality, sediment bulk chemistry, and/or toxicity if desired. This approach facilitates data interpretation.

2.4.2 Advantages/Disadvantages of Different Sampling Methodologies

No sampling method guarantees true interstitial water concentrations (Schults et al., 1992), and different sampling methods have been shown to differ in their accuracy (Bufflap and Allen, 1995), at least with respect to metals chemistry.

2.4.2.1 Centrifugation

Centrifugation is the most commonly used means of extracting sediment pore water. This method involves collection of a sediment core, using a polycarbonate tube or equivalent apparatus, and subsequently spinning the sediment in a high-speed centrifuge. This method is

appropriate for most studies, especially when a relatively large volume of pore water is necessary for analysis, such as toxicity testing studies, studies where multiple analyses are conducted, or low detection limits are required.

The principal disadvantage of centrifugation is that it is an ex situ method, and hence artifacts in the data may be introduced from sample handling. For example, oxidation of certain metals may occur, affecting analytical chemistry. It has also been suggested that in cases where solid and liquid phases are not in equilibrium, centrifugation may not be suitable for analysis of hydrophobic chemicals since they may partition back into the sediment (Baudo et al., 1990).

2.4.2.2 Seepage Meters

Sediment pore water can be collected by capturing groundwater seeping into standing surface waters by covering an area of the streambed with a bottomless cylinder vented to a deflated plastic bag (Lee, 1977). One-half of a 55-gallon drum is used to construct the seepage meter. A vent hole is fitted with a rubber stopper and tubing on the top side of the seepage meter. A deflated plastic bag is then attached to the vent hole in order to collect sediment pore water seeping into the riverbed.

The advantage of using a seepage meter to collect sediment pore water samples is that the rate of groundwater/surface water flux can also be determined using this method, which is often useful in determining groundwater/surface water dilution ratios. In addition, large sample volumes can be collected to facilitate toxicity testing or for multiple laboratory analyses by repeatedly filling the deflated plastic bag. Another advantage is the relatively low cost to construct, install, and sample the seepage meters. The limitation of seepage meters is that they are restricted to use in areas where groundwater is discharging into the surface water and in areas where the depth of the surface water is less than approximately 2 ft.

2.4.2.3 Dialysis

"Peepers" are another commonly used means of assessing sediment pore water quality. Since they are an in situ method, most potential sampling artifacts may be avoided. However, some oxidation of sediment contaminants may still occur via the water supplied in the peepers. Peepers generally do not provide sufficient volume for toxicity testing, and may not provide sufficient volume to achieve desired detection limits for analytical parameters. Moreover, it may take several days for the peepers to come into equilibrium with the surrounding pore water. However, they are inexpensive, and can be used in a variety of situations.

3. PROTOCOL FOR COLLECTION AND PROCESSING OF PORE WATER SAMPLES FOR TOXICITY IDENTIFICATION EVALUATION (TIE) AND ASSOCIATED ANALYTICAL CHEMISTRY

3.1 INTRODUCTION

This protocol describes the procedure, schedule, and logistics for collection and processing of pore water samples from Housatonic River sediments by centrifugal extraction for use in a toxicity identification evaluation (TIE) and associated analytical chemistry. Sediments for extraction of pore water will be collected from five locations, corresponding to the in situ sediment toxicity test locations (the pore water TIE study will not include the Dalton reference site, at which sediments are too coarse to allow efficient extraction of pore water). Based on preliminary processing of sediments from these locations, the approximate sediment volume necessary to generate sufficient pore water for completion of the TIE and water chemistry is 25 L. Only material from the top 5 cm of the bed sediment will be collected.

3.2 FIELD SAMPLE COLLECTION AND INITIAL PROCESSING

1. All sediment collection must be performed carefully to ensure that (1) only sediments from the 5-cm surface layer are collected, (2) no pore water is lost during collection, and (3) the sediment is not commingled with overlying surface water.

2. Push a 1-ft-long by 3-inch-diameter section of polycarbonate core tube into the sediment to a depth of at least 6 inches; depending on the nature of the sediment it may be necessary to insert the core deeper than 6 inches to ensure that material is not lost upon retrieval. Without disturbing the tube, carefully remove sediment from around the tube as necessary and insert a tight-fitting extrusion device into the bottom. If water is observed to be draining from the core before the extrusion device can be inserted, it should be rejected.

3. Maintain the core upright and slowly push the extruder up, allowing the supernatant water to flow out of the core. When the sediment surface reaches the top of the core, extrude the top 5 cm of sediment and its associated pore water into an appropriate compositing container (large pre-cleaned stainless steel bowl or equivalent).

4. Repeat steps 2 and 3, being careful to avoid areas that have been disturbed by prior sediment collection activities until sufficient material has been collected (a minimum of 65 cores).

5. Mix the entire 25-L sample volume to ensure homogeneity and place into nitrogen-purged 1-gallon wide-mouth glass jars; top off with nitrogen and ship on ice to Soil Technology (ST) of Bainbridge Island, Washington. Each station will be shipped as soon as the extrusion and compositing process has been completed. Sediments will be maintained in coolers at ST until all stations have been sampled, shipped, and received.

3.3 PORE WATER EXTRACTION

1. This phase of the process must be timed and managed to ensure that a minimum of 1 L of pore water can be extracted from all five stations, packaged, and shipped on the same day.

2. Place sediment material from the first station in the centrifuge and process to obtain pore water. Repeat as necessary to obtain a minimum 1-L sample volume. Return the remainder of the sediment to storage under nitrogen in the cooler. Record the time that extraction was started and completed.

3. Repeat Step 2 above with the remaining four stations.

4. Package the pore water in appropriate precleaned containers and ship samples to Wright State University, Dayton, Ohio.

3.4 TIE

The TIE procedure will be initiated simultaneously for all five stations as soon as practicable following receipt of the pore water. Wright State will communicate the starting time and date for the test to ST. The elapsed time between pore water extraction (mean of start and stop time for each station) and initiation of the TIE will be calculated for each station.

3.5 PORE WATER EXTRACTION FOR CHEMICAL ANALYSES

1. All pore water extraction for chemical analyses will be completed prior to expiration of the 21-day holding time limit for sediments, allowing sufficient time for the pause prior to preservation described below. Extraction will proceed in the same order in which the sediments were collected.

2. Pore water for chemical analyses will be extracted using procedures identical to those described above for TIE pore water extraction, except that all remaining sediment for each station will be processed. Following extraction, the pore water will be sealed in precleaned containers in the cooler for a time period identical to the time that transpired between the previous extraction of the 1-L pore water aliquots and initiation of TIE. This time will vary for each station and should be duplicated exactly for each station. At the appropriate time, all chemistry pore water samples will be preserved for subsequent analysis.

3.6 TIMING AND LOGISTICS

The following time factors are critical to successful completion of this protocol:

1. Once initiated, collection of sediments must proceed expeditiously to allow completion of all sampling (five stations) in one calendar week.

2. When sufficient sediment material has been collected from each station, the sediment will be processed as described in Subsection 3.2 and shipped on that day. Sediments will be stored by ST until all stations have been received, at which time extraction may be initiated.

3. The specified maximum holding time for sediment samples is 21 days. All activities from initial sediment collection through centrifugal extraction of pore water for analytical chemistry must be completed within the 21-day maximum holding time for each station.

4. The TIE will be initiated on all stations simultaneously. The initial centrifugal extraction of the 1-L aliquots must be completed on the same day and the pore water must be shipped that day.

5. Following extraction of the remaining pore water for analytical chemistry, the sample from each station must be held prior to preservation for a time corresponding to the delay between extraction of the initial 1-L aliquot and the initiation of the TIE.

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APPENDIX A.3

STANDARD OPERATING PROCEDURE FOR STORMFLOW SAMPLING

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APPENDIX A.3

STANDARD OPERATING PROCEDURE FOR STORMFLOW SAMPLING

This standard operating procedure (SOP) has been updated to reflect revisions to the stormflow sampling program as of 31 July 1999.

1. OBJECTIVE

Water samples will be collected from the Housatonic River and selected tributaries under conditions where water quality and suspended sediment transport are influenced by storm-induced flows. This will be termed "stormflow sampling" to distinguish it from stormwater sampling since samples will be collected from the river channel, not from stormwater drainage pipes or other conveyances. The term "high flow" sampling is not used because significant movement of suspended sediments and changes in water quality can occur under storm-induced flow conditions that are not necessarily "high flow" conditions.

The objective of the stormflow sampling effort is to provide data on the suspended sediments and water quality to support the calibration of a hydrodynamic and water quality model. The data will be used to assist in the determination of resuspension and redistribution of PCB-laden sediment within the study area and to determine the effects of storms on water quality and hydrodynamics of the Housatonic River. Suspended sediment samples will reflect the suspended sediment concentrations in the water column and not the bed load.

2. TECHNICAL APPROACH

2.1 SAMPLING LOCATIONS

Stormflow sampling will be performed at three primary locations and five secondary locations.

The three primary sampling locations are:

1. Pomeroy Avenue Bridge
2. New Lenox Road Bridge
3. Woods Pond (at the footbridge upstream of the dam)

The secondary stormflow sampling locations are:

1. Hubbard Avenue Bridge (Coltsville)
2. Unkamet Brook
3. West Branch of the Housatonic River
4. Sackett Brook

5. Roaring Brook

Water samples and suspended sediment samples will be collected from the three primary stormflow sampling locations during storm events. In addition, water samples will be collected from the five secondary stormflow sampling locations.

Activities that will be performed during each stormflow sampling event include water quality monitoring at the primary and secondary sampling locations in the river channels, collection of depth-integrated water quality samples at primary locations, high-volume suspended sediment collection at primary locations, and measurement of stream velocity and depth. The procedures to be used for these activities are described below.

2.2 PARAMETERS

2.2.1 Water Samples

The following parameters will be analyzed in the water quality samples collected from the primary stormflow sampling locations:

1. Ammonia-nitrogen
2. Nitrite-nitrogen
3. Nitrate-nitrogen
4. Total Kjeldahl nitrogen
5. Organic phosphorus
6. Ortho-phosphorus
7. Total phosphorus
8. Chlorophyll-a
9. Biochemical oxygen demand (5-day)
10. Chemical oxygen demand
11. Total organic carbon
12. Dissolved organic carbon
13. Particulate organic carbon
14. Total suspended solids
15. Total polychlorinated biphenyls (PCBs) (total, Aroclor, and congeners)
16. Dissolved PCBs (total, Aroclor, and congeners)
17. Alkalinity
18. Hardness
19. Turbidity (field measurement)
20. Temperature (field measurement)
21. pH (field measurement)
22. Dissolved oxygen (field measurement)
23. Specific conductivity (field measurement)

The following parameters will be analyzed in the water quality samples collected from the secondary stormflow sampling locations:

1. Ammonia-nitrogen
2. Nitrate-nitrogen
3. Nitrite-nitrogen
4. Total Kjeldahl nitrogen
5. Organic phosphorus
6. Ortho-phosphorus
7. Total phosphorus
8. Chlorophyll-a
9. Biochemical oxygen demand (5-day)
10. Total organic carbon
11. Dissolved organic carbon
12. Particulate organic carbon
13. Total suspended solids
14. Total PCBs (Unkamet Brook only)
15. Turbidity (field measurement)
16. Temperature (field measurement)
17. pH (field measurement)
18. Dissolved oxygen (field measurement)
19. Specific conductivity (field measurement)

2.2.2 Suspended Sediment Samples

Suspended sediment samples from the three primary stormflow sampling locations will be analyzed for the following parameters:

1. Grain size fractions for the following four size categories:

   - >5 - 10 µm
   - >10 - 62 µm
   - >62 - 250 µm
   - >250 µm

2. Total PCBs for each size fraction listed above except the >250 µm fraction (the >250 µm fraction may also be analyzed for PCBs after a preliminary data evaluation).

3. Total Organic Carbon (for each size fraction listed above).

Suspended sediment samples from the five secondary stormflow sampling locations will be analyzed for grain size fractions using laser analysis techniques. The results of these analyses will provide grain size fraction data similar to that obtained for the primary stormflow sampling locations.

2.2.3 Methods

Analytical methods, required containers, preservation techniques, and holding times for the above samples are contained in the *Quality Assurance Project Plan* (QAPP) (WESTON, 2000). The procedures for stream sampling and stream velocity measurements described in the *Field Sampling Plan* (FSP) (WESTON, 1999) apply to this SOP with the exception that manual verification samples will be collected with a depth-integrated sampler, model USDH-75Q, as described in Subsection 2.5, and velocity measurements will be taken at three representative locations at each monitoring station. These techniques will follow the general guidance recommended in Buchanan and Somers (1969).

2.3 NUMBER OF EVENTS

Sampling will be performed over at least three representative storm events. An event is defined as a minimum of 1.0 inch of rainfall; events must be separated by 48 to 72 hours from the conclusion of the previous storm event. It is estimated that it may require 2 or 2.5 times this number of storm events (i.e., six to eight events) to get the three representative events due to some of the storms not meeting the above criteria (personal communication, W. Tate, September 1998).

For sudden storms capable of generating substantial amounts of rain in short time periods, the full sampling protocol outlined in this SOP may not be implemented. A reduced data collection program may be followed and may include staff gauge measurements and TSS sample collection at selected stormflow sampling locations.

2.4 TIMING AND DURATION OF SAMPLING/MONITORING

Sampling will commence within 30 minutes to 1 hour of significant rainfall. Based upon a review of the hydrographs generated by rainstorms meeting the event criteria, monitoring may continue for 36 to 72 hours. Sampling will continue through the rising limb of the hydrograph and will include at least one sample near the peak of the hydrograph and one or more samples collected during the descending limb of the hydrograph.

2.5 WATER QUALITY SAMPLE COLLECTION METHODS

At all stormflow sampling locations, discrete samples will be collected every hour (wherever possible) throughout the stormflow sampling event from a fixed location in the river channel (mid-channel) for water quality analyses. At primary sampling locations, verification samples will be collected with a depth-integrated sampler model USDH-75Q at the same collection time. Based upon post-sampling evaluation of the stormflow hydrographs, five samples from each location, which are determined to best represent the conditions over the hydrograph, will be selected for analysis. Typically, two samples on the rising limb of the hydrograph, one sample at the peak of the hydrograph, and two samples on the descending limb of the hydrograph will be analyzed. Triple sample volumes will be collected for samples designated for matrix QC

analyses. The five samples selected for analysis will be analyzed separately and will not be composited. To collect sufficient volume for all analyses, a minimum of 12 liters (L) of water will be collected for each sample interval. PCB congener analyses will be performed on the samples representing the peak of the hydrograph and on other selected samples. Additionally, all hourly TSS samples will be analyzed.

At primary sampling locations, samples will be collected manually from a sample tap located at the pumping system described in Subsection 2.6. At secondary locations, samples will be collected manually using an ISCO sampler.

The sampling locations will be approximately 50 to 100 ft upstream or downstream of each bridge location to avoid any backwater effect (i.e., sediment deposition that may occur due to the bridge). The Woods Pond location will be sampled at the footbridge.

At the three primary sample locations, manual depth-integrated samples will be collected during the storm for comparison with the samples collected from a fixed depth. The manual samples will be collected at all three locations due to the differing characteristics of the river at each location. The manual samples will be collected with a depth integrated sampler model USDH-75Q. The model USDH-75Q sampler samples water isokinetically (i.e., at the same velocity as the stream), therefore the sample volumes are proportional to the velocity throughout the depth profile. The sample procedure involves connecting a 1-quart sample bottle to the device. The device is lowered from the water surface, at a constant/steady rate to the bottom, then brought to the surface at the same rate. The verification samples will be taken from stream locations and times closely corresponding to samples collected at the fixed depth. The results from the first round (i.e., first storm) of fixed depth samples, collected from a sample tap on the pumping apparatus, will be compared with the verification samples collected with the depth integrated samplers. Further manual sampling with the USDH-75Q device may not be required if a good correlation exists between the fixed withdrawal point data (0.6 Z_{max}) and the depth integrated data.

The water depth at stream gages will be monitored and recorded at each location, at intervals of approximately every 60 minutes. Also, representative velocity measurements will be taken approximately every 60 minutes. The progress of the hydrograph will be monitored through the stream gage (water depth) observations. Flow measurements will be based upon the stage-discharge curve (rating curve) developed for each stream gage wherever possible. Turbidity, pH, temperature, specific conductivity, and dissolved oxygen measurements will be taken every hour at each location. In addition, the time, gage height, and velocity measurement will be recorded when the river exceeds its banks (i.e., when bank-full stage is exceeded).

2.6 SUSPENDED SEDIMENT SAMPLE COLLECTION METHODS

At the three primary stormflow sampling locations, a sufficient quantity of suspended sediments will be collected to allow classification (fractionation) of the sediments by grain size. The separated grain size fractions will be analyzed for PCBs and TOC. Bulk suspended sediment samples will be collected by filtering a known volume of water through a bag filter system. Bulk sediment samples will be collected continuously for the duration of the sampling event. The bag

filter will be changed when the differential pressure across the filter bag exceeds 30 pounds per square inch (psi), indicating blinding of the filter bag. The suspended sediments collected in each individual bag filter will be combined to form a composite sample for analysis. A schematic process flow diagram of the bag filter system is shown in Figure 1. The following paragraph summarizes the principal components and operation of the bag filter system. Specific components and the piping configuration may change based on field conditions.

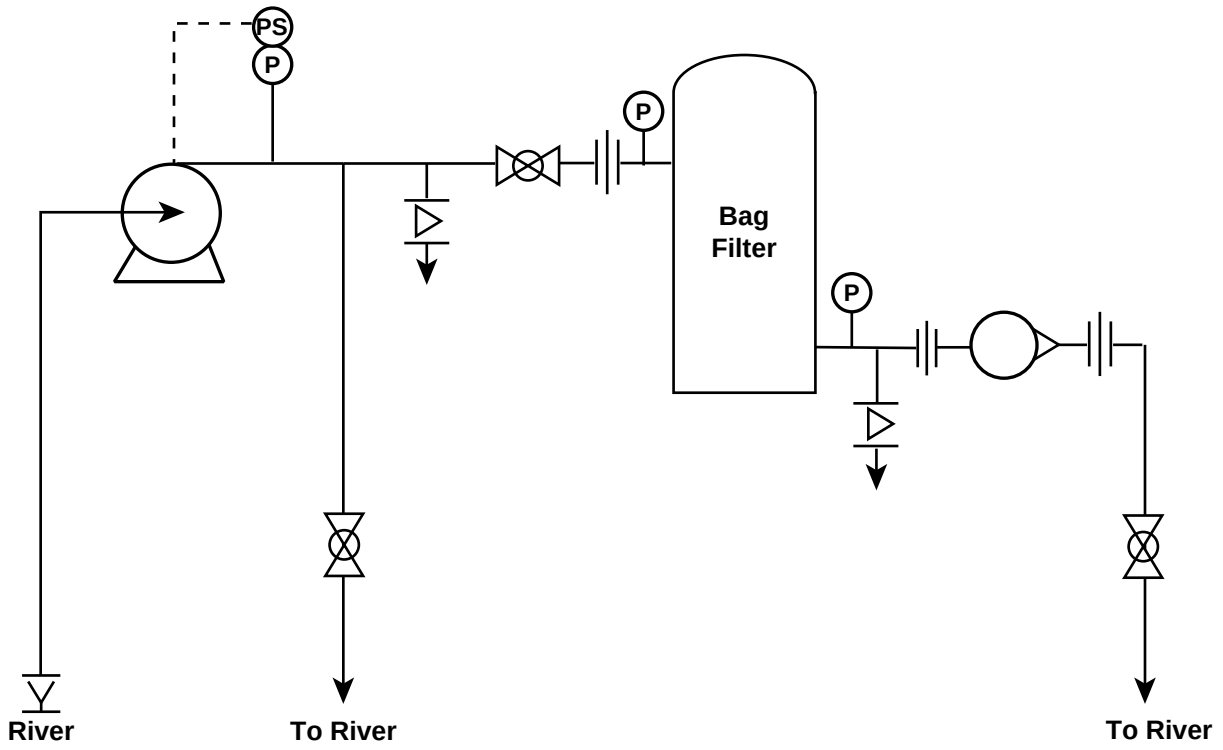
Water will be pumped from the river through a 1.5-inch diameter influent line attached to a centrifugal pump. The influent line will be mounted in the river at a horizontal location representative of the river flow at the sampling transect, at 6/10 of the maximum river depth (0.6 Z_{max}) at the sample location. The centrifugal pump will pump river water through a bag filter and flow meter. The bag filter will have a nominal size rating of 5 microns (µm). The bag filter sizes can be adjusted to 10, 25, 75, or 100 µm sizes, if necessary. The pump will be driven by a 2-hp 115V, 1Ø motor that will be powered by a gasoline-powered electric generator. Pump operation will initially be controlled manually, however, provision for other control strategies, such as continuous operation, manual intermittent operation, timer operation, and/or level or turbidity control could be applied. A high-pressure switch will be installed to shut down the pump if excessive pressure occurs due to blinding or overfilling of the filter bags. A totalizing flowmeter will be installed on the effluent side of the bag filter to record flow through the bag filter. Flow control valves and shutoff valves will be installed to allow flow control and flow balancing. Discharge from the flow control shall be downstream from the withdrawal location. Effluent from the bag filter system will be discharged to the river. This configuration will allow for long-term continuous sampling or discrete, storm event-driven sampling.

ISCO automated samplers will be used to collect samples from the water column at the five secondary stormflow sampling locations for laser analysis of particle size. These samples will be collected in 5-gallon carboys every 2 to 4 hours during the course of a storm event.

Based upon post-sampling evaluation of the stormflow hydrographs, three samples from each secondary location will be selected for analysis. The selected samples will be allowed to settle, the supernatant will be decanted, and the concentrated TSS will be submitted to an off-site laboratory for laser analysis of particle size.

2.7 VELOCITY MEASUREMENTS

Velocity measurements will be taken at seven sampling locations. Velocity measurements will not be taken at Hubbard Avenue because a USGS station (Coltsville, MA) is located at this sampling location. Velocity measurements, along with staff gage measurements, will be used to track the progress of the hydrograph. A representative location for velocity measurements will be chosen near and downstream from the water sample withdrawal tube in the river wherever possible. The velocity measurement location should be approximately the same distance from the river bank as the water sample withdrawal point and 10 to 20 ft downstream from the water sample withdrawal point. Due to safety requirements that prohibit personnel from entering the river, velocity measurements will be taken from bridges. Once selected, the velocity measurement location should be marked to ensure consistency and comparability between the measurements.



Direction

of River Flow

LEGEND

- Flow Control Valve
- Petcock Valve
- Check Valve
- Union
- Flow Totalizer
- Pressure Gauge
- Pressure Switch
- Centrifugal Pump

98P-3071 11/6/98

FIGURE 1 SEDIMENT COLLECTION SYSTEM SCHEMATIC PROCESS FLOW DIAGRAM

Velocity measurements will be made using a Marsh-McBirney Model 201D portable electronic current meter fixed to a hand-held vertical rod or a weight. The meter uses an electromagnetic sensor to measure the velocity in a conductive fluid such as water using Faraday's law of electromagnetic induction to measure fluid flow. As a conducting fluid, such as impure water, moves through a magnetic field produced by the meter a voltage will be induced (Gordon et al., 1992).

The total depth of water is measured. The position of the current meter is adjusted to the appropriate fraction of the total depth. The velocity measurement is recorded after waiting approximately 20 seconds from the start of the measurement in order to obtain a time average of the reading. When the total water depth at the velocity measurement location is 2.5 ft or less, the velocity measurement will be taken at 6/10 of the maximum depth (0.6 Z_{max}). When the depth is greater than 2.5 ft, velocity measurements will be taken at 2/10 maximum depth (0.2 Z_{max}) and 8/10 maximum depth (0.8 Z_{max}). The depth-averaged velocity will be taken as either the velocity at 0.6 Z_{max} or the average of the velocities measured at 0.2 Z_{max} and 0.8 Z_{max}. Velocity measurements will be taken at three locations across the river (left-channel, mid-channel, and right-channel). The velocity and stage data will be logged on a flow log sheet (Figure 2).

This flow log will document the following general information:

1. Flow monitoring location
2. Date
3. Time
4. Stage elevation from staff gage
5. Velocity at depth monitored

3. REFERENCES

Buchanan, T.J. and Somers, W.P. 1969. "Discharge Measurement at Gaging Stations." *Techniques of Water-Resource Investigations*, Book 3, Chapter A8, U.S. Geological Survey Washington, DC.

Gordon, N.D., McMahon, T.A. and Finlayson, B.L. 1992. *Stream Hydrology*. John Wiley & Sons. New York, NY.

WESTON (Roy F. Weston, Inc.). 2000. *Final Quality Assurance Project Plan*.

WESTON (Roy F. Weston, Inc.). 1999. *Final Field Sampling Plan*.

Tate, William, U.S. EPA. Personal Communication. 2 September 1998.

Figure 2 Housatonic River Storm Event Sampling Field Parameter Data Sheet

LOCATION: ______________________________

DATE: ____________

TIME	pH (std units)	SPECIFIC COND. (µs/cm)	DO (mg/L)	TEMP (^{o}C)	TURBIDITY (NTU)	STAFF GAUGE HEIGHT (ft)	VELOCITY (ft/s)

APPENDIX A.4

AMBIENT AIR MONITORING PROTOCOL

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APPENDIX A.4

AMBIENT AIR MONITORING PROTOCOL

1. INTRODUCTION

An ambient air monitoring program will be conducted to support a human health and ecological risk assessment for the Lower River. The monitoring program is designed to make seasonal measurements of polychlorinated biphenyls (PCBs) in ambient air and to supplement those measurements with meteorological data collected at a nearby Prevention of Significant Deterioration (PSD) air quality monitoring site. The technical approach and procedures that will be part of the ambient air and meteorological monitoring program are documented in the following sections of this report.

The design of the monitoring program is based on recommendations contained in the U.S. EPA technical guidance documents. Specifically, the ambient air monitoring programs will follow recommendations contained in the following documents:

- *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*, U.S. EPA-600/4- June 1998.
- *Ambient Monitoring Guidelines for Prevention of Significant Deterioration (PSD)*, U.S. EPA-450/87-007.
- *Quality Assurance Handbook for Air Pollution Measurement Systems; Volume II. Ambient Air Specific Methods*, U.S. EPA/6004-77/027a.

This protocol is organized as follows:

- Section 2—Description of the Proposed Monitoring Sites
- Section 3—Description of PCB Monitoring Equipment
- Section 4—Quality Control and Quality Assurance Procedures
- Section 5—Data Reporting

2. DESCRIPTION OF THE PROPOSED MONITORING SITES

The specific sites for the ambient air sampling program will be identified based on an upcoming site visit. It is intended that one of the air samplers will be located in the upper portion of Reach 5 where there is a greater concentration of residents, and the other would be at a location near Woods Pond to allow for a direct comparison to previous air sampling results.

3. DESCRIPTION OF PCB SAMPLING EQUIPMENT

3.1 AMBIENT AIR SAMPLING PROGRAM

The objectives of the ambient air monitoring program are as follows:

- Collect data by using time-integrated ambient air sampling methods.
- Establish seasonal measurements and annual average concentrations of PCBs.

3.2 TIME-INTEGRATED PCB SAMPLING

Two ambient air monitoring stations will be set up to sample for PCBs. All ambient air monitoring and analysis will be performed using a modified version of EPA Method TO-4 "Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air" (EPA/600/4-84-041, April 1984, Air Sampling for PCBs). The ambient air sampling component of the program was designed to collect air samples for PCB analysis during each of the four seasons. The following subsections discuss the sampling methodology and analytical methods. WESTON's Standard Operating Procedures are presented as Attachment 1 of this Appendix.

3.3 SAMPLING METHODOLOGY

Ambient air sampling for PCBs will be conducted using General Metals Works model GPS-1 (PUF) samplers for particulate and vapor collection of PCBs. The sample media used for the PCB sampling will be a PUF and a quartz glass fiber filter. The PUF plug is designed to collect the vapor phase of PCBs. The quartz glass fiber filter precedes the PUF plug and collects any particulate-bound PCBs. The samples will be collected by drawing ambient air at a rate of approximately 9 cubic feet per minute (cfm) through the filter and PUF plug. The sample flow rate will be monitored using a calibrated magnehelic gauge. The sample flow will be determined by the average of the pre- and post-sampling magnehelic gauge readings. After sampling is completed, both filter and PUF plug will be recovered and sent to the laboratory for extraction and analysis.

All ambient air monitoring and analysis will be performed using a modified version of the U.S. EPA Method TO-4, *Determination of Organochlorine Pesticides and Polychlorinated Biphenyls in Ambient Air* (EPA, 1984). Method TO-4 has been modified to allow for quarterly calibration of the PS-1 sampler instead of the daily calibration and the use of seasonal average barometric pressure and ambient temperature measurements to determine the volumetric flow for standard conditions.

3.4 ANALYTICAL METHODS

The analytical method to be used for the analysis of PCBs will be a modified EPA Method TO-4, using GC/ECD. This method specifies that all samples should be extracted within 1 week after

collection. The PUF cartridges are extracted for 14 to 24 hours at approximately four cycles/hour with methylene chloride. The quartz fiber filter can be placed in the extractor with the PUF cartridges.

Following the soxhlet extraction, the solvent is switched to hexane and the extract concentrated. Analysis is carried out on a HP 5890 GC/ECD and second column confirmation used to positively identify the pesticide results.

The method is Aroclor-specific. The following Aroclors will be analyzed:

- Aroclor 1016
- Aroclor 1221
- Aroclor 1232
- Aroclor 1242
- Aroclor 1248
- Aroclor 1254
- Aroclor 1260

4. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

Quality Assurance (QA) includes the planned and systematic actions necessary to provide adequate confidence that a measurement or process will satisfy a given requirement for accuracy. QA is typically achieved through the use of independent auditing procedures and duplicate measurement procedures. Quality Control (QC) consists of the operational techniques and activities that are used to fulfill requirements for quality. The QC procedures for the ambient air monitoring component of the program will include planned calibrations, preventive maintenance, and field/trip blanks.

4.1 QUALITY CONTROL FOR TIME-INTEGRATED PUF HIGH-VOLUME SAMPLING

4.1.1 Calibration

The calibration of the PUF sampler is a multipoint calibration of the flow indicator on the PUF sampler. A multipoint calibration is performed because the sampler is not equipped with a mass or volumetric flow controller. The calibration is performed at several flow rates to determine the actual air flow rates corresponding to readings on the flow indicator device (magnetic gauge) attached to the sampler venturi. An adapter plate, NIST-traceable orifice calibration unit, and a manometer are used to measure the pressure drop ( ) H) in inches of water across the calibration orifice. The) H for a calibration orifice corresponds to a specific flow rate. The calibration results are used to determine the flow rate of the PUF sample. Calibration of the sampler will be performed at the beginning and the end of program of the baseline air monitoring and at least once per quarter during the program.

4.1.2 Preventive Maintenance

The PUF samplers consist of three basic components. These components include the sampling media system, the flow controlling and measurement system, and the motor system. Of the three components, only the motor system requires any substantial preventive maintenance beyond normal general cleaning.

Prior to the start of each week-long sampling event, the motors on the PUF samplers will be checked. The condition of the motor brushes will be determined and if necessary replacement brushes will be installed. The condition of the armature and the windings will be checked to reduce the potential for motor failure.

4.1.3 Blank Samples

Two types of blank samples (field and trip) will be collected to measure the possible contamination introduced by field sampling procedures, sampling media, sampling equipment, or shipment of samples. Field blanks are handled in the same manner as actual samples, undergoing the same preparation, installation in the sampler module, and cleanup procedures. The only difference between a field blank and an actual sample is that there is no air volume drawn through the field blank. A trip blank is a sample that is handled similarly to an actual sample, but is not exposed to the environment (i.e., it is kept in the shipping container). One field blank and one trip blank will be collected during each week-long seasonal sampling event.

4.2 QUALITY ASSURANCE FOR TIME-INTEGRATED PUF HIGH-VOLUME SAMPLING

Precision and accuracy checks are both methods of QA. Precision checks are a measure of agreement among individual measurements of the same parameter, usually under prescribed similar conditions. Accuracy is the degree of agreement between an accepted reference measurement and the field measurement and is accomplished through independent auditing procedures. Accuracy may be expressed as a total difference, or percentage of the reference value, or a ratio.

4.2.1 Audits

An audit of the PUF sampler to determine the accuracy of flow measurements is performed using the same procedure as a calibration, but with a different orifice to ensure that the multipoint calibration was performed correctly. An audit of each sampler will be performed at the beginning and end of the program, and quarterly during the program. If the audit indicates the flow rate of the PUF sampler deviates by more than 7% from the calibration, recalibration of the sampler will be performed.

4.2.2 Precision

Precision checks will be performed by obtaining co-located measurements. One PUF sampler will be designated as a precision check sampler to collect a co-located or duplicate sample. The percentage difference between the concentrations measured by the reporting sampler and the precision sampler will be calculated and used to generate precision probability intervals as described in 40 Code of Federal Regulations (CFR) 58.

Samples will be collected at both sampling locations for 5 consecutive days in each of the four seasons of the program. A duplicate sample will be collected at one of the two locations each of the 5 days for a total of 15 samples.

5. DATA REPORTING

A Data and Quality Control Report (DQCR) will be prepared summarizing each of the week-long seasonal sampling events. The DQCR will contain the following information:

- Summary of the sampling events (comments on exceedences, problems with sampling equipment, site activities, laboratory problems, and results of QA samples).
- Wind rose for the 24-hour sample periods and obtained from the nearby meteorological station.
- Printed hourly meteorological data for all measured parameters for the sample periods.
- Summary table of results for the sampling period.
- The initial laboratory report.
- Sample data sheets.

ATTACHMENT 1

INSTALLATION AND OPERATION OF PUF HIGH VOLUME SAMPLER

STANDARD OPERATING PROCEDURES

SOP-003
PAGE 1 of 9
ORIGINATOR _____
APPROVED _____
EFFECTIVE _____

SUBJECT: INSTALLATION AND OPERATION OF PUF HIGH VOLUME SAMPLER

1.0 SCOPE:

The following procedures describe the installation and operation of the General Metal Work Polyurethane Foam (PUF) high volume sampler. This method uses a modified high volume sampler consisting of a glass fiber filter with a PUF backup absorbent cartridge to collect particles. Polychlorinated Byphenyls (PCBs) Dioxins/Furans and Polynuclear Aromatic Hydrocarbons (PAHs) in ambient air. This method and corresponding analytical analysis is designated by the EPA as Method TO-4, TO-9, and TO-13.

2.0 REFERENCES/FORMS

2.1 Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air. EPA 600/4-84-041, EPA Method TO-4, TO-9 and TO-13.

2.2 High Volume Sampling Data Form

3.0 INSTALLATION

The PUF sampler is generally shipped complete and requires no assembly with the exception of the sampler lid. There are several items that need to be checked before the sampler is put into operation (Figure 1).

3.1 Attach the sampler lid.

3.2 Connect the front and rear hasps to hold open and keep shut the sampler lid.

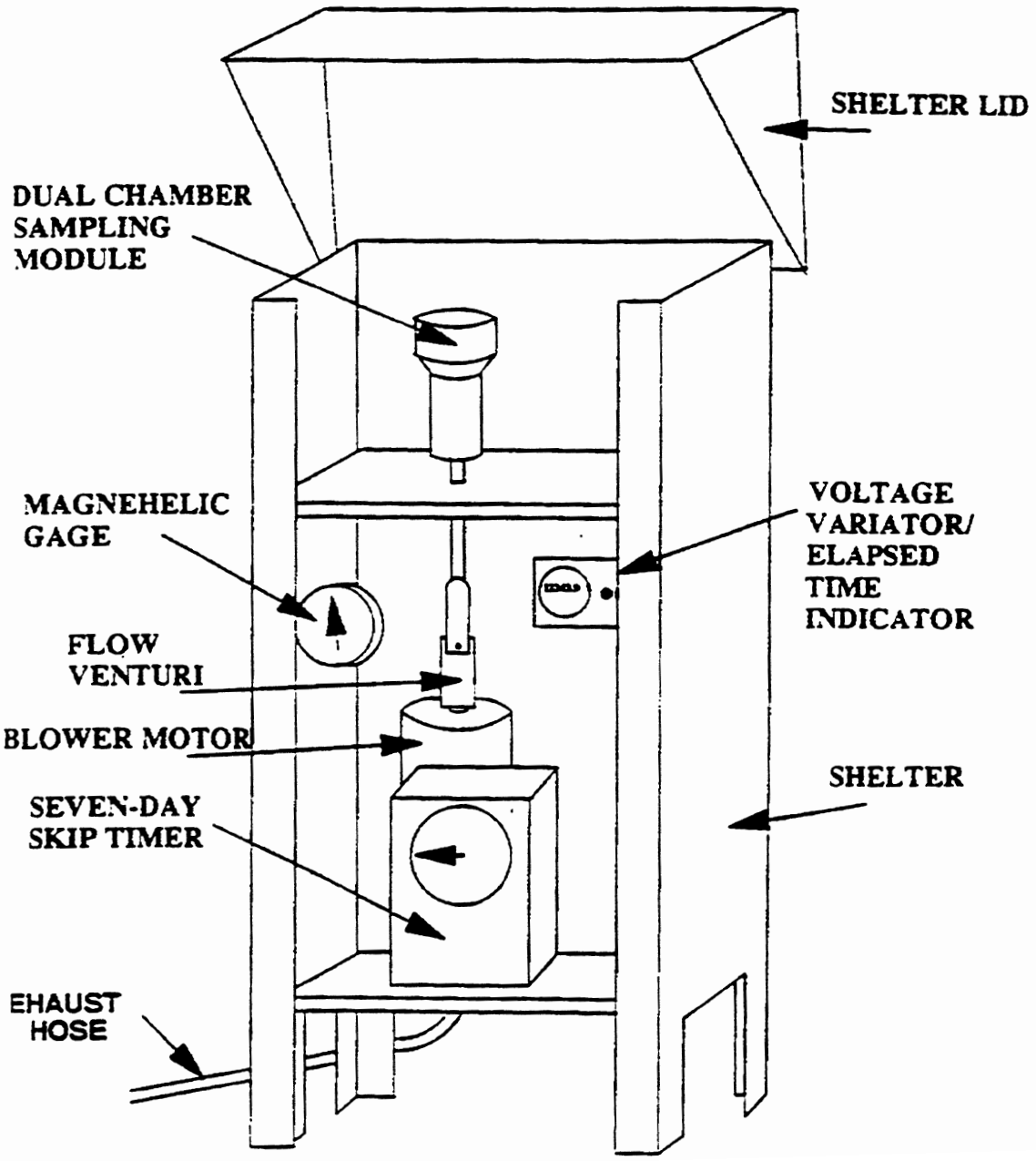
3.3 Check for the rubber gasket between the blower motor housing and housing assembly.

3.4 Connect the exhaust hose with the hose clamp and extend away from sampler.

3.5 Connect the timer controlled side of the plug to the voltage variator/elapsed timer and then to the blower motor.

FIGURE 1

HIGH VOLUME PUF SAMPLER



3.6 Connect 115 VAC, 10 amp power to the timer.

3.7 With the flow Venturi in the open position and the dual chamber sampling module removed, turn on the sampler.

3.8 Turn the voltage variator until you get the highest speed from the motor.

3.9 The magnehelic gauge should read over 100 in. H_2O. If not, check for obstruction in the tubing between the Venturi and magnehelic gauge and/or check that the motor is operating properly and at the highest speed.

3.10 Check for a small rubber gasket between the sampling module and the guide connection.

3.11 Check to see if all parts of the sampling module are present (Figure 2).

3.12 Secure the sampler to a solid and level surface.

4.0 OPERATION

After the sampling system has been assembled and calibrated as described in the SOP for Calibration of the PUF High Volume Sampler, it can be used to collect air samples as described below.

4.1 The samples should be located in an unobstructed area, at least two meters from any obstacle to air flow. The exhaust hose should be stretched out in the downwind direction to prevent recycling of air.

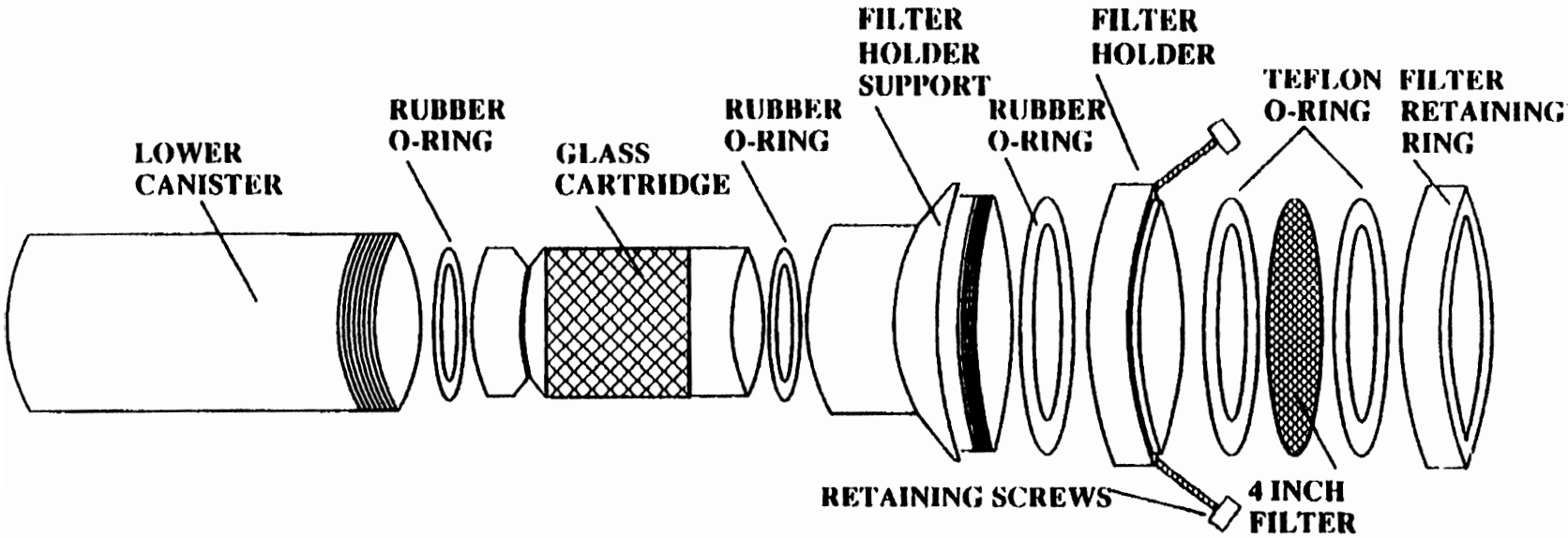
4.2 The Teflon o-rings and filter holder should be cleaned with Hexane between sampling events. The entire sampling module should be cleaned with Hexane on a as needed basis.

4.3 A clean glass cartridge and quartz fiber filter are removed from sealed transport containers and placed in the sampling module using hexane-rinsed forceps and new latex gloved hands. The module is tightly sealed into the sampling system as described below. The aluminum foil wrapping is placed back into a plastic 1-gallon zip-lock bag for later use.

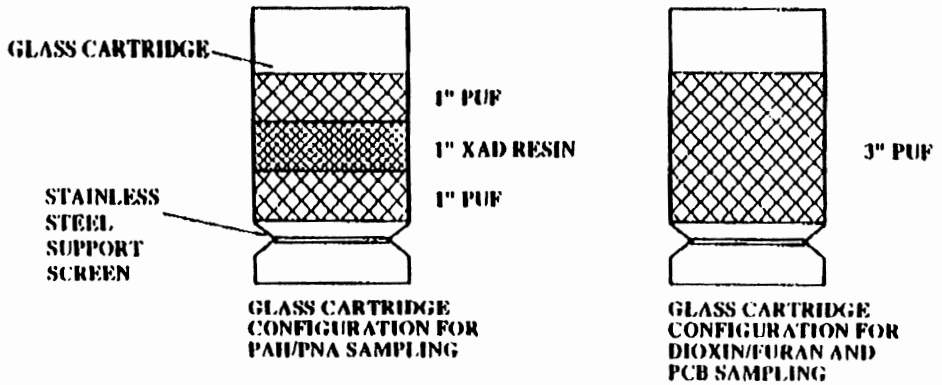
4.3.1 Place Glass Cartridge with PUF (Polyurethane Foam) into lower canister of Sampling Module:

FIGURE 2

SAMPLING MODULE SET-UP



GLASS CARTRIDGE CONFIGURATIONS



For PAH/PNA and Pesticides PCB sampling use One inch PUF, One inch XAD, One inch PUF (Figure 2).

For PCDD + PCDF (dioxin/furan), sampling use one whole PUF (Figure 2).

In some cases this glass cartridge will be prepared in the laboratory and sent to the site assembled.

4.3.2 Next place the glass fiber filter (coarse side up) on the filter holder of the sampling module, by placing the filter between the two Teflon® (white) O-rings (Figure 2). Seal the module as shown in Figure 2 and place the aluminum cover or aluminum foil over the module.

4.4 Check for a zero reading of the Magnehelic and then record the elapsed time meter setting, sampler serial number, filter and PUF sample number on the high volume sampler data form.

4.5 Turn on the PUF sampler. Ensure the flow venturi (yellow or orange handle) is in the up, open position (Figure 3).

4.6 Let the sampler warm up for ~15 minutes before installing sampling module. After ~15 minutes, remove the aluminum cover on the sampling module, install sampling module on to the sampler and record Magnehelic gauge reading (Mag). The Mag reading for a PUF should be ~60" - 70", and for a combined PUF resin between, 30" - 45". If your readings are higher, it means you have a leak. Check the sampling module for tightness (not too tight on the lower canister section, you can break the glass cartridge if it is over-tightened). Check that the sampling module is seated in the sampler correctly. (Module should not be loose when O-rings with tabs at base of module are fully depressed, Figure 4).

4.7 Once you have taken your mag reading, shut off the unit and record the setting on the elapsed time indicator. Record both setting and Mag readings on your data sheet (Figure 4).

4.8 Set the timer to the correct time and correct sampling period.

Example: Sampling period is 48 hours.
Sampling day is 11 Dec. (Friday), 1987.

FIGURE 3

FLOW VENTURI

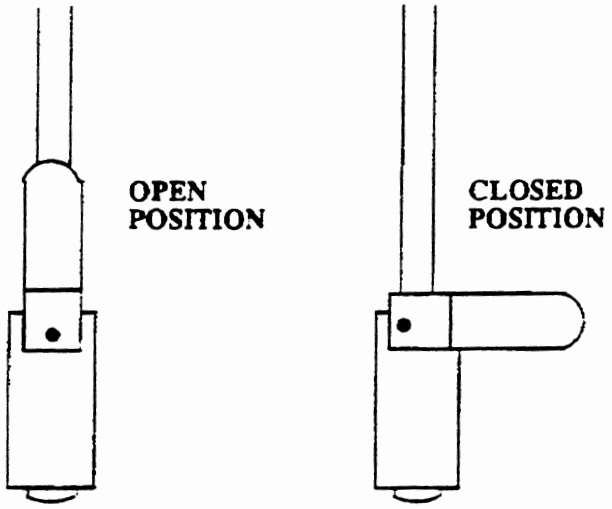
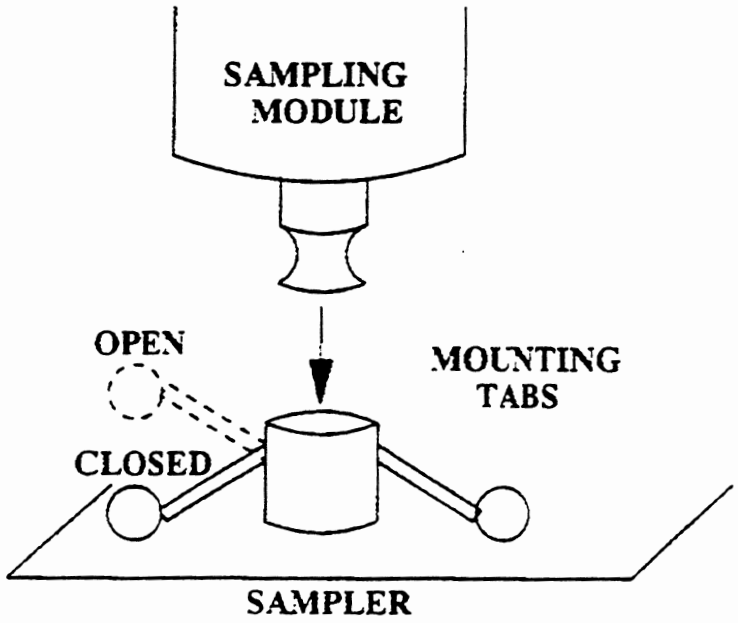


FIGURE 4

SAMPLING MODULE CONECTION



HIGH VOLUME SAMPLING DATA SHEET

Site _____ Performed by _____

Date	Sampling Location ID	Filter Number	Sampling Period, hr		Pump Hours,(min or hrs)			DIAL GAUGE READING		Indicated Flow		Sampler Number	Comments
			Start	Stop	Start	Stop	Elapsed	PRE	POST	PRE	POST		

CHECKLIST

LOADING

- ☐ 1. Load filter
- ☐ 2. Record filter number.
- ☐ 3. Turn sampler on, record dial gauge reading and indicated flow.
- ☐ 4. Turn sampler off and record pump hrs.
- ☐ 5. Set timer for operation.

UNLOADING

- ☐ 1. Record pump hours.
- ☐ 2. Turn sampler on and record final dial gauge reading and indicated flow.
- ☐ 3. Turn sampler off.
- ☐ 4. Unload filter and place in evelope.
- ☐ 5. Sampler may be reloaded for next sample at this time.

ADDITIONAL COMMENTS:

Data Checked by _____ Date _____

Sampling will begin on 10 Dec. at 12:00 noon and end on 12 Dec. at 12:00 noon.

(The sampling day is called the 11th of Dec. 1987, 12/11/87).

Rotate the timer one or two complete rotations to check that the timer trips work properly.

4.9 Turn on the unit to begin sample collection

4.10 Once the sampling period has begun and the unit is sampling, go back and check the sampler for proper operation, i.e., time correct, Mag reading normal, no unusual noises.

4.11 When the sampling period has ended, record the final setting from the elapsed time indicator on the data sheet and turn the flow venturi to the OFF position. Turn the sampler on.

4.12 Allow the sampler to run for ~ 15 minutes to warm up. Once the sampler has warmed up, turn the Venturi to the ON position, wait for the Mag reading to stabilize before recording the final Mag value on the data sheet. Turn the unit off.

4.13 Remove the sampling module and cover the module with the aluminum cover.

4.14 Remove the glass cartridge from the sampling module, wrap in Hexane rinsed foil and label with sampling date, location and sample number.

4.15 Remove the filter from the sampling module with Hexane rinsed spatula, place the filter inside glass cartridge with PUF plug, and place glass cartridge in Zip lock bag.

4.16 Automatic Operations - The above procedures are for automatic or timer operations which can start and stop the PUF sampler at pre-determined times.

4.17 Manual Operations - If the sampler is operated manually as for a 12-hour sample at a work site, the sampler can be turned on and off manually. The above procedures can be simplified.

4.17.1 Follow steps 4.1 through 4.5.

4.17.1 Load sampling module and start the sampler at the designated time. You shou have the correct Mag reading as indicated in 4.6.

4.17.2 Record the barometric pressure (uncorrected to sea level). If continuo temperature readings are not available, use a max/min thermometer to determi the average temperature during the sampling period. The barometric pressure a temperature are used to calculate the actual volumetric flow during the sampli period.

4.17.3 Once the flow has stabilized (after about 15 minutes), record the Mag reading

4.17.4 At the completion of sampling period record, the Mag reading, barometi pressure, max/min temperature.

4.17.5 Turn sampler off and record elapsed time and stop time.

4.17.6 Follow steps 4.13 through 4.15.

APPENDIX A.5

FIELD SAMPLING AND ANALYSIS PLAN FOR MACROPHYTES, PERIPHYTON, PLANKTON/DETRITUS, AND FILAMENTOUS ALGAE

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APPENDIX A.5

FIELD SAMPLING AND ANALYSIS PLAN FOR MACROPHYTES, PERIPHYTON, PLANKTON/DETRITUS, AND FILAMENTOUS ALGAE

1.0 INTRODUCTION

1.1 BACKGROUND

The Lower Reach of the Housatonic River has physical properties that are conducive to the propagation of vegetative and planktonic communities that contribute significantly to the nature of the aquatic ecosystem in this part of the river. Within the study area, a variety of lentic and lotic habitats are present in the river. Within these differing habitats, macrophytes, periphyton, plankton/detritus, and filamentous algae communities each contribute to the ecosystem structure and function, as described below.

1.1.1 Macrophytes

Submerged aquatic vegetation (hereafter called macrophytes) is a key component of many aquatic ecosystems. Macrophytes provide a food source and substrate for invertebrates and other biota; serve as habitat for fish, reptiles, and amphibians; and represent an important component in nutrient and chemical cycling in aquatic systems. Macrophytes also serve as a filter for water moving through aquatic ecosystems, removing suspended particles, nutrients, and contaminants. The biota (commonly referred to as epiphytes; see Subsection 1.1.2) and associated materials attached to the macrophyte surface also act as water column filters. Following senescence, decaying macrophytes and epiphytes enter the detrital food web. Both soluble and particulate components of decomposing macrophytes and epiphytes are released into the water column or accumulate in the sediment layer.

Macrophytes can accumulate certain contaminants from the surrounding aquatic environment. Understanding the levels of contaminants associated with macrophytes and the total contribution to the aquatic system being assessed provide insight into the potential effects that contaminants might have on the aquatic ecosystem of the Lower Reach of the Housatonic River. Portions of the Housatonic River study area have significant populations of macrophytes. These are primarily the areas mapped as riverine aquatic bed (RAB) by TechLaw, Inc. (1998), which includes sections of the Housatonic River, Woods Pond, and backwater areas.

1.1.2 Periphyton

Periphyton, also commonly known as aufwuchs (Ruttner, 1953), is a collective term used to describe a diverse assemblage of organisms that are firmly attached to, but do not penetrate, submerged substrate (plant and mineral) in aquatic habitats. The periphyton community is

complex and includes a wide range of algae, bacteria, fungi, protozoans, rotifers, and small macroinvertebrates. This diverse community is involved in many key processes in the aquatic food web, including primary productivity, decomposition, and nutrient and chemical cycling. Periphyton communities can be found in virtually every freshwater habitat occurring in the photic zone. Periphyton occurs on the gravel and cobble of stream riffles, on soft bottom sediments of shallow river channels and ponds, on snags and submerged debris, and on aquatic macrophytes.

In swift-flowing waters, periphyton attached to gravel particles and cobble of the streambed is the dominant converter of nutrients into biomass, forming a critical base to the food web in that habitat (Smith, 1966). The soft bottom of open pools, ponds, and slow-flowing streams also sustains a periphyton community that may contribute substantially to the biomass/primary productivity of these habitats (Hill, 1999). In open pools, ponds, and slow-flowing systems, the viable biomass of periphyton is limited to a fairly thin veneer (top centimeter or less) of the streambed by the rate of detritus deposition (Clark et al., 1979). Another key factor limiting primary production by periphyton in slow-flowing habitats is light attenuation caused by turbidity.

In lentic systems and slow-flowing streams, periphyton also occurs attached to the surfaces of macrophytes. These periphyton, commonly known as epiphytes, can contribute substantially to the primary production of the aquatic system.

Preliminary reconnaissance has confirmed that the major aquatic habitats occurring in the Housatonic River support a substantial biomass of periphyton.

1.1.3 Plankton/Detritus

The water column in freshwater habitats is a dynamic system wherein nutrients and xenobiotic chemicals are in constant state of flux among the various compartments of the aquatic ecosystem. Two key components are the plankton and detritus compartments. Both occupy important positions in the base of the food web of the Housatonic River and may have substantial influence on the extent to which polychlorinated biphenyls (PCBs) and related chemicals bioaccumulate in upper trophic level biota inhabiting or frequenting the Housatonic River ecosystem.

Plankton is a collective term used to describe a diverse assemblage of microscopic aquatic plants and animals that occur free-floating and suspended in surface waters and have limited or no resistance to current. Phytoplankton are the free-floating microscopic unicellular or colonial plants/algae that serve as major primary producers in open freshwater systems. Also in suspension are microscopic animal organisms (zooplankton) that graze upon the phytoplankton. In freshwater systems, zooplankton consist primarily of protozoans, cladocerans, copepods, and rotifers (APHA, 1995). Together, this assemblage comprises the base of the freshwater aquatic food web and serves as a key energy source to higher trophic level organisms. In addition to playing important roles as primary producers and consumers in the aquatic food web, plankton also serve as important points for xenobiotic chemicals such as PCBs to enter the food web (Thomann et al., 1992; Swackhammer and Skoglund, 1993; Sijm et al., 1998).

In swift-flowing waters, plankton represent a minor component of the aquatic food web. Plankton in this habitat consist primarily of benthic algal species released from periphyton as a result of scouring or some biological process; or discharged from ponds or impoundments upstream (Porter et al., 1993). As stream flow slows, the plankton biomass increases. In the still, open waters of ponds and impounded streams, plankton density may increase to such an extent that light penetration into the water column is reduced and their own growth is limited (Smith, 1966). Such high density is likely the case in the lower reaches of the Housatonic River near Woods Pond, where light attenuation (as measured by Secchi disk) occurs within the top 2 meters (m) of depth.

Detritus is a collective term for the organic matter (complex substances and particulate matter) that arises from non-living and decomposing organisms. Dissolved and particulate organic detritus are very important in controlling the direct and dietary uptake of PCBs by aquatic organisms. Association of PCBs with colloidal and dissolved organic matter (DOM) reduces bioavailability and uptake by planktonic organisms (Stange and Swackhammer, 1994). Association of PCBs with particulate organic matter also removes PCBs from availability for direct uptake, but makes them available to the detrital food web, which is an important pathway in riverine systems like the Housatonic River.

1.1.4 Filamentous Algae

Filamentous algae are multicellular algae that grow in the form of threads (filaments) where the cells are arranged in a simple linear series or chain. These algae can either be free-floating (phytoplankton) or attached to the surface of submerged rocks, wood, soil, or other structures. Filamentous algae serve as a food source and substrate for invertebrates and other aquatic biota, and can potentially have a significant influence on the bioaccumulation of PCBs and related chemicals in upper trophic level biota inhabiting aquatic systems. It is not uncommon for these filamentous algae to accumulate in large mats or blankets near the surface of the water and to be an important component of the aquatic ecosystem. Large mats of filamentous algae are frequently observed in the Lower Reach of the Housatonic River during the summer, particularly in Woods Pond and backwater areas. For these reasons, filamentous algae are considered a separate compartment from periphyton (Subsection 1.1.2) and plankton (Subsection 1.1.3).

1.2 OBJECTIVES

The sampling and analysis of aquatic macrophytes, periphyton, plankton, detritus, and filamentous algae, which make up the base of the food chain in aquatic ecosystems, is intended to provide two key pieces of information for the AQUATOX model (Donigian, et al., 1999): (1) biomass per unit area (standing crop) during a period when significant biomass is present in the Housatonic River study area, and (2) contaminant concentrations. The study is not intended to provide information on the seasonal and year-to-year variation that may exist for both biomass per unit area and contaminant concentration in the Housatonic River study area, nor is it intended to fully characterize these assemblages; however, voucher samples will be collected in case it is determined that the identification of dominant taxa in each sample group would provide useful information. Macrophyte species in samples will be identified and voucher samples collected. A

secondary objective is to provide insight into the potential for waterfowl exposure to PCBs through dietary uptake of relevant food items.

The AQUATOX model domain includes the Housatonic River from the confluence of the East and West Branches downstream to Woods Pond; therefore, the focus of this component of the Supplemental Investigation will be located in this area.

2.0 STUDY DESIGN

2.1 FIELD SAMPLING DESIGN

2.1.1 Sampling Areas

There are four major aquatic habitats that occur in the Housatonic River study area: shallow, swift stream; deep, slow river channel; backwater; and pond. Sampling areas for these dominant aquatic habitats have been established in the study area based on previous field reconnaissance. Additionally, two areas consisting largely of shallow, swift stream habitat have been identified for sampling to define boundary conditions at the upstream end of the study area.

Reach 5a transitions from shallow, swift stream habitat to deep, slow river habitat near the Pittsfield Wastewater Treatment Plant (WWTP) discharge. Therefore, samples of periphyton will be collected from both shallow riffle/swift stream habitat and deep, slow river lotic habitat in Reach 5a to allow for the comparison of similar habitats (deep, slow river lotic) upstream and downstream of the WWTP. Habitat definitions and AQUATOX modeling reach designations are provided below.

Shallow, Swift Stream Habitat

- **Reach 5A**—Confluence of the West Branch of the Housatonic River to Pittsfield WWTP outfall.

Deep, Slow River Habitat

- **Reach 5B**—Pittsfield WWTP outfall to transect 550 (below the confluence of Roaring Brook).

- **Reach 5C**—Below transect 550 to Woods Pond.

Backwater Habitat

- **Reach 5D**—Wide areas of open water adjacent to the river within 1 mile north of Woods Pond.

Pond Habitat

- **Reach 6**—Woods Pond.

Boundary Areas

- **Reach 4A**—The East Branch above the confluence, adjacent to Fred Garner Park, below Pomeroy Avenue.

- **Reach 4WB**—West Branch above the confluence, below South Street.

Within each reach, the occurrence of the community of interest will be mapped to provide estimates of areal distribution. Certain reaches lack sufficient appropriate physical conditions to support a significant community. Where a lack of suitable habitat occurs, no samples will be collected and the absence of suitable habitat will be documented in the field log.

2.1.2 Collection Methods

Collection of samples will occur within the reaches where the community of interest is present in sufficient amounts to be considered by trained observers a significant component of the aquatic ecosystem. The selection of the specific sampling areas will be based on wetland vegetation community maps prepared by TechLaw, Inc. (1998) and field reconnaissance. Field verification of the selected locations will be made prior to the start of sampling. Three sample stations will be selected in each of the reaches containing a significant community of interest as described below.

The major aquatic habitat types occurring in the study area (shallow, swift stream reaches; deep, slow river reaches; and backwater/pond reaches) have somewhat different sampling requirements (described below). The common goal for sampling these habitats is to characterize representative biomass per unit area and tissue residue concentrations in each major aquatic habitat type in the designated reaches. The collection techniques described below are consistent with U.S. EPA Rapid Bioassessment Protocols (EPA, 1997), U.S. Geological Survey Aquatic Biota Collection and Water Quality Assessment Protocols (USGS, 1987; Porter et al., 1993; Shelton and Capel, 1994), and American Public Health Association (APHA) Standard Methods (1995).

2.1.2.1 Sampling Shallow Stream Reaches

Periphyton attached to substrate in the shallow reaches will be collected quantitatively from 0.10-, 0.25-, or 1-m^2 quadrats, depending on density. Cobble and gravel riffle, soft bottom, and aquatic macrophyte bed locations will be selected to be representative of those areas occurring in the targeted major habitats.

In cobble riffles sampled for periphyton, all removable solid substrate within the quadrat will be collected (cobble, wood debris) into white plastic pails. Care will be taken also to collect all loosely associated algal mats and other soft-bodied algal forms encountered within the sampling

location. Using small knives, brushes, and other scraping tools, all periphyton will be removed from the substrate and rinsed with a minimum amount of site water into a collecting pan. The cleaned cobble will be discarded from the sample and returned to the sampling location. The consolidated sample will then be transferred quantitatively to large wide-mouth sample containers. The container will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis.

In shallow soft-bottom reaches and in gravel riffles sampled for periphyton, the top 0.5 to 1.0 centimeter (cm) of substrate matrix, as well as any larger cobble or other debris occurring within the sampling quadrat, will be collected in white plastic pails. A plexiglass collection chamber will be placed over the collection area to deflect current during sampling. Using small knives, brushes, and other scraping tools, all algae will be removed from any large substrate material (cobble, wood, macrophytes, etc.) and rinsed with a minimum amount of site water into a collecting pan. The cleaned cobble will be discarded from the sample and returned to the sampling location. The fine gravel and soft-bottom substrate, as well as the biomass removed from the larger substrate materials, will be combined and transferred quantitatively to large wide-mouth sample containers. The container will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis.

Rooted macrophytes occurring within an area of bottom approximating 0.10-, 0.25-, or 1-m^2 (depending on macrophyte density) will be harvested. Macrophytes will be severed above the root to minimize commingling sediments with the plant material. All macrophytes harvested will be immersed in a minimal volume of site water and shaken vigorously to free attached epiphytic periphyton. The plants will be rinsed over the collection pans and the rinsate quantitatively transferred to sample containers. The container(s) for epiphyte samples will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis. The rinsed macrophytes will be placed into container(s) that will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis. Voucher specimens of macrophytes will be collected from each sample location, dried in a plant press, and stored at WESTON's Pittsfield project office. Voucher specimens of epiphytes will be collected from each sample location, preserved in Lugol's solution, and stored at WESTON's Pittsfield project office.

At each sampling station, filamentous algae within an area of 0.1-, 0.25-, or 1-m^2 (depending on density) will be harvested and transferred quantitatively to large wide-mouth sample containers. The container(s) will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis. Voucher specimens will be collected from each sample location, preserved in Lugol's solution, and stored at WESTON's Pittsfield project office.

Within the shallow stream reaches, water will be collected for plankton/detritus samples from the main channel at a depth of 10 to 20 cm. If no channel is apparent, plankton and detritus samples will be collected at least 1 m from the streambank. All river water will be collected using a flow-calibrated peristaltic pump. The water will be discharged through decreasing aperture size plankton nets arrayed in series to allow the separation and collection of various classes of zoo- and phytoplankton (described in Table 1).

Table 1

Guidelines for Net Aperture Selection*

Net Order	Filter Size (micron)	Plankton Classification
1	560	Large zooplankton and ichthyoplankton
2	153	Small zooplankton, microcrustacea, and rotifers
3	63	Large phytoplankton
4	10	Small phytoplankton, including diatoms
	Filtrate	Total/dissolved detritus

*Wildlife Supply Co., 1999, p. 18.

A minimum of 100 liters (L) of stream water will be sieved at each sampling location. If an insufficient plankton biomass is collected at a sampling station (minimum of 10 grams (g) each of zoo- and phytoplankton), additional water may be pumped through the collection device to obtain an adequate sample. Collection may be discontinued if, based on professional judgment in the field, it is concluded that the reach does not support a significant plankton community. The volume of water pumped will be recorded and used to calculate biomass for the reach. An 8-L aliquot of the filtrate discharged from the plankton net array will be collected for detritus analysis.

On completion of the plankton harvest, the contents of each plankton net containing the biological sample will be quantitatively transferred to large wide-mouth sample containers. Each net will be rinsed with filtered water to ensure collection of all plankton. The contents of nets 1 and 2 will be combined as the zooplankton sample. The contents of nets 3 and 4 will be combined as the phytoplankton sample. Each container will be sealed, labeled, and stored immediately on ice in the dark.

2.1.2.2 Sampling Deep River Reaches

Deep-water river reaches provide limited solid substrate suitable for periphyton community development. In addition, stream turbidity limits the photic zone to the top 1 to 2 m of depth. Consequently, periphyton biomass tends to be limited, with suitable habitat in deep-water lotic reaches largely restricted to shallow areas along the shoreline and shoals where substrate and rooted macrophytes occur to which periphyton might anchor. These areas will be targeted for collection.

Sampling of shallow areas for periphyton attached to bottom substrate will be conducted as described in Subsection 2.1.2.1. Care will be taken to collect only the top 1 cm of substrate surface. The sample will then be transferred quantitatively to large wide-mouth sample containers. The containers will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis.

Rooted macrophytes occurring within an area of bottom approximating 0.10-, 0.25-, or 1-m^2 (depending on macrophyte density) will be harvested. Macrophytes will be severed above the root to minimize commingling sediments with the plant material. Procedures for the collection of macrophytes and epiphytic periphyton from the macrophytes, sample processing, and preservation are described in Subsection 2.1.2.2.

At each sampling station, filamentous algae within an area of 0.1-, 0.25-, or 1-m^2 (depending on density) will be collected, processed, and preserved as described in Subsection 2.1.2.2.

Preliminary reconnaissance of these reaches revealed that the photic zone, as determined by Secchi disk, is limited to the top 2 m of depth. Plankton and detritus samples will be collected in the river channel from a depth of approximately 1 m (mid-photic zone). Samples will be collected and processed as described above in Subsection 2.1.2.1. Voucher specimens will also be collected from each sample location, preserved in Lugol's solution, and stored at WESTON's Pittsfield project office.

2.1.2.3 Sampling Backwater and Pond Reaches

The backwater and pond reaches of the study area are largely depositional in nature and are covered with a layer of soft organic sediment. The rapid deposition rate of sediments, dense macrophyte growth, and limited photic zone in these reaches preclude the establishment of any significant periphyton community in pond and backwater reaches. It is not expected that soft-bottom periphyton communities in these reaches contribute substantially to the standing biomass. However, should a substantial area of soft-bottom substrate suitable for periphyton colonization be encountered, as is expected in portions of Woods Pond, that area will be sampled as described in Subsection 2.1.2.2.

Macrophyte development in these reaches is often dense and provides extensive surface area for colonization by periphyton (epiphytes). Periphyton communities established in the macrophyte beds will be targeted for harvesting.

Rooted macrophytes occurring within an area of bottom approximating 0.10-, 0.25-, or 1-m^2 (depending on macrophyte density) will be harvested. Macrophytes will be severed above the root to minimize commingling sediments with the plant material. Procedures for the collection of macrophytes and epiphytic periphyton from the macrophytes, sample processing, and preservation are described in Subsection 2.1.2.2.

At each sampling station, filamentous algae within an area of 0.1-, 0.25-, or 1-m^2 (depending on density) will be collected, processed, and preserved as described in Subsection 2.1.2.2.

Preliminary reconnaissance of these reaches revealed that the photic zone, as determined by Secchi disk, is limited to the top 2 m of depth. Plankton and detritus samples will be collected in the backwater, and epilimnion of Woods Pond from a depth of approximately 1 m (mid-photic zone). Water collected from the hypolimnion in Woods Pond will be taken from approximately 2 m above the pond bottom. Samples will be collected and processed as described in Subsection 2.1.2.1. A voucher specimen will also be collected from each sample location, preserved in Lugol's solution, and stored at WESTON's Pittsfield project office.

2.2 ANALYTICAL REQUIREMENTS

The three samples of each community collected from each study area will be analyzed individually for biomass, and will be composited and analyzed for tissue chemical residues as one sample.

2.2.1 Analyses

Tables 2 through 6 summarize the number of samples to be collected and the corresponding analyses to be performed.

All chemistry samples will be analyzed for PCBs (total, Aroclors, congeners, homologs), and one sample from each of the seven groups (macrophytes, periphyton from macrophytes, periphyton from substrate, filamentous algae, phytoplankton, zooplankton, and detritus) analyzed for dioxins/furans and organochlorine pesticides. A minimum of 10 g of sample material (total wet weight) will be required for these analyses.

Biomass samples will be analyzed as follows:

- Macrophyte plot samples will be analyzed for wet weight, dry weight, TOC, and ash-free dry weight.
- The filamentous algae and plankton samples will be analyzed for chlorophyll a and phaeophytin, wet and dry weight, TOC, and ash-free dry weight.
- For periphyton collected from cobble riffles and macrophytes, each sample will be analyzed for chlorophyll a, phaeophytin, wet weight, dry weight, ash-free dry weight, and TOC.
- For periphyton collected from soft-bottom and gravel substrates, samples will be analyzed only for chlorophyll a and phaeophytin.
- Detritus samples will be analyzed for chlorophyll a, phaeophytin, dry weight, ash-free dry weight, total organic matter (TOM), and DOM.

Analytical methods for tissue analysis are provided in Appendix C of the Quality Assurance Project Plan (QAPP) (WESTON, 2003).

Table 2

Macrophyte Sample Analyses

Habitat/Reach	Biomass[a]	Chemistry[b]	Duplicates	MS/MSD
Shallow Stream	3	1		
Deep River[c]	3	1		
Deep River[d]	3	1		
Backwaters	3	1		
Woods Pond	3	1		
Boundary area[e]	3	1		
Boundary area[f]	3	1		
See note[g]			1	1

Table assumes macrophytes are present in all habitats/reaches.
[a]Biomass analyses: wet weight, dry weight, ash-free dry weight, and total organic carbon.
[b]Chemical analyses: PCBs (total, Aroclor, congener, homolog) for all composited samples, and one sample for organochlorine pesticides, dioxins and furans.
Duplicate analyses conducted for biomass and chemistry; MS/MSD analyses conducted for chemistry only.
[c]Above transect 550.
[d]Below transect 550.
[e,f]Reach 4A and Reach 4WB, respectively.
[g]The location of QA/QC samples to be determined by the investigator at the time of sampling.

Table 3

Filamentous Algae Sample Analyses

Habitat/Reach	Biomass[a]	Chemistry[b]	Duplicates	MS/MSD
Shallow Stream	3	1		
Deep River[c]	3	1		
Deep River[d]	3	1		
Backwaters	3	1		
Woods Pond	3	1		
Boundary area[e]	3	1		
Boundary area[f]	3	1		
See note[g]			1	

Table assumes filamentous algae are present in all habitats/reaches.
[a]Biomass analyses: chlorophyll a/phaeophytin, dry weight, ash-free dry weight, and total organic carbon.
[b]Chemical analyses: PCBs (total, Aroclor, congener, homolog) for all composited samples, and one sample for organochlorine pesticides, dioxins and furans.
Duplicate analyses conducted for biomass and chemistry; MS/MSD analyses conducted for chemistry only.
[c]Above transect 550.
[d]Below transect 550.
[e,f]Reach 4A and Reach 4WB, respectively.
[g]The location of QA/QC samples to be determined by the investigator at the time of sampling.

Table 4

Periphyton Sample Analyses

Habitat	Biomass[a]	Chemistry[b]	Duplicates	MS/MSD
Shallow Stream	6	1		
Deep River[c]	6	1		
Deep River[d]	6	1		
Backwaters	6	1		
Woods Pond	6	1		
Boundary area[e]	6	1		
Boundary area[f]	6	1		
See note[g]			2	

[a]Biomass analyses: chlorophyll a, phaeophytin, dry weight, ash-free dry weight, total organic carbon.
Number of biomass samples collected is determined by the diversity of microhabitat types present in reach.
For periphyton from soft-bottom and gravel bottom samples, only chlorophyll a/phaeophytin will be analyzed.
[b]Chemical analyses: PCBs (total, Aroclor, congener, homolog) for all composited samples, and one sample for organochlorine pesticides, dioxins and furans.
Duplicate analyses conducted for biomass and chemistry; MS/MSD analyses conducted for chemistry only.
[c]Above transect 550.
[d]Below transect 550.
[e,f]Reach 4A and Reach 4WB, respectively.
[g]The location of QA/QC samples to be determined by the investigator at the time of sampling.

Table 5

Plankton Sample Analyses

Habitat	Biomass[a]		Chemistry[b]		Duplicates		MS/MSD	
	Zoo-plankton	Phyto-plankton	Zoo-plankton	Phyto-plankton	Zoo-plankton	Phyto-plankton	Zoo-plankton	Phyto-plankton
Shallow Stream	3	3	1	1				
Deep River[c]	3	3	1	1				
Deep River[d]	3	3	1	1				
Backwaters	3	3	1	1				
Woods Pond	3	3	1	1				
Boundary area[e]	3	3	1	1				
Boundary area[f]	3	3	1	1				
See note[g]					1	1		1

[a]Biomass analyses: chlorophyll a/phaeophytin, dry weight, ash-free dry weight, total organic carbon.
[b]Chemical analyses: PCBs (total, Aroclors, congeners, homologs); and one sample for organochlorine pesticides, lipids, dioxins and furans.
Duplicate analyses conducted for biomass and chemistry; MS/MSD analyses conducted for chemistry only.
[c]Above transect 550.
[d]Below transect 550.
[e, f]Reach 4A and Reach 4WB, respectively.
[g] The location of QA/QC samples to be determined by the investigator at the time of sampling.

Table 6

Detritus Sample Analyses

Habitat	Biomass[a]	TOM	DOM	Chemistry[b]	Duplicates	MS/MSD
Shallow Stream	3	3	3	1		
Deep River[c]	3	3	3	1		
Deep River[d]	3	3	3	1		
Backwaters	3	3	3	1		
Woods Pond	3	3	3	1		
Boundary area[e]	3	3	3	1		
Boundary area[f]	3	3	3	1		
See note[g]					1	1

[a]Biomass analyses: chlorophyll a, phaeophytin, dry weight, ash-free dry weight, total organic carbon.
[b]Chemical analyses: PCBs (total, Aroclors, congeners, homologs); and one sample for organochlorine pesticides, lipids, dioxins and furans.
Duplicate analyses conducted for biomass and chemistry; MS/MSD analyses conducted for chemistry only.
[c]Above transect 550.
[d]Below transect 550.
[e, f]Reach 4A and Reach 4WB, respectively.
[g] The location of QA/QC samples to be determined by the investigator at the time of sampling.
TOM = total organic matter; DOM = dissolved organic matter.

3.0 QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The two primary data quality objectives (DQOs) of the macrophyte, periphyton, plankton/detritus, and filamentous algae evaluation were outlined in Subsection 1.2 above. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Biomass for each community group: Biomass must be determined accurately and recorded to 1 milligram (mg) (.001 g) using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude. Accurate determination of biomass is also partly determined by following the field sampling methodologies discussed above.

- Tissue residue concentrations for PCBs and other contaminants of macrophyte, periphyton, plankton/detritus, and filamentous algae samples: Quality control considerations to ensure achievement of DQOs for this parameter will follow the QAPP.

- Taxonomic identification of macrophytes to LPIL (lowest practical identification level): Taxa must be identified to the species level whenever possible. When identification to the species level is not possible, the LPIL will be consistent with standard practice for taxonomy. Of equal importance is that the level of taxonomy is consistent for all samples.

3.1.2 Data Quality Indicators

Data developed in the macrophyte, periphyton, plankton/detritus, and filamentous algae study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2003), that are appropriate to the DQOs. Each of these data quality indicators, some of which are not readily quantifiable for macrophyte, periphyton, plankton/detritus, and filamentous algae data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Because of the spatial heterogeneity inherent in aquatic communities, it is not possible to take repeated independent measurements of the biological parameters. Precision will be evaluated by assessment of the degree to which sample collection procedures are able to ensure collection of consistent sample volumes. For the measurements that are not unique to the macrophyte, periphyton, plankton/detritus, and filamentous algae study, such as chemical analysis, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (taxonomy and biomass) accuracy is defined as meaning that the taxa are correctly represented and identified in each sample, correctly enumerated, and correctly weighed. Accuracy of sorting and identification are a function of consistent sample processing, and of consistent field sampling techniques. The data generated by this study will also be evaluated for accuracy via comparison with known and/or expected results from similar studies conducted in the Housatonic River or in similar New England systems. For parameters such as chemical constituents, accuracy is as defined in the QAPP. Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the macrophyte, periphyton, plankton/detritus, and filamentous algae program. To ensure achieving the planned statistical resolution, it is important that completeness of 100% be achieved for all components of this study with the exception of the tissue residue analyses. For this latter study component, the number of analyses will be determined by the material available for collection; therefore, establishment of an a priori completeness goal is not possible.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of representativeness will also be evaluated via comparison with known and/or expected results based on previous investigations of the Lower Housatonic River and other similar systems.

Comparability is a measure of the confidence with which the macrophyte, periphyton, plankton/detritus, and filamentous algae study data may be compared to another similar data set.

Comparability will be evaluated by examination of the variability in key parameters among samples to be collected at each sample site. Comparability will also be evaluated for this data set through comparison with previous macrophyte, periphyton, plankton/detritus, and filamentous algae work in the Lower Housatonic River and with known characteristics of these populations in similar stream systems in the Northeast.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is related for macrophyte, periphyton, plankton/detritus, and filamentous algae investigations to the ability of the taxonomic analysis to resolve the various samples into individual species and/or orders. Sensitivity is applicable and important for the chemistry parameters that will be analyzed as part of the study. For these parameters, the detection limits for chemistry specified in the QAPP will provide appropriate sensitivity for the purpose of providing insight into contaminant concentrations and standing crop among the sampled communities.

3.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical data are discussed in various sections of the QAPP (WESTON, 2003) and will be used whenever applicable in this study. For the biological data, usability will be largely determined by the experience of the senior investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

3.2 SAMPLING DESIGN

The rationale for selection of the locations to be sampled in the macrophyte, periphyton, plankton/detritus, and filamentous algae study was presented in Subsection 2.1.1. The locations are not intended to be representative of the entire river but rather are intended to characterize the four major aquatic habitats that occur in the study area in the Lower River between the confluence and Woods Pond. The quadrat size, random location in select habitats, and sample size (including compositing samples for each reach) will provide representative samples for the sampled communities in each reach.

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Sampling methods, as discussed in Subsection 2.1.2, were chosen to ensure unbiased (i.e., accurate) samples that will facilitate comparisons with other macrophyte, periphyton, plankton/detritus, and filamentous algae data, both from the Housatonic River and from other areas. Steps taken to ensure that sampling does not unnecessarily induce bias include: visual

inspection of each sample to confirm satisfactory collection, and confirmation of visual similarity of macrophyte, periphyton, plankton/detritus, and filamentous algae within a location. All samples will be collected by trained and experienced personnel; senior oversight of all aspects of the sampling and sample processing will further promote comparability and reduce potential bias. Subsamples for chemical analyses will be collected following procedures documented in the project QAPP and will, therefore, be comparable with procedures followed for all other similar efforts throughout the Supplemental Investigation.

3.3.2 Quality Assurance/Quality Control Samples

Quality Assurance/Quality Control (QA/QC) duplicate samples will be collected and analyzed for each medium sampled and analyzed for chemistry (Tables 2 through 6). An additional 10-g sample will be required for each set of analyses. A minimum of 10 L of filtrate water is required for TOM, DOM, and chemical analysis of detritus.

In addition, three matrix spike/matrix spike duplicate (MS/MSD) samples are required. MS/MSD samples were taken for macrophytes, phytoplankton, and detritus samples (Tables 2 through 6). The amount of sample material required for each set of MS/MSD analyses is twice that required for the original analyses.

3.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all macrophyte, periphyton, plankton/detritus, and filamentous algae samples to be collected as part of the study are provided in Section 4. Subsampling, homogenization, and decontamination between samples will follow procedures established in the QAPP (WESTON, 2003). All samples will be held on wet ice and returned to the field laboratory daily and will be either refrigerated, frozen (physical, chemical samples), or preserved (taxonomic samples) at that time. Holding time for chemical samples will follow procedures established in the QAPP; there is no holding time for taxonomic samples.

3.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the collection of macrophyte, periphyton, plankton/detritus, and filamentous algae samples. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Taxonomy Samples

Processing of macrophyte taxonomy samples will follow standard procedures established in previous investigations of the Housatonic River (TechLaw, Inc., 1998). All samples will be

processed by experienced staff who have received specific training in plant taxonomy and whose work is checked periodically by their supervisors and peers.

Quality of taxonomic identification will be ensured by maintaining voucher collections and requiring a consensus among all taxonomists prior to an identification becoming accepted as a determination for the voucher collection. In the event that the taxonomists are unable to agree on an identification, specimens will be sent to a third party for determination.

Voucher samples of periphyton, plankton, and filamentous algae will also be collected and maintained following standard procedures established in previous investigations of the Housatonic River (TechLaw, Inc., 1998). There are no plans for taxonomic evaluation of these samples. If such analysis should be conducted in the future, all samples will be processed by experienced staff who have received specific training in the taxonomy of the voucher samples and whose work is checked periodically by their supervisors and peers.

3.4.2 Physical/Chemical Samples

Samples for tissue chemistry will be processed following procedures and SOPs provided in the QAPP. These samples will be submitted in catalogs (sample delivery groups) and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP (WESTON, 2003).

3.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Subsection 2.2. The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations and will be prepared with specific reference to both the DQOs specific to the macrophyte, periphyton, plankton/detritus, and filamentous algae study (Subsection 3.1.1) and Subsection 4.1 of the QAPP (WESTON, 2003).

4.0 PROCEDURES

4.1 FIELD SAMPLING

General field sampling procedures are as follows:

- Working in teams, deploy boat with equipment or wading gear, as appropriate.
- Select the appropriate sampling location.
- Determine stream velocity using flow meter, obtain temperature, pH, specific conductivity, and DO. Record data, date, time, and a description of the location and number of sampling iterations in the field log.

- Record percent cover of macrophytes, periphyton, and filamentous algae within the sampling location.

- Deploy two 1-m^2 plexiglass chambers, adjacent to each other, over the streambed and anchor in place. One chamber will be used to collect biomass samples and the other for chemical samples. Place 0.10- or 0.25-m^2 frame (as appropriate) constructed of PVC tubing within the chamber.

- Within each reach, select and sample two additional sample stations and repeat the procedures outlined above. The samples for chemical analysis should be composited with the first sample.

Macrophytes, Epiphytes and Filamentous Algae—Collect samples of the macrophytes and filamentous algae to be submitted for chemical analysis from the adjacent plot selected for biomass sampling, and within each plot selected for biomass sampling. Collect the macrophyte samples by hand using a decontaminated stainless-steel knife or scissors sufficient to cut the macrophyte at the sediment-water interface. Collect filamentous algae using a decontaminated stainless-steel colander or scoop. If appropriate, use scissors to cut the algal mat if it extends beyond the quadrat edge. Care should be taken not to disturb sediments. Drain excess water from the samples.

- Epiphyte samples for biomass and PCB analysis will be obtained by immersing the sampled macrophytes in a minimal volume of site water and vigorously shaking to remove the epiphytes. The plants will be rinsed over the collection pans and the rinsate quantitatively transferred to sample containers. The container(s) for epiphyte samples will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis. The rinsed macrophytes will be placed into container(s) that will be sealed, labeled, and stored immediately on ice in the dark for transport to the laboratory for processing and subsequent analysis. Voucher specimens of macrophytes will be collected from each sample location, dried in a plant press, and stored at WESTON's Pittsfield project office. Voucher specimens of epiphytes will be collected from each sample location, preserved in Lugol's solution, and stored at WESTON's Pittsfield project office.

- Process and label duplicate and MS/MSD samples in the same fashion. Label these samples as either "Duplicate" or "MS/MSD" as appropriate.

- Macrophyte and filamentous samples will be packaged in labeled glass jars (or equivalent). Filamentous algae samples will be packaged in labeled glass jars (or equivalent).

- Voucher specimens will be collected from each sample location, preserved in Lugol's solution, and stored at WESTON's Pittsfield project office.

- Record Global Positioning System (GPS) coordinates at each sample location.

- For each macrophyte sample, determine the approximate plant species composition by identifying the plant species and estimating the percent of the total sample volume for

each species. Samples of macrophyte species that cannot be identified in the field will be saved for verification by a qualified botanist.

- Within each reach, estimate the distribution of macrophyte and filamentous algae communities to allow for a determination of total biomass (standing crop). A general survey of the river will be conducted to estimate the coverage of macrophyte communities in the entire reach and study area.

- Within each reach, select and sample two additional sample stations and repeat the procedures outlined above. The samples for chemical analysis should be composited with the first sample. Epiphyte samples for chemical analysis will be composited with any other periphyton samples for the same reach.

Periphyton—Collect all solid substrate (cobble, debris, etc.) (in cobble riffle) occurring within the grid, or collect the top 0.5 to 1.0 cm of substrate (in other substrates).

- Over a clean white plastic pan, thoroughly scrape or brush all algae and related biomass from solid substrate, rinsing with site water as necessary. Discard solid substrate after removing periphyton.

- Transfer the contents of the collection bucket and processing pan, rinsing with site water, as necessary, into 1-L glass sample containers and seal.

Plankton/Detritus—Deploy the weighted collection tubing to appropriate sampling depth, connect tubing to flow-calibrated peristaltic pump, and pump water through plankton collection device. Record total pump operating time. Record pump flow rate at the beginning of the collection period using 1-L graduated cylinder. Retain 1-L and 10-L aliquots of filtrate for chemical analysis of suspended and dissolved detritus in glass containers.

- On completion of the plankton collection, transfer quantitatively the contents of nets 1 and 2 to glass sample containers. Each net will be rinsed with filtrate water to ensure collection of all plankton. Identify container as the zooplankton sample. Likewise, transfer quantitatively the contents of nets 3 and 4 to similarly prepared glass sample containers. Identify container as the phytoplankton sample.

- Collect a voucher sample at each location for each community and place in a container with Lugol's solution, or plant press (as appropriate). Mark containers as described below.

- Samples will be labeled with project name, sampling location number, date, time, and type of analysis requested, then placed immediately in coolers with ice to maintain a temperature of 0 °C.

- Process and label duplicate and MS/MSD samples in the same fashion. Label these samples as either "Duplicate" or "MS/MSD" as appropriate.

- Record GPS coordinates at each location.

- Within each reach, select and sample two additional sample stations and repeat the procedures outlined above. The samples for chemical analysis should be composited with the first sample.

4.2 SAMPLE HANDLING

Keep all samples in the dark in a cooler with wet ice (for a maximum of 24 hours) until shipment to the laboratory.

4.2.1 Preliminary Processing of Plankton

For plankton samples follow the procedures outlined below:

- Transfer plankton samples to 1-L amber glass sample containers, seal, label, and store on wet ice.

- Composite 1 gallon of filtrate water sample from each sampling location. Transfer aliquots to appropriate containers and label as indicated below for total analyses.

Glassware	Total Analyses	Filtered Analyses
Two 1-L amber glass bottles	Aroclor	Aroclor
One 1-L amber glass bottle	Congener	Congener
One 1-L amber glass bottle	OC Pesticide	OC Pesticide
Two 1-L amber glass bottles	Dioxin/Furan	Dioxin/Furan

- When ready to ship, place the samples (in bottles or resealable plastic bags) in a cooler lined with vermiculite.

4.2.2 Shipping

Shipping procedures are as follows:

- Complete a chain-of-custody form listing the contents of each cooler, and place it into a resealable plastic bag. Tape the resealable plastic bag to the inside of the top lid of the cooler, or place it on top of the samples.

- Seal the cooler with two custody seals, and label the cooler with appropriate WESTON shipping labels, including the WESTON return address, and USFWS laboratory address.

- Samples will be delivered by courier or overnight delivery to the analytical laboratory.

- Voucher macrophyte specimens collected for identification by a botanist will be labeled and dried in a plant press to preserve the sample. All other voucher specimens will be preserved in Lugol's solution.

4.3 SAMPLE DOCUMENTATION

Sample documentation procedures are as follows:

- Use a field logbook to record the location, date and time, amount of time spent in collecting activities at each area, method of collection, name(s) of collector(s), and any other pertinent data or information such as problems encountered.

- Complete a sample attribute form for each tissue sample. Put the sample number for each sample, date, and processor's initials on the form.

- Complete a chain-of-custody form for each cooler of samples shipped to the USFWS laboratory. Provide copies to the task manager, who will retain them in the WESTON files.

5.0 EQUIPMENT LIST

5.1 FIELD COLLECTION

- First aid kit
- Life vests
- Boat, oars, anchor, rope for boat, trolling motor
- Cellular phone
- Flow meter
- DO/temperature/pH/conductivity meters
- Calibrated flow rate peristaltic pump
- Box of plastic pump tubing
- Plankton collection device with stand
- Ekman sampler with 10-ft extension
- Petite Ponar sampler
- Secchi disk
- 0.10-m^2 and 0.25-m^2 PVC sampling quadrats with four 4-ft-long steel rods
- Hydroscope
- Field logbook
- Waders
- White plastic collection pans
- 10 large white collection pails
- Stiff toothbrushes or other similar brushing scraping tools
- Stainless-steel knives, spoons, strainer
- Suction bulbs/pipettes

- Squeeze bottles
- Stainless-steel trowel and rake
- Polyethylene scoop
- Extension pole
- Stainless steel knife/razor
- Sample containers (16- or 32-ounce wide-mouth glass jars)
- Sample containers (40-mL plastic bottles) containing Lugol's solution (0.3 mL/100 mL sample)
- Sample containers (1-gallon amber glass bottles)
- Plant press
- Data sheets
- Flagging
- Coolers
- Ice to fill cooler, in plastic resealable bags or free
- Indelible markers (both fine and wide)

5.2 SAMPLE PROCESSING

- One roll plastic sheeting
- Large work table
- Lugol's solution
- 1-L wide-mouth amber glass containers
- One box of 30-gallon trash bags
- Four boxes of Nitrile gloves
- Four boxes of gallon-sized resealable plastic bags
- Shipping coolers
- Ice to fill cooler, in resealable plastic bags or free
- Indelible markers (both fine and wide)
- Ballpoint pens
- Large bucket for decontamination solutions
- Packaging tape
- Laboratory sample labels with unique sample numbers
- WESTON QA/QC labels

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APPENDIX A.5a

COLLECTION OF CORN AND FLOODPLAIN SOIL

APPENDIX A.5a

COLLECTION OF CORN AND FLOODPLAIN SOIL

1. SCOPE AND APPLICATION

The objective of this Standard Operating Procedure (SOP) is to provide guidance and general reference information for collection of corn (*Zea mays* Linnaeus) (both stalk and cob) and co-located floodplain soil for laboratory analysis.

2. EQUIPMENT

- Stainless steel shears or other cutting device
- Stainless steel trowel
- Stainless steel bowl
- Aluminum foil
- Disposable gloves
- 250-mL amber glass jars with Teflon-lined lids for soil samples
- Plastic resealable bags
- Deionized water

3. RELATED PROCEDURES

Note: The SOPs listed below are presented in the Field Sampling Plan, DCN GE-053001-AAMA, WESTON, 2001.

- G-1 Calibration of Field Screening Instruments
- G-2 Decontamination
- G-3 Field Documentation
- G-6 Field Sample Numbering
- G-7 Management of Investigation Derived Waste
- G-9 Quality Assurance/Quality Control Sampling
- G-10 Sample Documentation
- G-11 Sample Packing and Shipping
- G-13 Trimble Pathfinder Pro XL GPS Unit
- SS-5 Soil Sampling

4. PROCEDURE

Sampling locations will be selected to fulfill the following objectives:

- Locations will include and be representative of corn growing in proximity to the river and will be from areas where corn is grown as silage for dairy cows. Sampling will occur in PCB-contaminated areas as well as in a reference area known to have non-detected concentrations of PCBs and located outside the 10-year floodplain.

At each sampling location, document the following:

- Identify the location in a field book.
- Record the sample coordinates using the GPS system.
- Document interviews with farmers or property owners identifying the use of the corn.
- Document historical PCB soil data used to select the sampling location.

4.1 CORN STALK SAMPLING PROCEDURE

1. While wearing disposable gloves, remove two corn stalks near the base of the plant using the shears. Wrap the samples in aluminum foil and place the samples in a labeled resealable plastic bag.

2. Between sampling locations, decontaminate shears.

3. The analytical laboratory will separate the stalks and leaves from the ears. The laboratory will record the total stalk weight and the total ear weight.

4.2 CORN STALK CO-LOCATED FLOODPLAIN SOIL SAMPLING PROCEDURE

1. Collect three-point composite floodplain soils from the 0- to 6-inch interval from the area surrounding the corn stalk's growing location.

2. Describe the soil in the field book.

3. Homogenize each sample in a stainless steel bowl with a stainless steel trowel. Transfer an aliquot of homogenized soil to fill a 250-mL amber glass jar with Teflon-lined lid.

4. Seal the jar and complete a sample label according to the Field Sample Numbering protocol.

5. Place samples in cooler with ice for transport back to the office.

6. Use dedicated decontaminated sampling equipment for each location.

4.3 LABORATORY PREPARATION PROCEDURE

1. The laboratory will not wash samples prior to analysis.

2. The corn stalk samples are to be separated into cobs and stalks, ground up, and analyzed separately for PCBs according to the California Department of Food and Agriculture (CDFA) multi-residue extraction procedure and USEPA SW846 Method 8082.

3. Results are to be reported on a wet weight basis.

APPENDIX A.5b

COLLECTION OF FIDDLEHEAD FERNS AND FLOODPLAIN SOIL

APPENDIX A.5b

COLLECTION OF FIDDLEHEAD FERNS AND FLOODPLAIN SOIL

1. SCOPE AND APPLICATION

The objective of this Standard Operating Procedure (SOP) is to provide guidance and general reference information for collection of fiddlehead ferns and co-located floodplain soil for laboratory analysis.

2. EQUIPMENT

- Stainless steel trowel
- Stainless steel bowl
- Disposable gloves
- One-liter amber glass jars with Teflon-lined lids and sample labels for fiddlehead fern samples
- 250-mL amber glass jars with Teflon-lined lids for soil samples
- Balance (to ensure adequate sample quantity)
- Deionized water

3. RELATED PROCEDURES

Note: The SOPs listed below are presented in the Field Sampling Plan, DCN GE-053001-AAMA, WESTON, 2001.

- G-1 Calibration of Field Screening Instruments
- G-2 Decontamination
- G-3 Field Documentation
- G-6 Field Sample Numbering
- G-7 Management of Investigation Derived Waste
- G-9 Quality Assurance/Quality Control Sampling
- G-10 Sample Documentation
- G-11 Sample Packing and Shipping
- G-13 Trimble Pathfinder Pro XL GPS Unit
- SS-5 Soil Sampling

4. PROCEDURE

Sampling locations will be selected to fulfill the following objectives:

- Sample locations will include and be representative of fiddleheads growing in PCB-contaminated floodplain soils and will be representative of the range of possible PCB concentrations in the floodplain soils to provide some indication of how fiddlehead fern PCB concentrations might vary with soil PCB concentrations. Sampling will also occur outside the 10-year floodplain and in an area with non-detected concentrations of PCBs.

At each sampling location, document the following:

- Identify the location in a field book.
- Record the sample coordinates using the GPS system.

4.1 FIDDLEHEAD FERN SAMPLING PROCEDURE

1. While wearing disposable gloves, remove ferns from the fern stems and add to a 1-liter amber glass jar with a Teflon-lined lid.

2. Collect approximately 300 grams of material and verify the weight with the balance by weighing the sample bottle before and after collecting the sample.

3. Collect a total of 3 one-liter amber glass jars per location.

4. Seal the jar and complete a sample label according to the Field Sample Numbering protocol.

5. Place samples in cooler with ice for transport back to the office.

6. Change disposable gloves between locations.

4.2 CO-LOCATED FLOODPLAIN SOIL SAMPLING PROCEDURE

1. Collect approximately 80 grams to 100 grams of floodplain soil from the 0.0 inch to 6-inch interval, or to the maximum root depth of the ferns, from several locations within the boundary of where the co-located fiddlehead fern sample was collected.

2. Describe the soil in the field book.

3. Homogenize the sample in a stainless steel bowl with a stainless steel trowel. Transfer an aliquot of homogenized soil to fill a 250-mL amber glass jar with Teflon-lined lid.

4. Seal the jar and complete a sample label according to the Field Sample Numbering protocol.

5. Place samples in cooler with ice for transport back to the office.

6. Use dedicated decontaminated sampling equipment for each location.

4.3 LABORATORY PREPARATION PROCEDURE

1. Upon receipt, one fern sample from each location should be rinsed with deionized water. A rinsed and unrinsed fern sample from each location will be analyzed.

2. Ferns are approximately 90% water, and each fern sample will contain approximately 300 grams of material. Therefore, the samples should be homogenized with a tissuemizer and subsequently freeze dried.

3. Fiddlehead fern and soil samples will be analyzed for PCB homologs in accordance with EPA Method 680.

APPENDIX A.5c

COLLECTION OF PASTURE GRASS AND FLOODPLAIN SOIL

APPENDIX A.5c

COLLECTION OF PASTURE GRASS AND FLOODPLAIN SOIL

1. SCOPE AND APPLICATION

The objective of this Standard Operating Procedure (SOP) is to provide guidance and general reference information for collection of pasture grass and co-located floodplain soil for laboratory analysis. The most prevalent grass species, reed canary grass (*Phalaris arundinacea* Linneaus), is the grass to be sampled. Sampling is not to occur following heavy rains or during times of elevated air temperatures.

2. EQUIPMENT

- Stainless steel shears or other cutting device
- Stainless steel trowel
- Stainless steel bowl
- Aluminum foil
- Disposable gloves
- One-liter amber glass jars with Teflon-lined lids and sample labels for grass samples
- 250-mL amber glass jars with Teflon-lined lids for soil samples
- Teflon tape
- Balance (to ensure adequate sample quantity)
- Deionized water

3. RELATED PROCEDURES

Note: The SOPs listed below are presented in the Field Sampling Plan, DCN GE-053001-AAMA, WESTON, 2001.

- G-1 Calibration of Field Screening Instruments
- G-2 Decontamination
- G-3 Field Documentation
- G-6 Field Sample Numbering
- G-7 Management of Investigation Derived Waste
- G-9 Quality Assurance/Quality Control Sampling
- G-10 Sample Documentation
- G-11 Sample Packing and Shipping
- G-13 Trimble Pathfinder Pro XL GPS Unit
- SS-5 Soil Sampling

4. PROCEDURE

At each sampling location, document the following:

- Identify the location in a field book.
- Record the sample coordinates using the GPS system.

4.1 PASTURE GRASS SAMPLING PROCEDURE

1. The pasture grass will be clipped within 1 inch to 3 inches of the ground in order to simulate grazing patterns. Pasture grass samples will be collected from the current growing season. During sample collection, inspect the sample for dead material and remove any grass that is not current (green) growth.

2. Use solvent rinsed aluminum foil to collect grass clippings and transfer clippings into a one-liter amber glass sample jar with a Teflon-lined lid.

3. The total weight of each sample should range between 80 grams to 100 grams. Verify the weight of the sample is within the proper range by using the balance to weigh the sample bottle before and after collecting the sample.

4. Seal the jar and complete a sample label according to the Field Sample Numbering protocol.

5. Place samples in cooler with ice for transport back to the office.

6. Decontaminate sampling equipment between locations.

4.2 CO-LOCATED FLOODPLAIN SOIL SAMPLING PROCEDURE

1. Collect approximately 80 grams to 100 grams of floodplain soil from the 0.0-inch to 3-inch interval from an adjacent location where the co-located grass sample was collected.

2. Describe the soil in the field book.

3. Homogenize the sample in a stainless steel bowl with a stainless steel trowel and fill a 250-mL amber glass jar with a Teflon-lined lid.

4. Seal the jar and complete a sample label according to the Field Sample Numbering protocol.

5. Place samples in cooler with ice for transport back to the office.

6. Use dedicated decontaminated sampling equipment for each location.

4.3 LABORATORY PREPARATION PROCEDURE

1. The laboratory will thoroughly homogenize all samples prior to extraction.
2. The grass samples are not to be washed prior to extraction.
3. Soil and grass samples are to be analyzed for total PCBs, PCB congeners, and dioxins and furans.
4. The laboratory will report wet weight and dry weight concentrations for pasture grass.
5. The laboratory will report dry weight concentrations for soil.

APPENDIX A.5d

COLLECTION OF SQUASH

APPENDIX A.5d

COLLECTION OF SQUASH

1. SCOPE AND APPLICATION

The objective of this Standard Operating Procedure (SOP) is to provide guidance and general reference information for collection of acorn squash (*Cucurbita pepo*) for laboratory analysis.

2. EQUIPMENT

- Stainless steel shears or other cutting device
- Aluminum foil
- Disposable gloves
- Deionized water
- Plastic resealable bags

3. RELATED PROCEDURES

Note: The SOPs listed below are presented in the Field Sampling Plan, DCN GE-053001-AAMA, WESTON, 2001.

- G-2 Decontamination
- G-3 Field Documentation
- G-6 Field Sample Numbering
- G-9 Quality Assurance/Quality Control Sampling
- G-10 Sample Documentation
- G-11 Sample Packing and Shipping
- G-13 Trimble Pathfinder Pro XL GPS Unit

4. PROCEDURE

Sampling locations will be selected to fulfill the following objectives:

- Sampling will occur from three areas within the 10-year floodplain as well as in a reference area known to have non-detected concentrations of PCBs and located outside the 10-year floodplain.

At each sampling location, document the following:

- Identify the location in a field book.
- Record the sample coordinates using the GPS system.
- Document historical PCB soil data used to select the sampling location.

4.1 SQUASH SAMPLING PROCEDURE

1. While wearing disposable gloves, remove the fruit from the squash plant near the base of the fruit.
2. Remove any visible soil and wrap the squash in aluminum foil.
3. Collect several squash per location to ensure adequate sample volume for analysis.
4. Place the aluminum foil-wrapped squash from each location in a plastic bag and place a completed sample label on the bag.
5. Decontaminate shears by rinsing with deionized water and change disposable gloves between locations.

4.2 LABORATORY PREPARATION PROCEDURE

1. Three potential sample preparations may be conducted for analysis at the discretion of the technical study lead. Pulp and seed mass, flesh only, and whole squash aliquots from each location may be selected and prepared for analysis.
2. For each type of aliquot selected for analysis, prepare a composite sample of the media by homogenizing portions of the selected media from the multiple squash fruits submitted for that location.
3. Samples are to be analyzed in accordance with EPA SW-846 Method 8082 (Aroclor analysis).
4. Determine percent solids of each sample and report results on a dry weight basis.

APPENDIX A.6

WORK PLAN FOR THE STUDY OF RARE PLANTS AND NATURAL COMMUNITIES OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (TECHLAW, INC.)

APPENDIX A.6

WORK PLAN FOR THE STUDY OF RARE PLANTS AND NATURAL COMMUNITIES OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

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Regional Project Officer
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EPA Work Assignment No.:	R01074
Contract No.:	68-W4-0013
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LIST OF FIGURES

1. INTRODUCTION

Surveys for Priority Sites of Rare Species Habitats and Exemplary Natural Communities, as defined by the Massachusetts Natural Heritage and Endangered Species Program (MNHESP), will be conducted in the study area (Figure 1). Priority sites represent the most important natural communities and state-listed rare plant species habitats in Massachusetts (MNHESP, 1999). While the Massachusetts Endangered Species Act does not protect the priority sites themselves, that law protects the rare species using the habitats. Ecological risk decision makers may use information provided by this work effort.

1.1 OBJECTIVES

The objective of this survey is to document species of rare plants, and locations of rare natural communities, present in the study area.

2. METHODS

Rare plants and communities are defined as those with a state rank of S1, S2, S3, SU, S?, SH, or SX, or those with a state status of endangered, threatened, special concern, or watch list. These terms are described below.

Endangered - Native species which are in danger of extinction throughout all or part of their range or which are in danger of extirpation from Massachusetts, as documented by biological research and inventory.

Threatened - Native species which are likely to become endangered in the foreseeable future, or which are declining or rare as determined by biological research and inventory.

Special Concern - Native species which have been documented by biological research or inventory to have suffered a decline that could threaten the species if allowed to continue unchecked, or which occur in such small numbers or with such restricted distribution or specialized habitat requirements that they could easily become threatened within Massachusetts.

Watch List - Rare or uncommon species in Massachusetts that are not formally protected by legislation but are monitored by the Massachusetts Natural Heritage and Endangered Species Program. This category contains species that may have been dropped from the official rare plant list, are candidate species for listing, may have questions as to taxonomic identity or native range, or have had insufficient collection effort to ascertain rarity in Massachusetts.

S1 - Critically imperiled in Massachusetts because of extreme rarity (five or fewer or very few remaining individuals or hectares) or because some aspect of its biology makes it especially vulnerable to extirpation from the state of Massachusetts

S2 - Imperiled in Massachusetts because of rarity (6-20 occurrences or few remaining individuals or hectares) or because of other factors making it vulnerable to further decline.

S3 - Rare in Massachusetts (on the order of 20-100 occurrences).

SH - Occurred historically in Massachusetts, and could be rediscovered; not known to have been extirpated.

SX - Apparently extirpated in Massachusetts (historically occurring species for which habitat no longer exists in Massachusetts).

SU - Possibly in peril in Massachusetts, but status uncertain; need more information.

S? - Probably rare or historic in Massachusetts, based on status elsewhere in New England, but not yet reviewed or documented by MNHESP.

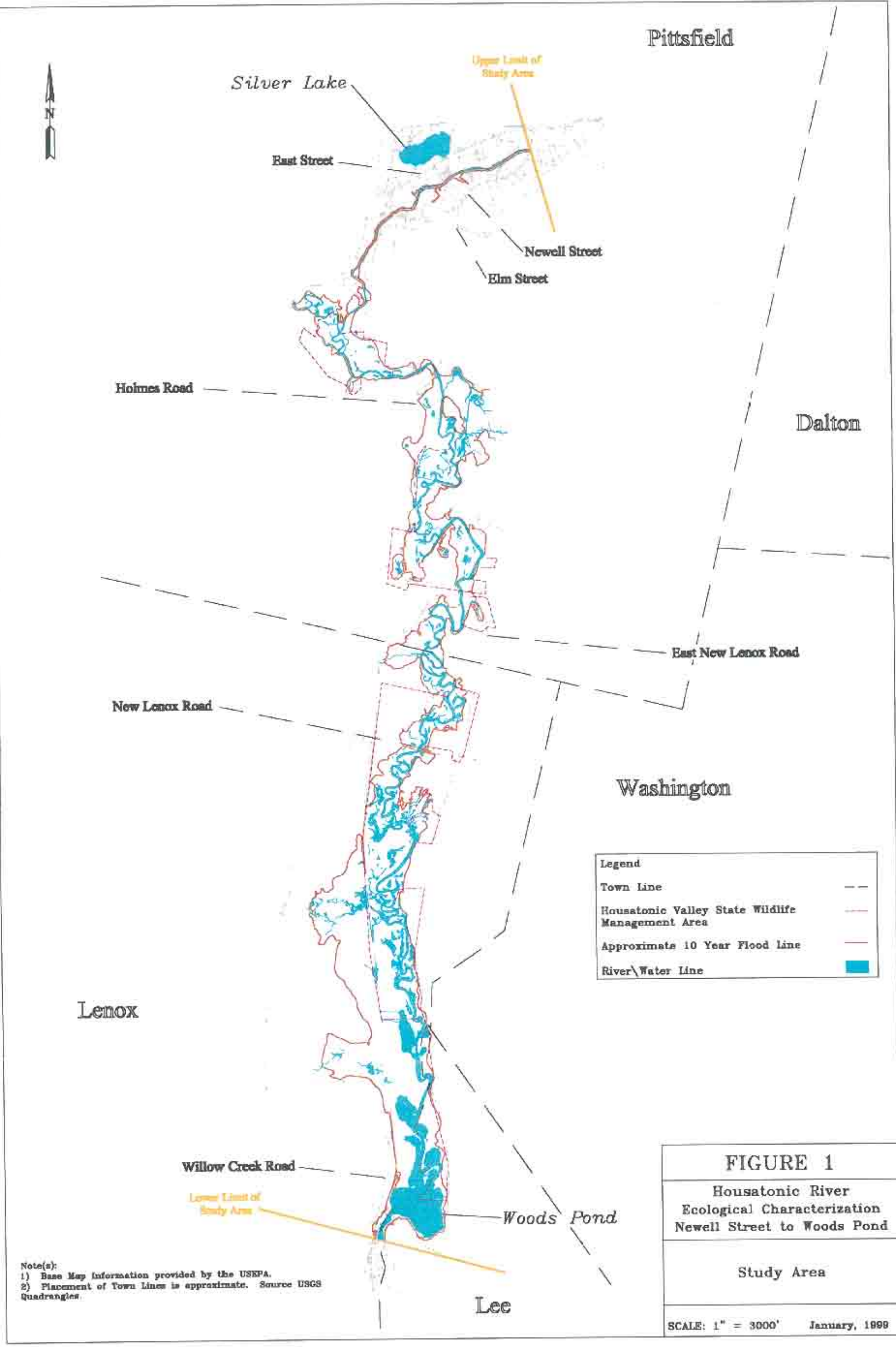
Global ranks (AG@ instead of AS@) follow the criteria for state ranks but refer to the entire range of a species, rather than just its statewide distribution.

Plant species and communities are also provided ranks based on the quality of the occurrence. The element occurrence (EO) rank is an average of four individual ranks: EO quality (size/ productivity); EO condition (pristineness/ability to recover from impacts); EO viability (long-term existence prospects); and EO defensibility (how protectable the occurrence is). The EO rank is a relative rating system based on range-wide observations. It primarily utilizes four classes of ranks: A (excellent); B (good); C (marginal); and D (poor). A ranking of E is sometimes provided for element occurrences that are extant, but information is inadequate to provide a qualitative score.

Landscape analysis, a multi-step process involving information collation, interpretation, and summarization, will be performed to provide a macroscopic view of the study area's history and ecology. The landscape analysis process identifies habitats in the study area that have moderate to high potential for containing a targeted feature (e.g., rare plants, animals, or natural communities). It involves using available natural resource information such as USGS 7.5 minute topographic maps, surficial and bedrock geology maps, aerial photographs, soils maps, wetlands maps, land use history information (e.g., fire, cutting, herbicide spraying), and Biologic Conservation Database information to develop a search image of the targeted feature (e.g., a rare plant) or its associated natural community. The search image is then compared to characteristics of habitats in the study area to those from areas known to contain the targeted feature. Areas with similar images, identified from aerial photos, are then identified as potentially containing the targeted feature. These areas are then field surveyed, at the appropriate time, to determine if the targeted feature exists.

2.1 LITERATURE REVIEW

Available information on rare plants and natural communities will be collected from published and unpublished sources. The Massachusetts Natural Heritage and Endangered Species Program will be contacted for rare plant and community information. The locations of known rare features will be located on study area base maps. Massachusetts Natural Heritage and Endangered Species Program



Note(s):
1) Base Map information provided by the USEPA.
2) Placement of Town Lines is approximate. Source USGS Quadrangles.

FIGURE 1

Housatonic River
Ecological Characterization
Newell Street to Woods Pond

Study Area

SCALE: 1" = 3000' January, 1999

botanists and natural community scientists will also be consulted regarding the availability of reports on plants and communities for the study area. Information on rare plant species taxonomy and biology will be collected from botanical texts (e.g., Flora of North America Editorial Committee, 1993 and 1997; Gleason and Cronquist, 1991; Fernald, 1950; Britton and Brown, 1970; Haines and Vining, 1998) and herbarium vouchers. All available information on rare plants and communities in and near the study area will be reviewed to predict whether additional rare plant or community sites might occur in the study area based on species ranges and habitat types.

2.2 AERIAL PHOTO INTERPRETATION

Stereo-pairs of aerial photos of the study area will be used to review the types and locations of natural communities in the study area. Wetland maps previously prepared by the EPA (TechLaw, 1998) will also be reviewed to identify the location of floodplain forest communities. Natural communities in the study area that appear to be in a natural state (i.e., they have not been altered by agricultural activities, development, man-induced flooding, or other factors) will be identified on maps and aerial photographs. In addition, based on information collected during the literature review, potential sites to field survey will be located on aerial photos and project maps.

2.3 FIELD SURVEYS

Field surveys to verify the existence of rare species or communities will be conducted by experienced botanists trained in plant taxonomy. Experienced botanists are individuals with a combination of academic training in plant taxonomy as well as several years of experience in performing rare plant and natural community surveys.

Using the list of rare plant species that are known or suspected to occur in the study area, and the aerial photos and base maps showing potential survey sites, field botanists will survey areas for rare species. Survey efforts will focus on areas identified during landscape analysis as having a moderate to high potential for containing a targeted rare plant or natural community. Survey sites will be carefully passed through to identify potential micro-habitat containing the plant species of interest.

In addition, most of the remainder of the study area will also be surveyed in a less intense manner during other field exercises to determine if potential habitats were missed during landscape analysis. When potential rare plant or natural community habitats are identified during these efforts, they will also be surveyed. Surveys will be performed during periods when individual species can be positively identified (e.g., in flower or with mature fruit). Multiple site visits will be used, as necessary, to ensure plants are observed in an appropriate stage for accurate identification. Taxonomic keys and herbarium specimens will be used to verify species identification.

At each extant rare plant or natural community site, information on population size, evidence of reproduction, likely persistence, location, and existing threats (e.g., on an all-terrain vehicle trail), will be collected in field notebooks. Photographs will be taken of rare plants and their habitats when possible. Voucher specimens will be collected for species when sufficient numbers exist such that collection would not harm the population (the number dependent of the species' biology). Locations of rare plant populations will be surveyed using Global Positioning System (GPS) equipment.

Massachusetts Natural Heritage and Endangered Species Program Rare Plant Observation Forms will be completed for rare plants observed in the study area.

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The objectives of surveys for priority sites of rare plant species habitats and exemplary natural communities are outlined in Section 1 and can be summarized as locating and documenting the occurrence of rare plants and natural communities, as defined by MNHESP, in the study and reference areas. To achieve these objectives, the following types of data will be required:

- Information concerning known and historic distributions of rare occurrences—Landscape analysis, as outlined in Section 2, will be performed to collate existing data from written and unwritten sources to identify species and communities known or suspected to occur in the study area.

- Taxonomic identification of rare occurrences—Rare plant species will be identified to subspecific level based on morphological, phenological, habitat, and distributional information. Identifications will be performed by plant biologists with formal training and experience in field taxonomy and systematics. Collection of voucher specimens and photographs, if appropriate, will allow confirmation of identification by other project scientists.

- Natural community and population data—Each rare occurrence (plant or community) will involve collection of descriptive information (e.g., associated plant species, population area, canopy height, areal cover of species, canopy tree age and diameter, substrate). Data gathered will provide information on the biology and quality of the occurrence, as well as identified existing threats.

3.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. Each of these data quality indicators that are applicable to this study are discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Precision for this study will be achieved through the consistent identification of plants species throughout the study area through the use of taxonomic keys, investigator expertise, photographs, and the collection of voucher specimens.

Accuracy is defined as the agreement of a measurement with its true value. For parameters of concern to this study, accuracy is defined as meaning that plant taxa will be correctly identified and descriptive information will be collected properly. Rare species identification will be corroborated through examination of plant specimens and photographs by project scientists other than the collector. Accuracy of descriptive information will be controlled through careful supervision by the senior scientist to ensure that measurement devices (e.g., Biltmore Stick – tree diameter, clinometer – canopy height and ground slope) are used correctly. Areal cover of plants species will be estimated through use of estimation proportion diagrams (Color Communications, Inc., 1997) and will be performed by a single researcher to eliminate observer bias.

Completeness is defined as the percentage of the planned samples actually collected and processed. When performing studies to identify new occurrences of rare species or communities, it is not known whether or not new occurrences actually exist in the study area. Therefore, there is not a pre-defined sample number for collection. However, historically known occurrences in the study area will be surveyed using the same field methods that were applied to locating new occurrences. This will test the effectiveness of the field methods and hence, their completeness in determining all the rare occurrences in the study area.

Representativeness is defined as the degree that data collected during this study accurately reflect the true characteristics of the population that occurs in the study area. Descriptive information collected during the natural community surveys will be performed in a manner that ensures site variability is captured on field forms (e.g., multiple plots per community type). Additionally, surveys will be performed during appropriate times of the year to accurately describe and catalog the vegetation and physical characteristics of the rare occurrences.

Comparability is defined as the measure of confidence with which the results from this study may be compared to data from a similar study. Descriptive information for rare occurrences will be recorded on standard field forms acquired from the MNHESP and the Maine Natural Areas Program (MNAP). This will ensure comparability of the data collected in the study area to other rare plant and natural community surveys.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because detailed collection of site soil and water samples will not occur. Species identifications will be verified through examination of voucher specimens and photographs by other project scientists. Usability of information gathered during this study will be based on: (1) the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan and to accurately identify specimens collected at survey locations; and (2) an evaluation of the taxonomic data collected in the study area compared to previous studies from the biophysical region.

3.2 SAMPLING DESIGN

Survey locations for rare occurrences will be selected by two processes. The first process for determining survey location is through examination of data collected during landscape analysis compared to physical features and existing natural communities in the study area. For example, black maple (*Acer nigrum*) is known to occur in rich, alluvial forests and is associated with mesic forest species such as sugar maple (*Acer saccharum*), basswood (*Tilia americana*), white ash (*Fraxinus americana*), and wild leek (*Allium tricoccum*). Areas known or thought to harbor these associated species will be searched carefully for the occurrence of black maple. The second process for determining survey location is through serendipity while performing other research tasks in the study and reference areas. For example, during crayfish studies on the West Branch and main stem Housatonic River, a careful eye will be maintained while traveling over muddy accretion bars to reach sample locations. This specific type of shoreline is known to harbor mudflat spikesedge (*Eleocharis intermedia*) in Berkshire County, MA, a state-listed species.

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Study methodology was chosen to reliably obtain the study objectives. Trained and experienced personnel will perform plant identifications and collection of descriptive information. In addition, senior oversight will serve to promote comparability and reduce researcher bias.

3.3.2 Quality Control Samples

The nature of ecological characterization studies often does not allow for the establishment of typical quality control samples (e.g., duplicate and blank samples) commonly used in studies that collect samples for chemical and detailed physical analysis. Rare species voucher specimens and tree cores (for age determination) are the only samples normally collected during studies of this type. Both of these samples will be retained and can be viewed by other project scientists, where needed, to verify accuracy of determination.

3.3.3 Sample Processing and Preservation

Plant specimens will be collected in large, resealable plastic bags in the field (to prevent desiccation) and transferred to a plant press that day. Upon drying (determined by inspection of specimen and enclosing paper folder), the specimens are mounted on acid-free paper stock using herbarium glue (acid-free). Labels with collection information are affixed to the herbarium sheet. The specimens are stored in a standard herbarium cabinet until shipment to regional herbaria.

Tree cores collected in the field will be stored in aluminum foil folded accordion-style and labeled with a permanent marker. Tree cores will be carried from the field in metal clipboards, to avoid breakage, until delivery to the laboratory. There, the cores will be sanded using medium grain (220)

sandpaper to remove marks left by the increment borer and to facilitate observation of the growth rings. After determination of age, the cores will be stored in the aluminum foil (dried if necessary to avoid fungal growth) and maintained for verification purposes.

3.3.4 Training

All field and laboratory work will be directed by the principal investigator. Supporting staff will be trained by and work closely with the principal investigator for all data collection.

3.4 SAMPLE ANALYSIS

3.4.1 Taxonomy Samples

Plant samples will be identified to subspecific level by the principal investigator. Taxonomy primarily follows Haines and Vining (1998) and Flora of North America Editorial Committee (1993 and 1997). Accuracy of identification will be verified through examination of voucher specimens and photographs by other project scientists. Specimens that cannot be identified will be compared with collections at the Harvard Herbaria or sent to North American experts in that plant group for determination.

3.4.2 Physical Samples

No soil, air, or water samples will be collected for physical or chemical analysis for this study. Tree cores will be collected, enumeration of growth rings performed, and samples saved for verification if needed.

3.5 DATA ANALYSIS AND REPORTING

No numerical analysis for this study will be conducted. As stated in Section 2, descriptive information concerning the site location, biology, associated species, and existing threats will be reported for rare occurrences. Massachusetts Natural Heritage and Endangered Species Program rare plant forms and MNAP natural community forms will be completed and submitted to the MNHESP. Plant voucher specimens will be deposited at Harvard, and pending space limitations, a subset of specimens may be sent to the University of Maine Herbarium.

4. EQUIPMENT LIST

- Binocular stereomicroscope
- Aerial photographs
- Wetland base maps
- Binoculars

- Large sealable plastic bags
- Field notebooks
- Plant taxonomy keys
- Plant press
- Small magnifying lens
- Rubber boots
- Insect repellant
- Camera

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APPENDIX A.7

WORK PLAN FOR THE STUDY OF DRAGONFLIES ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (WOODLOT ALTERNATIVES, INC.)

APPENDIX A.7

WORK PLAN FOR THE STUDY OF DRAGONFLIES ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

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Submitted by:

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1. INTRODUCTION

No state-listed Threatened or Endangered dragonfly species are known to occur in Berkshire county; however, four species listed as Special Concern by the Massachusetts Natural Heritage and Endangered Species Program (MNHESP) are historically recorded as occurring in Berkshire County. These are the skillet clubtail (*Gomphus ventricosus*), brook snaketail (*Omphiogomphus aspersus*), ringed emerald (*Somatochlora cingulata*), and slender emerald (*Somatochlora elongata*) (MNHESP, 1999). Habitat used by these species includes medium to large streams with good water quality; clear, cold, rapid streams with gravelly bottom; large calcareous rivers and forest streams with intermittent rapids, lakes, and ponds (Legler et al., 1998, Walker, 1953). All four of these species have very broad emergence dates ranging from mid-May through August with the most active emergence period being the months of June and July (Legler et al., 1998; Walker, 1953). Dragonfly survey techniques have a high probability of documenting these species should they be present in the study area (Figure 1 in Appendix A.6).

1.1 OBJECTIVES

The objective of this survey is to document species of dragonflies present in the study area, with particular attention focused on the occurrence of rare species.

2. METHODS

A review of existing information on dragonflies of Massachusetts and field surveys will be used to collect information on dragonflies that occur, or may occur, in the study area.

2.1 LITERATURE SEARCH

The scientific and technical literature will be reviewed to determine the historic distribution of dragonflies in the Housatonic River drainage system. As part of this effort, local and regional experts will be consulted to obtain unpublished records regarding the historic and current distribution of dragonflies in the Housatonic River drainage. The MNHESP and the U.S. Fish and Wildlife Service (USFWS) will also be consulted to determine if any records of dragonflies from the Housatonic River drainage are available from surveys sponsored or conducted by these agencies.

2.2 EXUVIA COLLECTION AND IDENTIFICATION

When larval dragonflies leave the water to transform into their adult form, they shed their outer exoskeleton. This shed exoskeleton is called an exuvia. Dragonfly surveys in the study area will consist of exuvia collection along the riverbanks. Larvae very rarely travel far (3 ft to 4 ft maximum) from the water when they shed the exuvia, so surveys will be conducted by foot in the shallow upstream reaches and by canoe in the deeper downstream reaches. Two observers will

walk or float slowly along the shore and collect exuvia from vegetation, rocks, logs, and exposed substrates. Exuvia will be placed in round paperboard containers and sent to a contracted lab for identification. Surveys will be conducted over a 2-day period and be repeated five times throughout the field season. Surveys will commence in mid-May, depending on weather.

2.3 ADULT COLLECTION AND IDENTIFICATION

Opportunistic aerial netting will also be conducted during the course of exuvia collections and other field surveys. Adult dragonflies will be netted, killed in a killing jar, and then mounted as reference specimens. These specimens will be sent to a contracted lab for verification. Since this method is more destructive to the individual dragonflies, it will be limited and only one to two specimens of each common species and one specimen of listed species (if found) will be retained in this way. Field identification will be aided by the use of several dragonfly keys (Legler et al., 1998; Needham and Westfall, 1954; Walker, 1953; Walker, 1958; Walker and Corbet, 1975).

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The data quality objectives for the dragonfly study are outlined in Subsection 1.1. To achieve these objectives, two types of data will be collected:

- Taxonomic identification of dragonfly specimens to the lowest practical identification level (LPIL)—Taxa (both adults and exuvia) will be identified to the specific level when possible. When identification to species is not possible (i.e., collected exuvia are in poor condition), the LPIL will be consistent with standard practice for dragonfly taxonomy.

- Enumeration (counts) for each taxon in exuvia transects—Counts of the number of exuvia per species will be recorded from each transect. Accurate counts are readily achievable in the laboratory as the samples are macroscopic and limited in number.

3.1.2 Data Quality Indicators

Data developed in the dragonfly study must meet the standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (dragonfly taxonomy), accuracy means that the taxa are correctly identified

and counted. Dragonflies, both exuvia and adults, will be sent to an expert in Odonate taxonomy for determination.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness, in the strict sense, cannot be evaluated for this study because the actual number of exuvia or adults present at a location is unknown. However, during exuvia collection events, researchers will look carefully over all river shore habitat in a survey area for all dragonfly samples. Thorough collection effort will be important for documenting all species potentially occurring on the Housatonic River.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study will be ensured through establishment of an approved, thorough sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of the representativeness will also be evaluated via comparison with known and expected results based on previous investigations in the biophysical region.

Comparability is defined as the measure of confidence with which the dragonfly collection data may be compared to another similar data set. Comparability will be attained through use of sampling procedures that are used by the dragonfly researcher community and by careful oversight of the project during collection events. As with representativeness, the comparability of the data will also be evaluated through a study of results from previous dragonfly investigations in the biophysical region.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to identify dragonfly samples from the study area. The method employed for determination will need to occur at a high level of sensitivity in order to characterize the dragonfly fauna of the Housatonic River as well as to ensure that rare occurrences are identified.

3.1.3 Data Validation, Verification, and Usability

Usability of the biological data will be determined primarily by the experience of the senior investigator in establishing that field sampling will be conducted following the study plan and that accuracy and precision will not be compromised by an inability to control sampling procedures in the field.

3.2 SAMPLING DESIGN

The rationale for using nine transects distributed throughout the study area is to ensure that field collection events of dragonfly exuvia occur in a manner providing representation of the communities available. This means that sampling from different vegetation, substrates, and stream velocities will be incorporated in the collection study design. In addition, five separate surveys will provide the opportunity to identify species with different emergence times. Aerial netting of adult dragonflies will further add to creating a complete Odonate faunal inventory for the study area.

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Sampling methods, as discussed in Subsection 2.2, have been selected to ensure that the objectives of the study are met. In addition, procedures are intended to facilitate comparisons with other similar studies from the biophysical region. Samples will be collected by personnel that are familiar with the study objectives and are trained in sample methodology. Senior oversight will further promote comparability and reduce potential bias during collection events.

3.3.2 Quality Control Samples

Dragonfly samples will not be tested for chemical and physical properties. Therefore, quality control samples will not be needed for these purposes.

3.3.3 Sample Processing and Preservation

Procedures for collection and initial processing are described in Subsection 2.2. Exuvia samples will be collected and stored in round paperboard containers, which allows the exoskeletons to dry in order to prevent fungal growth. Adult specimens captured by aerial netting will be euthanized in killing jars. Killing jars contain a porous material at the base impregnated with ethyl acetate. Samples will then be stored in small wax paper envelopes and mailed to an expert for identification. Methods for capture, killing, and storage largely follow protocols detailed in Bioquip (1992).

3.3.4 Training

Sample collection will be directed by scientists with experience in the collection and processing of dragonfly samples. Supporting staff will receive training in the overall goals and techniques to be followed to ensure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Taxonomy Samples

Processing of taxonomy samples will be performed by an independent contractor with expertise in the systematics of Odonates. Because of the difficulty of identification in some genera and potential poor quality exuvia (specimens may be silt covered or damaged during emergence), some samples may require additional examination and comparison with reference collections at regional museums.

The quality of taxonomic identifications will be assured through maintaining a voucher collection of the species collected during the dragonfly survey effort. Specimens that cannot be accurately determined will be sent to another party for identification if a quality specimen exists.

3.4.2 Physical/Chemical Samples

No physical or chemical samples will be collected as part of the dragonfly study.

3.5 DATA ANALYSIS AND REPORTING

The report contents are described in Section 5. No statistical analyses will be performed as part of this study. Massachusetts Natural Heritage and Endangered Species Program reporting forms will be completed and submitted for state-listed species identified during this study.

4. EQUIPMENT LIST

- Killing jars
- Small cardboard containers for protecting exuvia
- Collecting nets
- Dragonfly taxonomic keys
- Study area natural community maps and aerial photos
- Compass
- Binoculars
- Camera
- GPS equipment

5. RESULTS

The following materials will be prepared at the conclusion of this survey: maps showing the location of dragonfly surveys and the location of captures for species of interest; a list of species collected per survey area; and draft and final reports describing methods and results. Global Positioning System (GPS) equipment will be used to collect information on transect locations and, as needed, on capture and/or collection data for dragonflies of interest. No statistics will be performed on the results from this survey.

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APPENDIX A.8

WORK PLAN FOR THE STUDY OF FRESHWATER MUSSELS OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (TECHLAW, INC.)

FINAL

APPENDIX A.8

WORK PLAN FOR THE STUDY OF FRESHWATER MUSSELS OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

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1.0 Introduction

The United States Environmental Protection Agency (EPA) is in the process of characterizing the natural resources found in and adjacent to the Housatonic River in portions of Pittsfield, Lenox, and Lee, Massachusetts (see Figure 1 in Appendix A.6). This reach of river is approximately 12 miles long and extends from Newell Street in Pittsfield downstream to Woods Pond dam in Lee. Elevated levels of polychlorinated biphenyls (PCBs), which originated from the General Electric (GE) facility in Pittsfield, have been found in this reach of the Housatonic River (Blasland, Bouck, & Lee, Inc., 1996).

Freshwater mussels are bivalve mollusks (Class Pelecypoda) that belong to the order Unionoida. There are two families of mussels in Massachusetts: 1) Margaritiferidae, represented by the single species *Margaritifera margaritifera*, and 2) Unionidae, which includes 11 species in Massachusetts (Table 1). Species of Unionoida produce a larva, called a glochidium, that is parasitic on fish or amphibians. Metamorphosis into the adult form occurs while attached to the host (i.e., an intermediate host is required to complete the life cycle). Adult freshwater mussels are infaunal filter feeders, living in or on the substrate, that only rarely move (Smith, 1995).

Freshwater mussels in the Housatonic River could potentially be directly or indirectly influenced by PCBs through several mechanisms, including: 1) direct mortality due to accumulation of PCBs in tissues, 2) impaired physiological function, 3) loss of food supplies, 4) loss of fish or amphibian intermediate hosts, and 5) loss or degradation of habitat. These effects, if they exist, could have occurred historically or may be ongoing. Mussels are also prey items for a variety of wildlife species, including otters *(Lutra canadensis)*, mink *(Mustela vison)*, and muskrat *(Ondatra zibethicus)*.

1.1 Objectives

The objectives of the study are to: 1) determine the historic distribution of mussels in the Housatonic River drainage; 2) determine the current distribution of mussels below the Newell Street to Woods Pond study area, within the study area, and above the study area; 3) identify the host fish and/or amphibians, if known, for the mussels that occurred or still exist within the study area; and 4) identify the wildlife species that are known or expected to prey upon the mussel species found in the project area. This information will be used in the problem formulation in the ecological risk assessment, in the ecological characterization for the study area, and to guide future study needs and design.

1.2 Project Approach

To accomplish the objectives of the study, the scientific and technical literature will be reviewed to determine the historical distribution of mussels in the Housatonic River drainage. If available, modern studies that have included surveys of mussel distribution in the Housatonic River drainage will also be reviewed. This analysis will involve consultation with regional and local malacological experts. Once these tasks are completed, the Newell Street to Woods Pond study area will be surveyed to document the current distribution of mussels in the study area. Similar surveys will be conducted both downstream and upstream of the study area to determine if mussels are

Table 1
Mussels of Massachusetts

Name	*State (Federal) Status*	*Distribution in Mass.*[1]
Alasmidonta heterodon Dwarf wedge mussel	Endangered (Endangered)	Very rare. Confined to a few streams in the Connecticut River drainage system. A few populations may still occur in the Merrimack River. Thought to be historically absent from Housatonic, but museum specimens from the 1840s recently found.
Alasmidonta undulata Heavy-toothed wedge mussel	Special Concern	Found in most drainage systems of the state, but no longer common. Occurs in most types of permanent aquatic habitats.
Alasmidonta varicosa Brook floater	Endangered	Confined to low-lying streams and natural ponds of most major drainages. Never abundant so distribution is uncertain. Most populations reported from the Connecticut River, Merrimack River, and Charles River drainage systems.
Anodonta implicata Alewife floater		Historically confined to streams, rivers, and natural ponds in coastal drainages. Expanding its range in the Connecticut River basin and the Merrimack River drainage.
Elliptio complanata Eastern elliptio		Common and widespread.
Lampsilis cariosa Yellow lampmussel	Endangered	In the past, found in both the Merrimack and Connecticut Rivers. Now only occurring in the Connecticut, where it is rare.
Leptodea [=Lampsilis] ochracea Tidewater mucket	Special Concern	Known only from “Great” ponds and their outlets in the South Shore, Buzzards Bay, and Cape Cod coastal drainages.
Lampsilis radiata Eastern lamp mussel		Present in all drainage systems except the Hoosic and Housatonic. Occurs in most permanent habitats, but prefers larger streams and natural ponds.
Ligumia nasuta Eastern pond mussel	Special Concern	Uncommon in natural “Great” ponds and lowland streams of most coastal drainage systems and the Connecticut River basin.
Margaritifera margaritifera Eastern pearl shell		Relatively common in small to medium sized streams of the Connecticut River system, but uncommon in the Merrimack drainage. Thought to be absent from Housatonic and Hoosic River drainage systems.
Pyganodon cataracta Eastern floater		Very common and found in every drainage system. This species prefers standing or slow moving water and is often the only mussel found in impounded sections of streams and rivers. It is the only species known from the Hoosic River drainage.
Strophitus undulatus Squaw foot	Special Concern	Lives in almost every drainage system.

[1]Smith (1995)

discontinuously distributed in this reach of the Housatonic (i.e., is a mussel species found above and below, but not in, the study area).

2.0 Methods

2.1 Literature Review

Freshwater mussel surveys were conducted with some regularity during the 1800s and many specimens were placed in museum collections. The scientific and technical literature, including museum records, will be reviewed to determine the historic distribution of mussels in the Housatonic River drainage system. Several recent documents are available that specifically reference mussels in the Housatonic River drainage (e.g., Smith, 1995; and Strayer and Jirka, 1997). As part of this effort, local and regional experts in the field of malacology will be consulted to obtain unpublished records regarding the historic and current distribution of mussels in the Housatonic River drainage. The Massachusetts Natural Heritage Program and the U.S. Fish and Wildlife Service will also be consulted to determine if any records of mussels from the Housatonic River drainage are available from surveys sponsored or conducted by these agencies.

2.2 Field Surveys

To characterize the mussel community in the Newell Street to Woods Pond study area, surveys will be conducted in areas that are shallow enough to observe live mussels through viewing scopes. This generally is limited to water that is less than 3 feet deep, depending on water clarity. Mussel inventories frequently include snorkel and/or SCUBA surveys to reach water that is deeper than 3 feet. Such surveys will not be conducted in this project area, however, due to the presence of PCBs in the river sediments. In addition, shorelines in these same areas will be searched for shell middens of individual relict mussel shells.

Within the study area, as much of the river will be surveyed as practical. Habitats that will be surveyed will be areas of shallow, fast-flowing water over sands, silty sands, and gravels as well as areas of deeper, slower moving water along silt loam banks. In general, the fast water surveys will be conducted throughout the river channel in the upper half of the study area and the slow water surveys will be conducted along the riverbanks in the lower half of the study area. Areas of unsuitable habitat, such as ledge bottoms and coarse rip-rapped shorelines will not be surveyed. During mussel surveys, biologists will walk slowly along suitable shoreline and riverine habitat in a systematic manner to search for mussel shells and live specimens. Attempts will be made to survey all accessible, suitable habitat at each survey site. Water depth and turbidity will limit the extent of visual searches. At each site where mussels are found, the number of each species present will be determined, and a Global Positioning System (GPS) receiver will be used to locate the site.

Similar surveys will be conducted in five representative areas downstream of the study area from below the Woods Pond dam to and including the Housatonic River reaches found in Great Barrington. Representative areas are places in the river where mussel habitats are similar, e.g., fast-flowing water over sands, silty sands, and gravels, to those found in the study area. An additional survey will be conducted in Konkapot Brook, a tributary to the Housatonic River,

which has a known population of eastern elliptio (D. Smith, pers. comm., 1998). Upstream surveys will also be conducted at five representative areas extending to and including the reaches found in Dalton. Surveys will generally occur within the river itself, although the mouths and downstream sections of some tributary streams may also be surveyed. In general, these surveys will take place upstream and downstream of road bridges, a survey method often used by state natural heritage and fish and wildlife agencies to assess the local distribution of riverine mussel populations. At each site where mussels are found, the number of each species and general habitat characteristics will be recorded. Existing information on fish species known to occur in each area surveyed for mussels will be reviewed to identify fish that could potentially serve as hosts for larval mussels. Sources of information on fish will include published reports and unpublished electrofishing data obtained from the U. S. Fish and Wildlife Service.

2.3 Habitat Characterization

At each site where mussels are found, the habitat will be characterized in terms of substrate, water depth, and water velocity. The condition of the entire study area, as well as the surveyed reaches downstream and upstream of the study area, will also be generally characterized with regard to suitability for freshwater mussels.

3.0 Quality Assurance/Quality Control

3.1 Data Quality Objectives, Indicators, and Assessment

3.1.1 Data Quality Objectives

The objectives of the freshwater mussels study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- Historic distribution of freshwater mussels in the Housatonic River Drainage—A thorough literature review is required to identify mussel species historically known or suspected to occur within the study area as well as within the Housatonic River Drainage as a whole.

- Taxonomic identification of samples—Mussels found in the study area must be identified to the species level. Relatively few mussel species potentially occur in Massachusetts relative to other states in the Eastern United States, making identification relatively simple. Identification of these species will be based on shell morphology and will be conducted using a key to Massachusetts species. If needed, information from other taxonomic keys will be used to corroborate information presented in the Massachusetts key.

- Characterization of habitat at mussel locations and survey sites—Will be characterized: data collected must allow for general habitat suitability determinations. Freshwater mussels inhabit a variety aquatic habitats in lotic and lentic environments. Data gathered will provide information on the physical components of the river stretches surveyed, thereby allowing for determinations of

the general suitability of survey sites for freshwater mussel colonization. Data will not be collected on habitat parameters such as flow rates, water temperature, and water chemistry.

3.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. These data quality indicators are discussed below.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will not be possible because specimens will not be collected. However, agreement between surveyors regarding species identification must be obtained for verification. This will occur either in the field as surveys are conducted, or in the office using photographs of individuals showing distinguishing features (e.g., size, color, shell morphology). Precision may also be evaluated by assessing the degree to which surveys are consistent among sites. For measurements that are not unique to freshwater mussels, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy means that any freshwater mussels observed during the course of the study are correctly identified and counted. Mussels will be identified by the principal investigator and will be verified by a regional expert.

Completeness is defined as the percentage of the planned samples actually collected and processed. For this study, completeness will be a measure of the number of sites visited and surveys completed compared to the number planned.

Representativeness refers to the degree to which the data collected during this study accurately reflect the true characteristics of the population that occurs in the study area, with respect to study objectives. For example, a principal objective of this study is to identify species occurring within the study area. Sample design should therefore employ methods that will allow for the discovery and accurate identification of species in the study area. Measures of population characteristics are not an objective of this study. Therefore, methods to determine demographic information on the mussel population (e.g., sex ratios, densities) are not required.

Comparability is a measure of the confidence with which results from this study may be compared to another similar study. This comparability, however, will be limited by natural and anthropogenic variability in habitat conditions that will likely be encountered at each sample site for this study and other studies. Therefore, comparability will be limited largely to general statements on species composition relative to habitat conditions and historical information.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to identify freshwater mussels from the study and reference areas.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because detailed collection of site soil or water samples will not occur. The usability of the information gathered during this study will be based on: 1) the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan and to accurately identify specimens collected at survey locations; and 2) an evaluation of the taxonomic data collected in the study area with respect to similar taxonomic communities in the Housatonic River and the New England area.

3.2 Sampling Design

The sample design for field surveys was developed to encompass as much of the Housatonic River from Newell Street to Woods Ponds and even farther downstream as possible and to survey that area during time periods and in areas that would more likely result in the discovery of mussels, given the limitations of certain survey methodologies. Surveys in areas upstream and downstream of the Newell Street to Woods Pond study area will employ similar search methods, and will be timed similarly, but occur in more finite areas due to the unconstrained limits of river reaches in these areas, particularly downstream.

3.3 Sampling Methodology

3.3.1 Sampling Procedures

The study methodology, as described in Section 2.0, was chosen to reliably attain study objectives. The simplicity of this study allows for accurate species identifications based on shell morphology and literature information to provide additional characteristics for species found in the study area. Field surveys employ a methodology commonly used for mussel population characterization studies and include a number of ways that mussels can be discovered, including direct observation of live individuals in shallow water areas, and the discovery of relict shells resulting from natural mortality and predation located along the shoreline.

3.3.2 Quality Control Samples

The nature of ecological characterization studies often does not allow for the establishment of typical duplicate and blank samples, commonly found in studies that collect samples for chemistry analysis. This study, however, will rely on the discovery of shell materials of freshwater mussels. Individual samples discovered, therefore, will be retained after identification until verification can be made. The verification of identified samples will be conducted by a regional expert in the field of freshwater mussels. If differences arise between identifications by the principal investigator and the regional expert, a resolution of that species identification will be made and the specimen will be retained. However, if no differences arise, specimens need not be retained.

3.3.3 Sample Processing and Preservation

No processing or preservation activities are required for this study.

3.3.4 Training

All field work will be directed by senior field scientists with experience in survey techniques for freshwater mussels. Supporting staff will have some experience in these types of surveys but will be trained, as needed, with respect to the specific methodology and species identification. Final on-site identification of samples, however, will be conducted by the principal investigator, with follow-up verification by the regional expert.

3.4 Sample Analysis

3.4.1 Taxonomy Samples

Samples will be identified to species using a taxonomic key for freshwater mussel species of Massachusetts. A consensus on species identification between the principal investigator and the regional expert will be required. If a consensus is not reached, the sample in question will be sent to a third party for determination.

3.4.2 Physical/Chemistry Samples

No physical or chemical samples will be collected for this survey.

3.5 Data Analysis and Reporting

No numerical analysis for this study will be conducted. However, as stated in Section 2.0, descriptive information on species presence, habitat requirements, and ecology will be presented.

4.0 Equipment List

Equipment that will be needed as part of the field component of the study includes:

- Camera
- Field notebooks
- Viewing scopes
- Rubber knee and hip boots
- Rubber gloves
- GPS receiver
- Canoe
- Ziploc-type bags
- Resealable

5.0 Results

A table of mussel species known or suspected to occur in the study area per habitat type will be prepared. This table will include the species of mussels observed in the study area during this study. Maps showing the location of mussel populations will be produced. Draft and final narrative reports, which describe the methods used to survey mussels and the results of surveys, will also be prepared and included as part of the draft and final ecological characterization report.

6.0 Literature Cited

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APPENDIX A.9

WORK PLAN FOR THE STUDY OF REPTILE AND AMPHIBIAN USE OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (TECHLAW, INC.)

FINAL

APPENDIX A.9

WORK PLAN FOR THE STUDY OF REPTILE AND AMPHIBIAN USE OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

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Attachments

1.0 Introduction

The United States Environmental Protection Agency (EPA) is in the process of characterizing natural resources found in and adjacent to the Housatonic River in portions of Pittsfield, Lenox, and Lee, Massachusetts (see Figure 1 in Appendix A.6). The study area is approximately 12 miles in length and extends from Newell Street in Pittsfield to Woods Pond Dam in Lee. In this stretch of river elevated levels of polychlorinated biphenyls (PCBs) have been found, which originated from the GE facility in Pittsfield (Blasland, Bouck, and Lee, Inc., 1996a). Areas being surveyed include riverine habitat, adjacent floodplain wetlands, and uplands associated with the main stem of the river. This work plan provides an approach for collecting baseline data pertaining to the reptiles and amphibians (collectively called herps) present within the approximate 10-year floodplain.

Floodplains in the study area provide habitat for a wide variety of reptiles and amphibians (hereafter referred to as herps). In fact, as many as 40 different species may occur in the area based on published range maps (Attachment 1), although the actual number is likely lower. Breeding amphibians use temporary and permanent pools for courtship and egg-laying. These pools then support larval amphibians for periods ranging from several months to more than one year depending on the species (i.e., wood frogs [*Rana sylvatica*] metamorphose in several months, while green frogs [*Rana clamitans*] take over a year). Several species of turtles use permanent pools and the Housatonic River.

Documentation of the use of the study area by reptiles and amphibians is being conducted because these animals may be exposed to PCBs present in floodplain soils and river sediment, and therefore may be directly affected by these conditions. They may also be bioaccumulating PCBs, which then may be passed on to other animals through the food chain. In addition, several species that may occur in the study area are of management concern (i.e., they are listed as State-Endangered, Threatened, Special Concern, or Watch List [Massachusetts Natural Heritage and Endangered Species Program, 1997]). These include Jefferson salamander (*Ambystoma jeffersonianum*), spotted salamander (*Ambystoma maculatum*), marbled salamander (*Ambystoma opacum)*, spring salamander *(Gyrinophilus porphyiticus*), four-toed salamander (*Hemidactylium scutatum*), and wood turtle (*Clemmys insculpta*).

1.1 Objectives

The objectives of this task are to provide an estimate of amphibian and reptile species richness in the study area per habitat type; to sample larval amphibians in breeding habitats that are expected to have different sediment concentrations of PCBs based upon evaluations of previously collected PCB data (Blasland, Bouck & Lee, 1996b); and, should incidental mortality occur during pit trap sampling, to collect herp samples to be submitted for PCB analysis. These data will be used in the Ecological Characterization for the study area, development of the Problem Formulation in the Ecological Risk Assessment, and design of future data collection efforts.

1.2 Project Approach

Several steps will be taken to meet the objectives of the project. These include literature review, wetlands mapping, visual encounter surveys, aquatic funnel trapping, pit trap array operation, and data analysis. The literature review will be used to identify available information on present and historic use of the study area by herps. Habitat types present in the study area will be determined by reviewing the results of the preliminary wetlands mapping effort, performed in the spring of 1998 (TechLaw, 1998). This review will identify the location, type, and juxtaposition of wetlands in the study area, particularly those areas most likely to be used by breeding amphibians. This information will then be used to design field surveys. Visual encounter surveys, and aquatic funnel and pit trapping, will be used to identify herp presence in the study area per habitat type and location. In addition, morphometric data will be collected from a sample of amphibians, to compare herp growth rates between different locations within the study area. Finally, data analysis and report preparation will be performed to document the methods and results of the herp investigations.

2.0 Methods

Both qualitative and quantitative sampling of herps will occur in the study area. Qualitative studies will include visual encounter surveys (Crump and Scott, 1994), dipnetting (Shaffer et al., 1994), and acoustic monitoring (Zimmerman, 1994), while quantitative surveys will consist of using aquatic funnel traps (Shaffer et al., 1994) and pit traps (deMaynadier, 1996) (in conjunction with small mammal surveys). Qualitative data will provide information on species presence per habitat type and area and relative abundance. Quantitative sampling will occur in selected pools within the floodplain that contain or could contain breeding amphibians. Quantitative sampling will provide information on species richness; numbers per species captured per unit effort (abundance); and minimum, maximum, and mean size of individuals captured in pools. In addition, physical information on vernal pools in the study area will be collected using established criteria (Kenney, 1995). Any malformations present on amphibians will be documented (Northern Prairie Wildlife Research Center, 1997).

2.1 Literature Review

To help focus field efforts, a list of species likely to occur in the study area, and the micro-habitats in which they occur, will be prepared. This information will be obtained from published field guides and manuals, peer-reviewed scientific papers, available masters and Ph.D. dissertations, and discussions with scientists familiar with herps in the study area. This information will be compiled into a table with common and scientific names per species, state- and federal-list status, breeding and other habitats, proposed survey methods, and references. Macro- and micro-habitat descriptions will be used to focus field surveys in areas most likely to contain each species. Existing literature will also be used to develop species identification protocol for eggs, larvae, recently metamorphosed juveniles, and adults.

2.2 Mapping Temporary and Permanent Pools

Temporary and permanent pools in the floodplain provide potential breeding sites for several species of frogs (e.g., wood frogs, northern spring peepers [*Pseudacris crucifer*]) and salamanders (e.g., spotted salamander, Jefferson salamander), and potential foraging areas for several species of reptiles (e.g., painted turtle [*Chrysemys picta*], Eastern garter snake [*Thamnophis sirtalis*]). To determine the location of temporary and permanent pools in the study area, preliminary wetland mapping performed in the spring of 1998 will be reviewed (TechLaw, 1998). Locations of pools will be documented and visited by the field crew. The preliminary wetlands maps will be modified, if needed, to reflect locations and sizes of temporary and permanent pools.

2.3 Visual Encounter Surveys

During the spring of 1998, visual encounter surveys and acoustic surveys (Crump and Scott, 1994) will be performed during wetlands mapping and other reconnaissance surveys of the study area. Because the objective of this task is to develop a list of species seen or heard per site searched, survey efforts will not be timed per area. In each site searched, a list of species encountered per habitat will be recorded in field notebooks.

Turtles will be sampled by visually surveying temporary and permanent pools for basking individuals. All turtle sightings will be recorded in field notebooks along with information on date, time, location, behavior, estimated size, and sex (if possible). All sightings of snakes in the study area will be similarly recorded. Visual searches for snakes in appropriate habitat and micro-habitat will be made throughout the growing season. If any rare species, or state- or federal-listed species, are encountered, an attempt will be made to locate additional individuals in similar habitat. Massachusetts Division of Fisheries and Wildlife, Natural Heritage and Endangered Species Program Rare Animal Observation Forms (Attachment 2) will be completed for each rare animal observation.

To supplement visual encounter and acoustic surveys, an attempt will be made to visit all temporary and permanent pools in the study area in the spring of 1998 when amphibians would be breeding. For each pool visited, information on reptiles and amphibians using the pool will be collected using Vernal Pool Field Observation Forms (Attachment 3) developed by Kenney (1995). General pool descriptive data will also be collected; however, information on water quality will not be gathered. Qualitative dipnetting (Shaffer et al., 1994) surveys will be used in each pool visited. All reptiles and amphibians encountered will be identified to species, while invertebrates will be identified to the lowest practical taxonomic level.

2.4 Aquatic Funnel Trapping

Before any herp trapping begins, a Scientific Collecting Permit will be obtained from the Massachusetts Division of Fisheries and Wildlife. Once a permit is obtained, Aquatic Funnel Trap surveys (Shaffer et al., 1994) will be performed throughout late spring and summer on a sample of 15 to 20 pools in the study area (Figure 1). Pools that are known, or believed, to contain PCB

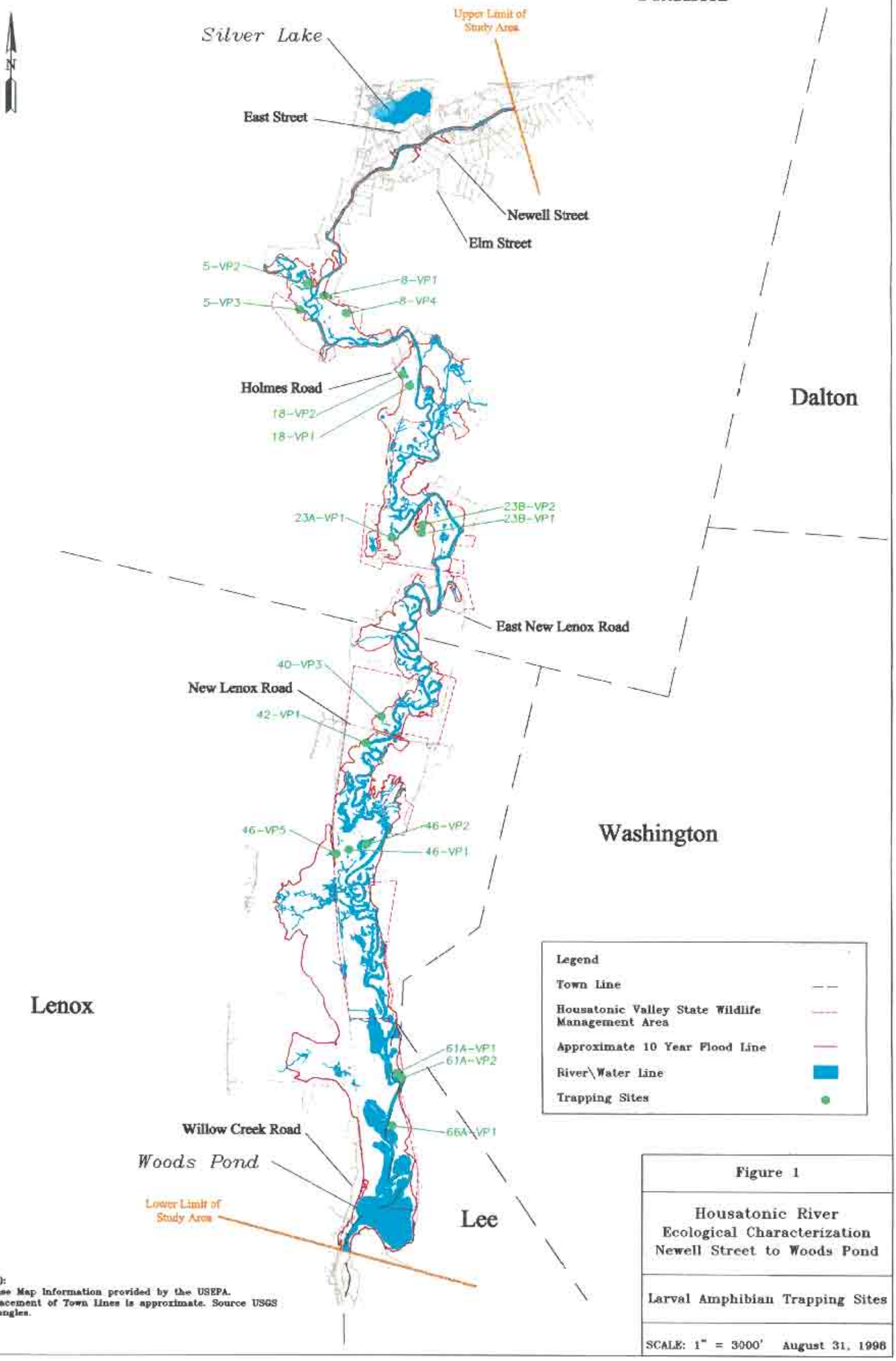
levels greater than, and less than, 10 ppm, based on their proximity to soil and sediment PCB sample locations identified in Blasland, Bouck, and Lee, Inc. (1996a and 1999b) will be surveyed.

Pools that occur directly on soil and sediment that have previously been sampled for PCBs will be considered to have "known" PCB levels. The levels will be determined based on review of existing data (Blasland, Bouck, and Lee, Inc., 1996b). For pools that occur within 400 feet of soil and sediment sample sites, the surrounding floodplain configuration and topography will be examined to classify them as having "assumed" PCB levels greater than, or less than, 10 ppm.

For example, if a pool is located downstream of a transect with PCB levels less than 10 ppm and is adjacent to a fast water section of river (i.e., non-depositional) with high (approximately 8 - 10 feet above the normal high water line), stable banks, it is assumed to have PCB levels similar to the nearby upstream transect. Similarly, if a pool is located just downstream of a transect where PCB levels were greater than 10 ppm and is adjacent to a slow-moving section of the river (i.e., depositional) in low floodplain forest habitat, or is adjacent to a low bank (approximately less than 8 feet above the normal high water line) that could frequently become overtopped by high water, it is assumed to have PCB levels similar to the nearby transect. Pools that are greater than 400 feet from soil and sediment sample sites, or for which the surrounding topography does not provide conclusive information regarding potential PCB levels, will be identified as having unknown PCB levels. To confirm PCB levels, additional soil and sediment sampling will be performed in the pools sampled with aquatic funnel traps.

As stated earlier, 15 to 20 pools will be sampled with aquatic funnel traps. This number of pools represents approximately 25% of the total number of pools in the study area and provides a sample of several types of pools. All pools will be trapped within a single week period, and surveys will be repeated every other, or every third week, starting in mid-May and ending in mid-July. Pools will be selected by preparing a list of all pools surveyed during the temporary and permanent pool mapping exercise detailed in Subsection 2.2. These pools will then be stratified into: 1) those with known or assumed soil and substrate PCB levels greater than 10 ppm, 2) those with known or assumed soil and substrate PCB levels less than 10 ppm, and 3) those with unknown soil and substrate PCB levels. A random sample of pools with assumed soil and sediment PCB levels greater than 10 ppm, and less than 10 ppm, will then be surveyed with the aquatic funnel traps. These classifications will be revisited upon review of actual data from the pools.

Ten aquatic funnel traps will be placed in each pool selected for sampling. The number of funnel traps (i.e., survey effort) needed for capturing amphibians is not known based on previous surveys; however, ten traps are expected to provide a suitable sample because of the small size of pools. Keeping the level of effort the same in all pools will insure that the survey effort is uniform and unbiased. Traps will be randomly placed by establishing a transect along the long axis of each pool, selecting random distances along the transect, and then selecting random distances laterally from the transect. Traps will be placed in the pools in the evening and collected the following morning. If too many individuals are being captured, which results in trap mortality, the amount of time the traps are operating will be reduced. In addition, the time of day during which the traps are



Note(s):
1) Base Map Information provided by the USEPA.
2) Placement of Town Lines is approximate. Source USGS Quadrangles.

Figure 1

Housatonic River
Ecological Characterization
Newell Street to Woods Pond

Larval Amphibian Trapping Sites

SCALE: 1" = 3000' August 31, 1998

operated may be changed to reduce the numbers of individuals captured. At each trap location, water depth and micro-habitat information will be recorded, as will the trap entry and exit date, and time. In addition, water quality data will be collected while installing and collecting traps at one representative area within pools with water depths not greater than 1 foot, and two representative locations within pools with water depths greater than 1 foot (one location at less than 1 foot and one location greater than 1 foot deep). Information recorded will include temperature, dissolved oxygen, conductivity, and pH.

The total number of larval amphibians of each species will be recorded for each trap, and the body, tail, and total length of up to 25 individuals of each species will be recorded (Attachment 4). In addition, general notes on trap mortality, condition of larvae, associated invertebrates, and any other observations will be recorded. Malformations of amphibians, if observed, will be documented (Northern Prairie Wildlife Research Center, 1997). Aquatic macroinvertebrates from each trap will be collected and preserved in 80% ethanol for identification to lowest practical taxonomic unit.

Finally, additional physical information for each pool sampled with aquatic funnel traps will be collected. Changes in pool size over time will be described using field notes collected during different visits to each pool, and the maximum pool size will be estimated in the field using micro-topography, vegetation, and water stained leaves. The boundaries of each pool may be surveyed using GPS equipment in the summer of 1998. This may be used to estimate pool size and amend wetland mapping if necessary.

2.5 Pit Trap Arrays

Pit trap arrays, which will be used to sample herps and small mammals, will be installed in the floodplain, according to methods described by Campbell and Christman (1982), deMaynadier (1996), and Stockwell (1985). The location of each array will be based on the species targeted for capture, location and availability of habitat for the target species, and proximity to soils and sediments with and without PCB levels greater than 10 ppm. Pit trap arrays will be installed in the late summer to early fall of 1998 and may be repeated in subsequent seasons as necessary. Arrays will consist of four plastic sheeting drift fences, 7.5 m long and 0.5 m high, arranged in a plus formation with a 15 m gap in the center of the plus. At either end, and at both sides, of each drift fence, a pit trap consisting of two Number 10 tin cans taped together will be sunk flush to the ground. In the middle of each fence, and also on both sides, a single funnel trap, consisting of a 0.75 m by 0.2 m diameter tube with two entrance funnels at either end, will be placed. All pit traps will be sheltered with a cedar shingle placed approximately 0.1 m above the ground. Similarly, funnel traps will be sheltered by leaning cedar shingles against the drift fence and over the funnel trap. Finally, when not in operation, all pit traps will be covered with a tight fitting lid, and funnel traps will be made inoperable to prevent accidental captures.

Quantitative natural community data will be collected near each pit trap array to describe representative habitat where herp surveys were conducted. This information will be used when comparisons between species occurrence and abundance per area are made. Natural Community

Survey Forms (Maine Natural Areas Program, 1997) (Attachment 5) will be completed in each area to be described. Information on macro- and micro-topography, plant species composition, tree size and distribution, soils, and hydrology will be recorded. Massachusetts Division of Fisheries and Wildlife, Natural Heritage and Endangered Species Program Rare Plant Observation Forms (Attachment 4) will be completed for each rare plant observation.

Incidental mortality specimens will be collected for PCB analysis according to the methods in Attachment 6.

2.6 Data Analysis

Data collected includes visual encounter survey data, acoustic survey data, temporary and permanent pool field observation data, dipnetting data, pit-trapping data, and aquatic funnel trap survey data. Data from surveys will be summarized, and a list of species known to occur in the study area per habitat type will be prepared. Species presence data may be mapped. Narrative descriptions of when, where, and how the species were observed will be documented in a report. Further information on breeding behavior and apparent levels of success, and a discussion of natural factors believed to be influencing breeding success will be presented. These will include pool size and persistence in relation to weather. Catch per unit effort (i.e., trap night) will be reported. Due to variability, data evaluated will primarily be qualitative rather than quantitative. Recommendations for future surveys may be presented.

3.0 Quality Assurance/Quality Control

3.1 Data Quality Objectives, Indicators, and Assessment

3.1.1 Data Quality Objectives

The objectives of the study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- Occurrence data for species within the various study area habitat types—Accurate species identification is required to prepare a list of reptile and amphibian species that occur in each of the habitats within the study area.

- Taxonomic data from vernal pools—Accurate identification of larval amphibians is needed during the processing of aquatic funnel trap samples.

3.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. These data quality indicators are usually not applicable to general ecological characterization investigations.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will not be possible because specimens will not be collected. However, agreement between surveyors regarding species identification must be obtained for verification. This will occur either in the field as surveys are conducted, or in the office using photographs of individuals showing distinguishing features (e.g., size, color, cloacal swelling) Precision may also be evaluated by assessing the degree to which surveys are consistent among sites. For measurements that are not unique to herps, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy means that herps are correctly identified to species and counted.

Completeness is defined as the percentage of the planned samples actually collected and processed. For this study, completeness will be a measure of the number of sites visited and surveys completed compared to the number planned.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study will be ensured through establishment of an approved, thorough sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of the representativeness will also be evaluated through comparison with known and expected results based on previous investigations in the biophysical region.

Comparability is defined as the measure of confidence with which the data may be compared to another similar data set. Comparability will be attained through the use of sampling procedures that are commonly used by herp researchers and by careful oversight of the project during collection events. As with representativeness, the comparability of the data will also be evaluated through study of results from previous investigations in the biophysical region, if available.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to identify herps from the study area.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because collection of site soil or water samples will not occur under this SOP, any sampling will be conducted as outlined in Section 5 of the Work Plan. The usability of the information gathered during this study will be based on: 1) the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan and to accurately identify specimens collected at survey locations; and 2) an evaluation of the taxonomic data collected in the study area with respect to similar taxonomic communities along the Housatonic River and the New England area.

3.2 Sampling Design

Ecological characterization investigations, including this study, often rely on habitat-based assessment of species assemblages that are expected to occur in a given area. These studies rely on detailed habitat mapping and characterization to determine the types and conditions of habitats available to the species communities and on species' natural history, ecology, and range data to determine if a given species would be expected to occur within any of those available habitats. Field surveys for species are then used to supplement this information.

3.3 Sampling Methodology

3.3.1 Sampling Procedures

Field investigations for this study consist of the compilation of observational data to create species-habitat associations. No physical samples of site soil or water will be collected under this SOP; therefore, QA/QC procedures are not discussed here.

3.3.2 Quality Control Samples

The nature of ecological characterization studies often does not allow for the establishment of typical duplicate and blank samples, which are commonly found in studies that collect samples for chemistry analysis. This study, however, will rely on the accurate identification of species while conducting observations in the field. Therefore, a consensus on species identification between the two field investigators conducting field surveys will be required. If a consensus is not reached in the field, the sample in question will be retained in order to determine its identification in the lab using scientific species identification keys. If a consensus is still not reached, the sample in question will either be sent to a third party for identification or will not be used in the data set, particularly if identification is made difficult by physical limitations of the sample (such as a lack of the development of physical characteristics in recently hatched larvae, or decomposition of dead animals).

3.3.3 Sample Processing and Preservation

Processing and preservation of animals found dead will be in accordance with standards identified in Attachment 6.

3.3.4 Training

Field work will be directed by senior field scientists with experience in survey techniques for and identification of all life stages of reptiles and amphibians. Supporting staff will have some experience in these types of surveys but will be trained, as needed, with respect to the specific methodology and species identification.

3.4 Sample Analysis

3.4.1 Taxonomy Samples

Samples will be identified to species using available taxonomic keys for the representative species groups. A consensus on species identification between investigators will be required. If a consensus is not reached in the field, the sample in question will be retained in order to determine its identification in the lab using scientific species identification keys. If a consensus is still not reached, the sample in question will either be sent to a third party for identification or will not be used in the data set, particularly if identification is made difficult by physical limitations of the sample (such as a lack of the development of physical characteristics in recently hatched larvae, or decomposition of dead animals).

3.4.2 Physical/Chemistry Samples

Water temperature, dissolved oxygen, conductivity, and pH data will be collected in the field during aquatic funnel trapping surveys, as described in Subsection 2.4. No water samples will be collected for processing in the laboratory for this survey. Protocols for using field instruments as supplied by the manufacturer will be followed.

3.5 Data Analysis and Reporting

Information collected in this study can be used to identify species in the study area that most likely could come in contact with PCB-contaminated sediments or potential PCB-contaminated prey items, and identify species in the study area of management concern to the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife. For each state-listed species observed, Massachusetts Natural Heritage and Endangered Species Program Rare Animal Reporting Forms will be completed and submitted. No statistical analysis for this study will be conducted. However, as stated in Section 2.0, description information on species presence, habitat requirements, and ecology will be presented.

4.0 Equipment List

Equipment that will be needed as part of the field component of the study includes:

- Camera
- Binoculars
- Field notebooks
- Rubber knee and hip boots
- Heavy duty rain gear
- Eye protection
- Rubber gloves
- GPS receiver
- D-ring dipnet

- Funnel traps with small rope
- Sample jars, both chemically and non-chemically clean
- Plastic sheeting
- Hammer
- No. 10 cans with covers
- Duct tape
- Wooden shingles
- Wooden stakes
- Heavy duty stapler
- Ethanol
- Survey flagging
- Resealable bags
- Wet and dry ice
- Aluminum foil
- Coolers for shipping samples
- Conductivity, DO, pH meters
- Thermometer

5.0 Results

The information collected on herps in the study area will be presented in several formats. Maps showing the location of temporary and permanent pools will be prepared, and individual pools will be labeled for referencing data. A narrative report will be prepared explaining the methods used to collect herp data and the results of data analysis. Qualitative information concerning species presence/absence per area and habitat will be presented in narrative and tabular form.

6.0 Literature Cited

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FINAL

ATTACHMENT 1

POTENTIAL REPTILES AND AMPHIBIANS PRESENT IN THE HOUSATONIC RIVER STUDY AREA

Potential Reptiles and Amphibians Present in the Housatonic River Study Area

Common Name	Scientific Name	State-List Ranking	Federal-List Ranking	Breeding and Other Habitats	Potential Survey Method	Reference
Snapping Turtle	Chelydra s. serpentina	NL	NL	Clutch size of 20-40 eggs laid between April and Nov. in open areas with loose sand or loam. Adults prefer muddy bottoms of shallow, slow-moving fresh water.	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Stinkpot (Musk) Turtle	Sternotherus odoratus	NL	NL	Clutch size of 3-9 eggs laid in shallow nests formed by scraping vegetative matter or rotting wood from May-July. Adults prefer shallow, clear-water lakes, ponds, and rivers.	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Spotted Turtle	Clemmys guttata	SC	NL	Clutch size of 2-8 eggs are laid between May and July in flask-shaped nests that are exposed to full sunlight in well-drained areas. Adults usually found in shallow swamps, bogs, and marshes with soft substrate and some aquatic vegetation	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Bog Turtle	Clemmys muhlenbergi	E	NL	Clutch size of 1-6 eggs are laid in elevated sedge tussocks or sphagnum moss above the water line from March-June. Adults prefer spring-fed sphagnum bogs or tamarack and black spruce bogs with clear, slow-moving brooks with an organic sub	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4
Wood Turtle	Clemmys insculpta	SC	NL	Clutch size average 7-9 eggs laid in well-drained, moist sand or soil in areas of ample sunlight from May-July. Adults prefer terrestrial habitat during summer months with firm sandy or gravelly streams nearby.	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Eastern Box Turtle	Terrapene c. carolina	NL	NL	Clutch size of 3-8 eggs are laid from May to July in open, elevated patches of sand or loam. Adults very terrestrial and prefer open woodlands.	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Painted Turtle	Chrysemys picta	NL	NL	Clutch size of 5-11 eggs are laid from late May until mid-July in flask-shaped nests. Nests are located in open areas of sand or loam, usually within 200 meters of water. Adults prefer slow-moving shallow water of ponds, marshes, lakes,	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Blanding's Turtle **	Emydoidea blandingii	T	NL	Clutch size of 12-13 eggs are laid in flask-shaped nests from late May until early July in sandy soils of upland areas. Adults prefer shallow waters of marshes, ponds, bogs, and swamps with an abundance of aquatic vegetation. Basks on l	Live traps baited with tuna or catfood. Visual basking turtle surveys.	1,2,4,7
Northern Water Snake	Nerodia s. sipedon	NL	NL	Average litter size of 20-40. Young are born from August to early October. Found in virtually every swamp, marsh, or bog, of every stream, pond, or lake border within its range. Frequently found basking.	Visual survey.	1,4,6,7
Northern Brown Snake	Storeria d. dekayi	NL	NL	Litter size of 3-27 young are born late July to August. Ubiquitous, found in urban and rural areas. Prefer damp woods, swamps, bogs, and open fields. Hides under stones, banks, logs, and leaves.	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Northern Red-bellied Snake	Storeria o. occipitomaculata	NL	NL	Anywhere from 1-21 young are born in August to September. Adults prefer moist woods, hillsides, sphagnum bogs, under surface debris. Most frequently found in upland woody ridges.	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Eastern Garter Snake	Thamnophis s. sirtalis	NL	NL	Average litter size of 14 to 40 are born from July to early September. A common, ubiquitous snake found in moist areas, forest edges, stream edges, bogs, and swamps.	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Eastern Ribbon Snake	Thamnophis s. sauritus	NL	NL	An average of 10 young are born from late July to September. Adults are semiaquatic, inhabiting stream edges, swampy areas, wet meadows, ponds, and bogs. Also found in damp or wet deciduous forests. Seldom far from cover.	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Eastern Hognose Snake	Heterodon platyrhinos	NL	NL	Average clutch size of 22 eggs are laid in June to July with most clutches hatching in August. A locally common snake that prefers areas where sandy soils predominate, such as beaches, open fields, and dry, open pine or deciduous forests.	Placement of boards in typical basking snake areas - visual survey.	1,4,6
Northern Ringneck Snake	Diadophis punctatus edwardsi	NL	NL	An average of 3-4 eggs are laid in late June to early July, with young hatching in late August through September. A common, secretive snake that prefers cover in moist, shady woodlands, rocks, stone walls, logs, and old woodland junk pile	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Northern Black Racer	Coluber c. constrictor	NL	NL	An average of 16 or 17 eggs hatch late August to September. Habitat preference includes moist or dry areas, forests and wooded areas, fields, swamps and marshes. Partially aboreal. Basks on ledges.	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7

Potential Reptiles and Amphibians Present in the Housatonic River Study Area

Common Name	Scientific Name	State-List Ranking	Federal-List Ranking	Breeding and Other Habitats	Potential Survey Method	Reference
Eastern Smooth Green Snake	Opheodrys v. vernalis	NL	NL	A clutch size of 3-12 eggs hatch from August to early September. Can be found in upland areas, grassy fields, mountain meadows, and open spots. Also found in sphagnum bogs, marshes, and in vines and brambles.	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Black Rat Snake	Elaphe o. obsoleta	E	NL	Clutch size of 6-24 eggs are laid in loose soil, decaying wood, or sawdust piles in July or August. This snake has a variety of habitats including woodlands, thickets, field edges, rocky hillsides, and river bottoms. Readily climbs trees	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Eastern Milk Snake	Lampropeltis t. triangulum	NL	NL	Average clutch size of 13 eggs hatch from late August to October from under soil piles, sawdust or manure, and other cover. Prefers habitats with brushy or woody cover, and found from sea level to mountain elevations. Found in woods, mea	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Northern Copperhead	Agkistrodon contortrix	E	NL	Typically 5 to 6 live young are born in September. An uncommon snake usually associated with deciduous forests. Prefers areas with damp leaf litter. Also found in habitats with large rocks, talus slopes, ledges, and open woods.	Placement of boards in typical basking snake areas - visual survey.	1,4,6
Timber Rattlesnake	Crotalus horridus	E	NL	An average of 5 to 17 young are born from late August to September. Uncommon to rare, these snakes are found in timbered areas with rocky outcroppings, and second growth coniferous and deciduous forests with high rodent populations. Usua	Placement of boards in typical basking snake areas - visual survey.	1,4,6,7
Jefferson Salamander	Ambystoma jeffersonianum	SC	NL	Eggs are laid in March or early April in small, cylindrical masses, averaging 16, and are found attached to slender twigs below the surface of quiet pools and ponds. Adults are found in mixed and deciduous woods with swamps, pools, and s	Placement of drift fences and pit traps.	1,3,4
Blue-spotted Salamander	Ambystoma laterale	SC	NL	300-500 eggs are laid on the bottoms of temporary shallow forest ponds, drainage ditches, and kettle holes, attached to litter or bottom detritus. Adults found in wooded, swampy or moist areas. Found under logs and other forest debris.	Placement of drift fences and pit traps.	1,3,4,7
Spotted Salamander	Ambystoma maculatum	NL	NL	An average of 100-150 eggs are deposited in large, compact masses attached to submerged objects. Average number of eggs deposited is 125. Adults are most abundant in deciduous and mixed forests where ponds, slow streams, or vernal poo	Placement of drift fences and pit traps.	1,3,4,7
Marbled Salamander	Ambystoma opacum	T	NL	Eggs are deposited in the fall in small depressions on the ground beneath surface materials, old bark, logs, moss. 50 to 200 eggs are laid singularly. Adults prefer dry, sandy or gravelly areas bordering ponds and slow streams.	Placement of drift fences and pit traps.	1,3,4
Northern Dusky Salamander	Desmognathus f. fuscus	NL	NL	10-30 eggs are deposited in small, compact clusters of 12 to 26 from June until August beneath logs, stones, or bark in the vicinity of water. Adults frequent margins of streams and springs, springy banks where soil is constantly moist.	Placement of drift fences and pit traps.	1,3,4,7
Red-backed Salamander	Plethodon cinereus	NL	NL	4-17 eggs are deposited in June and July in small clusters of 8 to 10. The eggs are found suspended to the nest cavity roof of well rotted logs. Adults are found beneath old logs, bark, moss, stones, and leaf mold in evergreen, mixed, or	Placement of drift fences and pit traps.	1,3,4,7
Four-toed Salamander	Hemidactylium scutatum	SC	NL	Approximately 19 to 50 eggs are deposited in close proximity to water, usually in cavities in sphagnum moss or among the roots of grass. Approximately 30 eggs are laid singly , not aggregated. Adults are generally terrestrial, inhabiting	Placement of drift fences and pit traps.	1,3,4,7
Northern Spring Salamander	Gyrinophilus p. porphyriticus	SC	NL	An average of 40-60 eggs are laid in running water under logs and stones, usually in groups. Uncommon to rare, adults may be found in forested areas with clear, cold water, springs, mountain streams, creeks, and boggy areas. Also in depr	Placement of drift fences and pit traps.	1,3,4,7
Two-lined Salamander	Eurycea b. bislineata	NL	NL	12-36 eggs are laid singly, attached to the lower surface of a support in running water. Adults prefer to hide under stones and logs in well saturated soils of brooksides.	Placement of drift fences and pit traps.	1,3,4,7
Red-spotted Newt	Notophthalmus v. viridescens	NL	NL	80-450 eggs are laid in water, attached singly to the leaves of aquatic plants. Adults are found in ponds, particularly water with abundant submerged vegetation, and in weedy areas of lakes, marshes, backwaters, and pools of shallow slow-	Placement of drift fences and pit traps.	1,3,4,7
Eastern American Toad	Bufo a. americanus	NL	NL	An average of 3200 eggs are laid in long curling strings amidst aquatic vegetation. Common, found in almost any habitat: moist upland woods, gardens, areas with cover from sea level to mountainous elevations.	Placement of drift fences and pit traps.	4,5,6,7
Fowler's Toad	Bufo woodhouseii fowleri	NL	NL	Up to 8,000 eggs are laid in long strings in aquatic vegetation. Adults prefer areas with sandy soil such as shorelines, river valleys, beaches, and roadside areas. Usually found in lowland areas, but frequently in pine and oak forests.	Placement of drift fences and pit traps.	4,5,6
Northern Spring Peeper	Hyla c. crucifer	NL	NL	As many as 900 eggs are laid singly near the bottom of shallow weedy ponds, attached to submerged plants. Adults are common to abundant and are found in marshy or wet woods, sphagnum bogs, second growth woodlots, near ponds and swamps.	Placement of drift fences and pit traps.	4,5,6,7

Potential Reptiles and Amphibians Present in the Housatonic River Study Area

Common Name	Scientific Name	State-List Ranking	Federal-List Ranking	Breeding and Other Habitats	Potential Survey Method	References
Gray Tree Frog	Hyla versicolor	NL	NL	Up to 2000 eggs are laid loosely attached to vegetation on the surface of shallow water. Adults found in forested regions with small trees, shrubs, and bushes near or in shallow water. Commonly inhabit moist areas in hollow trees, under	Placement of drift fences and pit traps.	4,5,6,7
Pickerel Frog	Rana palustris	NL	NL	2,000 to 3,000 eggs are laid in firm globular masses attached to submerged plants and branches. Adult habitat includes colder waters of lakes, ponds, clear streams, sphagnum bogs. In MA, fairly ubiquitous along streams and shores of perm	Placement of drift fences and pit traps.	4,5,6,7
Northern Leopard Frog	Rana pipiens	NL	NL	2,000-4,000 eggs are laid in masses in shallow water, sometimes attached to twigs. Adults commonly found in wet open meadows and fields, and wet woods during summer months.	Placement of drift fences and pit traps.	4,5,6,7
Wood Frog	Rana sylvatica	NL	NL	An average of 1750 eggs are laid attached to submerged twigs or free on the bottom in globular masses. Adults prefer temporary woodland pools and backwaters of slow-moving streams. A common and terrestrial frog, often far from water duri	Placement of drift fences and pit traps.	4,5,6,7
Green Frog	Rana clamitans melonata	NL	NL	1,500 to 5,000 eggs are deposited in floating masses of jelly attached to underwater twigs and stems in permanent water. Adults are riparian, inhabiting margins of shallow permanent or semipermanent fresh water, shores and banks of lakes	Placement of drift fences and pit traps.	4,5,6,7
Bullfrog	Rana catesbeiana	NL	NL	12,000 to20,000 eggs are laid in floating films of jelly in water of lakes, quiet streams, and ponds. Adults are found near deep permanent water with emergent vegetation. Highly aquatic, will occupy floating logs far from shore.	Placement of drift fences and pit traps.	4,5,6,7

Notes:
** Blanding's Turtle found in limited scattered pockets in New England.
NL = No Listing
SC = Special Concern
E = Endangered
T = Threatened
MA = Massachusetts

References:

1 = A Field Guide to Reptiles and Amphibians of Eastern and Central North America, Third Edition, R. Conant and J. Collins - 1991.
2 = Turtles of the United States and Canada, C. Ernst, R. Barbour, and J. Lovich - 1994.
3 = Handbook of Salamanders, S. Bishop - 1994.
4 = National Heritage Program of MA.
5 = MA Audubon Society - Amphibians and Reptiles List, Lincoln, MA.
6 = New England Wildlife: Habitat, Natural History, and Distribution, R. DeGraaf and D. Rudis, 1992.
7 = The Amphibians and Reptiles of Maine, July 1992.

ATTACHMENT 2

RARE ANIMAL AND RARE PLANT OBSERVATION FORMS
PART I - RECONNAISSANCE

Commonwealth of Massachusetts

Division of Fisheries & Wildlife

Wayne F. MacCallum, *Director*

NOTICE TO SCIENTIFIC COLLECTING PERMITTEES

Enclosed are copies of rare animal/plant observation forms. These forms are to be completed for any state-listed rare species encountered while working under the authority of a scientific collecting, salvage or bird banding permit. The completed forms are then to be submitted with your annual report at the end of the year. Please make additional copies if needed, or call the permit office at (617) 727-3151. Thank you for your cooperation.

Division of Fisheries & Wildlife
Leverett Saltonstall Building,

Natural Heritage
and Endangered Species
Program

1995

RARE ANIMAL OBSERVATION FORM

Observer's name: _____ Species observed:

Address: _____ _____

_____ Date and Time of observation:

Phone: _____ (H) _____ (W) _____

1. Location where species was observed:

a) Town: _____ County: _____ USGS Topo: _____

b) Please attach a photo copy of the appropriate section of a USGS topo map (or similar map if a topo map is unavailable). Please carefully mark the site in red where you observed this rare species.

c) Please explain in writing how to get to this spot: _____

_____

_____

_____

_____

2. Number of animals observed: _____

3. Was a positive ID possible? _____ Based on what field marks? _____

_____

Were photographs or slides taken? _____
If yes, please submit one clear photograph or slide of the animal.

4. Age and sex of animals observed: _____

_____

5. Evidence (if any) of breeding activity at this site: _____

_____

_____

(Over)

Commonwealth of Massachusetts

Division of Fisheries & Wildlife Route 135, Westborough, MA 01581 (508) 792-7270 ext. 200

6. Have you observed this species at this site in previous years? _____

   If yes, please give details: _____

   _____

7. Description of habitat at this site: _____

   _____

   _____

8. Observed or potential threats to the species or its habitat at this site:

   _____

   _____

9. Landowner's name and address, if known: _____

   _____

10. Additional observations/comments: _____

    _____

    _____

11. Qualifications of observer (check all that apply):
    _____ Amateur naturalist. Years of experience: _____
    _____ Conservation Commission member
    _____ Biology/science teacher
    _____ Environmental Consultant
    _____ Degree in Biology: ___ Bachelor's ___ Master's ___ Ph.D.
    _____ Other. Please specify: _____

12. Briefly explain your previous field experience with this species: _____

    _____

13. List names and qualifications of other observers (if any): _____

    _____

14. I hereby certify under pains and penalities of perjury that the information contained in this report is true and complete to the best of my knowledge.

    Signature _____ Date: _____

Please submit field forms and all supporting documentation (USGS map, photo, etc.) to:

Natural Heritage and Endangered Species Program
MA Division of Fisheries & Wildlife
Route 135
Westborough, MA 01581
(508) 792-7270 ext. 200

Natural Heritage & Endangered Species Program

Please submit field forms, a copy of a USGS map, and all supporting documentation to the State Botanist at:
Massachusetts Natural Heritage
and Endangered Species Program
Division of Fisheries & Wildlife
Route 135
Westborough, MA 01581
(508) 792-7270 ext. 200

RARE PLANT OBSERVATION FORM

Species Scientific Name:_____

NHESP Element Occurrence Number (if known):_____

Observation Date(s):_____

USGS Quad Name and Series (7.5"x7.5" or 7.5"x15"):_____

County:_____ Town:_____

Directions to location of observations (please attach USGS map):_____

Habitat: (Describe plant communities, associated vegetation, physical characteristics, geological features and surrounding land use. Are other rare species present?) _____

Areal Distribution, Numbers, Phenology

Population found:_____ No. of mature plants:_____
not found:_____ No. of immature plants:_____
Presumed extirpated:_____ No. of plants age unknown:_____
(give reasons under "Comments")

Population area:_____

Percent of population in leaf:_____ bud:_____ flower:_____
with immature fruit:_____ mature fruit:_____ senescent:_____

Observations of floral visitors, fruit dispersers:_____

Vigor of individuals and population:_____

Evidence of expansion or decline:_____

Have you observed this species at this site in previous years? Please give details:_____

Site Physiography

Elevation:_____

Topographic position	Aspect	Slope	Light	Moisture
crest_____	N___	0-20____	open____	inundated ____
upper slope_____	E___	20-45____	filtered____	wet ____
mid slope_____	S___	45-75____	shade____	mesic____
lower slope_____	W___	vert.____		dry ____
bottom_____	Flat___			xeric ____

Soil types:_____

Surficial rock types (with percent of ground covered):_____

Bedrock/parent material:_____

Surficial water (distance away):_____Source:_____

Management / Ownership

NHESP Site Name (if any):_____ Managed Area:_____

Comments/Management recommendations: _____

_____

_____

Disturbance or threats (natural or unnatural) to population:_____

_____

Owner's Name:_____ Telephone: (___)_____

Address:_____

Owner Comments:_____

Observer Information

Observer's Name:_____ Telephone: (___)_____

Address:_____

Plant ID by:_____

Specimen # & Herbarium:_____

Element Occurrence (EO) Summary (Circle one choice in each category)

EO Quality: (How representative is this occurrence? Consider the size and productivity of the population and the vitality and vigor of the individuals.)

A - Excellent B - Good C - Marginal D - Poor

Comments:_____

EO Condition: (Is the habitat supporting the EO pristine or degraded? Is there a potential for the habitat to recover from disturbances? A - Excellent B - Good C - Marginal D - Poor

Comments:_____

EO Viability: (What are the long-term prospects for continued existence of this occurrence at the indicated level of quality?) A - Excellent B - Good C - Marginal D - Poor

Comments:_____

EO Defensibility: (Can this occurrence be protected from extrinsic human factors?)

A - Excellent B - Good C - Marginal D - Poor

Comments:_____

EO RANK: (A summary of all factors listed above.) A - Excellent B - Good C - Marginal D - Poor

Comments:_____

Signature:_____ Date:_____

ATTACHMENT 3

VERNAL POOL OBSERVATION FORMS

Vernal Pool Field Observation Forms

IMPORTANT: This form must be signed on the back prior to submittal

Observer information: Name:_____

Address:_____

_____

Telephone:_____

Submit completed forms to:
Vernal Pools
MNH&ESP
Route 135
Westboro, MA 01581

* REQUIRED *

- ▸Photographs of the pool and of species observed must be submitted for certification
- ▸USGS Topographic map, Town Assessor's map

Refer to the "Guidelines for Certification of Vernal Pool Habitat" (1988) for required documentation for certification of vernal pool habitat

---

Mapping Sources Submitted: (Check all submitted)

REQUIRED	At Least ONE Additional
_____USGS	_____Metes and Bounds (see "Guidelines")
_____Assessor's	_____Professional Survey
	_____Aerial Photograph
	_____Other:_____

YES / NO Were any rare state-listed species observed utilizing this pool?

YES / NO Is documentation of this/these species included with this filing?

Are fish present?

YES / NO Pool observed dry (date)_____

YES / NO Obligate vernal-pool breeding species observed

Other evidence:

Vernal Pool Data

County:_____ Town:_____

USGS Quadrangle Name:_____ Series: 7.5' X 7.5' 7.5' X 15' (Circle one)

Written Directions to pool:_____

_____

_____

_____

Observation Dates: Last date biota observed:_____

Last date pool observed (if different):_____

First date biota/pool observed (if different):_____

Observations:
Obligate Species BF-4

	Wood Frog	Spotted Salamander	Blue-spotted Salamander	Jefferson's Salamander
Date, Evidence Code				
	Marbled Salamander	Unidentified Mole Sal.	Spadefoot Toad	Fairy Shrimp
Date, Evidence Code				

Codes
1. Breeding chorus
2. Mated pairs
3. Courting adults
4. Spermatophores
5. Egg masses
6. Frog tadpoles
7. Salamander larvae
8. Transforming juveniles

Facultative Species (See Guidelines)

Species	Date	Observations
1.		
2.		
3.		
4.		

Attach extra sheets if needed

General Description:

Wetlands Resource Area Type: _____ILSF _____BLSF _____BVW _____Upland _____Unknown
_____10 YR FLOOD PLAIN _____100 YR FLOOD PLAIN

Is there an inlet or outlet to the pool? Inlet: Y / N Outlet: Y / N
Is the inlet/outlet permanent? Inlet: Y / N Outlet: Y / N

Vegetation: Tree canopy closure over pool (circle one): Open Complete Partial _____%

Dominent vegetation *type* within pool:_____
Approximate percent cover:_____

Dominent vegetation *type* within 100 feet of pool:_____
Approximate percent cover:_____

Mosses
Submergents
Emergents (grasses, sedges, rushes)
Shrubs
Forested

Pool Bottom / Soil Type: (Circle all that apply)

PEAT LEAF LITTER MUD/MUCK SAND GRAVEL COBBLES BOULDERS/BEDROCK

Size of pool: Length:_____ Width:_____

Area:_____

Depth (Ave.):_____

Property Owner(s): Name_____

Address:_____

I hereby certify under pains and penalties of perjury that the information contained in this report is true and complete to the best of my knowledge.

Signature _____ Date _____

FINAL

ATTACHMENT 4

AQUATIC FUNNEL TRAP DATA SHEET

AQUATIC FUNNEL TRAP DATA SHEET

Pool No.: _____ AFT No.: _____ Start Date: _____ End Date: _____
Start Time: _____ End Time: _____

Water Depth: _____
Microhabitat: _____

Weather: In: _____
Out: _____

Pool Description/Characteristics: _____

	IN		OUT	
	<1'	>1'	<1'	>1'
Temperature:				
Conductivity:				
Dis. Oxygen:				
pH:				

RESULTS					
Species	Total Number	Body	Tail	Total	Notes

Record the total number of individuals for each species. For each species record the body and total lengths of 25 individuals (if greater than 25 occur).

FINAL

ATTACHMENT 5

NATURAL COMMUNITY SURVEY FORMS

INSTRUCTIONS
NATURAL COMMUNITY SURVEY FORM

Maine Natural Areas Program

3 June 1997 draft

For the past several years the Maine Natural Areas Program has been trying to improve the quality and quantity of data on natural community occurrences in Maine. This form is the latest iteration in our attempts to standardize data collection for unusual occurrences of natural communities and move towards quantitative documentation.

When to use this form: Fill out at least the first part of the natural community survey ("Reconnaissance") any time you're surveying an area with any natural attributes. (The natural community survey supplements but does not replace the Site Survey Summary. For every site you visit, even ones with no unusual natural features, you fill out a Site Survey Summary.) For example, if you were checking an area which turned out to have been clearcut a few months ago, you would just fill out the Site Survey Summary. But if you were checking an area which had 50-year old pine forest, a red maple swamp, and an oak-pine woodland, you'd fill out the community reconnaissance form to generally describe each community type. The reconnaissance is useful to keep track of the different vegetation types you encounter in an area, even if none of them turn out to be particularly unusual.

How this form is structured: The Natural Community Survey has three parts.
Part I is the Reconnaissance, which serves as an overview of all of the natural communities or vegetation types in your survey area. What communities are present, and where are they in relation to changes in topography? What are the community boundaries? Even if this is the only part of the community form that you complete (e.g. if the area has nothing unusual), it will serve as a record of the visit and provide some community information, but probably will not be mapped or entered into the database.
Part II is the Description of a particular natural community. This is to be completed for all unusual natural community occurrences. An unusual natural community can either be an occurrence of a highly-ranked type (S1, S2, or S3, see the MNAP's natural community classification) or an outstanding example of a more common community type. Part II contains all the basic information fields needed for minimum documentation of community occurrences. It combines environmental descriptors for the community with vegetation data collected from nested plots arrayed on one or more transects through the community. Note that if your reconnaissance of an area turns up three unusual natural communities (say a pristine red maple swamp [!], a pitch pine - scrub oak barren, and a high-quality sedge meadow), you will fill out three Part IIs-- one for each community occurrence in that area. If your reconnaissance turns up only marginal red maple swamp and typical 80-year-old mixed forest, you won't fill out any Part IIs (unless you have lots of time on your hands and want to help us supplement our data on lower-quality occurrences...).
Part III is the Summary Notes. This can apply to one or more of the communities you've described, at your discretion. You can fill out one Part III to apply to all of the natural communties in the area, or fill out separate ones for each community type; whichever seems to make the most sense for that particular site. Like Part II, fill this out only if you encounter unusual natural communities.

After you've filled out the various forms for an area, staple them all together along with a copy of the topo map showing the area you surveyed, the location of your observation points (from the reconnaissance page), and the approximate boundaries of any unusual natural communities, as well as the location of any rare species.

Part 1: Reconnaissance

Note: This form has boxes separating each data item. It's designed to cue you to **put something in each of the boxes,** except those with their names in parentheses (these are to be filled in by MNAP staff when logging in the data). If a box does not apply, note that; to us in the office, blank boxes can mean "don't know", "didn't pay attention to this", or "not applicable here", all of which have very different meanings.

IDENTIFIERS/LOCATION:

Survey Area - provisional name assigned by field worker; should represent an identifiable feature on topographic map. If you're dealing with a specific portion of a larger identifiable area, note both: "Pemetic Mountain, Acadia National Park."

Date - date of the fieldwork.

(Site Name) - "Official" name; field workers ignore.

Surveyors - your name(s).

Town and **County**

USGS Quad - the name of survey map used. Assumed to be 7.5' (1:24000) unless you note otherwise.

(Quadcode:) Field workers ignore.

Airphoto: - type, scale, date, and source of imagery used (e.g. airphoto, 1:20K, 1980, MGS).

Directions - precise directions in words; or if the topo or Maine Atlas shows directions clearly, attach copy with route of access marked. Access notes can be extremely important and are often not apparent simply from a topographic map.

VEGETATION/HABITAT:

Community type - appropriate name from the MNAP natural community classification; or assign provisional name of your own. (Also O.K. to leave this blank– MNAP staff can fill in later– as long as your other information is sufficient!)

Soil - describe texture, moisture regime, and origin, as appropriate. Give soil series, if known.

Slope, aspect, topography - describe.

Strata (Dominant Species & Total Cover) - Enter the total coverage (estimated %) of a particular layer (stratum), and 1 or 2 dominant species (i.e. those with greatest coverage or abundance). Strata are defined by a combination of dominant plant type and height; for example, the herb layer includes all vascular plants less than 1 m tall, as well as any herbs more than 1 m tall.
The strata are defined as follows:

TREE = canopy (if emergents present, note as "E");
SAPLING / TALL SHRUB = > 2 m: woody plants not forming tree canopy but > 2 m tall;
SHRUB = 1 - 2 m: woody plants 1 - 2 m tall;
HERB = < 1 m: all herbaceous vascular plants plus any woody plants < 1 m tall;
BRYOID = all ground-layer non-vascular plants.

Condition - How much human impact is apparent? Is there evidence of natural disturbance that's notable? This is a very important bit of information for us as we interpret your reconnaissance forays.

Additional data - indicate if plots were sampled for this community or if reconnaissance only.

Part II: Description

Note: Again, this form has boxes separating each data item. It's designed to cue you to put **something in each of the boxes,** except those with their names in parentheses (these are to be filled in by MNAP staff when logging in the data).

IDENTIFIERS/LOCATION

Area - same as on Reconnaissance part.

Date - date of the fieldwork.

Observation Pt. # - Essential cross reference to your reconnaissance page.

Adjacent communities - list communities bordering this one (helps understand community transitions on the landscape.)

Community type - from your reconnaissance page.

(Lat.) - field workers ignore.
(Long.) - field workers ignore.

CLASSIFICATION HIERARCHY

This is used to cross-reference to TNC's national vegetation classification, ordered by physiognomy, phenology, and leaf type. This can be left blank IF it's obvious from the accompanying data.

Physiognomy

forest (trees forming 60-100% cover, generally > 5 m tall)
woodland (open stands of trees, 25-60% canopy cover; generally > 5 m tall)
shrubland (shrubs or small trees, usually 1-5 m tall, with > 25% cover; trees at < 10% cover
dwarf shrubland (shrubs and dwarf trees < 1 m tall, usually < 0.5 m tall, with > 25% cover; any taller strata have < 10% cover)
herbaceous (graminoids &/or forbs forming > 10% cover; any taller strata have < 10% cover)

sparse vascular / non-vascular (each vascular layer is < 10%; non-vascular vegetation anywhere from absent to continuous).

Phenology– what best describes the leaf form of the uppermost stratum (ignoring those with < 10% cover):

evergreen (> 75% of the total woody cover)
deciduous (>75% of the total woody cover)
mixed (evergreen and deciduous species each contribute 25% - 70% of the total woody cover)
perennial (herbaceous vegetation with > 50% cover of perennial spp.)
annual (herbaceous vegetation with > 50% cover of annuals).

Leaf type– applies to the uppermost stratum, as above:

broad-leaf woody
needle-leaf woody
graminoid
forb
pteridophyte
non-vascular

Alliance - field workers ignore.

ADDITIONAL DATA FOR FORESTS

Tree canopy height - measure with a clinometer.

Supercanopy trees? - indicate species, if present, e.g. "few, white pine 80-100 cm dbh"

Core data - list cores with an identifier so that person who later counts the rings can enter data here.

Deadwood - characterize, especially downed wood (standing dead will be counted in plots).

HISTORY

Fire, e.g. "charcoal bbs common in soil, no fire scars above ground"
Wind, e.g. "some pine with broken tops"
Cutting, e.g. "few stumps, mostly rotted"
Agriculture, e.g. "stone fences present"
Impoundment, e.g. "water level maintained by dam at bridge"

This information is really important to interpreting the field data. If a box does not apply, please mark it out or put n/a– do not leave it blank.

ADDITIONAL SPECIES LIST

List additional plant species - Species recorded in the plot data should capture the overall character of the community, but there will always be plant species that don't occur in your quadrats; these should be listed here. This will give us a more-or-less complete species list, another useful interpretive and comparative tool.

Species list sketchy? - Note whether your species list covers just the dominants or is basically complete. This will be important in interpreting your data. Note if particular groups are missing

because of season or your level of expertise (e.g., "most composites not yet identifiable" or "not confident of grass i.d.s").

VEGETATION PLOT DATA

Once you have a sense of the area, decide on a transect direction and put in 5 - 10 plots spread through the community. Heterogeneous communities will require more plots than uniform ones. [Add sampling specification details here, duplicating field sheet.]

TOPOGRAPHY & SOILS

Elevation - note whether in feet or in meters.

Aspect - Please record as 0-360°; give range if appropriate. Note magnetic or true reading.

Slope - Notice this is in percent. A 45° slope = 100% slope.

Microtopography - hummocks and hollows, etc.

Habitat patchiness - describe pattern or patches. Are they substrate related?

Topographic Position - circle most appropriate descriptor.

pH - record substrate pH and note how it was obtained,

Soil Profile Description - sketch the soil profile representative of the community. If you don't have a complete soil profile, describe as deep as you dug or probed. For mineral soils, give depth, color, presence of mottling, etc. of each horizon as appropriate. For organic soils, indicate peat depth if it is less than 1 m, or just check off the "> 1 m" blank if appropriate. Indicate degree of decomposition using the Von Post scale (see Appendix 2). Note depth to water table and depth to obstruction, if those can be determined. If possible, take a soil temperature reading at about 10 cm depth.

Surficial deposit - circle the appropriate descriptor, or more than one if surficial geology is unknown/unclear.

Surface - percentage of surface covered by each category. What would you see if you took all of the vegetation away (a geologist's dream!)?

Average texture - These are general soil texture classes. The attached "Simplified Key to Soil Texture" (Appendix 1) may be helpful.

Bedrock type - name, if known; or check off the closest type of those listed. Indicate whether you confirmed the type in the field, or if this was taken off of the bedrock map.

Igneous Rocks

- Granitic (Granite, Schyolite, Syenite, Trachyte)
- Dioritic (Diorite, Dacite, Andesite)
- Gabbroic (Gabbro, Basalt, Pyroxenite, Peridotite)

Sedimentary Rocks	Metamorphic Rocks
Limestone (and Dolomite)	Gneiss
Sandstone	Schist
Siltstone	Slate / Phyllite
Shale	Marble
Marl	Serpentine

Soil stoniness - average stoniness of soil or deposit up to 1 m in depth.

Drainage & moisture regime - These classes indicate the amount of moisture available to plants, and are defined in terms of (1) actual moisture content (in excess of field capacity), (2) the extent of the period during which excess water is present in the plant-root zone, and (3) soil structure/texture. Permeability, level of groundwater, and seepage are factors affecting moisture status, but because these may not be directly observed or measured in the field, they are limited as criteria of moisture status. Soil profile morphology, for example mottling, normally reflects soil moisture status (indirectly), but because it does not always do so, it should not be the overriding criterion. Topographic position and vegetation as well as mottling or other morphological characteristics are useful field criteria for assessing soil moisture status. The key provided is from the Maine Association of Professional Soil Scientists.
If soils are strongly influenced by seepage waters, please note.

Hydrologic regime - circle appropriate descriptor.

PART III. Summary Notes

This should be self-explanatory.

APPENDIX 1. MINERAL SOIL TEXTURE KEY

Simplified Key to Texture

A1	Soil does not remain in a ball when squeezed	**sand**
A2	Soil remains in a ball when squeezed	B
B1	Squeeze the ball between your thumb and forefinger, attempting to make a ribbon that you push up over your finger. Soil makes no ribbon	**loamy sand**
B2	Soil makes a ribbon; may be very short	C
C1	Ribbon extends less than 1 inch before breaking	D
C2	Ribbon extends 1 inch or more before breaking	E
D1	Add excess water to small amount of soil; soil feels at least slightly gritty	**loam or sandy loam**
D2	Soil feels smooth	**silt loam**
E1	Soil makes a ribbon that breaks when 1-2 inches long; cracks if bent into a ring	F
E2	Soil makes a ribbon 2+ inches long; doesn't crack when bent into a ring	G
F1	Add excess water to small amount of soil; soil feels at least slightly gritty	**sandy clay loam or clay loam**
F2	Soil feels smooth	**silty clay loam or silt**
G1	Add excess water to a small amount of soil; soil feels at least slightly gritty	**sandy clay or clay**
G2	Soil feels smooth	**silty clay**

APPENDIX 2. VON POST SCALE OF PEAT DECOMPOSITION

H1: Completely undecomposed peat; only clear water can be squeezed out.

H2: Almost undecomposed and mud-free peat; water that is squeezed out is almost clear and colorless.

H3: Very little decomposed and very slightly muddy peat; when squeezed water is obviously muddy but no peat passes through fingers. Residue retains structure of peat.

H4: Poorly decomposed and somewhat muddy peat; when squeezed, water is muddy. Residue muddy but it clearly shows growth structure of peat.

H5: Somewhat decomposed, rather muddy peat; growth structure visible but somewhat indistinct; when squeezed some peat passes through fingers but mostly very muddy water. Press residue muddy.

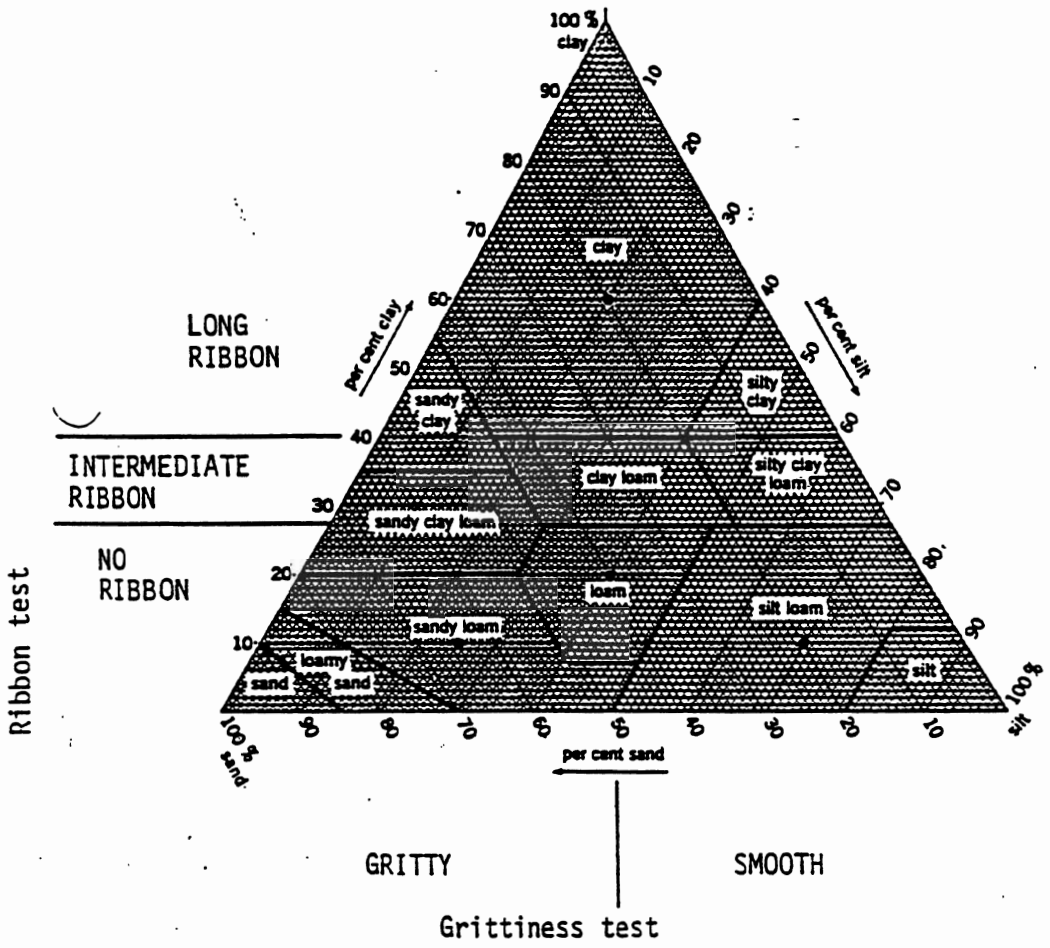
H6: Somewhat decomposed, rather muddy peat; growth structure indistinct; less than 1/2 of peat passes through fingers when squeezed. Residue very muddy, but growth structure more obvious than in unpressed peat.

H7: Rather well-decomposed, very muddy peat; growth structure visible, about 1/2 of peat squeezed through fingers. If water is squeezed out, it is porridge-like.

H8: Well-decomposed peat; growth structure very indistinct; about 2/3 of peat passes through fingers when pressed, and sometimes a somewhat porridge-like liquid. Residue consist mainly of roots and resistant fibers.

H9: Almost completely decomposed and mud-like peat; almost no growth structure visible. Almost all peat passes through fingers as a homogeneous porridge if pressed.

H10: Completely decomposed and muddy peat; no growth structure visible; entire peat mass can be squeezed through fingers.



Dots shown represent (by percent):

Sand	Silt	Clay
20	70	10
20	20	60
65	25	10
40	40	20

Figure 25. Soil texture classification (after Donahue et al. 1971). Interpretation for any point in the graph is read as follows: % Clay, move horizontally to the left; % Silt, move parallel to the Clay Axis, up and to the right; % Sand, move parallel to the Silt Axis, down and to the right.

NATURAL COMMUNITY SURVEY PART I: RECONNAISSANCE

Maine Natural Areas Program

IDENTIFIERS / LOCATION

Survey area:			Date:
(Site name:)		(Quadcode:)	Airphoto (#, scale, date):
Surveyors:	Town: County: (Biophysical Region:)	USGS 7.5' Quad:	

Mark all observation points on a copy of the topo. Add any comments or sketches here if necessary to clarify the topo.	Directions (if not obvious from topo or Maine Atlas):

VEGETATION / HABITAT

Observation Point 1	Observation Point 2	Observation Point 3
Community type:	Community type:	Community type:
Soil:	Soil:	Soil:
Slope, aspect, topography	Slope, aspect, topography:	Slope, aspect, topography:
STRATA: cover & 1-2 dominant spp. for each	STRATA: cover & 1-2 dominant spp. for each	STRATA: cover & 1-2 dominant spp. for each
Tree layer: Total cover (%):_____	Tree layer: Total cover (%):_____	Tree layer: Total cover (%):_____
Sapling / tall shrub layer: Total cover (%)_____	Sapling / tall shrub layer: Total cover (%)_____	Sapling / tall shrub layer: Total cover (%)_____
Shrub (1-2 m) layer: Total cover (%)_____	Shrub (1-2 m) layer: Total cover (%)_____	Shrub (1-2 m) layer: Total cover (%)_____
Herb layer: Total cover (%)_____	Herb layer: Total cover (%)_____	Herb layer: Total cover (%)_____
Bryoid layer: Total cover (%)_____	Bryoid layer: Total cover (%)_____	Bryoid layer: Total cover (%)_____
Other diagnostic or notable species:	Other diagnostic or notable species:	Other diagnostic or notable species:
Condition / evidence of human use:	Condition / evidence of human use:	Condition / evidence of human use:
Additional data collected / COMMENTS plots (size)? tree cores? photos?	Additional data collected / COMMENTS plots (size)? tree cores? photos?	Additional data collected / COMMENTS plots (size)? tree cores? photos?

Observation Point 4	Observation Point 5	Observation Point 6
Community. type:	Community type:	Community type:
Soil:	Soil:	Soil:
Slope, aspect, topography:	Slope, aspect, topography:	Slope, aspect, topography:
STRATA*: cover & 1-2 dominant spp. for each	STRATA*: cover & 1-2 dominant spp. for each	STRATA*: cover & 1-2 dominant spp. for each
Tree layer: Total cover (%):_____	Tree layer: Total cover (%):_____	Tree layer: Total cover (%):_____
Sapling / tall shrub layer: Total cover (%)_____	Sapling / tall shrub layer: Total cover (%)_____	Sapling / tall shrub layer: Total cover (%)_____
Shrub (1-2 m) layer: Total cover (%)_____	Shrub (1-2 m) layer: Total cover (%)_____	Shrub (1-2 m) layer: Total cover (%)_____
Herb layer: Total cover (%)_____	Herb layer: Total cover (%)_____	Herb layer: Total cover (%)_____
Bryoid layer: Total cover (%)_____	Bryoid layer: Total cover (%)_____	Bryoid layer: Total cover (%)_____
Other diagnostic or notable species:	Other diagnostic or notable species:	Other diagnostic or notable species:
Condition / evidence of human use:	Condition / evidence of human use:	Condition / evidence of human use:
Additional data collected / COMMENTS plots (size)? tree cores? photos?	Additional data collected / COMMENTS plots (size)? tree cores? photos?	Additional data collected / COMMENTS plots (size)? tree cores? photos?

* STRATA are defined as:

TREE = canopy (if emergents present, note as "E");
SAPLING / TALL SHRUB = > 2 m tall and < 5 cm dbh: woody plants not forming tree canopy but > 2 m tall;
SHRUB = 1 - 2 m: woody plants 1 - 2 m tall;
HERB = < 1 m: all herbaceous vascular plants plus any woody plants < 1 m tall;
BRYOID = all ground-layer non-vascular plants.

NATURAL COMMUNITY SURVEY PART II: DESCRIPTION

-> complete separate description forms for **each** notable natural community on reconnaissance page.

IDENTIFIERS / LOCATION

Area (specific/general):			Obs. Pt. #
Community type:			Adjacent communities:
Quad:	(Lat.:)	Size (acres) of community EO (not site):	BE SURE TO MAP EXTENT OF COMMUNITY ON TOPO. Distinguish between portions ground-truthed vs. portions presumed to be part of community based solely on photo/map interpretation, where applicable.
(Quadcode:)	(Long:)		

CLASSIFICATION HIERARCHY

Physiognomy (Class)	Phenology (Subclass)	Leaf type (Group)
forest	evergreen woody	broad-leaf woody
woodland	deciduous woody	needle-leaf woody
shrubland	mixed woody	graminoid
dwarf shrubland	perennial	forb
herbaceous	annual	pteridophyte
sparse vascular/nonvascular		non-vascular

(ALLIANCE:)

ADDITIONAL DATA FOR FORESTS

Tree canopy height:	Core data: ring counts (~ 5 cores) of larger trees (give sp. & dbh)	Deadwood (describe distribution, abundance, degree of decay):	
supercanopy trees?			

HISTORY (describe evidence or lack thereof; please do not leave boxes blank. Indicate approximately how recent where possible.)

Fire:	Wind:	Cutting:	Agriculture:	Impoundment:

comment:

ADDITIONAL SPECIES LIST

List additional plant species in community not included in the plot data that follows.	Species list sketchy or basically complete? Comment:

VEGETATION PLOT DATA

Area:	Obs. pt. #:
Community type:	(Regional alliance/community:)

LAYER	plot #			
TREE list species and dbh for all trees >= 5 cm dbh; count standing dead as 1 species. note units: QUAD SIZE: note which size used 5.64 m radius for 1/100th ha 7.98 m radius for 2/100th ha use same size throughout!				
SAPLING / TALL SHRUB cover class by species of: trees > 2 m tall but < 5 cm dbh; and shrubs > 2 m tall QUAD SIZE: 2.8 m radius or 25 m^2				
SHRUB cover class* by species of shrubs/trees 1 - 2 m tall. QUAD SIZE: 2.8 m radius or 25 m^2				
HERB cover class* by species for all herbaceous plants plus any woodies < 1 m tall QUAD SIZE: 1 m^2 , 2-4 herb quads per tree plot. Enter individual values in left-hand column and average in right-hand column. Remember the zeros for spp present in some but not all herb quads when figuring averages!				
BRYOID ground-layer mosses, liverwort, lichens in herb quads. resolution (check one): __"moss"/"liverwort"/"lichen" only; __identified to major group; __identified to genus; __identified to species.				
REMARKS				

In box on previous page, list plant spp. present in the community but not in the sample plots so we have a complete species lis

* cover classes (record midpoint): < 2 1 2-5% 3 6-12% 9 13-24% 19 25-49% 37 50-74% 63 75-100% 8

TOPOGRAPHY / SOILS

Area: | Obs. pt. #:

Community type: | (Regional alliance/community:)

Elevation: | Aspect: magnetic or true? | Slope: measured or estimated? | Microtopography:

pH (note kit or meter type)

Topographic position:
P low plain, level
T toe of slope
LS lower slope
MS middle slope
TB hillside terrace/bench
US upper slope
E cliff/ledge
C crest
M high plateau
N narrow valley
D drainage channel

Habitat patchiness (describe zones or patches if present):

Mineral Soil Profile:

horizon | depth (cm) | color | mottling | other

O

A

E

B

C

Organic Soil Profile:

peat depth:_____cm OR > 1 m_____

vonPost decomposition:_____

ALL SOILS:

DEPTH TO WATER TABLE:_____

DEPTH to OBSTRUCTION:_____

Soil temperature reading _____ F/C at _____ (depth)

Surficial deposit:
bedrock
talus slope
glacial till
moraine
esker/outwash
glacial delta
lacustrine/fluvial
marine
aeolian
other:

Surface:
_____% Bedrock
_____% Boulders (>50 cm)
_____% Cobbles/Gravel
_____% Bare mineral soil
_____% Organic soil
_____% Litter (note type)
_____% Water
_____% Total vegetation
_____ Other:

Average Texture:
gravel
sand
loamy sand / sandy loam
loam
silt loam
clay loams
sandy clay / clay
peat
muck

Bedrock type:
Igneous
granite
dioritic
gabbroic
other igneous
_____
Metamorphic
slate/phyllite
schist/gneiss
other metamorphic
_____
Sedimentary
limestone
other sedimentary
_____
details?

Soil stoniness:
v. little (< 1%)
moderate (2-25%)
very (26-100%)

Drainage & moisture regime (see MAPPSS key):
very poorly drained
poorly drained
somewhat poorly drained
moderately well drained
well drained
somewhat excessively drained
excessively drained

Hydrologic regime:
upland
nontidal wetland:
permanently flooded
semiperm'ly flooded
seasonally flooded
saturated
tidal - irregularly
tidal - regularly
saltwater
brackish
freshwater
unknown

NATURAL COMMUNITY SURVEY PART II: DESCRIPTION

-> complete separate description forms for each notable natural community on reconnaissance page.

IDENTIFIERS / LOCATION

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Tree canopy height:	Core data: ring counts (~ 5 cores) of larger trees (give sp. & dbh)	Deadwood (describe distribution, abundance, degree of decay):	
supercanopy trees?			

HISTORY (describe evidence or lack thereof; please do not leave boxes blank. Indicate approximately how recent where possible.)

Fire:	Wind:	Cutting:	Agriculture:	Impoundment:

comment:

ADDITIONAL SPECIES LIST

List additional plant species in community not included in the plot data that follows.	Species list sketchy or basically complete? Comment:

FINAL

ATTACHMENT 6

SAMPLE HANDLING FOR HERPETOFAUNA PROTOCOL

Sample Handling for Herpetofauna - Housatonic River. Pittsfield, MA Spring/Summer 1998

1. Mortality specimens (samples) from pitfall traps should be collected on a daily basis, preferably early each morning.. The date and time of collection should be noted.

2. Samples should be retained in the field in chemically clean jars. Each jar (8, 16 or 32 ounce dependent on biomass) should be representative of one trapline area or one pitfall. Jars should be labeled with the site name, trap line/pitfall number, date and time of collection. Samples should be kept on wet ice in the field. The wet ice should be retained in double ziploc bags to avoid contact with samples. If sample biomass is too large for a 32 ounce jar then dedicated decontaminated stainless steel or aluminum buckets should be used for transport.

3. Processing of samples should be as follows: individual specimens should first be thoroughly rinsed with deionized water for removal of all external organic matter, identified to species, weighed (to the nearest 0.1 gram), measured (nose to vent, to the nearest millimeter), and examined for gross external pathology. All information should be recorded on a separate data sheet for each sample.

4. Each individual should be assigned a specific sample number, based on species, trap line and pitfall number. If samples are composited, the composite should consist of the same species and preferably the same size class. To the extent possible, the number of individuals per composite should remain consistent and be recorded.

5. Individuals should be wrapped in aluminum foil (shiny side out, dull side next to sample). Aluminum foil can be hexane-rinsed or a field blank of representative unrinsed aluminum foil from each roll used should be submitted for analysis. The field blank should be prepared in the same method as biota samples.

6. A sample label should be placed on the exterior of each individually wrapped sample or sample composite. The sample label should be securely taped with clear tape. The sample label should contain the sample number, sample type, analysis to be performed, site name, and date.

7. Aluminum-wrapped, labeled samples should be placed in a ziploc bag and sealed. That bag should be placed in another ziploc bag containing a separate sample tag with the sample number clearly showing.

8. Samples should be kept on dry ice until they are shipped (with dry ice) to the analytical laboratory (or the USFWS field office for freezer storage). For large cooler/numerous sample shipments, layering of samples with dry ice and newspapers allows for better sample preservation and longer dry ice persistence.

9. All pertinent information regarding each sample (sample number, sample type, date collected, etc.) should appear on a standard EPA chain-of-custody form and be included

in a sealed ziploc bag, taped to the lid, inside each sample cooler.

10. Samples should be sent Federal Express Overnight (Next morning delivery). Samples sent to the USFWS should be shipped to:

Ken Carr/Ken Munney
USFWS — Phone: 603-225-1411
22 Bridge St., Unit 1
Concord, NH 03301 — Fed Ex Acct #: 1510-1036-9

Shippers should call ahead to the receiving laboratory or the USFWS and notify that samples are being sent for next day delivery. Samples should not be sent to USFWS if Ken Munney, Ken Carr, or Drew Major are not available for receipt of the shipment. Samples need to be sent for arrival on a weekday only. Therefore, Thursday is the last day of the week to ship samples. Shippers should also call the receiving laboratory or USFWS the day of delivery to verify receipt of samples.

APPENDIX A.10

WORK PLAN FOR THE STUDY OF RAPTORS AND WATERFOWL ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (WOODLOT ALTERNATIVES, INC.)

FINAL

APPENDIX A.10

WORK PLAN FOR THE STUDY OF RAPTORS AND WATERFOWL ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

Roy F. Weston, Inc.
1400 Weston Way
West Chester, PA 19380-1499

Submitted by:

Woodlot Alternatives, Inc.
122 Main Street
Topsham, Maine 04086

Table of Contents

1.0 Introduction

In 1998, during reconnaissance-level surveys being performed as part of an ecological characterization of the study area (see Figure 1 in Appendix A.6), 9 out of a possible 19 raptors were observed, primarily during migration. Several species were not seen, either because they were not present or because they are secretive and difficult to observe. Of the species that were not seen, or seen only during migration, several are of interest and worthy of further survey efforts. These include the northern harrier (*Circus cyaneus*) - state-threatened; sharp-shinned hawk (*Accipiter striatus*) - state-special concern; Cooper's hawk (*Accipiter cooperii*) - state-special concern; and long-eared owl (*Asio otus*) - state-special concern, all of which could occur in the study area based on habitat availability (Block et al., 1993; Palmer, 1988; Palmer, 1988; Evans, 1982; Johnsgard, 1988; Veit and Peterson, 1993). There were no observations during the nesting season in the study area indicative of locally breeding individuals. To determine if raptors are not breeding in, or near, the study area in suitable habitat, more-intensive surveys are needed. Similar surveys in reference areas can be used for comparison of breeding raptors outside of the study area.

1.1 Objectives

The objective of this task is to identify the raptors breeding in the study area. This information can be used to help develop fate and effects models and identify species in the study area of management concern by the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife. Use of the study area by raptors is of importance because these higher order predators are potentially exposed to high levels of PCBs due to bioaccumulation.

1.2 Project Approach

Broad-wing hawks (*Buteo platypterus*) and red-shouldered hawks (*Buteo lineatus*) consume reptiles and amphibians (Bent, 1937; Palmer, 1988), small mammals, birds, and insects (Goodrich et al. 1996). Both of these species could be useful when reviewing PCB food web interactions. Red-shouldered hawks are reported to accumulate PCBs (in Ehrlich et al., 1988). Both sharp-shinned hawks (*Accipiter striatus*) and Cooper's hawks (*Accipiter cooperii*) are known to occur in, or near, the study area, and could possibly breed in the area. While both species feed on small birds, both also eat small mammals, reptiles, and amphibians (in Ehrlich et al., 1988). To help develop a more complete picture of higher trophic-level bird use of the study area during the growing season, surveys will be conducted to solicit responses from nesting hawks and owls. If active nests are found, then attempts will be made to determine nestling fledge rates. Efforts recommended by Grier and Fyfe (1987) to minimize disturbance will be followed during all survey and nest monitoring activities.

2.0 Methods

Several methods will be used to identify and survey potential raptor habitat in the study area and in nearby reference areas. Literature searches will be used to identify existing information on hawk and owl occurrences in Lenox, Lee, and Pittsfield, and possibly in some adjacent towns with similar, suitable nesting habitat. Surveys for courting and nesting individuals will then be performed, and if possible, nesting success will be estimated.

2.1 Literature Search

The scientific and technical literature will be reviewed to determine the distribution of hawks and owls in the Housatonic River drainage system. As part of this effort, local and regional experts will be consulted to obtain unpublished records regarding the historic occurrence of raptors in the area. The Massachusetts Natural Heritage Program and the U.S. Fish and Wildlife Service will also be consulted to determine if any records of raptors from the Housatonic River drainage are available from surveys sponsored or conducted by these agencies.

2.2 Raptor Surveys

Playback point counts (Kennedy and Stahlecker, 1993) will be used to census raptors within the study area and in reference areas. To identify potential reference areas, USGS topographical maps, aerial photos, and windshield surveys will be used to search for areas with open water habitat, emergent marsh, scrub-shrub wetland and forested wetland located adjacent to land with a minimum of several hundred acres of undeveloped forested and active or former agricultural land similar to that of the study area. Additionally, similar land use patterns will be searched for, including Wildlife Management Areas (WMAs), which exist in the study area. Potentially suitable areas identified using maps and windshield surveys will be visited on foot to determine if they were suitable for use in raptor surveys based on similarity of surrounding forests, riparian habitat, and wetland habitat.

Potential reference areas include the Three-Mile Pond WMA, Hinsdale Flats WMA (Muddy Pond), and Washington Mountain Lake. Three-Mile Pond WMA, approximately 15.6 miles south of the study area, consists of a shallow pond dominated by submerged aquatic vegetation with pockets of emergent and scrub-shrub wetlands around the periphery of the pond. Surrounding the pond is a mixture of upland habitats including old field, and deciduous and mixed deciduous/coniferous forest. This habitat contains similar water levels, similar amounts of submerged aquatic vegetation, emergent marsh, and surrounding undeveloped habitat, as that of the lower portions of the study area near and upstream of Woods Pond. Washington Mountain Lake is located approximately 2 miles east of the study area in the middle of October Mountain State Forest. The lake now contains palustrine open water, emergent marsh, and scrub-shrub habitat similar to that found to the west along the Housatonic River. The lake is surrounded by mixed forests containing trees of similar size as those found in the study area. Muddy Pond and its outlet in Hinsdale, approximately 7.2 miles east-northeast of the study area, is the headwaters of the East Branch of the Housatonic River. This area contains similar wetland habitats and surrounding upland habitat as both the study area and the two other reference areas.

Survey transects will be established along the river or waterbody (for reference areas) and adjacent roads with point counts being taken at intervals of 300 meters. At each point, calls will be broadcast in all directions for 10 seconds followed by 30 seconds of silence for each call. This will be repeated with calls from each of the hawks and owls expected to occur within the study area (Fuller and Mosher, 1981; Veit and Peterson, 1993). Approximately 10 minutes will be spent at each point during which time all raptors observed will be identified to species and recorded along with type of observation (call, visual, or both). Hawk surveys will be conducted between one half hour before sunrise to sunset. Transects will be visited a minimum of 3 times during the breeding and post-breeding seasons (Fuller and Mosher, 1981), at least once during

mating season and once during the nestling-fledgling period. Owl surveys will be conducted one half hour after sunset to sunrise. Transects will be visited a minimum of 3 times during the breeding and post-breeding season (Fuller and Mosher, 1981). The location of transects and survey points will be recorded and plotted on maps of the study area.

Birds will be identified by sight and sound. Observers performing surveys will be trained in identifying each species' call by listening to bird tapes. Similarly, observers will be trained to identify each species of raptor that could potentially occur in the study area through the use of field guides, slides and videos. Sight identification will be aided by the use of *A Field Guide to the Birds of Eastern and Central North America* (Peterson and Peterson, 1980) and by the use of *Field Guide to the Birds of North America* (National Geographic Society, 1983). Sound identification will be aided by use of the Cornell Laboratory of Ornithology (1990) *A Field Guide to Bird Songs*. Nest, eggs and nestlings identification will be aided by the use of *A Guide to the Nests, Eggs, and Nestlings of North American Birds* (Baicich and Harrison, 1997).

When conducting bird studies, disturbance can create biases that affect the gathering and analysis of data, and can have effects on the birds being studied themselves (Gaunt et al., 1997). As Gaunt et al. (1977) point out, in field ornithology, adverse affects are most commonly associated with nest visits, which can result in biased data and decreased reproductive success. Investigators can cause nest desertion, damage to eggs and young from frightened adults, thermo-damage to eggs or young, mortality from missed feedings or predation, or accidental death from mishandling (Fyfe and Olendorff, 1976).

During the surveys, all efforts will be made to minimize disturbance to nesting raptors. These efforts will include minimizing the number of surveys, nests visits, and the type of nest visits, particularly during the early parts of incubation (Grier and Fyfe, 1987). To the extent possible remote observation of nests will be performed using binoculars or spotting scopes. If sufficient data cannot be obtained remotely, then inspection from a short distance (i.e., walking up to the nesting tree) or direct inspection (i.e., actually climbing a tree) will occur, only, however, if these activities are not believed to be detrimental to the nesting birds. Raptors are believed to be most sensitive to disturbance just prior to egg laying up to the onset of incubation; from first hatching until the young become endothermic; and just prior to fledging (Steenhof, 1987). Nest visits during these periods will be avoided. Additionally, nest visits will not be performed when weather conditions could prove detrimental to eggs or young (e.g., during a cold, rainy day, or during the middle of a hot, sunny day). Nest visits will be also kept as short as possible. Numbers of young fledged per nest will be measured by visually counting the number of young that leave a nest. These birds will be identified as they follow adults and beg for food. As stated above, visual observations will be aided by the use of binoculars and spotting scopes.

2.3 Waterfowl Surveys

In 1998 mallard (*Anas platyrhynchos*) and wood duck (*Aix sponsa*) tissues were collected for analysis to determine if waterfowl in the study area were accumulating PCBs. Depending on the results of tissue analysis, additional waterfowl surveys may be performed to better characterize PCB accumulation in waterfowl tissue. These surveys may involve monitoring nesting, egg-laying, hatching rates, fledgling rates, juvenile feeding, and accumulation of PCBs by young-of-

the-year ducks. A detailed study plan will be developed, if necessary, once the results of the tissue analysis are available.

3.0 Quality Assurance/Quality Control

3.1 Data Quality Objectives, Indicators, and Assessment

3.1.1 Data Quality Objectives

The objectives of the study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- Taxonomic data—Accurate species identification is required to prepare a list of raptors and waterfowl species that occur in each of the habitats within the study area.

3.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will not be possible because specimens will not be collected. However, agreement between surveyors regarding species identification must be obtained for verification. This will occur in the field as surveys are conducted. Precision may also be evaluated by assessing the degree to which surveys are consistent among sites. For measurements that are not unique to birds, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy means that the target animals are correctly identified and counted.

Completeness is defined as the percentage of the planned samples actually collected and processed. Although no sample sizes can be determined for characterization studies, observations must be conducted throughout the season that raptors and waterfowl are present within the study area and in each habitat that these species could use. The full distribution of study efforts within those parameters is a measure of the completeness of this study.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study will be ensured through the establishment of a thorough literature review to identify all species potentially occurring, and completing field investigations in a manner to determine if those species are present. Since a study objective is to identify the species occurring in the study area for possible use in future ecological risk studies, the study design must employ methods that will allow for identification of species known or expected to occur in the study area.

Comparability is defined as the measure of confidence with which results from this study may be compared to another similar data set. Comparability will be attained through use of sampling procedures that are used by the scientific community and by careful oversight of the project during field surveys.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to identify raptors and waterfowl from the study area.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because detailed collection of site soil or water samples will not occur. The usability of the information gathered during this study will be based on the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan and to accurately identify specimens collected at the survey locations.

3.2 Sampling Design

This study employs standard, repeatable methodology available in the scientific literature for collecting songbird occurrence data. Since the data are intended primarily for characterization, sites were located according to standard transect survey methods.

3.3 Sampling Methodology

3.3.1 Sampling Procedures

Field investigations for this study consist of the compilation of observational data to create species-habitat associations and provide location data for potentially nesting raptors. No physical samples of site soil or water will be collected for this study; therefore, QA/QC procedures for physical samples are not required.

3.3.2 Quality Control Samples

No QA/QC samples will be collected for this study.

3.3.3 Training

All field work will be directed by a senior field scientist with experience in raptor survey techniques. Field crew members will be able to identify each species of raptor potentially occurring in the study area by sight and sound. Prior to conducting actual surveys, field crews will be required to study and listen to previously recorded bird calls.

3.4 Sample Analysis

No physical, chemical, or biological sample analysis will be conducted for this study.

3.5 Data Analysis and Reporting

Information collected in this study can be used to develop fate and effects models, identify species in the study area that most likely could come in contact with PCB-contaminated sediments or potential PCB-contaminated prey items, and identify species in the study area of management concern to the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife and will be reported as such. Any state-listed rare species observed in the study area will have MNHESP Rare Animal Reporting Forms completed and submitted.

4.0 Equipment List

The following equipment will be used during the bird survey:

- Cover type maps and aerial photos of the study area
- Bird visual and song field guides
- Camera
- Binoculars, spotting scope
- Mirror and extension pole
- Field notebooks and data forms
- Rubber knee and hip boots
- Tape player
- Canoe

5.0 Results

5.1 Raptor Surveys

A table of raptor species known or suspected to occur in the study area and reference areas per habitat type will be prepared. Following the completion of surveys, this table will be edited to include the species of birds observed during this study, number of nests observed, location of nests, and success of nests. Maps showing the location of survey points in relation to natural community types will be produced. Data forms (see Table 1) will be appended to the draft and final narrative reports, which will describe the methods used to survey birds and the results of surveys. This information will be included as part of the draft and final ecological characterization report.

5.2 Waterfowl Surveys

Methods to be used to describe the results of waterfowl surveys will be prepared at a later date, if necessary.

6.0 Literature Cited

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Veit, R.R. and W.R. Peterson. 1993. *Birds of Massachusetts*. Massachusetts Audubon Society. Lincoln, MA. 514 pp.

WESTON (Roy F. Weston, Inc.). 2000. *Final Quality Assurance Project Plan*.

Table 1

Raptor Survey Data Form

Survey Area: Date: _____
Study Area North
Study Area South Observers: _____
Washington Mt. Res. _____
Three-mile Pond
Visit Number: 1 2 3

Weather Observations					
	Time	Temp. (C)	Cloud Cover (%)	Wind Speed	Wind Direction
Start					
Middle					
End					

	Station										Total
	1	2	3	4	5	6	7	8	9	10	
Start Time of Survey Station											
American Kestrel											
Sharp-shinned Hawk											
Cooper's Hawk											
Northern Goshawk											
Northern Harrier											
Broad-winged Hawk											

Table 1

Raptor Survey Data Form

	Station										Total
	1	2	3	4	5	6	7	8	9	10	
Start Time of Survey Station											
Red-shouldered Hawk											
Red-tailed Hawk											

Type of observation: C = call, V = visual

Record species observed, number of individuals seen or heard, and the species on the tape that it responded to.

Other Observations (including non-target species)

APPENDIX A.11

WORK PLANS FOR THE STUDY OF FOREST BIRDS AND MARSH AND WADING BIRDS (TECHLAW, INC.)

APPENDIX A.11-1

WORK PLAN FOR THE STUDY OF FOREST BIRD USE OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

FINAL

APPENDIX A.11-1

WORK PLAN FOR THE STUDY OF FOREST BIRD USE OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

Ms. Kathy Castagna
Regional Project Officer
U.S. Environmental Protection Agency
Region 1, New England
Waste Management Division (HPC CAN-7)
JFK Federal Building
Boston, Massachusetts 02203

Submitted by:

Christina Bramante
Regional Manager
TechLaw, Inc.
160 N. Washington Street, Suite 400
Boston, MA 02114

EPA Work Assignment No.:	R01074
Contract No.:	68-W4-0013
TechLaw WAM:	Travis Kline
Telephone No.:	617/720-0320
EPA WAM:	Bryan Olson
Telephone No.:	617/573-5747

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List of Figures

1.0 Introduction

The United States Environmental Protection Agency (EPA) is characterizing the natural resources found in and adjacent to the Housatonic River in portions of Pittsfield, Lenox and Lee, Massachusetts (see Figure 1 in Appendix A.6). The study area includes an approximately 12-mile section of the Housatonic River in Pittsfield, Lenox, and Lee, Massachusetts. This area includes riverine, adjacent floodplain wetlands, and uplands associated with the main stem of the river, and to a lesser degree, sections of the East Branch and West Branch of the Housatonic, from Newell Street in Pittsfield downstream to the Woods Pond Dam in Lee. Elevated levels of polychlorinated biphenyls (PCBs), which originated from the General Electric (GE) facility in Pittsfield, have been found in this reach of the Housatonic River (Blasland, Bouck, & Lee, Inc., 1996).

1.1 Objectives

The objective of this task is to identify birds that use the study area floodplain forests and scrub-shrub habitats. This information can be used to identify species in the study area that most likely could come in contact with PCB-contaminated sediments or potential PCB-contaminated prey items, and identify species in the study area of management concern to the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife. This information will be used in the problem formulation in the ecological risk assessment and in the ecological characterization of the study area.

2.0 Methods

Information available on historic and recent bird use of the study area will be collected from the Massachusetts Audubon Society, Massachusetts Division of Fisheries and Wildlife, and from published and unpublished books and manuscripts. This information will be used to prepare a table of birds known or suspected to occur in the study area per habitat and season.

Maps and aerial photos of the study area will be reviewed to identify the location and juxtaposition of forested, scrub-shrub, emergent, and riverine habitats. Potential bird survey sites will then be located on study area base maps, previously prepared as part of a wetland delineation and characterization effort (TechLaw, Inc., 1998). The survey sites will be chosen so that all habitat types in the floodplain will be surveyed for bird use, and so that sample points are approximately 500 m apart (Figure 1). This amount of separation should preclude counting the same individual more than once (Blondel et al., 1981). The amount of effort per habitat type will be stratified with habitat type abundance (Ralph and Scott, 1981) (based on acreage of each habitat type from the wetland maps mentioned above); therefore, the most abundant habitat types will have the greatest survey effort.

Two types of bird use data will be collected during this study: 1) miscellaneous observations while performing other surveys, and 2) observations while performing point counts. During other survey work, all birds encountered will be recorded in field notebooks along with the date and location. This qualitative information will be used to supplement other bird use survey data. Point counts with unlimited distance using a frequency sampling method (Blondel et al., 1981) will be used to census forest birds in different habitat types in the floodplain. At each survey

station, a surveyor will record each bird species seen or heard during a 20-minute sampling period. Each survey station will be visited once in the morning hours during June or the first half of July. This sampling protocol is an adaptation to both extensive and patchy environments (Blondel et al., 1981), like those found in the study area.

At each survey station the following information will be collected: date, start and end time of survey, percent cloud cover, amount of precipitation, wind speed and direction, temperature, bird species seen or heard, natural community type, dominant (>20 percent areal coverage or basal area for trees) species in the overstory, sapling, shrub, herb and woody vine strata, and estimated maximum and mean tree height. Five 35-mm photographs will be taken at each survey station, one of each in the cardinal directions, and one straight up to show canopy closure. Birds will be identified by sight and sound. Sight identification will be aided by the use of *A Field Guide to the Birds of Eastern and Central North America* (Peterson and Peterson, 1980) and by use of *Field Guide to the Birds of North America* (National Geographic Society, 1983). Sound identification will be aided by use of the Cornell Laboratory of Ornithology (1990) *A Field Guide to Bird Songs*.

3.0 Quality Assurance/Quality Control

3.1 Data Quality Objectives, Indicators, and Assessment

3.1.1 Data Quality Objectives

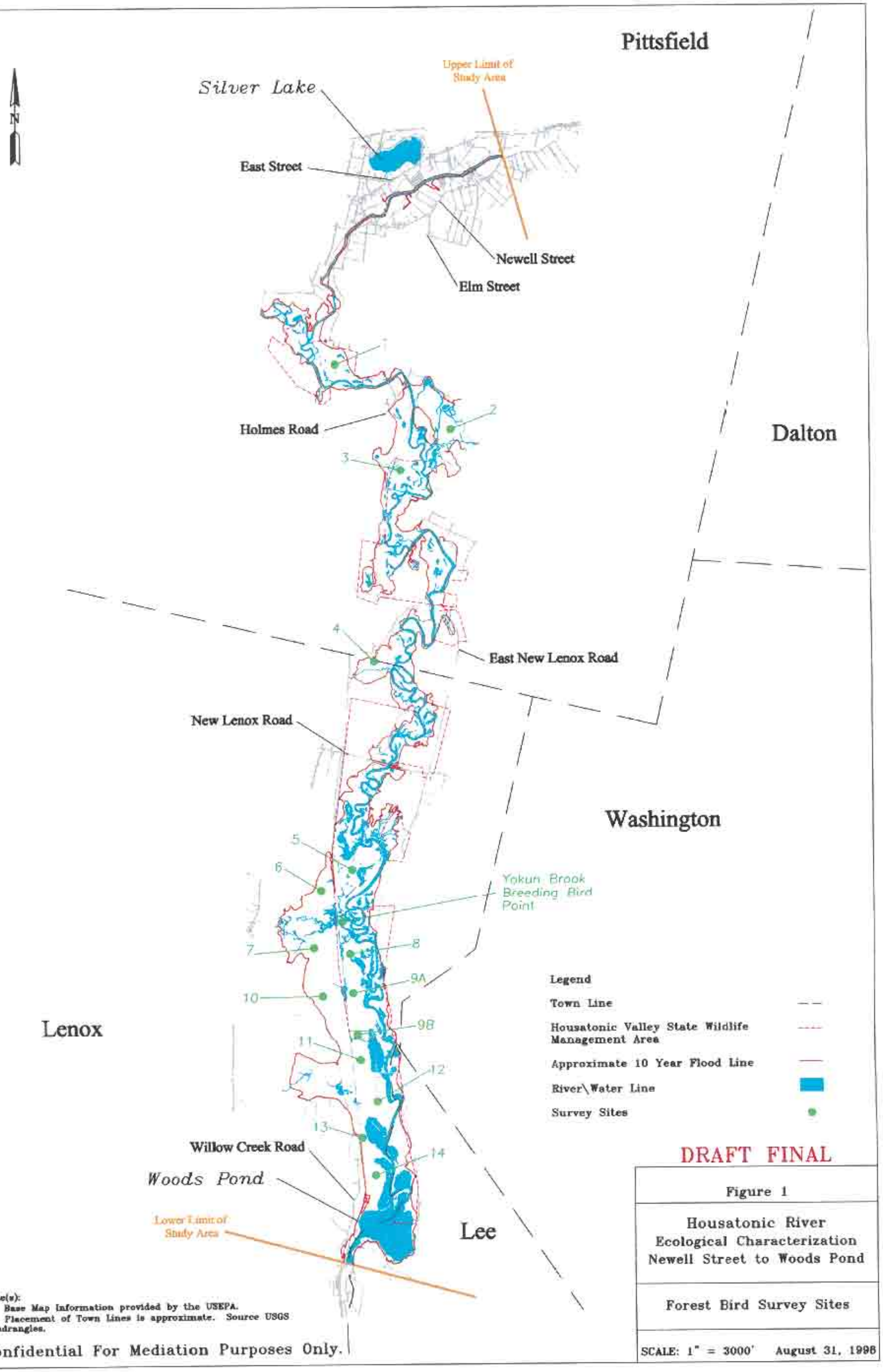
The objectives of the study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- Taxonomic data: accurate species identification is required to prepare a list of bird species that occur in the forested habitats of the study area.

3.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. These data quality indicators are usually not applicable to general ecological characterization investigations.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will assessing the degree to which surveys are consistent among sites. For measurements that are not unique to forest birds, precision is evaluated as defined in the QAPP.



DRAFT FINAL

Figure 1

Housatonic River
Ecological Characterization
Newell Street to Woods Pond

Forest Bird Survey Sites

SCALE: 1" = 3000' August 31, 1998

Note(s):
1) Base Map Information provided by the USEPA.
2) Placement of Town Lines is approximate. Source USGS Quadrangles.

Confidential For Mediation Purposes Only.

Accuracy is defined as the agreement of a measurement with its true lue. For the parameters unique to this study, accuracy means that birds observed are correctly identified to species. Species identification is aided by the use of bird song identification materials, including tapes and CDs of bird calls and written descriptions of calls in field guides.

Completeness is defined as the percentage of the planned samples actually collected and processed. For this study, completeness will be a measure of the number of sites visited compared to the number of planned sites.

Representativeness refers to the degree that the data collected during this study accurately reflect the true characteristics of the population that occurs in the study area, with respect to the study objectives. Since a study objective is to identify the species occurring in the study area for possible use in future ecological risk studies, the study design must employ methods that will allow for identification of species observed in the study area. This data quality objective is addressed through proper implementation of the sampling methods and will be evaluated through comparisons with known and expected lists of birds believed to occur in the study area.

Comparability is defined as the measure of confidence with which this study's results may be compared to another similar data set. Comparability will be attained through use of standard sampling procedures that are used by the scientific community and by careful oversight of the project during collection events. As with representativeness, the comparability of the data will also be evaluated through study of results from previous investigations in the region.

Sensitivity is the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. Field biologists performing this study will have passed hearing and eye tests performed as part of their annual physicals.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because collection of site soil or water samples will not occur. Furthermore, no biological samples will be collected. The usability of the characterization information gathered during this study will be based on the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan.

3.2 Sampling Design

Ecological characterization investigations, including this study, often utilize habitat-based assessment of species assemblages that are expected to occur in a given area. These studies utilize detailed habitat mapping and characterization to identify the types and conditions of habitats available to the species communities. The studies also use species' natural history, ecology, and range data to identify if a given species would be expected to occur within any of those available habitats. Field surveys for species are then used to supplement this information.

3.3 Sampling Methodology

3.3.1 Sampling Procedures

Field investigations for this study consist of the compilation of observational data to create species-habitat associations. No samples of site soil or water will be collected for this study; therefore, QA/QC procedures for analytical samples are not required.

3.3.2 Quality Control Samples

No QA/QC samples will be collected for this study.

3.3.3 Training

All field work will be conducted by a senior field scientist with experience in forest bird survey techniques. In addition, personnel performing bird surveys will have previous experience performing surveys of this type and will be capable of identifying by sight and sound all of the species that are likely to occur in the study area based on range maps (DeGraaf and Rudis, 1992; Veit and Peterson, 1993). Prior to initiating surveys, personnel will review lists of species likely to occur in the study area, listen to calls of species that they are not familiar with, and review photographs and illustrations of these species. To facilitate this review, the Birds of North America CD-ROM (Thayer Birding Software, 1997) with photos and calls of species expected to be found in the study area will be reviewed.

3.4 Sample Analysis

No physical, chemical, or biological sample analysis will be conducted for this study.

3.5 Data Analysis and Reporting

Information collected in this study can be used to identify species in the study area that most likely could come in contact with PCB-contaminated sediments or potential PCB-contaminated prey items, and identify species in the study area of management concern to the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife. For each state-listed species observed, Massachusetts Natural Heritage and Endangered Species Program Rare Animal Reporting Forms will be completed and submitted.

4.0 Equipment List

The following equipment will be used during the bird survey:

- Cover type maps and aerial photos of the study area
- Bird visual and song field guides
- Camera
- Binoculars
- Field notebooks and data forms
- Rubber knee and hip boots
- Thermometer

5.0 Results

A table of bird species known or suspected to occur in the study area per habitat type will be prepared. This table will include the species of birds observed in the study area during this study. Maps showing the location of survey points in relation to natural community types will be produced. Data forms will be appended to draft and final narrative reports, which describe the methods used to survey birds and the results of surveys (example forms are appended to this Work Plan). This information will be included as part of the draft and final ecological characterization report.

6.0 Literature Cited

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APPENDIX A.11-2

WORK PLAN FOR THE STUDY OF MARSH AND WADING BIRD USE OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

FINAL

APPENDIX A.11-2

WORK PLAN FOR THE STUDY OF MARSH AND WADING BIRD USE OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

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Regional Project Officer
U.S. Environmental Protection Agency
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JFK Federal Building
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Submitted by:

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160 N. Washington Street, Suite 400
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EPA Work Assignment No.:	R01074
Contract No.:	68-W4-0013
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List of Figures

1.0 Introduction

The Housatonic River and floodplain wetlands from Pittsfield downstream to Lee, Massachusetts (see Figure 1 in Appendix A.6), contain habitat suitable for use by a number of marsh and wading birds, which if present would nest and feed in the floodplain of the river. Marsh birds include several species of rails, pied-billed grebes (*Podilymbus podiceps*), common moorhen (*Gallinula chloropus)*, and American coot (*Fulica americana*); wading birds include bitterns, night-herons, other herons, and egrets. Of the birds that may use the area, several are state-listed rare species (Massachusetts Division of Fisheries and Wildlife 1997): American bittern (*Botaurus lentiginosus*) - Endangered[1]; common moorhen - Special Concern; least bittern (*Ixobrychus exilis*) - Endangered; pied-billed grebe - Endangered; and king rail (*Rallus elegans*) - Threatened.

1.1 Objectives

The objective of this task is to identify the species of marsh and wading birds that occur in the study area and to identify the areas that these birds use for nesting and feeding.

2.0 Methods

Maps and aerial photos of the study area will be reviewed to identify the location and juxtaposition of open water, aquatic bed, emergent, and scrub-shrub habitats. Potential marsh and wading bird survey sites will then be located on study area base maps, previously prepared as part of a wetland delineation and characterization effort (TechLaw, Inc. 1998). Potential survey sites will be chosen based on habitat descriptions for each species contained in the literature (Veit and Peterson, 1993; Gibbs et al., 1991; Griscom, 1949; Ehrlich et al., 1988; Terres, 1982), and based on reconnaissance surveys of the study area performed during March, April, and May 1998. All areas chosen for survey will contain habitat potentially suitable for one or more species of marsh and wading birds.

Potential survey sites will be established at a density of 1 station for approximately 5 ha of wetland (Gibbs and Melvin, 1993). Wading bird survey routes will be developed and plotted in AutoCAD® on study area base maps (Figure 1). These maps will be used during field surveys to locate individual survey stations and to record site-specific marsh and wading bird observations. Wading bird use of wetlands in the study area will be measured using the methods described by Gibbs and Melvin (1993). Each survey route will be visited 2 to 3 times from late May through July, between 30 minutes before and 4 hours after sunrise. Before each survey begins, all marsh and wading birds seen or heard will be recorded during a 15-minute period. Thereafter, the number of individuals and times each species was heard or observed while broadcasting playback

[1] Endangered species are native species which are in danger of extinction throughout all or part of their range, or which are in danger of extirpation from Massachusetts, as documented by biological research. Threatened species are native species which are likely to become endangered in the foreseeable future, or which are declining or rare as determined by biological research and inventory. Special Concern species are native species which have been documented by biological research or inventory to have suffered a decline that could threaten the species if allowed to continue unchecked, or which occur in small numbers or with such restricted distribution or specialized habitat requirements that they could easily become threatened within Massachusetts (Massachusetts Division of Fisheries and Wildlife, 1997).

calls will be recorded. Playback calls will be broadcast from portable cassette players. Approximately 50 seconds of calls per species will be broadcast, interspersed with 10 seconds of silence. Calls for least bittern, sora rail (*Porzana carolina*), Virginia rail (*Rallus limicola*), American coot, American bittern, pied- billed grebe, common moorhen, king rail, and green-backed heron (*Butorides striatus*) will be broadcast at each survey station.

During each survey the following information will be collected: wetland name, town, start and end time, observer, date, and visit number. At each survey station information on wind speed, cloud cover, precipitation, start and end time, responses per species, and all other wildlife sightings will be recorded.

3.0 Quality Assurance/Quality Control

3.1 Data Quality Objectives, Indicators, and Assessment

3.1.1 Data Quality Objectives

The objectives of the study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- Taxonomic data—Accurate species identification is required to prepare a list of marsh and wading birds that occur in the study area.

3.1.2 Data Quality Indicators

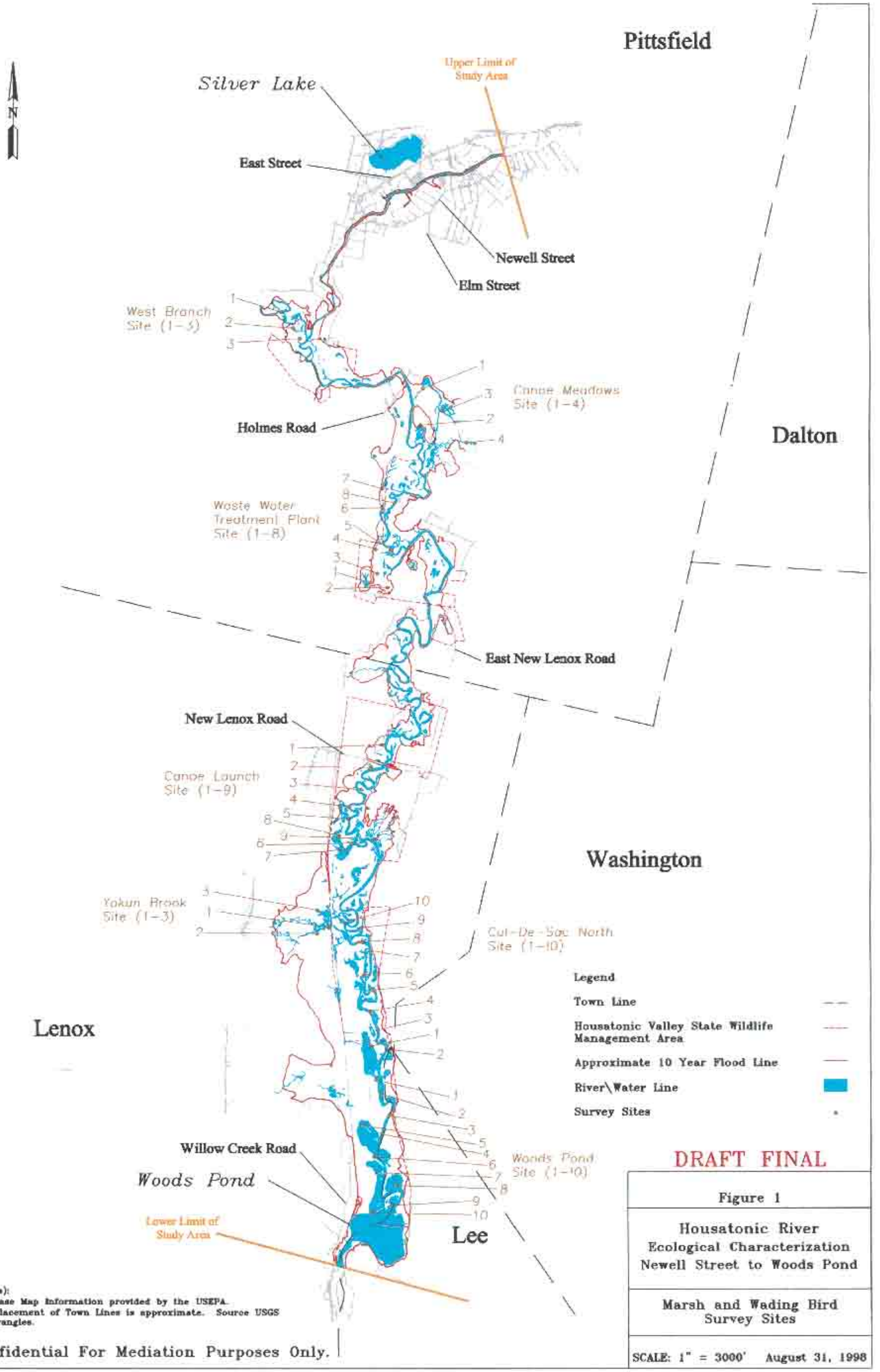
Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. These data quality indicators are usually not applicable to general ecological characterization investigations.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will not be possible for each observation because specimens will not be collected. However, if a species is observed on more than one occasion in the same area, but by different surveyors, the observation will be validated. Precision may also be evaluated by assessing the degree to which surveys are consistent among sites. For measurements that are not unique to marsh and wading birds, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy means that birds observed are correctly identified to species.

Species identification is aided by the use of bird song identification materials including tapes and CDs of bird calls and written descriptions of calls in field guides.

Completeness is defined as the percentage of the planned samples actually collected and processed. For this study, completeness will be a measure of the number of sites visited compared to the number of planned sites.



Note(s):
1) Base Map Information provided by the USEPA.
2) Placement of Town Lines is approximate. Source USGS Quadrangles.

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DRAFT FINAL

Figure 1

Housatonic River
Ecological Characterization
Newell Street to Woods Pond

Marsh and Wading Bird Survey Sites

SCALE: 1" = 3000' August 31, 1998

Representativeness refers to the degree to which the data collected during this study accurately reflect the true characteristics of the population that occurs in the study area, with respect to the study objectives. Since a study objective is to identify the species occurring in the study area for possible use in future ecological risk studies, the study design must employ methods that will allow for identification of species observed in the study area. This data quality objective is addressed through proper implementation of the sampling methods and will be evaluated through comparisons with known and expected lists of birds believed to occur in the study area.

Comparability is defined as the measure of confidence with which this study's results may be compared to another similar data set. Comparability will be attained through use of standard sampling procedures that are used by the scientific community and by careful oversight of the project during collection events. As with representativeness, the comparability of the data will also be evaluated through study of results from previous investigations in the region.

Sensitivity is the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. Field biologists performing this study will have passed hearing and eye tests performed as part of their annual physicals.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because collection of site soil or water samples will not occur. Furthermore, no biological samples will be collected. The usability of the characterization information gathered during this study will be based on the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan.

3.2 Sampling Design

Ecological characterization investigations, including this study, often utilize habitat-based assessment of species assemblages that are expected to occur in a given area. These studies utilize detailed habitat mapping and characterization to identify the types and conditions of habitats available to the species communities and on species' natural history, ecology, and range data to identify if a given species would be expected to occur within any of those available habitats. Field surveys for species are then used to supplement this information.

3.3 Sampling Methodology

3.3.1 Sampling Procedures

Field investigations for this study consist of the compilation of observational data to create species-habitat associations. No samples of site soil or water will be collected for this study, therefore, QA/QC procedures for analytical samples are not required.

3.3.2 Quality Control Samples

No QA/QC samples will be collected for this study.

3.3.3 Training

All field work will be conducted by a senior field scientist with experience in marsh and wading bird survey techniques. In addition, personnel performing bird surveys will have previous experience performing surveys of this type and will be capable of identifying by sight and sound all of the species that are likely to occur in the study area based on range maps (DeGraaf and Rudis, 1993; Veit and Peterson, 1993). Prior to initiating surveys, personnel will review lists of species likely to occur in study area, listen to calls of species that they are not familiar with and review photographs and illustrations of these species. To facilitate this review, the Birds of North America CD-ROM (Thayer Birding Software, 1997) with photos and calls of species expected to be found in the study area will be reviewed.

3.4 Sample Analysis

No physical, chemical, or biological sample analysis will be conducted for this study.

3.5 Data Analysis and Reporting

Information collected in this study can be used to identify species in the study area that most likely could come in contact with PCB-contaminated sediments or potential PCB-contaminated prey items, and identify species in the study area of management concern to the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife. For each state-listed species observed, Massachusetts Natural Heritage and Endangered Species Program Rare Animal Reporting Forms will be completed and submitted.

4.0 Equipment List

The following equipment will be used during the bird survey:

- Cover type maps and aerial photos of the study area
- Cassette player and playback tape
- Canoe, paddles, life jackets
- Headlight and/or flashlight
- Bird visual and song field guides
- Camera
- Binoculars
- Field notebooks and data forms
- Thermometer

5.0 Results

A list of wading bird species known or suspected to occur in the study area per habitat type will be prepared. This list will indicate which species were observed during this survey. Maps showing the location of marsh and wading bird survey routes, survey stations, and sightings of marsh and wading birds will accompany a narrative report describing survey methods and results.

6.0 Literature Cited

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APPENDIX A.12

WORK PLAN FOR THE STUDY OF RIVER OTTER, MINK, AND BATS ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (WOODLOT ALTERNATIVES, INC.)

FINAL

APPENDIX A.12

WORK PLAN FOR THE STUDY OF RIVER OTTER, MINK, AND BATS ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

Roy F. Weston, Inc.
1400 Weston Way
West Chester, PA 19380-1499

Submitted by:

Woodlot Alternatives, Inc.
122 Main Street
Topsham, Maine 04086

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1.0 Introduction

In 1998 an ecological characterization of the study area was performed, in part, to identify all of the species of mammals that could potentially use the area based on published range maps (e.g., DeGraaf and Rudis, 1992; Godin, 1977; Burt and Grossenheider, 1976) and habitat availability. A literature review and reconnaissance-level field surveys were conducted as part of the characterization (TechLaw, Inc., 1999). During these surveys a total of 22 out of a possible 52 mammals that could possibly occur in the area based on published range maps, were observed in the study area (see Figure 1 in Appendix A.6). A number of species were not seen, either because they were not present or because they are secretive and difficult to observe during general reconnaissance-level surveys. Of the species that were not seen, several are of interest and worthy of further survey efforts. Mink and otter, two species known to be susceptible to PCB contamination (Auerlich et al., 1971; Bleavins et al., 1980; Eisler 1986; Organ, 1989), were not observed in the study area, even though there is suitable habitat throughout the study area (Dubuc et al., 1990; Newman and Griffin, 1994). While it may be difficult to directly observe these two species, their sign, tracks, and scats are easy to detect when individuals are present. In an effort to determine if these species occur in the study area, additional, more-intensive surveys will be performed in 1999.

Besides mink and otter, a number of species of bats should occur in the study area; however, they are difficult to detect and identify to species. Bat use of the study area is of interest for several reasons. Up to 9 species may occur in the area (Whitaker and Hamilton 1998), several of which are poorly understood and possibly rare - Leib's myotis (*Myotis leibii*), northern myotis (*Myotis septentrionalis*), and Indiana myotis (*Myotis sodalis*). The Indiana bat is listed as endangered in Massachusetts and 19 other states, whereas Leib's myotis is listed as endangered in Kentucky and New Hampshire and as threatened in Pennsylvania and Vermont (Whitaker and Hamilton 1998). In addition to reasons of rarity, bat use of the study area is of interest because of the feeding and roosting habitats of several species. Floodplain forests and riverine areas have been found to have high density of bat populations (Kunz 1988). The silver-haired bat (*Lasionycteris noctivagans*), for example, roosts in trees and feeds over watercourses, as does the little brown bat (*Myotis lucifugus*) (Fenton and Bell 1979), northern myotis, Indiana myotis, and big brown bat (*Eptesicus fuscus*) (Whitaker and Hamilton 1998). Bats feeding on emergent insects in the study area could potentially be exposed to contaminants if the insects contain PCBs. The information collected during this investigation will be used in the ecological characterization, in refining the Conceptual Model in the Ecological Risk Assessment, and in determining and designing future studies, if warranted.

1.1 Objectives

The objectives of this study are to determine if mink and otter are present in the study area and nearby reference areas; and to determine what species of bats are present in the study area and the habitats they are using for feeding and possibly roosting.

1.2 Project Approach

1.2.1 Mink and Otter

In 1998, field surveys for mink and otter in the study area did not result in any indication that either species was present. No observations of individuals, scats, or tracks were made, despite the availability of suitable habitat. These surveys, however, could be characterized as reconnaissance-level, that is, for the most part, they did not specifically target mink or otter as they were conducted as part of the initial ecological characterization effort. Because neither of these species was observed, efforts to detect them in 1999 will be increased. In addition, surveys for mink and otter in nearby reference areas with suitable habitat will also be conducted to help estimate how effective survey methods are for detecting these two species. Potential mink and otter habitat in the study area and nearby reference areas will be identified using several methods including literature surveys, aerial photo interpretation, and field surveys.

Scent stations have been widely used to determine seasonal and annual trends in mammalian carnivore populations. The technique was originally developed to monitor red and grey fox populations (Conner et al., 1983). It has since been applied to coyote (Linhart and Knowlton 1975), bobcat, raccoon, and opossum (Conner et al., 1983), wolves (Roughton and Sweeney 1982), river otter and mink (Humphrey and Zinn, 1982), and bears (Lindzey et al., 1977). The technique used to set up scent stations differs widely for individual studies and target species. Roughton and Sweeney (1982) suggest setting up scent stations along parallel transects throughout a study site. Distances between stations and transects should be determined based on the mobility of the target species. Humphrey and Zinn (1982) used transects spaced 180 m apart, with each transect containing 10 scent stations at intervals of 60 m to conduct their mink and otter study.

Scent stations are most often a circle of smooth dirt at the center of which the attractant, or lure, is placed. A 0.9 m circle of sifted dirt is the most commonly used method (Phillips, 1982; and Linhart & Knowlton, 1975). Roughton and Sweeney (1982) recommends using fine dust sifted over the site and avoiding sand as a tracking medium. However, moist sand has been used successfully (Conner et al., 1983). Chalk dust spread over 0.4 m square masonite board was found to be a successful medium for recording tracks (Humphrey and Zinn, 1982).

There are many different lures used to attract animals to the scent station. Day et al., (1980) suggests a similar scent could be used for all species. Ingredients widely used are rotten eggs, decomposed meat, fish oil, seal oil, musk oil, beaver castor, and skunk musk. Commercial scents are a good choice as they are readily available from hunting stores and have been proven to work on the target species. Commercial mustelid scents were successfully used to attract both mink and otter by Humphrey and Zinn (1982). When attempting to attract more than one species a common lure can be used (Conner et al., 1983) or species-specific lures can be alternated between stations (Humphrey and Zinn, 1982).

Lure can be deposited at the scent station in variety of ways. Scented pastes can simply be smeared in the area (Proulx et al., 1993) or the attractant can be placed on a stick in the center of

the circle (Linhart and Knowlton, 1975). Liquids can be poured directly onto the ground (Roughton and Sweeney, 1982), saturated cotton balls can be used (Conner et al., 1983), or wooden dowels can be saturated in the liquid scent (Humphrey and Zinn, 1982). Most studies sampled the stations for several consecutive days (Phillips, 1982; Bull et al.,1992; Linhart and Knowlton, 1975; Lindzey et al., 1977). Humphrey and Zinn (1982) found 2 consecutive nights to be adequate. Roughton and Sweeney (1982) suggest operating lines for only 1 night. They found no difference in coyote visitation during longer operation periods.

The date the study is conducted can be important. It is best to time carnivore studies when there is the least human disturbance in the area, generally not during hunting season. The best time to conduct scent post studies, according to Roughton and Sweeney (1982), is when juveniles disperse because this is when animals have the widest range of movement. Both mink and otter disperse during the fall (Whitaker and Hamilton, 1998). Breeding season is also a good time to set up scent stations as animals are more likely to be drawn to scents during this time (Humphrey and Zinn, 1982). Mink can breed from November to March with the most activity in late winter. Otter breed from December to May with peak activity during March and April (Whitaker and Hamilton, 1998).

1.2.2 Bats

Bats are usually active at dawn and dusk, or at night, and are difficult to detect visually and identify. Their behavior poses some censusing difficulty that is possible to overcome using some recent advancements in echolocation technology. Fenton et al. (1973) demonstrated the use of an ultrasonic sensing system to monitor the activity of bats. Other studies have since shown the reliability and ease of use allowed by monitoring the ultrasonic echolocation calls of bats (Bell, 1980; Fenton and Bell, 1981; Fenton, 1983; Barclay, 1984). Currently, sampling bat populations by monitoring their echolocation calls has become the preferred method in many studies. Barclay (1984) demonstrated that echolocation calls give a much better picture of the presence and abundance of the various species than does trapping. The echolocation calls of all bats in an area at a given time can be recorded while mist netting only allows for a sample of the bat population to be taken. Monitoring echolocation calls also allows scientists to determine foraging behavior as feeding buzzes of bats are readily distinguishable from their echolocation calls (Fenton et al., 1983).

2.0 Methods

Several methods will be used to determine if mink and otter are in the study area and reference areas, and to determine the species of bats found in the area. Specific survey techniques are described below.

2.1 Literature Search

Scientific and technical literature will be reviewed to determine the historic distribution of mammals in the Housatonic River drainage system. As part of this effort, local and regional experts will be consulted to obtain unpublished records regarding the historic and current distribution of mammals in the Housatonic River drainage. The Massachusetts Division of Fisheries and Wildlife (MDFW) and the U.S. Fish and Wildlife Service will also be consulted to determine if any records of mink, river otter, or bats from the Housatonic River drainage are available from surveys sponsored or conducted by these agencies.

2.2 Mammal Snow Tracking

Mammal snow track counts (Halpin, 1984; Halpin and Bissonette, 1988) will be conducted in the various habitat types encompassed by the study area as well as in reference areas. Several 500-m-long transects will be established so that each habitat type (shoreline, palustrine forested, shrub-scrub, emergent, wet meadow, and old field) is represented. Transects in the study and reference areas will be walked in fresh snowfall for a minimum of two to three snow events to ensure representative surveys are done in both the winter of 1999 and 2000. Mammal tracks will be recorded according to species and habitat type. Transect locations will be recorded and plotted onto a map of the study site. Survey level of effort in study and reference areas will be the same. In 1999, two study areas will be utilized, Three-Mile Pond and Ashley Lake, while in 2000, two additional reference areas will be added to the study, Washington Mountain Lake and Muddy Pond.

2.3 Scent Stations for Mink and Otter

Scent stations will be used in December 1998, and in the late summer or early fall 1999, and during the winter snow tracking in 2000 to detect the presence or absence of mink and otter in the study area and in two reference areas located within the Housatonic River Watershed. In the study area, three transects will be located parallel to the river; one each in the upper, middle, and lower study area. Transects in reference areas will also be located parallel to the water body. Each transect will be 600 m long, and will contain 10 scent stations at 60-m intervals. Each scent station will consist of a 0.9-m diameter circle of moist sand (i.e., for summer and fall surveys), or of snow (i.e., for surveys that take place in the winter). A 10-cm wooden dowel will be smeared with an attractant and placed in the center of the circle. *Leon Lures Mink #1 Super All Call* and *Otter Super All Call* will be used as lures. These lures will be purchased from Chagnon's Trapping Supply, Manistique, MI. Lures will be alternated between stations so that half of the stations on each transect are baited with each lure.

Transects will be visited for the three days following setup, weather permitting. During each visit mammal tracks present will be identified to species and recorded. Presence of small mammal and bird tracks will be noted, without tracks being identified to species. Presence of any other animal

sign in the area will also be noted. At the end of each visit, if necessary, sand will be smoothed in preparation for the next visit.

2.4 Bat Surveys

Bat species will be surveyed using echolocation (De Oliveira, 1998; Fenton and Bell, 1981) in July and/or August 1999. Three transects will be set up along the river bank at the upper, central, and lower parts of the study area. Transects will run parallel with the river for approximately 1 km. Bat surveys will be conducted starting 15 minutes after sunset to take advantage of the period of highest bat activity (Crampton and Barclay, 1998). While echolocation surveys may continue through the night, most surveys are expected to be completed within two hours, the period during which bat activity is highest (De Oliveira, 1998). Surveys will be performed in each area for three consecutive nights as recommended by De Oliveira (1998). Transects will be walked or paddled and echolocation noise of bats will be recorded using the Anabat System, which transforms ultrasound to an audible output capable of being recorded by normal-speed tape recorders (De Oliveira, 1998). Recorded calls will be analyzed to identify species using Analook, a PC sound analysis software. The number of flyover passes will be recorded for each species. Location of transects will be recorded and plotted onto maps of the study area.

2.5 Data Analysis

No statistical analysis of data is proposed. Presence and absence data collected will be presented in reports.

3.0 Quality Assurance/Quality Control

3.1 Data Quality Objectives, Indicators, and Assessment

3.1.1 Data Quality Objectives

The objectives of the study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- Taxonomic data—Accurate species identification (tracks, sightings, and sound analysis for bats) is required to prepare a list of mammal species that occur in each of the habitats within the study area.

3.1.2 Data Quality Indicators

Data developed must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives of this study.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will not be possible because specimens will not be collected. However, agreement between surveyors regarding species identification must be obtained for verification. This will occur either in the field as surveys are conducted, or in the office using photographs of tracks with measurement scales showing distinguishing features (e.g., numbers of toes, foot pad size, length of stride). Precision may also be evaluated by assessing the degree to which surveys are consistent among sites. For measurements that are not unique to mammals, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy means that mammal observations are correctly identified to species and counted.

Completeness is defined as the percentage of the planned samples actually collected and processed. For this study, completeness will be a measure of the number of sites visited compared to the number of planned sites.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study will be ensured through establishment of an approved, thorough sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of the representativeness will also be evaluated via comparison with known and expected results based on previous investigations in the biophysical region.

Comparability is defined as the measure of confidence with which the data may be compared to another similar data set. Comparability will be attained through use of sampling procedures that are commonly used by the mammal researcher community and by careful oversight of the project during collection events. As with representativeness, the comparability of the data will also be evaluated via study of results from previous investigations in the biophysical region, if available.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to identify mammals from the study area.

3.1.3 Data Validation, Verification, and Usability

The validation and verification of physical data will not be required for this study because collection of site soil or water samples will not occur as part of this SOP. Furthermore, no biological samples will be collected. The usability of the occurrence data gathered during this study will be based on the experience of the senior investigator to competently oversee field investigations and ensure that field surveys are conducted following the established plan and to accurately identify mammals or mammal sign observed at survey locations.

3.2 Sampling Design

Specific scientific sampling methods were chosen to target these mammal species groups and detect their presence in the study area. Selection of survey locations was conducted in accordance with scientific sampling methodology designed to collect species occurrence data and usually employ transect survey methods.

3.3 Sampling Methodology

3.3.1 Sampling Procedures

No physical or biological samples of site soil or water will be collected for this study, therefore, QA/QC procedures for physical samples are not described in this SOP.

3.3.2 Quality Control Samples

No QA/QC samples will be collected for this study.

3.3.3 Training

All field work will be overseen and coordinated by a senior field scientist with experience in mammal survey techniques. Scientists performing surveys will be trained to use mammal track keys and to use the Anabat system (De Oliveira, 1998).

3.4 Sample Analysis

No physical, chemical, or biological sample analysis will be conducted for this study.

3.5 Data Analysis and Reporting

Information collected in this study will be used to identify the presence/absence of mink, otter, and bat species in the study area, and to identify species in the study area of management concern to the U.S. Fish and Wildlife Service or the Massachusetts Division of Fisheries and Wildlife and will be reported as such. Observations of State-listed mammals will be documented through completion and submittal of MNHESP rare animal reporting forms.

4.0 Equipment List

- Cover type maps of the study area
- Warm clothing for winter surveys
- Measuring tape
- GPS receiver

- Maps and aerial photos
- Survey flagging
- Compass
- Binoculars
- Keys to mammal tracks
- Bat and echolocation field guides
- Plastic bags for collecting scats
- Mink and otter lures
- Wooden dowels
- Sand
- Small hammer
- Camera
- Headlight
- Anabat V Bat detector
- Professional-level tape recorder
- Analook sound analysis software
- Anabat V Zero-crossing Analysis Interface Module
- Field notebook and data forms

5.0 Results

5.1 Mammal Snow Tracking

The results of mammal snow tracking surveys will be produced in several formats. Maps showing the locations of tracking surveys will be prepared. In addition a list of mammals observed during each survey will be prepared. This information will be documented in a report which will summarize the methods used to conduct surveys and the results.

5.2 Scent Stations for Mink and Otter

Maps showing the location of scent stations, both in and out of the study area, will be produced. The locations of otter and mink observations will be plotted on maps. In addition a list of mammals observed during each survey will be prepared. This information will be documented in a report summarizing the methods used to conduct surveys and the results.

5.3 Bat Surveys

A report will be prepared that summarizes the methods used to conduct the bat surveys as well as the results. Survey locations will be plotted on maps of the study area.

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APPENDIX A.13

FIELD SAMPLING AND ANALYSIS PLAN FOR BENTHIC MACROINVERTEBRATE COMMUNITY EVALUATION AND TISSUE ANALYSIS

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APPENDIX A.13

FIELD SAMPLING AND ANALYSIS PLAN FOR BENTHIC MACROINVERTEBRATE COMMUNITY EVALUATION AND TISSUE ANALYSIS

1. INTRODUCTION

1.1 BACKGROUND

The benthic macroinvertebrate community in streams, rivers, and ponds plays a key role in ecosystem functions, such as nutrient cycling and organic matter processing, and is an important food source for instream consumers, as well as for some bird and mammal species. Benthic macroinvertebrates are relatively sedentary organisms that inhabit or depend on bottom sediments or other substrates for their various life functions. Therefore, they are sensitive to both long-term and short-term changes in habitat, sediment, and water quality and, because they spend most of their lives in a single location, can serve as effective indicators of environmental conditions in that location (Davis and Lathrop, 1992).

Benthic macroinvertebrate community structure and function have been used extensively to evaluate the quality of water resources and characterize causes and sources of impacts in lotic (flowing water) and lentic (standing water) freshwater ecosystems. The individual organisms that make up benthic communities respond to both biotic and abiotic environmental variables; therefore, the structure of these communities reflects the integration of the influence of these variables. Biotic variables may include competition, predation, and food availability, whereas abiotic variables may include sediment grain size distribution, temperature, dissolved oxygen, flow characteristics, and pollutants.

Because of the long-recognized importance of benthic community structure in evaluating the health and condition of aquatic habitats, a wide variety of community metrics have been used to capture and represent key community characteristics. Such metrics include species richness, faunal density, evenness, equitability, and diversity. A body of statistical techniques has also been developed to compare these and other metrics across communities. In addition to the normal parametric and non-parametric hypothesis testing tools, there are more subjective techniques that fall under the general headings of classification (cluster analysis) and ordination (factor analysis). Because of the relatively non-motile lifestyle of benthic invertebrates, the tissue residue concentration of contaminants in benthic species is also integrator of levels of chemical contaminants at a particular location.

1.2 OBJECTIVES

A focused assessment of the benthic macroinvertebrate community is an important component of the Sediment Quality Triad approach (Long and Chapman, 1985; Chapman, 1992) used in the

Ecological Risk Assessment for the Lower Housatonic River. This study has three primary data quality objectives; in addition, all data will also support the DQOs outlined in Subsection 4.1 of the Final Quality Assurance Project Plan. The three primary objectives of the study are to:

- Evaluate the potential effects of PCBs, other contaminants, and the Pittsfield WWTP effluent on the benthic macroinvertebrate community in the Lower Housatonic River.

- Determine tissue residue concentrations in benthic macroinvertebrate trophic groups relative to the corresponding sediment PCB concentration for use in the AQUATOX model and as a component of the tree swallow study.

- Provide one of the three components of the Sediment Quality Triad approach to evaluating the potential ecological risk across a range of PCB concentrations in the sediment.

This document provides a detailed discussion of the design for the benthic macroinvertebrate study and provides background on the rationale for station selection, analysis of biological and chemical samples, and data reduction and presentation.

2. STUDY DESIGN

2.1 FIELD SAMPLING

2.1.1 Study Areas

The benthic macroinvertebrate investigation is sharply focused on providing information specific to the three data quality objectives outlined above, rather than providing a full characterization of the benthic macroinvertebrate community, which is beyond the scope of this study.

2.1.2 Station Locations

Sampling of benthic macroinvertebrates inhabiting soft (depositional) sediments will be conducted at 13 locations in the Housatonic River watershed (Figure 1). Three reference stations will be established upstream of the GE Pittsfield facility, one in the East Branch in the Town of Dalton (Location 011) and two in the West Branch (Locations 398 and 161). These three locations provide a similar and therefore appropriate habitat unaffected by releases from the GE facility to serve as reference areas for much of the Lower River habitat. An additional reference location will be established in Three-Mile Pond in the Town of Sheffield, which provides a reference habitat for the softer sediments found in the river closer to Woods Pond. All eight "target" locations are in Reach 5 of the main stem of the Housatonic River between the confluence of the East and West Branches and Woods Pond.

Two of the reference locations and four of the target locations are co-located with the in situ and laboratory toxicity testing to provide data for the Sediment Quality Triad evaluation across a

New York

H0-SEEC0011

HW-SE000161

HW-SE000398

H3-SE000051

H3-SE000060

H3-SEEC0019

H3-SD043702

H3-SE000428

H3-SE000116

H3-SE000389

H3-SEEC0031

H3-SE000246

H9-SE000615

NOTE: Station identifications shown are those of the associated sediment chemistry sample.

LEGEND:

- Macroinvertebrate Sampling Locations
- Surface Hydrology
- Major Roads
- Housatonic River Basin, MA

N

Scale In Feet

8000 0 8000 16000

SI Work Plan
Lower Housatonic River
Massachusetts

FIGURE 1
BENTHIC MACROINVERTEBRATE SAMPLING LOCATIONS

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range of PCB concentrations. The remaining two reference locations will be selected in proximity to locations currently being investigated in the upper West Branch and Three-Mile Pond in conjunction with the Swallow Study. All four reference stations will provide data on benthic macroinvertebrate community structure in locations that are known to have near-background levels of chemical contamination.

Of the remaining five target stations, four will be selected to (1) provide data from locations with PCB concentrations that expand upon the range of concentrations represented at the Triad locations and (2) investigate the potential effects of the WWTP discharge on benthic communities in the river. Two pairs of stations, each pair with a similar concentration of PCBs, will have one station located upstream and the other located downstream of the WWTP (see Table 1*)*. All stations will be located based upon expected PCB concentrations determined by previous sampling efforts. All nine of the target stations will be used to provide data on tissue residue concentrations of PCBs and other contaminants in macroinvertebrates for integration into the swallow study, AQUATOX model, and Sediment Quality Triad evaluation.

A summary of the 13 proposed sampling locations and their associated contaminant levels is provided in Table 1.

2.1.3 Sampling Site Selection

The benthic macroinvertebrate study will focus on suitable soft bottom (i.e., depositional) habitats. At each of the 13 locations discussed above, soft bottom sediments will be located by inspection prior to sampling. To the extent practicable in the field, sediment composition and additional habitat characteristics such as flow regime and water depth will be evaluated and matched to reduce the impact of small-scale variation on differences in benthic community characteristics.

For three of the six stations co-located with the toxicity testing, the sample locations for the benthic invertebrate collections will be visually placed in sediments similar to those used in the toxicity tests (see Appendix A.14). The other three toxicity test stations are located in water depths that will preclude simple visual inspection of the sediments; at these locations, extra care will be taken to examine each grab sample, and samples with atypical sediment characteristics will be rejected.

2.1.4 Sampling Procedure

The sampling methods described below follow EPA (1983, 1989, 1990) procedures for collecting quantitative samples of benthic macroinvertebrates to be used for evaluating community structure and tissue residue analysis. Twelve replicate samples will be collected at each of the 13 locations (thus providing a total of 156 individual samples) with a Wildco Petite Ponar grab sampler that has been modified to facilitate removal of one of the two covering screens. The Petite Ponar samples an area of 0.023 m^2, to an approximate depth of 10 cm. Immediately upon retrieval, the screen cover will be removed from one of the grab buckets and the contents of the sampler inspected to ensure adequate penetration. Samples that have greater than a 1-inch space between the top of the sediment and the top of the grab bucket will be

Table 1

Proposed Sampling Locations

Sample Location ID	Location	PCB (ppm)	TOC (%)	In Situ Toxicity	Location in Relation to WWTP
H0-SEEC0011	Dalton reference	ND	0.01	Y	Above
HW-SE000161	Upper WB reference	ND	0.15		Above
HW-SE000398	Lower WB reference	ND	1.5	Y	Above
H9-SE000615	3-Mile Pond reference	ND	2.8		NA
H3-SEEC0019	¼ mile below Holmes Rd.	8.7	0.3	Y	Above
H3-SE000428	Immediately above WWTP discharge	31.7	0.9	Y	Above
H3-SEEC0031	½ mile above Woods Pond	72	5.2	Y	Below
H3-SE000389	2 miles below New Lenox Rd.	213	4.7	Y	Below
H3-SD043702	1 ¼ mile below Holmes Rd.	48.7	1.9		Above
H3-SE000246	Immediately above Woods Pond	41	1.3		Below
H3-SE000060	¼ mile below Holmes Rd.	215	1.9		Above
H3-SE000116	¼ mi below New Lenox Rd.	165	0.7		Below
H3-SE000051	At Holmes Rd.	614	0.1		Above

discarded and recollected. If the hardness of the sediment at an established location does not allow this level of penetration of the sampler, the acceptance criterion will be modified for that location to ensure comparability among replicates, and the modified criterion will be noted in the field log. Sediment type will also be examined visually to ensure that each grab sample is similar to the others at each location.

Two core subsamples will be removed from each undisturbed grab sample for analysis of sediment chemistry, grain size, and TOC. These subsamples will be collected by inserting an approximately 1-ft-long section of 37.5-mm diameter polycarbonate core liner into the undisturbed sediment surface to a depth of 5 cm, capping the top of the core, and extracting the 55 mL of sediment. The two core subsamples from each grab will be composited in a clean stainless-steel bowl and separated into two aliquots of approximately 30 mL and 80 mL for total

PCB/TOC analysis and grain-size determination, respectively. Preservation and analysis techniques for these subsamples will follow the QAPP (WESTON, 2000).

The remainder of each sample will be sieved in the field using a standard 0.5-mm mesh sieve bucket. The sample will then be placed in a 500-mL (or larger, if necessary) glass or polyethylene jar and preserved with 70% denatured ethanol.

Separate qualitative benthic macrofauna samples will be collected for tissue residue analysis at each location using a standard kick-net. A minimum of 0.1 g (per functional group, see below) is required for each of the following analyses:

- PCBs (total, Aroclors, congeners, homologs), total lipids, and percent moisture.
- Dioxins/furans.
- OC pesticides.

Detection limits for tissue residue analyses using this reduced sample volume are provided in Table 4.2-3. If possible, enough material will be collected to perform all analyses (i.e., 0.3 g of tissue). If this is not possible, the analyses will be prioritized in the order shown above. Also if possible, only a single functional feeding group (i.e., only predators) or a single ecologically equivalent group (i.e., burrower) will be composited. If adequate numbers of an individual taxon, functional feeding group, or ecologically equivalent group are present, these organisms/groups will be separated and analyzed separately. If the amount of biomass in the samples allows this separation, consideration will also be given to separating taxa that comprise swallow prey species (i.e., in general, taxa that will develop a flying adult life stage) from non-prey (e.g., annelids and molluscs). If it is not possible to conduct such a separation due to limitations on the biomass present in the samples, all individuals will be composited for a single analysis, making note of the relative proportions of the various taxa.

Samples for residue analysis will be placed into precleaned 4-ounce glass jars, or equivalent, and the jar will be placed on wet ice for return to the laboratory where the separation into functional groups will be conducted. Samples will be frozen at approximately 0^{o} F following separation and shipped frozen to the analytical laboratory.

2.2 TAXONOMY AND BIOMASS ANALYSIS

Preserved macroinvertebrate samples will be sorted in the laboratory using low-power stereoscopic microscopes, and organisms will be identified to Lowest Practical Identification Level (generally genus level, but species identifications will be made when age and condition of the organisms allow) using stereoscopes and compound microscopes as necessary. Species/taxa names and counts will be entered onto laboratory logsheets and subsequently transferred onto electronic spreadsheets for calculation of community summary parameters.

All specimens will be appropriately labeled and preserved as a voucher collection. Biomass (as blotted wet weight) will be determined from the voucher collections following preliminary analysis of the taxonomic results.

2.3 DATA REDUCTION AND ANALYSIS

The following metrics will be calculated for each replicate:

- Number of individuals (N).
- Number of species/taxa (S).
- Diversity (Shannon-Wiener H').
- Evenness (Pielou's J').
- Hilsenhoff Biotic Index (HBI) as modified for soft-bottom communities (Hilsenhoff, 1987; Bode, 1988).

Differences in each of the community parameters among the 13 stations will be tested using statistical techniques described below. If the data satisfy the necessary assumptions of normality and homoscedasticity, ANOVA will be used to test the null hypothesis that there are no significant differences at $p = .05$ among means in the data set for these four parameters. If the data do not allow the use of parametric tests, data transformation and/or non-parametric analogues will be used to examine the same null hypothesis. If the overall analysis indicates significant differences, *a posteriori* comparisons, specifically the Student-Newman-Keuls Multiple Range Test, will be used to further examine the pattern of these differences. If the hypothesis testing indicates significant differences among locations, regression analysis will be used to examine the relationship of the community parameters to varying levels of PCBs and/or other contaminants in the sediments.

In addition to the hypothesis testing described above, a multivariate classification (cluster) analysis will be conducted to investigate patterns of similarities in community structure among replicates and locations. The analysis will be conducted using both a binary (presence/absence) coefficient such as Jaccard's and a quantitative coefficient such as Common Percentage Overlap (equivalent to Bray-Curtis or Czekanowski coefficient) and/or Euclidean Distance. Results of the analysis will be presented as dendrograms constructed using unweighted pair-group (UPGMA) sorting for both coefficients (Boesch, 1977).

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The three primary data quality objectives of the benthic macroinvertebrate community evaluation are outlined in Subsection 1.2. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Taxonomic identification of benthic organisms to LPIL (lowest practical identification level)—Taxa must be identified to the species level whenever possible. When identification to the species level is not possible, the LPIL will be consistent with standard practice for aquatic taxonomy. Although it is always preferable that each taxon is correctly identified, arriving at the correct name is less important than correctly distinguishing and separating each taxon - community summary parameters such as species richness and diversity are not impacted by incorrect taxonomic names but are affected by inadvertent lumping of taxa. Of equal importance is that the level of taxonomy is consistent for all samples.

- Enumeration (counts) for each taxon in each replicate sample—Counts must be made and recorded accurately. Accurate counts are readily achievable in the laboratory - a more important factor in correct enumeration is the collection of consistent volumes in the field sampling effort. Procedures have been established (see Subsection 2.1.4) to ensure that consistent volumes are collected. Care will also be taken in processing samples to ensure that no sample volume is lost.

- Biomass (wet weight) for each taxon or larger taxonomic group—Biomass must be determined accurately and recorded to 1 mg (.001 g) using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude. Accurate determination of biomass is also partly determined by following the field sampling methodologies discussed above.

- Sediment chemistry for PCBs and selected other contaminants—Analysis of sediment for chemical constituents must result in data that are consistent in all respects with other sediment contaminant data collected as part of the larger project. Satisfactory results will be ensured by submitting samples to the same laboratories that are analyzing samples for other components of the program. Quality control specifications for these data are delineated in the projectwide QAPP.

- Sediment grain size distribution—Quality control considerations to ensure achievement of DQOs for this parameter will follow the QAPP.

- Tissue residue concentrations for PCBs and other contaminants by trophic group—Quality control considerations to ensure achievement of DQOs for this parameter will follow the QAPP.

3.1.2 Data Quality Indicators

Data developed in the benthic macroinvertebrate study must meet standards of precision, accuracy, completeness, representativeness, comparability and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. Each of these data quality indicators, some of which are not readily quantifiable for benthic community data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Because of the small-scale spatial heterogeneity inherent in benthic

communities, it is not possible to take repeated independent measurements of the biological parameters. Rather than control and measure precision, the study design includes an increase in the number of replicates to increase the statistical resolution; for this study the large number of replicates (12) is used in this manner. Precision may also be evaluated by assessment of the degree to which sample collection procedures are able to ensure collection of consistent sample volumes. For the measurements that are not unique to the benthic macroinvertebrate study, such as sediment chemistry and grain size, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (benthic taxonomy and biomass) accuracy is defined as meaning that the taxa are correctly represented and identified in each sample, correctly enumerated, and correctly weighed. Accuracy of sorting and identification are a function of each sample being processed under a microscope rather than by eye, and of consistent field sampling techniques. The data generated by this study will also be evaluated for accuracy via comparison with known and/or expected results from similar studies conducted in the Housatonic River or in similar New England systems. For parameters such as sediment contaminants and sediment grain size, accuracy is as defined in the QAPP.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the benthic macroinvertebrate program. To ensure achieving the planned statistical resolution, it is important that completeness of 100% be achieved for all components of this study with the exception of the tissue residue analyses. For this latter study component, the number of analyses will be determined by the material available for collection, therefore establishment of an *a priori* completeness goal is not possible (see Subsection 2.1.4).

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved through sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of representativeness will also be evaluated via comparison with known and/or expected results based on previous investigations of the Lower Housatonic River and other similar systems.

Comparability is a measure of the confidence with which the benthic macroinvertebrate data may be compared to another similar data set. Comparability will be evaluated by examination of the in-station variability in key parameters as determined from the large numbers of replicates to be collected at each location. Comparability will also be evaluated for this data set through comparison with previous benthic work in the Lower Housatonic River and with known characteristics of benthic populations in similar stream systems in the Northeast.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is related for benthic invertebrate investigations to the ability of the taxonomic analysis to resolve the various benthic invertebrates into individual species. This data quality indicator will be evaluated by comparing the number of species-specific separations against the number of unresolved larger taxonomic groups. As the number of unresolved groups increase, the community metrics such as species richness and diversity are less able to resolve differences between samples. Sensitivity is applicable and important for the

chemistry parameters that will be analyzed as part of the benthic macroinvertebrate study. For these parameters, the detection limits for chemistry and grain-size parameters specified in the QAPP will provide appropriate sensitivity for the purpose of providing insight in to factors controlling abundance and distribution of the benthic taxa and populations.

3.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical and physical data are discussed in various sections of the project QAPP and will be used whenever applicable in this study. For the biological data, usability will largely be determined by three factors: (1) the experience of the senior investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field; (2) an evaluation of the taxonomic data both within the study and compared with previous studies in the River and in the New England area; and (3) a direct comparison between the chemistry and grain-size data and similar data developed from co-located samples that have been collected as part of other project components.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

3.2 SAMPLING DESIGN

The rationale for selection of the 13 locations to be sampled in the benthic macroinvertebrate study is presented in Subsection 2.1.2. The locations are not intended to be representative of the entire river but rather are intended to encompass the range of sediment PCB concentrations in the Lower River between the Confluence and Woods Pond; four appropriate reference locations with near-background PCB levels will also be sampled.

Benthic macroinvertebrate community data are typically highly variable in nature. To achieve acceptable statistical resolution it is necessary to collect large numbers of replicate samples. Data will be collected from 12 replicate samples at each of 13 stations. This number of replicates was selected based on power analyses conducted for GE (Chadwick Ecological Consultants, 1994).

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Sampling methods, as discussed in Subsection 2.1.4, were chosen to ensure unbiased (i.e., accurate) samples that will facilitate comparisons with other aquatic benthic data, both from the Housatonic River and from other areas. Steps taken to ensure that sampling does not unnecessarily induce bias include: visual inspection of each sample to confirm satisfactory grab penetration, and confirmation of visual similarity of sediment type within a location. All samples will be collected by trained and experienced personnel; senior oversight of all aspects of the sampling and sample processing will further promote comparability and reduce potential bias.

Subsamples for physical and chemical analyses will be collected following procedures documented in the project QAPP and will therefore be comparable with procedures followed for all other similar efforts throughout the Supplemental Investigation.

3.3.2 Quality Control Samples

The nature of benthic macroinvertebrate sampling does not allow the incorporation of typical duplicate and blank samples as part of the study design. For community metrics, there is no acceptable method of obtaining such samples in a manner analogous to that for duplicates and blanks collected for chemistry analysis.

Duplicate samples for chemistry will not be collected in this study in order to minimize the volume of material removed from each grab. Quality control of chemistry analyses will be provided by co-located samples taken at the benthic macroinvertebrate locations and processed in accordance with the QAPP and via comparisons with results from split samples provided to GE.

3.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all samples to be collected as part of the benthic macroinvertebrate study are provided in Section 4. Subsampling, homogenization, and decontamination between samples will follow procedures established in the QAPP. All samples will be held on wet ice and returned to the field laboratory twice daily and will be either frozen (physical, chemical samples) or preserved (taxonomic samples) at that time. Holding time for physical and chemical samples will follow procedures established in the QAPP; there is no holding time for taxonomic samples.

3.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the collection of benthic macroinvertebrate samples. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Taxonomy Samples

Processing of taxonomy samples will follow procedures established by Lotic, Inc., as documented in the attached Lotic SOP. All samples will be processed by experienced staff who have received specific training in the SOP and whose work is checked periodically by their supervisors and peers. Depending on sample volume and other factors, samples will be sorted by eye or under low-power microscopes. Because the possibility of overlooking organisms in the

samples processed by microscope is very low, no formal quality control procedures are necessary. For samples processed by eye, 5% of the sample volume will be reviewed by someone other than the original sorter. Corrective action, including reprocessing of samples and retraining of staff, will be instituted if these QC checks produce unsatisfactory results.

Quality of taxonomic identification will be assured by maintaining voucher collections and requiring a consensus among all taxonomists at the processing laboratory prior to an identification becoming accepted as a type for the voucher collection. In the event that the taxonomists are unable to agree on an identification, specimens will be sent to a third party for determination.

3.4.2 Physical/Chemical Samples

Samples for sediment grain size, sediment chemistry, and tissue chemistry will be processed following procedures and SOPs provided in the QAPP. These samples will be submitted in catalogs (sample delivery groups) and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP.

3.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Subsection 2.4. The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations, and will be prepared with specific reference to both the data quality objectives specific to the benthic macroinvertebrate study (see Subsection 2.3.1) and Subsection 4.1 of the QAPP.

4. PROCEDURES

4.1 FIELD SAMPLING

4.1.1 Collection of Taxonomy, Grain Size, and Sediment PCB Samples Using the Ponar Grab Sampler

1. Ensure that the equipment necessary for collecting and field-processing the samples is available and accessible. Open the Ponar buckets by pressing on the lever arms and secure the locking pin, maintaining pressure on the lifting line so that the buckets do not close. Lower the device slowly until it comes into contact with the sediment surface, then lift approximately 1 ft and allow to free-fall to the bottom, allowing the lifting line to go slack so that the locking pin will disengage.

2. Slowly retrieve the Ponar sampler for the first 2 ft to allow the buckets to close. Continue to raise the sampler rapidly but smoothly, placing it carefully on top of a

0.5-mm mesh sieve bucket while maintaining tension on the lever arms to ensure that the buckets remain closed. While continuing to maintain tension, unfasten the wing nuts holding one of the two mesh screens and remove the screen to allow inspection of the bucket contents.

3. Visually examine the contents to ensure appropriate sediment type and adequate penetration, and bucket closure to ensure that the contents have not washed excessively out of the buckets. If these conditions are not satisfied, reject the sample and repeat Step 1.

4. If the sample meets the retention criteria, insert a cleaned 37.5-mm-diameter polycarbonate tube vertically into the sediment surface to a depth of approximately 5 cm and withdraw the contained core sample, depositing it into a precleaned stainless-steel bowl. Repeat this procedure and rinse the core tube into the sieve bucket. Mix the two core samples well in the stainless-steel bowl using a precleaned stainless-steel spoon and place approximately 75% of the volume in one 4-oz precleaned glass jar and label for grain-size analysis. The remaining 25% of the sample volume is to be placed in a second 4-oz precleaned glass jar and labeled for PCB analysis.

5. Open the Ponar buckets, allowing the sample to fall into the sieve bucket, and rinse any sediment adhering to the bucket into the sieve with site water. Sieve the sample using site water, place into an appropriately sized glass or plastic jar(s), label, and secure for return to the laboratory. The sample may be preserved with 70% denatured ethanol in the field or upon return to the laboratory if the unpreserved time will not exceed one-half day.

6. Repeat until the 12 replicate samples have been achieved. Thoroughly decontaminate the sampler by flushing with several volumes of site water between samples.

4.1.2 Collection and Initial Processing of Benthic Macroinvertebrates for Tissue Residue Analysis

1. Select a shallow-water location in proximity to the Ponar grab sampling location and of similar sediment type. Deploy the kick-net along the bottom and rinse any excess sediment through the net to reduce the sample volume, taking care not to eliminate any organisms from the sample.

2. Place the contents of the net in a shallow white plastic tray or bowl and mix with site water to assist in separating the organisms from the sediment and plant matrix.

3. Remove organisms from the tray using stainless-steel forceps and place in a precleaned 4-oz glass jar. Repeat the collection as necessary to obtain an estimated minimum of 0.1 g (dry weight) of biomass for analysis. Place the final sample jar on ice and return to the laboratory for initial processing.

4. Upon return to the laboratory, remove the organisms from the collection jar and place in a clean dish or other suitable container. Determine if there is enough biomass

present to separate organisms into functional or trophic groups with at least 0.1 g dry weight per group. If enough material is present, separate organisms into primary consumers and secondary consumers (predators). If material is available for additional splits, organisms having a flying adult life stage (e.g., dipterans, mayflies, caddisflies) should be separated from organisms that would not be potential prey for swallows (e.g., molluscs, annelids).

5. Place the separated organisms into appropriately sized precleaned glass jars or other suitable containers, freeze, and maintain at approximately 0 ^{o}C pending shipment to the designated analytical laboratory.

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ATTACHMENT 1

LABORATORY TECHNIQUES AND TAXONOMIC REFERENCES FROM:

Quality Assurance/Quality Control Document for Macroinvertebrate Evaluations of Surface Waters (Lotic, Incorporated)

LABORATORY TECHNIQUES: BIOMONITORING

Sample Inspection

Samples shipped to LOTIC are examined for any shipping damage. Any noticeable damage (i.e. broken or leaking containers) is reported immediately to the client. Upon arrival, samples are represerved in 80% ethanol if necessary.

Sample Tracking

All appropriate chain-of-custody forms are signed and sample numbers are assigned for in-house use. A Sample Tracking and Custody Worksheet (Figure 12) is initiated for each sample; this worksheet follows the sample through sorting, subsampling and identification procedures. All information must be completed and signed by the appropriate laboratory personnel. Each worksheet is reviewed by the Senior Entomologist for completeness and accuracy.

Sample Sorting

Sample sorting may be performed by the Senior Entomologist, Senior Biologist or a trained laboratory technician. Samples which are to be processed in their entirety are sorted under a dissecting microscope to remove organisms from debris. The debris is retained and represerved in ethanol in a labeled container. One out of every ten samples will be evaluated by a second biologist for sorting completeness.

Subsampling

Benthic samples frequently contain unusually large numbers of macroinvertebrates which render the samples unmanageable. In such cases, subsampling techniques may be used to reduce the volume of the material processed. It is imperative that the personnel

which perform the subsampling be familiar with macroinvertebrate taxonomy, in order to avoid the addition of rocks, sticks and seeds to subsamples. For this reason, subsampling will be performed by the Senior Entomologist, Senior Biologist or a trained laboratory technician.

MEDEP Subsampling Method - Subsampling is performed using the following procedures specified by the Maine DEP. The sorted macroinvertebrate sample is placed into an Imhoff-type settling cone and water is added to bring the total volume to one liter. The sample is then agitated with an aquarium air stone sealed in the bottom of the container and connected to compressed air supply. Large or dense organisms, such as crayfish or some Trichoptera, should be removed from the material to be subsampled and counted separately.

After several minutes, 25% of the sample is removed in five aliquots with a wide mouth dipper and placed in a sample vial. At this point the subsampler should ascertain whether the required 100 organisms have been removed.

EPA Caton Method - Subsampling performed following the EPA approved "Caton Method" involves the use of a standardized gridded screen which contains 30 uniform squares of $6cm^2$ each. The screen portion fits into a slightly larger white tray so that water may be added to the sample. Once water is added, the sample is dispersed over the screen. Special care is taken to completely distribute the sample over all the grids; each grid has an equal probability of being selected. The screen is then removed from the tray, causing the organisms to settle on the screen.

Biologists use a random numbers table to choose grid numbers for organism selection. Organisms adhering to the screen are subsampled using a $6cm^2$ metal square to delineate sample size. A special scoop is used to remove the organisms from the

screen. Organisms which occupy more than one grid are considered to be in the grid which contains the head. For organisms with no discernable head, the organism is considered to be in the grid containing the largest portion of the organism.

Each randomly-selected grid is sorted in its entirety until the designated number of organisms is obtained. The person subsampling records the number of grids needed to achieve the appropriate subsample number. If the number of organisms on a grid is greater than that needed for the subsample, the entire grid is sorted, regardless of the number of remaining organisms. A subsample, therefore, may contain a very large number of organisms, depending on the original sample. Occasionally, samples with very low abundance will be designated for subsampling. In such a case, the sample will be sorted under a dissecting microscope to ensure that all organisms are selected.

Regardless of subsample method, each subsample fraction will be placed in a vial and labeled with the client name, site location, sample date, sample and subsample numbers (if appropriate). The remaining sample is represerved and placed in the original labeled container.

Sample Processing

Trained entomologists will use a stereo microscope to identify macroinvertebrates to the lowest practical level. Organisms are identified, enumerated and returned to the original labeled vial. Chironomidae (midges) are mounted on slides in solutions of CMCP 9/9AF following the methods of Beckett and Lewis (1982)*. Oligochaeta (worms) are mounted in polyvinyl lactophenol. All slide material is identified with a

* Beckett, D.C., and P.A. Lewis. 1982. An Efficient Procedure for Slide Mounting of Larval Chironomids. Trans. Am. Microsc. Soc. 101(1): 96-99.

compound microscope, labeled with the site location, sample number and sample date and placed in slide boxes. All identifications and enumerations are noted on separate macroinvertebrate data sheets.

Target Levels for Identifications

Many macroinvertebrate studies require identifications to the genus level. Some clients, however, may specify identifications be made to the species level. Identifications can be made to the species level in some cases, but may not practical or possible in certain groups. Many macroinvertebrate immatures cannot be accurately identified to species without rearing the specimen to the adult stage. This is, of course, impossible with preserved material. Many macroinvertebrates are known in literature only as adults, and the immature stages are not described. Another obstacle to species identifications is the lack of published dichotomous keys for many insect groups.

LOTIC entomologists will attempt to comply with the desired level of taxonomy for each study. Species level identifications, however, depend upon the availability of published reference material, as well as the age and overall condition of the individual organism.

Taxonomic Consistency

Consistency when processing benthic samples is achieved by following standardized techniques and using approved taxonomic references. The science of taxonomy is constantly changing, necessitating the use of current dichotomous keys to ensure accurate identifications. A list of taxonomic references used by LOTIC is included in Section 14.0.

Frequently, specimens collected in the field are either damaged from collection and shipment or too immature to accurately identify. It is important, therefore, to have personnel on staff with experience in identifying local fauna so that identifications can be made to the lowest possible taxonomic level. Entomologists at LOTIC have processed benthic samples from throughout New England, and are familiar with the taxonomy of state and regional fauna.

Taxonomic QA/QC

Identifications are verified by a second taxonomist or by comparison to LOTIC's reference collection. Identifications in question may be verified by subcontracting to an independent taxonomic expert. Taxonomic names are checked for errors in spelling and phylogenetic classification by the Senior Entomologist. Changes in taxonomy are tracked through time by contacting regional experts, attending regional and national meetings, and reviewing recently published literature.

Sample Processing QA/QC

The following measures are taken to ensure the accuracy of sample processing: one of every ten samples is checked to evaluate the quality of the identification and enumeration of macroinvertebrates. Samples to be reevaluated are chosen randomly after the entire set of samples have been processed. Samples found to vary by 5% in either the number of individual insects or types of taxa are considered to be unacceptable.

If the sample fails to meet the required QA/QC criteria, a second sample from the set is randomly chosen and reevaluated. If this sample also fails to meet the established QA/AC criteria, the entire sample set is reprocessed. The same QA/QC acceptance criteria apply when processing subsamples.

Other quality assurance measures vary with the type of sampling and/or subsampling, and specifics can be provided on an individual basis.

Voucher Collections

Voucher collections containing material from each taxa can be provided on an individual basis, depending on individual client needs. Vials contain several representative specimens of each taxa and are preserved in 80% ethanol. Vials are labeled with the correct taxonomic nomenclature and all pertinent collection information. Reference material mounted on slides is assembled separately in slide boxes. Unless specifically requested, LOTIC reserves the right to retain specimens for its in-house reference collection. When this occurs, it will be marked on the individual data sheets.

Evaluations

Analysis techniques used to evaluate biological communities depend on the scope and goals of each individual study. LOTIC biologists are proficient with techniques which measure the biological integrity of communities and, if needed, can provide guidance to clients regarding appropriate analysis procedures.

Statistical computations are performed by the Senior Biologist or Senior Entomologist and the results of these computations are rechecked for accuracy. Community evaluations are based upon the results of various analysis techniques, knowledge of historical data, and best professional judgement of the biologist performing the evaluations.

Report Format

At project completion, each client receives a detailed report which, depending on the scope of work, includes some or all of the following:

1. A phylogenetically arranged list of macroinvertebrates collected at each site, in addition to their corresponding abundances
2. A review of all sampling, subsampling, identification and analysis procedures
3. QA/QC procedures followed
4. Copies of raw data and custody sheets
5. Results and discussion of each evaluation
6. A hard copy and electronic format of the report

Prior to issuance, data forms, QA/QC reports, computerized data files and project reference collections will be reviewed by the Senior Entomologist or Senior Biologist for accuracy, consistency and completeness.

The client may also receive a voucher collection containing reference specimens from each project. Benthic samples will be retained at LOTIC for five years, or returned at the client's request.

FIGURE 2: SAMPLE TRACKING AND CUSTODY WORKSHEET

LOTIC, INC.
SAMPLE TRACKING AND CUSTODY WORKSHEET*

Client_____ Site_____

Job#_____ Substrate #_____

Sample ID #_____ Date Collected_____

Preservative_____ Collected by_____

Presorted? Y_____ N_____

SORTING

Date Sorted_____ Sorter ID_____

Coarse Sort_____ Full Sort_____

Material Retained? Y____ N_____ Retain #_____

SUBSAMPLING

Performed? Y_____ N_____ On Whole Sample? Y___ N___

Date Subsampled_____ Subsample ID_____

Number of organisms per subsample_____

(check groups subsampled below)

IDENTIFICATION
(initial and date when complete)

COLEOPTERA_____
DIPTERA (Misc.)_____
CHIRONOMIDAE_____
EPHEMEROPTERA_____
MEGALOPTERA_____
NEUROPTERA_____
ODONATA_____
PLECOPTERA_____
TRICHOPTERA_____
MISCELLANEOUS INSECTS_____
NON INSECT TAXA_____
OLIGOCHAETA_____

**This form must remain with the sample information at all times.*

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APPENDIX A.14

ASSESSMENT OF IN SITU STRESSORS AND SEDIMENT TOXICITY IN THE LOWER HOUSATONIC RIVER (BURTON)

Assessment of In Situ Stressors and Sediment Toxicity in the Lower Housatonic River

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APPENDIX A.14

ASSESSMENT OF IN SITU STRESSORS AND SEDIMENT TOXICITY IN THE LOWER HOUSATONIC RIVER

1. INTRODUCTION

Aquatic ecosystems experience disturbances and stress that originate from natural and anthropogenic sources. These stressors commonly include flow, suspended solids, habitat, temperature, light, food, nutrients, ammonia, metals, and organics. The importance of these stressors depends on the sensitivity of the species, populations and communities, and their degree of exposure. Exposure can be split into three quantitative components: magnitude, frequency, and duration. Each of these three components can be a primary contributor to adverse responses. While each of these components are constant and controlled in traditional laboratory toxicity testing, exposures in the field (in situ) are rarely constant and fluctuate through time. Isolating the source of stress to aquatic ecosystems requires a comprehensive evaluation of physical, chemical, and biological profiles, determination of natural and anthropogenic stressors and their spatial and temporal characteristics, and analysis of exposure pathways and patterns. Exposures of aquatic organisms can be separated into three media: overlying water, bedded sediments, and suspended solids. These three media represent major exposure pathways for a large number of stressors. The following work plan outlines approaches for identifying major stressors in the Lower Housatonic River by using a weight-of-evidence approach that combines laboratory and field assessments of physical, chemical, and biological conditions with innovative and traditional toxicity testing.

2. STUDY DESIGN

The study will consist of assessments of: 1) sediment toxicity using draft EPA chronic test methods for *Hyalella azteca* and *Chironomus tentans* (Task 1)*;* 2) in situ exposures of *Daphnia magna, C. tentans, H. azteca,* and *Lumbriculus variegatus* (2 to 10 day) to determine effects and/or contaminant uptake from overlying water, suspended solids, and bedded sediment (Task 2); and 3) laboratory Toxicity Identification Evaluations using *Ceriodaphni a dubia* to fractionate chemical stressor types (Task 3). Task 3 will only be initiated if significant adverse effects are observed in Task 1 or Task 2. These studies form one of the three principal components of the Sediment Quality Triad, and will be coordinated with, and support the outcomes of, other WESTON project tasks, including: 1) mussel transplants; 2) storm flow and transport modeling; 3) habitat and physical-chemical sampling; and 4) fate and effects modeling.

2.1 SEDIMENT COLLECTION, TRANSPORT, STORAGE, AND CHARACTERIZATION

Sediment samples will be collected by WESTON personnel or their subcontractors following project-approved protocols. These methods will include standard quality assurance and quality control measures to ensure that the sediment samples are not significantly altered and that cross contamination does not occur. Site selection criteria will include the following: sediments with grain sizes typical of the site; sediments from sites where PCB concentrations are likely to be in the appropriate concentration range; site of previous analysis; and site near proposed sampling for indigenous benthic communities; physicochemical characterizations, and habitat. Data will be collected for these parameters to help determine which sites will be selected and subsequently sampled. The following site locations will be sampled for sediment toxicity testing:

- Site with high PCB contamination.
- Three sites with chronic (low to moderate) PCB contamination.
- Two background reference sites with very low to non-detectable PCB concentrations.
- In addition, a control treatment will be included. Split samples will be collected for PCB, TOC, and grain size analyses by WESTON's contract laboratories. Samples will be held on ice and shipped within 24 h of collection to Dr. Burton's laboratory. Chain-of-custody procedures will be followed. Samples will be stored at 4 EC until testing. Testing will commence within 2 weeks of sample receipt.

2.2 CULTURING

In all toxicity tests, early life stages of test organisms will be used as prescribed. Culturing procedures follow EPA methods for *H. azteca* and *C. tentans* (EPA, 1994).

2.3 TASK 1. SEDIMENT MACROINVERTEBRATE LABORATORY TOXICITY TESTING

Currently, there are no standardized test methods for chronic toxicity testing of sediments. EPA and the American Society for Testing and Materials (ASTM), however, have identical draft methods that are expected to be finalized during 1999. The testing for this project will follow the latest draft guidance for chronic toxicity testing of *H. azteca* and *C. tentans* (EPA, 1998).

H. azteca are routinely used to assess the toxicity of chemicals in sediments (e.g., Burton et al., 1989; Burton, 1991; Burton et al., 1996a and 1996b). Test duration and endpoints recommended in previously developed standard methods for sediment testing with *H. azteca* include 10-day (d) survival and 10- to 28-d survival and growth. Short-term exposures that only measure effects on survival can be used to identify high levels of contamination, but may not be able to identify moderately contaminated sediments. This method can be used to evaluate potential effects of contaminated sediment on survival, growth, and reproduction of *H. azteca* in a 42-d test.

The sediment exposure starts at Day 0 with 7- to 8-d-old amphipods. On Day 28, amphipods are isolated from the sediment and placed in water-only chambers where reproduction is measured on Day 35 and 42. Typically, amphipods are first in amplexus at about Day 21 to 28 with release of the first brood between Day 28 to 42. Endpoints measured include survival (Day 28, 35, and 42), growth (dry weight measured on Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). EPA and ASTM state that a subset of endpoints may be measured with minor method modifications (EPA, 1999; ASTM, 1999).

Reproduction in amphipods is measured by exposing them in sediment until a few days before the release of the first brood. The amphipods are then sieved from the sediment and held in water to determine the number of young produced. This test design allows a quantitative measure of reproduction. One limitation to this design is that amphipods might recover from effects of sediment exposure during this holding period in clean water; however, amphipods are exposed to sediment during critical developmental stages before release of the first brood in clean water.

The midge *C. tentans* has been used extensively in the short-term assessment of chemicals in sediments (e.g., Burton, 1991; Burton, et al. 1996a and 1996b), and standard methods have been developed for testing with this midge using 10-d exposures (EPA, 1994). *C. tentans* is also a good candidate for long-term toxicity testing because it normally completes its life-cycle in a relatively short period of time (25 to 30 d at 23 EC), and a variety of developmental (growth, survivorship) and reproductive (fecundity) endpoints can be monitored. In addition, emergent adults can be readily collected so it is possible to transfer organisms from the sediment test system to clean, overlying water for direct quantification of reproductive success. In Europe and Canada, the chronic midge method ends after emergence and EPA gives the option for test termination (EPA, 1999). Survival is determined at 20 d and at the end of the test (about 50 to 65 d). Growth is determined at 20 d, which corresponds to the 10-d endpoint in the 10-d *C. tentans* growth test started with 10-d old larvae. From Day 23 to the end of the test, emergence is monitored daily. Each treatment of the life-cycle test is ended separately when no additional emergence has been recorded for 7 consecutive days (the 7-d criterion). When no emergence is recorded from a treatment, ending of that treatment should be based on the control sediment using this 7-d criterion. EPA and ASTM state that minor modifications to the basic methods and a subset of endpoints may be used (EPA, 1999; ASTM, 1999). Interlaboratory comparisons of these test methods suggest the following variances should be expected in measurement endpoints with control sediments: survival (< 20% CV), dry weight (<15% CV), emergence (<30% CV), reproduction (<20% CV), and percent hatch (<10%).

2.4 TASK 2. IN SITU TOXICITY AND BIOACCUMULATION

There are no standardized methods for toxicity and bioaccumulation testing of aquatic organisms in the field (in situ). However, there have been many investigators who have shown the usefulness of in situ testing of caged organisms for determinations of site toxicity and bioaccumulation (see citations in Burton et al., 1996c). In situ testing of indicator organisms provides the following advantages to laboratory testing or surveys of indigenous community structure:

Exposures are more accurate/realistic than laboratory testing, which reduces laboratory to field extrapolations and uncertainties with assumptions, and thereby provides simplified interpretations;

Stressor types and exposure pathways can be better determined, looking at:

- Different species (e.g., amphipods, midges, oligochaetes, mussels, and/or fish);
- Water, suspended solids, and bedded sediment exposures can be separated to determine dominant pathways of stress;
- Stressor categories, such as flow and suspended solids, photoinduced toxicity, ammonia, metals, and nonpolar organics, can be roughly separated to allow for ranking stressor importance;
- Exposure partitioning and resulting effects can be used to link effects in the indigenous communities; and
- Information provided from in situ exposures can be used to validate and refine contaminant transport, fate, and bioaccumulation models.

This information is coupled with laboratory toxicity, indigenous communities, tissue residues, habitat, physicochemical characterizations, and modeling predictions to provide a high degree of certainty in the weight-of-evidence approach.

Testing Design: The Task 2 activities will be divided into two testing periods. Testing periods will include a low- and high-flow exposure period, with subsampling at Day 2 and 10 of each sample period on a species-specific basis. Four test species (*D. magna*, *H. azteca*, *C. tentans*, and *L. variegatus*) will be evaluated in situ in exposure chambers. The exposure chambers are constructed on plastic core tubes ~ 3 inches diameter and 4 inches long. Two windows are cut on opposite sides of the chamber and covered with nylon mesh. The mesh size varies with the experimental treatment, ranging from 10- to 1,000-micron openings. Chambers are placed in the stream, either in the overlying water or buried partially in the sediment with exposures varying with the treatment. For high-flow testing, only water column chambers will be exposed. One duplicate set of chambers will have reduced mesh size openings to allow determinations of flow and suspended solids effects.

Organisms are acclimated to site water temperatures slowly and then added to each test chamber (10 organisms/chamber). The age of the organisms, handling, and culturing follows EPA toxicity test methods (EPA, 1994). For bioaccumulation testing, additional organisms are placed to provide enough tissue mass. For the oligochaete assay, 5 g of tissue are used in each chamber. Chambers are placed in the stream in replicates of four and secured with netting and steel stakes. At Day 2 and 10, chambers will be retrieved and organisms enumerated within 2 h of collection. Test endpoints include survival and tissue residue (*L. variegatus* only).

L. variegatus sampled for tissue analyses will be allowed to depurate sediment particles for several hours. Following that time, organisms will be counted, weighed, and frozen. Tissue analyses will be conducted by the Brehm Research Laboratory of Wright State University. The tissue mass requirements and associated detection limits are provided in the WESTON QAPP (WESTON, 2000). Lipid and total PCB analyses will be conducted, with a subset being analyzed for PCB congeners. The Brehm Research Laboratory and WESTON contract labs will also analyze water, and sediments from within the exposure chambers for PCB concentrations.

Sample treatments will be at the same locations as in Task 1, including a control for each organism, two site references, and four downstream sites.

The effects of water quality during high-flow events will be measured at all test sites. This will involve exposures using chambers with small and large mesh sizes to vary the organism exposure to suspended solids. Exposures will be for 48 h (and 10 days if conditions dictate) and include *D. magna, H. azteca, C. tentans,* and *L. variegatus.* Testing will only be conducted when organisms can be exposed to a significant first flush event.

No standardized interlaboratory variance or power analyses have been conducted with these assays. Testing by the principal investigator's laboratory, however, has shown these tests (using four replicates) to be similar in sensitivity and variability to laboratory toxicity and bioaccumulation testing, with coefficients of variation typically in the 20 to 40% range. Statistically significant differences between controls, references, and test sites will be determined using Dunnett's Test and analysis of variance (ANOVA).

2.5 TASK 3. TOXICITY IDENTIFICATION EVALUATION

The Toxicity Identification Evaluation (TIE) is a process by which water, effluent, or pore water samples are fractionated into various classes of contaminants and then tested for toxicity. This process allows one to determine which group of contaminants (e.g., metals, organics, ammonia) are primarily responsible for toxicity (EPA, 1991a and 1991b). These groups of contaminants include pH sensitive and volatile compounds (such as ammonia), metals, and nonpolar organics. Toxicity is determined by exposing *C. dubia* for 24 h and then measuring survival. The TIE will be conducted following modified draft EPA guidelines for TIEs of sediments (EPA 1991b). The pore water fractions will include initial toxicity tests (within 24 h of sample receipt); baseline ambient pore water; pH adjusted with aeration; pH adjusted with filtration; pH adjusted with C18 filtration; thiosulfate addition; and ethylene diamine tetra-acetate (EDTA) addition fractions. The fraction treatments will target the following toxicant groups: ammonia, metals, and nonpolar organics. As previously discussed, Task 3 will only be initiated if significant adverse effects are observed in either Task 1 or Task 2.

No standardized interlaboratory variance or power analyses have been conducted for these assays. Initial testing by the principal investigator's laboratory indicates that these tests are slightly more variable than traditional laboratory toxicity tests (using four replicates). Statistically significant differences between controls, references, test sites, and treatments will be determined using Dunnett's Test and ANOVAs.

3. DATA ANALYSES

Data analysis will include summary statistics, determinations of variance, and significant ($p<0.05$) differences between sites and the control. Data will be entered into Excel and parametric and nonparametric correlations determined with other physicochemical parameters. An ANOVA will be used to determine significant differences among levels of factors.

4. QUALITY ASSURANCE

Detailed protocols for the chronic toxicity test methods are presented in Attachments 1 and 2 and follow draft ASTM and EPA guidance (EPA, 1998). Other quality assurance procedures are addressed in the Quality Assurance Project Plan for the U.S. Environmental Protection Agency's Freshwater Sediment Toxicity Methods Evaluation (Burton, 1997).

The following discussion presents an overview of several key components associated with the QA/QC procedures incorporated in the laboratory chronic toxicity testing (Task 1); in situ toxicity and bioaccumulation testing (Task 2); and sediment toxicity identification evaluation (Task 3).

4.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

4.1.1 Data Quality Objectives

The primary data quality objectives for the assessment of in situ stressors and sediment toxicity are outlined in the preceding task descriptions. To achieve these objectives the following types of data and specific quality criteria will be required:

- Laboratory chronic and acute toxicity testing data for *Hyalella azteca* and *Chironomus tentans* will be collected after exposing organisms to a range of PCB-contaminated sediments collected within the study area and reference locations. Testing procedures and endpoint evaluations for laboratory testing will in general adhere to guidelines provided in EPA's and ASTM's draft methods for life-cycle assessment tests (EPA, 1998, 1999; and ASTM, 1999) and are presented in detail in Attachments 1 and 2. Age of organisms, handling, and culturing procedures will follow standard EPA toxicity testing methods (EPA, 1994).

- In situ toxicity and bioaccumulation tests will be conducted at locations corresponding to previous sediment sampling locations in an effort to reduce laboratory to field extrapolation uncertainty; to better identify stressor types and exposure pathways; and to validate and refine contaminant transport and bioaccumulation mechanisms. Procedures to be followed for in situ testing are described in Subsection 2.4.

- TIE testing will be used to evaluate classes of chemicals present in sediment pore water that may be responsible for any observed toxicity. Testing procedures that will be followed for TIE testing are modified from EPA's draft guidelines for TIEs of sediments (EPA,1991a&b)

- Sediment, tissue and water chemistry; and sample handling and shipping procedures will follow guidelines presented in the project QAPP (WESTON, 2000). Sediment, water, and tissue analyses for the majority of samples collected in support of this study (except those associated with Task 2) will be analyzed by Wright State University's Brehm Research Laboratory. Sediment pore water extraction will be conducted by Soil

Technology, Inc. All sample processing procedures specifically required for the TIE testing are provided in the QAPP (WESTON, 2000).

- For Tasks 1 and 2, water quality parameters such as temperature, alkalinity, turbidity, hardness, conductivity, flow, pH, dissolved oxygen (DO), and total ammonia will be measured. Procedures that will be used to evaluate these parameters follow guidelines established in SOPs developed by Wright State University's Institute for Environmental Quality and are included in the project QAPP. Instrument-measured parameters such as pH, DO, temperature, conductivity, and turbidity will be taken in accordance with manufacturer-recommended quality assurance steps and control specifications presented in the QAPP (WESTON, 2000).

4.1.2 Data Quality Indicators

Data developed in the assessment of in situ stressors and sediment toxicity must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. Each of these data quality indicators is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Throughout the three tasks incorporated in this evaluation, replicated samples are used to increase the statistical resolution; the number of replicates per task is provided in the corresponding task descriptions. For measurements that are not unique to this study, such as sediment, water, and tissue chemistry, precision is evaluated as defined in the project QAPP (WESTON, 2000).

Accuracy is defined as the agreement of a measurement with its true value. For parameters unique to this study (invertebrate growth, reproduction, and survival), accuracy is defined as meaning: invertebrates were correctly weighted; reproduction was correctly determined; and surviving organisms were correctly enumerated. Accuracy associated with these endpoints is primarily a function of the investigators to weigh organisms using sensitive scales, assess reproduction, and correctly identify all surviving organisms. The data generated by this study will also be evaluated for accuracy by comparisons with on-site reference areas and, where appropriate, laboratory controls. For parameters such as sediment, water, and tissue chemistry, accuracy is as defined in the QAPP (WESTON, 2000).

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the assessment of in situ stressors and sediment toxicity. To ensure achieving the planned statistical resolution, it is important that completeness of as close to 100% as possible be achieved for all components of this study with the exception of the tissue residue analyses. For this latter study component, the number of analyses will be determined by the tissue available after acute, in situ exposures; therefore, the establishment of an a priori completeness goal is not possible.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved sampling design and through careful implementation of the sample processing and analytical methods. This study also included an in situ evaluation that can be used to help identify uncertainties associated with laboratory-based testing methods.

Comparability is a measure of the confidence with which the assessment of in situ stressors and sediment toxicity data may be compared to another similar data set. Comparability in this study will be primarily evaluated by the examination of location-specific variability in key parameters as determined from the large number of replicates collected at each location for each task. Where possible, comparability will also be assessed through comparisons with findings present in the scientific literature.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is related to the investigator's ability to accurately assess endpoint values at a level that allows for a meaningful interpretation of any potential relationships present with the parameters evaluated. In the biological parameters that are being assessed in this study (i.e., survival, growth, and reproduction), sensitivity is applicable and important for the numerous chemical parameters that will be analyzed as part of this study. These parameters include analytical chemistry analyses for which detection limits are specified within the QAPP (WESTON, 2000) and water quality parameters such as temperature, alkalinity, pH, DO, conductivity, ammonia, and hardness, which will be assessed with the sensitivity required by protocols established by Wright State University's Institute for Environmental Quality.

4.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical data are discussed in various sections of the project QAPP (WESTON, 2000) and will be used whenever applicable in this study. For biological data, usability will largely be determined by two factors: (1) the experience of the principal investigator in establishing that the field sampling and sample processing was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling and handling procedures, and (2) the control sample analysis results remaining within established testing procedures norms.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

4.2 SAMPLING DESIGN

The rationale for selection of the six locations to be sampled in this study was presented in the section on Sediment Collection, Transport, Storage, and Characterization. The locations are not intended to be representative of the entire river but rather are intended to encompass the general range of sediment PCB concentrations within the study area; two appropriate reference locations with very low to non-detectable sediment PCB concentrations will also be sampled.

To achieve acceptable statistical resolution it is necessary to have sufficient replicates per sample location; sample replicates vary by task and associated test (see previous text). The number of replicates per location was dictated primarily by peer-reviewed protocols (U.S. EPA 1991a, 1991b, 1994, and 1999) and the professional judgment of the principal investigator.

4.3 SAMPLING METHODOLOGY

4.3.1 Sampling Procedures

Sampling methods are briefly discussed by task in previous sections of this study plan and/or follow methods provided in the QAPP (WESTON, 2000). These methods were chosen to ensure unbiased samples that will facilitate comparisons with other locations sampled within the Housatonic River. Steps taken to ensure that sampling does not necessarily induce bias include: visual inspection of each sample to confirm satisfactory grab penetration; and if one grab is insufficient to collect adequate sample volume, confirmation of visual similarity of sediment type within a location. All samples will be collected by trained and experienced personnel; senior oversight of all aspects of the sampling and sample processing will further promote comparability and reduce potential bias. Subsamples for physical and chemical analysis, and pore water samples for the Task 2 TIE will be collected following procedures documented in the project QAPP (WESTON, 2000).

4.3.2 Quality Control Samples

Wherever sample volumes permit, duplicate samples for chemistry or matrix spikes will be collected in accordance with the QAPP (WESTON, 2000). Duplicate tissue residue will not be collected due to limited sample mass. Laboratory control samples will be evaluated as part of the testing procedures for all three tasks. The number and type of control samples that will be evaluated is presented in previous sections of this study plan or in the standard protocols referenced therein.

4.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all samples to be collected as part of the in situ stressors and sediment toxicity study are provided in previous sections of the study plan. Subsampling, homogenization, and decontamination between samples will follow procedures established in the QAPP (WESTON, 2000). All samples will be held on wet ice and returned to the field laboratory twice daily and will be frozen for subsequent shipping. Holding times for physical, chemical, and pore water extraction samples will follow procedures established in the QAPP (WESTON, 2000).

4.3.4 Training

All sampling will be directed in the field by senior scientist(s) or field crew leader(s) with experience in the collection of sediment or in situ chamber samples. Supporting staff will receive training from

the senior scientist(s) or field crew leader(s) in the overall goals of the study and in the techniques to be followed to ensure collection of quality data.

4.4 SAMPLE ANALYSIS

4.4.1 Sediment Toxicity and TIE Samples

Processing of sediment toxicity and TIE samples will follow procedures recommended in previously cited protocols or (in the case of the in situ testing) procedures developed by the principal investigator. All samples will be processed by experienced staff who have received specific project-related training and whose work is checked periodically by their supervisors and peers. Where required, organisms will be sieved, sorted, and evaluated under low-power microscope.

4.4.2 Physical, Chemical, and Water Quality Samples

Samples for grain size, water quality analysis, sediment, water, and tissue chemistry will be processed following procedures, SOPs, and protocols provided in the QAPP (WESTON, 2000) or as outlined in standard laboratory procedures followed by Wright State University's Institute for Environmental Quality.

4.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in task-specific writeup. The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations and will be prepared with specific reference to both the data quality objectives and Subsection 4.1 of the project QAPP (WESTON, 2000).

5. REPORTING

A report will be submitted to WESTON upon completion of all three tasks. The report will contain summary and raw data tables, and data interpretation in both hard copy and electronic forms. The data will be submitted in Excel and the report in Word 97. Study updates will be provided at project meetings during 1999 and 2000.

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ATTACHMENT 1
METHODS FOR CONDUCTING LONG-TERM SEDIMENT TOXICITY TESTS WITH HYALELLA AZTECA

ATTACHMENT 1

METHODS FOR CONDUCTING LONG-TERM SEDIMENT TOXICITY TESTS WITH *HYALELLA AZTECA*

Conditions for evaluating sublethal endpoints in a sediment toxicity test with H. azteca are summarized in Table A-1. A general activity schedule is outlined in Table 1-2.

The 42-d sediment toxicity test with H. azteca is conducted at 23 °C with a 16L:8D photoperiod at an illuminance of about 500 to 1000 lux (Table 1-1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Amphipods in each test chamber are fed 1.0 mL of YCT (yeast-cerophyl-trout chow) daily (EPA 1994). Each test chamber receives two volume additions/d of overlying water.

Controls will include sand and Trout Farm sediment. In addition to laboratory controls, two field references will be used for evaluating test performance and determining site differences.

A total of 12 replicates, each containing ten 7- to 8-d-old amphipods, are tested for each sample. For the total of 12 replicates the assignment of beakers is as follows: 12 replicates are set up on Day '-1' of which four replicates are used for 28-d growth and survival endpoints, and eight replicates are used for measuring survival and reproduction on Day 35, and survival, reproduction, or growth on Day 42.

Placement of Sediment into Test Chambers: The day before the sediment test is started (Day '-1'), each sediment is thoroughly homogenized and added to the test chambers. Sediment is visually inspected to judge the degree of homogeneity.

Each test chamber will contain the same amount of sediment, determined by volume. Overlying water is added to the chambers on Day '-1' in a manner that minimizes suspension of sediment. Renewal of overlying water is started on Day '-1.' A test begins when the organisms are added to the test chambers (Day 0). Hardness, alkalinity, and ammonia concentrations in the water above the sediment within a treatment should not vary by more than 50% during the test.

Acclimation: Test organisms are cultured and tested at the same temperature. Test organisms will be cultured in the same water that will be used in testing, as recommended by EPA; therefore, no acclimation will be necessary.

Table 1-1. Test Conditions for Conducting a 42-d Sediment Toxicity Test with *Hyalella azteca*

Parameter	Conditions
1. Test type:	Whole-sediment toxicity test with renewal of overlying water
2. Temperature:	23 ± 1 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	500 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	300-mL high-form lipless beaker
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL in the sediment exposure from Day 0 to Day 28 (175 to 275 mL in the water-only exposure from Day 28 to Day 42)
9. Renewal of overlying water:	2 volume additions/d; continuous or intermittent (e.g., one volume addition every 12 h)
10. Age of organisms:	7- to 8-d old at the start of the test
11. Number of organisms/chamber:	10
12. Number of replicate chambers/treatment:	12 (4 for 28-d survival and growth; 4 for 35-d survival and reproduction; and 4 for 42-d survival, growth, and reproduction). Reproduction is more variable than growth or survival; therefore, more replicates might be needed to establish statistical differences among treatments
13. Feeding:	YCT food, fed 1.0 mL (1800 mg/L stock) daily to each test chamber
14. Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15. Overlying water:	Culture water, well water, surface water or site water. Use of reconstituted water is not recommended.
16. Test chamber cleaning:	If screens become clogged during a test; gently brush the outside of the screen.
17. Overlying water quality:	Hardness, alkalinity, conductivity, and ammonia at the beginning and end of a sediment exposure (Day 0 and 28). Temperature daily. Conductivity weekly. Dissolved oxygen (DO) and pH three times/ week. Concentrations of DO should be measured more often if DO drops more than 1 mg/L since the previous measurement.
18. Test duration:	42 d
19. Endpoints:	28-d survival and growth; 35-d survival and reproduction, and 42-d survival, growth, and reproduction.
20. Test acceptability:	Minimum mean control survival of 80% on Day 28.

Table 1-2. General Activity Schedule for Conducting a 42-d Sediment Toxicity Test with *Hyalella azteca*

Day	Activity
Pre-Test	
-7	Remove adults and isolate <24-h old amphipods.
-8	Separate known-age amphipods from the cultures and place in holding chambers. Begin preparing food for the test. The <24-h amphipods are fed 10 mL of YCT (1800 mg/L stock solution) and 10 mL of *Selenastrum capricornutum* (about 3.0 x 10^7 cells/mL) on the first day of isolation and 5 mL of both YCT and *S. capricornutum* on the 3rd and 5th d after isolation.
-6 to -2	Feed and observe isolated amphipods, monitor water quality (e.g., temperature and dissolved oxygen).
-1	Feed and observe isolated amphipods, monitor water quality. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
Sediment Test	
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, and ammonia). Transfer ten 7- to 8-d old amphipods into each test chamber. Release organisms under the surface of the water. Add 1.0 mL of YCT (1800 mg/L stock) into each test chamber. Archive 80 amphipods for dry weight determination. Observe behavior of test organisms.
1 to 27	Add 1.0 mL of YCT to each test beaker. Measure temperature daily, conductivity weekly, and dissolved oxygen (DO) and pH three times a week. Observe behavior of test organisms.
28	Measure temperature, dissolved oxygen, pH, hardness, alkalinity, conductivity, and ammonia. End the sediment-exposure portion of the test by collecting the amphipods with a #40 mesh sieve (425-µm mesh; U.S. standard size sieve). Use four replicates for growth measurements: count survivors and preserve organisms in sugar formalin for growth measurements. Eight replicates for reproduction measurements: Place survivors in individual replicate water-only beakers and add 1.0 mL of YCT to each test beaker/d and two volume additions/d of overlying water.
Reproduction Phase	
29 to 35	Feed daily. Measure temperature daily, conductivity weekly, DO and pH three times a week. Measure hardness and alkalinity weekly. Observe behavior of test organisms.
35	Record the number of surviving adults and remove offspring. Return adults to their original individual beakers and add food.
36 to 41	Feed daily. Measure temperature daily, conductivity weekly, DO and pH three times a week. Measure hardness and alkalinity weekly. Observe behavior of test organisms.
41	Same as Day 1. Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, and ammonia).
42	Record the number of surviving adults and offspring. Surviving adult amphipods on Day 42 are preserved in sugar formalin solution. The number of adult males in each beaker is determined from this archived sample. This information is used to calculate the number of young produced per female per replicate from Day 28 to Day 42.

Placing Organisms in Test Chambers: Amphipods will be introduced into the overlying water below the air-water interface. Weight will be measured on a subset of 20 amphipods used to start the test.

Feeding: For each wet beaker, 1.0 mL of YCT is added from Day 0 to Day 42. The amount of food added to the test chambers is kept to a minimum to avoid microbial growth and water fouling. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding will be suspended for one or more days. A drop in dissolved oxygen below 2.5 mg/L during a test may indicate that the food added is not being consumed. Feeding will be suspended for the amount of time necessary to increase the dissolved oxygen concentration. If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface will be made daily.

Monitoring a Test: All chambers will be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system will be monitored daily.

Overlying water quality characteristics monitored during the assays include: conductivity, hardness, alkalinity, and ammonia will be measured in all treatments at the beginning and at the end of the sediment exposure portion of the test. Water quality characteristics will also be measured at the beginning and end of the reproductive phase (Day 29 to Day 42). Conductivity will be measured weekly and DO and pH three times/week.

Dissolved oxygen will be measured a minimum of three times a week and will be at a minimum of 2.5 mg/L. Aeration will be used to maintain dissolved oxygen in the overlying water above 2.5 mg/L.

Temperature will be measured at least daily in at least one test chamber from each treatment. The daily mean test temperature must be within ±1 °C of 23 °C. The instantaneous temperature must always be within ±3 °C of 23 °C.

Ending a Test: Endpoints monitored include 28-d survival and growth of amphipods, 35-d survival and reproduction, and 42-d survival, growth, and reproduction (number of young/female) of amphipods. Growth or reproduction of amphipods may be a more sensitive toxicity endpoint compared to survival.

On Day 28, 4 of the replicate beakers/sediment are sieved with a #40 mesh sieve (425-µm mesh; U.S. standard size sieve) to remove surviving amphipods for growth determinations. Growth of amphipods will be reported as dry weight. Dry weight of amphipods in each replicate will be determined on Day 28 and 42. Dry weight of amphipods will be determined by: (1) transferring rinsed amphipods to a pre-weighed aluminum pan; (2) drying these samples for 24 h at 60oC; and (3) weighing the pan and dried amphipods on a balance to the nearest 0.01 mg. Average dry weight of individual amphipods in each replicate is calculated from these data.

On Day 28, the remaining eight beakers/sediment are also sieved and the surviving amphipods in each sediment beaker are placed in 300-mL water-only beakers containing 150 to 275 mL of overlying water and a 5 cm x 5 cm piece of Nitex screen or 3M fiber mat. Each water-only beaker receives 1.0 mL of YCT stock solution and about two volume additions of water daily.

Reproduction of amphipods is measured on Day 35 and Day 42 in the water-only beakers by removing and counting the adults and young in each beaker. On Day 35, the adults are then returned to the same water-only beakers. Adult amphipods surviving on Day 42 are preserved in sugar formalin. The number of adult

females is determined by simply counting the adult males (mature male amphipods will have an enlarged second gnathopod) and assuming all other adults are females. The number of females is used to determine number of young/female/beaker from Day 28 to Day 42. Growth will also be measured for these adult amphipods.

Interpretation of Results: Endpoints measured in the 42-d H. azteca test include survival (Day 28, 35, and 42), growth (Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). Reproduction is often more variable than growth. Some investigators have shown growth provides unique information that can help discriminate toxic effects of exposure to contaminants in sediment, while others have not seen differences from survival information.

On rare occasions, test organism responses in control sediments may exhibit responses that are less than reference or test sediments. This may be due to the poor nutritional content of the control sediment or other unknown physicochemical factors. Currently, there are no standard control sediments that can be strongly recommended for chronic toxicity testing due to a lack of testing and research. Should poor responses be observed in a control sediment, a secondary control or reference sediment may be substituted for comparisons of significance. Reference sediment substitution will not invalidate the test, but simply adds some degree of uncertainty in the determination of ecological significance.

EPA (EPA 1999) recently conducted interlaboratory variance testing with the 42-day H. azteca assay. In these tests, the draft standard methods were used. The minimum detectable differences for amphipod survival at 28-d and 42-d ranged from 8 to 12% in moderately contaminated sediments. Minimum detectable differences for reproductive endpoints were higher, as expected, ranging from 19 to 25%.

ATTACHMENT 2
METHODS FOR CONDUCTING LONG-TERM SEDIMENT TOXICITY TESTS WITH CHIRONOMUS TENTANS

ATTACHMENT 2

METHODS FOR CONDUCTING LONG-TERM SEDIMENT TOXICITY TESTS WITH *CHIRONOMUS TENTANS*

Conditions for conducting a long-term sediment toxicity test with *C. tentans* are summarized in Table 2-1. A general activity schedule is outlined in Table 2-2.

The long-term sediment toxicity test with *C. tentans* is conducted at 23 °C with a 16L:8D photoperiod at an illuminance of about 500 to 1000 lux (Table 2-1). Test chambers, sediment addition, water renewal, and water quality monitoring are as described in Attachment 1 for *H. azteca*.

A total of 8 replicates, each containing 10, <24-h-old larvae, are tested for each sample. For the total of 8 replicates the assignment of beakers is as follows: initially, 8 replicates are set up on Day '-1,' of which four replicates are used for 20-d growth and survival endpoints and 4 replicates for determination of emergence and mortality. It is typical for males to begin emerging 4 to 7 d before females. Midges in each test chamber are fed 1.5 mL of a 4-g/L Tetrafin® suspension daily. Endpoints monitored include 20-d survival and growth, emergence, and mortality. Reproduction and egg hatchability will not be assessed. Control samples include: alpha-cellulose formulated sediment (EPA 1999), Florissant soil, and Trout Farm sediment. The reference samples are as used for the amphipod assays.

Collection of Egg Cases: Egg cases are obtained from adult midges held in a sex ratio of 1:3 male:female. Adults are collected 4 days before starting a test. The day after collection of adults, six to eight of the larger "C" shaped egg cases are transferred to a petri dish with culture water and incubated (at 23 °C). Hatching typically begins around 48 h and larvae typically leave the egg case 24 h after the first hatch.

Table 2-1. Test Conditions for Conducting a Long-Term Sediment Toxicity Test with *Chironomus tentans*

Parameter	Conditions
1. Test type:	Whole-sediment toxicity test with renewal of overlying water
2. Temperature:	23 ±1 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	500 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	300-mL high-form lipless beaker
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	2 volume additions/d; continuous or intermittent (e.g., one volume addition every 12 h)
10. Age of organisms:	< 24 h old larvae
11. Number of organisms/chamber	10
12. Number of replicate chambers/treatment:	8
13. Feeding:	Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber (1.5 mL contains 6.0 mg of dry solids); starting Day '-1'
14. Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L
15. Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16. Test chamber cleaning:	If screens become clogged during a test; gently brush the outside of the screen
17. Overlying water quality:	Hardness, alkalinity, conductivity, and ammonia at the beginning and end of a test. Temperature daily (ideally continuously). Dissolved oxygen (DO) and pH three times/ week. Concentrations of DO should be measured more often if DO has declined by more than 1 mg/L since previous measurement.
18. Test duration:	About 40 to 50 d; each treatment is ended separately when no additional emergence has been recorded for seven consecutive days. When no emergence is recorded from a treatment, termination of that treatment should be based on the control sediment using this 7-d criterion.
19. Endpoints:	20-d survival and growth; female and male emergence, and adult mortality.
20. Test acceptability:	Minimum average size of C. tentans in the control sediment at 20 d must be at least 0.6 mg/surviving organism as dry weights or 0.48 mg/surviving organism as AFDW. Emergence should be > 50%.

Table 2-2. General Activity Schedule for Conducting a Long-Term Sediment Toxicity Test with *Chironomus tentans*

Day	Activity
-4	Start reproduction flask with cultured adults (1:3 male:female ratio). For example, for 15 to 25 egg cases, 10 males and 30 females are typically collected. Egg cases typically range from 600 to 1,500 egg/case.
-3	Collect egg cases (a minimum of 6 to 8) and incubate at 23 °C.
-2	Check egg cases for viability and development.
-1	1. Check egg cases for hatch and development.
	2. Add 100 mL of homogenized test sediment to each replicate beaker and place in corresponding treatment holding tank. After sediment has settled for at least 1 h, add 1.5 mL Tetrafin slurry (4 g/L solution) to each beaker. Overlying water renewal begins at this time.
0	1. Transfer all egg cases to a crystallizing dish containing control water. Discard larvae that have already left the egg cases in the incubation dishes. Add 1.5 mL food to each test beaker with sediment before the larvae are added. Add 12 larvae to each replicate beaker (beakers are chosen by random block assignment). Let beakers sit (outside the test system) for 1 h following addition of the larvae. After this period, gently immerse all beakers into their respective treatment holding tanks.
	2. Measure temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity, and ammonia at start of test.
1-End	On a daily basis, add 1.5 mL food to each beaker. Measure temperature daily. Measure the pH and dissolved oxygen three times a week during the test. If the DO has declined more than 1 mg/L since previous reading, increase frequency of DO measurements and aerate if DO continues to be less than 2.5 mg/L. Measure hardness, alkalinity, conductivity, and ammonia weekly.
6	For auxiliary male production, start reproduction flask with culture adults (e.g., 10 males and 30 females; 1:3 male to female ratio).
7-10	Set up schedule for auxiliary male beakers (4 replicates/treatment) the same as that described above for Day '-3' to Day 0.
19	In preparation for weight determinations, ash weigh pans at 550 °C for 2 h. Note that the weigh boats should be ashed before use to eliminate weighing errors due to the pan oxidizing during ashing of samples.
20	Randomly select four replicates from each treatment and sieve the sediment to recover larvae for growth and survival determinations. Pool all living larvae per replicate and dry the sample to a constant weight (e.g., 60 °C for 24 h). Install emergence traps on each reproductive replicate beakers.
21	The sample with dried larvae is brought to room temperature in a dessicator and weighed to the nearest 0.01 mg. The dried larvae in the pan are then ashed at 550oC for 2 h. The pan with the ashed larvae is then reweighed and the tissue mass of the larvae determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.
23-End	On a daily basis, record emergence of males and females, and pupal and adult mortality for previously collected adults.
33-End	Transfer males emerging from the auxiliary male replicates to individual inverted petri dishes. The auxiliary males are used for mating with females from corresponding treatments from which most of the males had already emerged or in which no males emerged.
40-End	After 7 d of no recorded emergence in a given treatment, end the treatment by sieving the sediment to recover larvae, pupae, or pupal exuviae. When no emergence occurs in a test treatment, that treatment can be ended once emergence in the control sediment has ended using the 7-d criterion.

Hatching of Eggs: Hatching of eggs should be complete by about 72 h. Hatched larvae remain with the egg case for about 24 h and appear to use the gelatinous component of the egg case as an initial source of food. After the first 24-h period with larvae hatched, egg cases are transferred from the incubation petri dish to another dish with clean test water. The action of transferring the egg case stimulates the remaining larvae to leave the egg case within a few hours. These are the larvae that are used to start the test.

Placing Organisms in Test Chambers: To start the test, larvae are collected with a Pasteur pipette from the bottom of the incubation dish with the aid of a dissecting microscope. Test organisms are pipetted directly into overlying water. Larvae are transferred to exposure chambers within 4 h of emerging from the egg case.

Feeding: Each beaker received a daily addition of 1.5 mL of Tetrafin® (4 mg/mL dry solids). Feeding is curtailed under circumstances described in the amphipod methods.

Dissolved Oxygen: Routine chemistries on Day 0 should be taken before organisms are placed in the test beakers. Excursions of DO as low as 1.5 mg/L did not seem to have an effect on midge survival and development (EPA 1999). Based on these findings, periodic depressions of DO below 2.5 mg/L (but not below 1.5 mg/L) are not likely to adversely affect test results, and thus should not be a reason to discard test data. Nonetheless, tests should be managed toward a goal of DO > 2.5 mg/L to ensure satisfactory performance. If the DO level of the water falls below 2.5 mg/L for any one treatment, aeration will be conducted in all replicates for the duration of the test.

Monitoring Survival and Growth: At Day 20, 4 of the initial 12 replicates will be selected for use in growth and survival measurements. Using a #40 sieve (425-µm mesh) to remove larvae from sediment, *C. tentans* is collected. Surviving larvae are kept separated by replicate for weight measurements; if pupae are recovered, these organisms are included in survival data but not included in the growth data.

The ash-free dry weight (AFDW) of midges is determined for the growth endpoint. All living larvae per replicate are combined and dried to a constant weight (e.g., 60 °C for 24 h). All weigh boats are ashed before use to eliminate weighing errors due to the pan oxidizing during ashing. The sample is brought to room temperature in a desiccator and weighed to the nearest 0.01 mg to obtain mean weights per surviving organism per replicate. The dried larvae in the pan are then ashed at 550 °C for 2 h. The pan with the ashed larvae is then re-weighed and the tissue mass of the larvae is determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.

Monitoring Emergence: Emergence traps are placed on the reproductive replicates on Day 20 (emergence traps for the auxiliary beakers are added at the corresponding 20-d time interval for those replicates). At 23 °C, emergence in control sediments typically begins on or about Day 23 and continues for about 2 weeks. However, in contaminated sediments, the emergence period may be extended by weeks.

Two categories are recorded for emergence: complete emergence and partial emergence. Complete emergence occurs when an organism has shed the pupal exuviae completely and escapes the surface tension of the water. If complete emergence has occurred but the adult has not escaped the surface tension of the water, the adult will die within 24 h. Partial emergence occurs when an adult has only partially shed the pupal exuviae. These adults will also die, an event that can be recorded after 24 h.

Between Day 23 and the end of the test, emergence of males and females, pupal and adult, and mortality for adults is recorded daily for the reproductive replicates.

Ending a Test: The point at which the life-cycle test is ended depends upon the sediments being evaluated. In clean sediments, the test typically requires 40 to 50 d from initial setup to completion if all possible measurement endpoints are evaluated. However, test duration will increase in the presence of environmental stressors that act to reduce growth and delay emergence. Where a strong gradient of sediment contamination exists, emergence patterns between treatments will likely become asynchronous, in which case each treatment needs to be ended separately. For this reason, emergence is used as a guide to decide when to end a test. Testing will be terminated with completion of emergence.

For treatments in which emergence has occurred, the treatment (not the entire test) is ended when no further emergence is recorded over a period of 7 d (the 7-d criterion). At this time, all beakers of the treatment are sieved through a #40 mesh screen (425 µm) to recover remaining larvae, pupae, or pupal castes. When no emergence is recorded in a treatment at any time during the test, that treatment can be ended once emergence in the control sediment has ended using the 7-d criterion.

Interpretation of Results: Endpoints measured in the *C. tentans* test include survival, growth, and emergence. On rare occasions, test organism responses in control sediments may exhibit responses that are poorer than reference or test sediments. This may be due to the poor nutritional content of the control sediment or other unknown physicochemical factors. Currently, there are no standard control sediments that can be strongly recommended for chronic toxicity testing due to a lack of testing and research. Should poor responses be observed in a control sediment, a secondary control or reference sediment may be substituted for comparisons of significance. Reference sediment substitution will not invalidate the test, but will simply add a degree of uncertainty in the determination of ecological significance.

Recently, EPA conducted interlaboratory variance testing with the chronic *C. tentans* assay (EPA 1999). In these tests, the draft standard methods were used (EPA 1998). The minimum detectable differences have not been calculated at this time, but will be available in the near future to provide a point of comparison for the test assays. It is expected that the minimum detectable difference for 28-d survival and emergence endpoints will be in the 15 to 30% range.

APPENDIX A.15

AN IN SITU ASSESSMENT OF CHEMICAL STRESSORS IN THE LOWER HOUSATONIC RIVER, MA, USING BIOACCUMULATION AND CONDITION IN TRANSPLANTED FRESHWATER MUSSELS (NEVES AND PATTERSON)

Appendix A.15

An In Situ Assessment of Chemical Stressors in the Lower Housatonic River, Massachusetts, Using Bioaccumulation and Condition in Transplanted Freshwater Mussels

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1. INTRODUCTION

1.1 BACKGROUND

A mussel inventory of the Lower Housatonic River in Massachusetts conducted in 1998 indicated an almost complete absence of mussels. Monitoring of habitats in the river where mussels would be expected to occur was not successful in locating significant populations of these molluscs. The decline of mussels in rivers of New England, as well as elsewhere in the United States since the late 1800s, has been well documented (Bogan, 1993). Studies suggest that this decline is a direct response to a degradation of the water quality in the nation's rivers. Several factors have been implicated in this degradation, including the industrialization and urbanization of watersheds, which has resulted in the point and non-point discharge of chemical pollutants to these waters, the agricultural runoff of nutrients and silt into waterways, and the damming of rivers for various uses. In recent monitoring studies of riverine systems, bivalve molluscs have demonstrated utility as biological indicators of chemical exposure because of their ability to concentrate and integrate chemicals from water and sediment in their tissues. Toward that end, in situ monitoring of transplanted mussels to the Lower Housatonic River is proposed to elucidate the extent to which chemical contamination may be responsible for the absence of mussels from the river in this area.

1.2 OBJECTIVES

The primary data quality objectives of the mussel transplant study are:

- Determine the effect of chemical stressors in surface water and sediment to mussel populations that are either resident in the Lower Housatonic River or are believed to have occurred historically in that area.

- Determine the bioaccumulation potential of PCBs and other select chemicals in mussels potentially resident in the Lower Housatonic River.

- Demonstrate the extent of bioavailable PCBs and other select chemicals in the Lower Housatonic River.

The data generated in this study will be used in the assessment of ecological risk both to the mussel community itself as well as to animals that may forage mussels as part of their diet.

2. STUDY DESIGN

The Eastern elliptio, *Elliptio complanata*, will be transplanted to five monitoring stations in the Lower Housatonic River. *E. complanata* is a freshwater mussel common to the rivers of New England and has been widely used in in situ monitoring of effects of chemical contamination in mussels. The general location of the monitoring stations in the lower Housatonic River include:

- One reference station located upstream of the influence of the GE facility in the East Branch of the Housatonic River near Dalton.
- One station located downstream of the GE facility in the vicinity of Holmes Road.
- One station located between Holmes Road and New Lenox Road.
- One station located in Housatonic River immediately above Woods Pond.
- One station located downstream of Woods Pond in Great Barrington.

In addition to the monitoring locations to which the mussels are transplanted in the Housatonic River, the Connecticut River, from which the resident mussels are collected, will also serve as a reference monitoring location. Sample testing and sample design will be replicated at that location.

Specific monitoring locations in the Housatonic River will be determined following a review of the available PCB data for the river. The three mussel exposure locations in the study target area will be selected to reflect a PCB gradient in the sediments (e.g., low, medium, high PCB concentrations). Mussels will also be placed at one upstream reference area and one location downstream in Great Barrington. The upstream reference location and one of the target exposure locations will be co-located with two of the Sediment Quality Triad locations (see Appendix A.13). This design provides a total of five exposure locations in the Housatonic River and one additional reference location in the Connecticut River.

To conduct this study, several hundred *E. complanata* will be collected from the Connecticut River, which is known to contain substantial beds of resident mussels expected to be free of chemical contamination. Prior to the harvesting of a bed, several mussels will be collected and examined for general condition by an experienced malacologist. In addition, a sample will be submitted for chemical analysis for PCBs as well as other chemical constituents to ensure the use of non-contaminated mussels. Should the condition of the mussels indicate a pathology or a stressed condition, mussels from that bed will not be used for the study. To limit any potential effects of shell length on glycogen levels, only mussels ranging from 60 to 80 mm in shell length (approximately 2 to 3 inches) will be collected for use in this study. Mussels will be grouped in 10-mm increments and placed in aerated coolers filled with water from the Connecticut River. Fresh water from the Connecticut River will be added to the coolers approximately every 2 to 4 hours until the mussels are distributed for deployment in the Housatonic River.

In addition, three sediment samples and one surface water sample will be collected from representative locations on the bed from which the mussels were obtained. Sediment samples will be analyzed for the following chemicals:

- PCBs (total and Aroclors)
- Ammonia
- Total organic carbon
- Grain size

In addition, one composite sediment sample will also be analyzed for the following:

- PCBs (total and Aroclors)
- PCBs (congeners and homologs)
- Dioxins/furans
- Appendix IX OC pesticides
- Total organic carbon
- Grain size

These sediment samples will be taken immediately after conclusion of the exposure period, so as not to disturb the sediments during the deployment. Surface water samples will be analyzed for conventional parameters (e.g., temperature, dissolved oxygen, pH, suspended solids, alkalinity, and specific conductance), as well as the organic compounds described above.

To establish the background chemical load in mussel tissue prior to assessing bioaccumulation at the monitoring stations, composite mussel samples will be harvested for tissue analysis of PCB (total and Aroclors), lipid, and moisture. Additional mussel samples will be submitted for analysis of PCB congeners/homologs, dioxins/furans, and Appendix IX OC pesticides. Chemical concentration in mussel tissues will be reported in terms of wet weight and dry weight. In addition to harvesting tissue from these samples, whole-animal wet weight as well as whole animal wet weight minus the shell will be recorded.

Visual examination of the outer shell of freshwater mussels often gives little or no indication of general condition. Measurement of growth, a traditional indicator of condition, also is problematic due to slow growth rates of adult mussels, chipping of the shell margins, and measurement error. Consequently, biochemical analysis of the soft tissues is often needed to determine mussel condition. Patterson et al. (1997) have shown glycogen changes to be a sensitive indicator of the physiological condition of freshwater mussels. Glycogen, the primary energy reserve in bivalves, drives many important physiological processes and may be used to endure short-term exposure to anoxia, emersion, and reduced food supplies (Bayne, 1976; Gabbott, 1983; Bayne et al., 1985; and Hummel et al., 1988). Glycogen stores also have been shown to change in response to environmental perturbations such as temperature extremes, anaerobiosis, chemical pollutants, and general nutrition (Hummel et al., 1989; de Zwann and Wijsmann, 1976). While drastically reduced glycogen levels may lead to reduced survival, smaller reductions in the glycogen levels of adult bivalves also may have sublethal effects on reproduction including reduced fecundity and reduced growth rates of developing offspring (Bayne, 1972; Helm et al., 1973; and Bayne et al., 1975).

The glycogen content of a subset of 20 mussels from the reference area as well as the deployment sites will be determined using the technique described by Keppler and Decker, 1974 (as cited in Patterson et al., 1997). A 50- to 100-mg sample of preserved mantle tissue will be removed from the anterior/ventral portion of the mantle, blotted dry to remove the excess ethanol, and weighed. Tissue samples will then be homogenized at 4 °C for 2 hours in 3M perchloric acid ($HClO_4$). After homogenization, the perchloric acid will be neutralized with 2M $KHCO_3$. The extracted glycogen will be converted to glucose with the enzyme amyloglucosidase (Sigma Chemical Co.), combined with a dye solution (0-dianisidine dihydrochloride), and absorbance measured in a Beckman spectrophotometer at 450 nm. Total glycogen will be determined from a standard curve of glycogen extracted from the blue mussel, *Mytilus edulis*, and expressed in milligrams glycogen per gram preserved mantle tissue. Using experimentally

determined conversion factors, glycogen also will be expressed as milligrams glycogen per gram dry weight of mantle tissue.

Mussel deployment cages (17 in by 17 in by 10 in) will be constructed of plastic-coated metal with 1-in^2 mesh to permit normal transport of food to the mussels. Cages will be placed into the sediment 4 to 5 inches in areas of adequate current. Cages will be anchored on all four sides with rebar and attached to cinder blocks to prevent movement of the cages during high flow. Mussels will be hand-placed in the sediment by divers to ensure proper burrowing and feeding behavior. For each deployment cage, approximately 50 mussels will be used. At each station, three mussel cages will be deployed, resulting in a total of 150 mussels per station.

At each station, sediment samples will be taken for chemical analyses as described above.

Mussels will be deployed for a total exposure period of 84 days. An 84-day exposure period was chosen because 1) this time period has been widely used in mussel transplant experiments (Salazar and Salazar, 1995); and 2) Patterson et al. (1997) have shown significant changes in mussel glycogen levels in as little as 40 days during starvation experiments.

Mid-test measurements will be made after approximately 42 days to determine mussel glycogen levels and to determine the extent of chemical accumulation that has occurred over that period. Mussel deployment cages will be retrieved after an additional 42 days. Mortality will be noted and dead mussels removed. At each monitoring station, up to 10 mussels will be taken from each cage and placed in a bucket containing water equating to a total of up to 30 mussels collected from each station. This represents a subset of approximately 20% of the mussels deployed at each station. For each monitoring location, the mussel tissues will be combined in several composite samples (six mussels per composite) for analyses. The number of composites may be modified based on the rate of survival at each station. All samples will be analyzed for PCBs (total, Aroclors, congeners, and homologs), wet and dry weight, and lipid levels. Of these, a subset will receive additional analyses for dioxins/furans, and Appendix IX OC pesticides.

In addition, 20 individual mussels will be harvested from each station for glycogen analysis. At the Connecticut River reference site, an additional 20 samples will be collected from the bed adjacent to the cages to evaluate the potential effects of caging on the glycogen levels.

Note that the total number of samples that will be processed at each station will be contingent on the survival of the mussels.

In summary, the mid-test analyses will include the following:

- Composite mussel samples (approximately five per station) for PCBs, whole tissue wet and dry weight, and lipid concentrations.

- Composite mussel samples (one per station) for dioxins/furans, and Appendix IX OC pesticides. Note that these additional analyses are conducted on two of the five samples discussed above.

- 120 individual mussel samples (20 per station) for glycogen analysis. Note that an additional 20 mussels will be collected outside the cages at the Connecticut River reference site.

At the end of the test, the mussel deployment cages will be removed and processing procedures will be similar to that described above for the mid-test sampling. Again, mortality will be noted and remaining animals will be collected. All measurements and analyses will be similar to that described above.

Expected total analyses will include the following:

- Composite mussel samples (approximately five per station) for PCB and lipid concentrations.

- Composite mussel samples (one per station) for dioxin/furans and Appendix IX OC pesticides.

- 300 samples (20 per station plus the initial 20 mussels; 40 outside the cages in the Connecticut River) for glycogen.

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The three primary data quality objectives of the mussel bioaccumulation/condition study were outlined in Subsection 1.2. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Sediment chemistry for PCBs, selected other contaminants, and physical parameters: Analysis of sediment for chemical constituents and other properties must result in data that are consistent in all respects with other sediment contaminant data collected as part of the Housatonic River project. Satisfactory results will be ensured by submitting samples to the same laboratories that are analyzing samples for other components of the program. Quality control specifications for these data are delineated in the project QAPP (WESTON, 2000).

- Water chemistry for PCBs, selected other contaminants, and physical parameters: Analysis of water for chemical constituents and other properties must also result in data that are consistent in all respects with other comparable data collected as part of the Housatonic River project. Satisfactory results will be ensured by submitting samples to the same laboratories that are analyzing samples for other components of the program and using the same instruments and procedures for field measurements. Quality control specifications for these data are delineated in the project QAPP.

- Tissue chemistry for PCBs and selected other contaminants: Analysis of mussel tissue for chemical constituents must similarly result in data that are consistent in all respects with other tissue residue data collected as part of the Housatonic River project. Satisfactory results will be ensured by submitting samples to the same laboratories that are analyzing samples for other components of the program. Quality control specifications for these data are delineated in the project QAPP.

- Glycogen content of mussels: The glycogen measurement is unique to the mussel study and therefore data consistency with the remainder of the project is not a concern. Glycogen data must, however, be comparable within the mussel study (across locations) and must have sufficient accuracy and precision to distinguish any effects due to differing exposure at the test locations. The data quality for the glycogen determinations will be ensured by following the established procedure for this analysis as delineated in this Work Plan.

3.1.2 Data Quality Indicators

Data developed in the mussel study must meet standards of precision, accuracy, completeness, representativeness, comparability and sensitivity, as defined in Section 15 of the QAPP, that are appropriate to the data quality objectives. Each of these data quality indicators is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. The design of the study is based on replicate measurements of the important parameters at each sampling and location. The number of mussels selected for analysis at each location was based on previous similar experiments conducted by the principal investigators. Precision will be evaluated using standard calculation of statistical resolution, commonly referred to as power analysis. Each evaluation of a hypothesis will be accompanied by the corresponding power analysis, which will facilitate review of the precision of the test, which is in turn governed by the precision, or agreement, among the replicates.

Accuracy is defined as the agreement of a measurement with its true value. The major parameters to be evaluated in this study have defined standards of accuracy delineated in the QAPP. All analyses for these parameters will be conducted following procedures established in the QAPP. The accuracy of the analytical procedure for glycogen (Attachment 1) has been verified in previous investigations through analysis of samples of known glycogen content.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the mussel bioaccumulation/ condition study. Completeness goals are to achieve 100% of the planned samples. However, mussel survival may constrain the attainment of the 100% goal. There is no lower limit at which completeness would invalidate the study. As lower levels of completeness are reached, the ability of the data to resolve differences statistically decreases. This will be evaluated in the *a posteriori* power analysis discussed above.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is

ensured through establishment of an approved sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of representativeness will also be evaluated via comparison with known and/or expected results based on previous investigations of bioaccumulation in mussels.

Comparability is a measure of the confidence with which the data collected as part of the mussel study may be compared to themselves and to another similar data set. Comparability will be evaluated by examination of the variability in key parameters among replicates.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, will largely be a function of the precision of the chemistry analyses. Data quality objectives, particularly MDLs, will therefore be as delineated in the QAPP.

3.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical (except glycogen) and physical data are discussed in various sections of the project QAPP and will be used whenever applicable in this study. For the glycogen analysis, usability will be largely be determined by two factors: (1) the experience of the investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field; (2) a direct comparison between the glycogen data and similar data developed from other studies of biochemical stressors in mussels.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

3.2 SAMPLING DESIGN

The rationale for selection of the six locations to be sampled in the mussel bioaccumulation/ condition study was presented in Section 2 above. The locations will be chosen to be representative of a gradient of sediment PCB concentrations and also to be similar in other physico-chemical parameters. Because of the apparent lack of viable mussel populations in the Lower Housatonic River, a reference location will be established in the Connecticut River as well as one in the East Branch of the Housatonic River above the GE facility. Up to 10 replicate composite samples will be collected at each location. This number of samples has provided reasonable statistical resolution in previous similar studies conducted by the principal investigators.

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

The issue of sampling methods is not a major factor in controlling the quality of data to be collected in the mussel study. Mussels will be hand-placed at the initiation of the study and hand-collected at each of the two sampling events. Other than to collect only living mussels, no bias will be introduced via this procedure. Any unforeseen potential for the introduction of bias will be addressed through the reference (control) exposure in the Connecticut River.

3.3.2 Quality Control Samples

The nature of the mussel study does not allow the incorporation of typical duplicate and blank samples for the glycogen evaluation. Quality control and assessment of chemistry analyses will be provided by the replication of all chemistry analyses as specified in the project QAPP.

3.3.3 Sample Processing and Preservation

This study requires minimal sample processing, as described above in Section 2. All mussels will be promptly returned to the field laboratory and preserved by freezing. Samples will be stored frozen pending analysis, as delineated in the QAPP.

3.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the conduct of mussel exposure experiments. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Glycogen Analysis

Processing of samples for glycogen analysis will be personally supervised by and will follow procedures established by one of the principal investigators. Sample processing will be conducted as documented in Section 2 of this study plan. All samples will be processed by experienced staff who have received specific training in the procedure.

3.4.2 Physical/Chemical Samples

Samples for tissue chemistry (other than glycogen) will be processed following procedures and SOPs provided in the QAPP. These samples will be submitted in catalogs (sample delivery

groups) and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP.

3.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Section 4, below. The study findings will be reported with specific reference to both the data quality objectives specific to the mussel bioaccumulation/condition study and Subsection 4.1 of the QAPP.

4. DATA ANALYSES

Data collected as part of the study will be used to demonstrate site-specific differences as well as temporal and spatial variability. Data analyses will include summary statistics and determinations of variance and significant ($p<0.05$) differences between monitoring stations.

Differences in survival, glycogen levels, and bioaccumulation will be investigated using a variety of statistical approaches to test several hypotheses including:

1. H_0 = There is no significant difference in survival of mussels among stations.

2. H_0 = There is no significant difference in glycogen content of mussels among stations.

3. H_0 = There is no significant difference in the bioaccumulation of PCBs (and other chemicals) among stations.

Analysis of variance (ANOVA) will be used to determine significant differences among stations. In addition, ANOVA, regression, and correlation analysis will be used to determine significant differences among several factors. Prior to conducting statistics, all data sets will be analyzed for homogeneity in variances. If data do not meet the requirements for parametric analyses, non-parametric equivalents will be used (e.g., Kruskal-Wallis nonparametric ANOVA and Dunnett's multiple comparison test).

5. RESULTS

The results of this study will be presented in report form with all pertinent data provided, including mid-study and end-of-study tissue residue concentrations, percent survival, and glycogen concentrations. Maps showing exact locations for mussel deployment will also be prepared.

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ATTACHMENT 1

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF GLYCOGEN CONTENT IN FRESHWATER UNIONID TISSUES

ATTACHMENT 1

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF GLYCOGEN CONTENT IN FRESHWATER UNIONID TISSUES

Matthew A. Patterson

1. Determine the blotted wet weight of a sample of mantle tissue. Cut the tissue into thin slices and add 120 microliters of cold, 3M $HClO_4$. Homogenize the tissue and leave in the perchloric acid for 2 hours in the refrigerator.

2. Neutralize the acid with 200 microliters of 2M $KHCO_3$. Centrifuge the homogenate at 13,000 rpm for 2 minutes. Remove 5 microliters of the supernatant and add to 45 microliters of Gluco-amylase Solution (SIGMA). Vortex to ensure mixing of reactants and place in a water bath (40 degree C) for 2 hours.

3. After 2 hours in water bath, add 500 microliters of Reaction Mixture (SIGMA), vortex, and place in a water bath (37 degree C) for 30 to 40 minutes.

4. Record the absorbance reading on a spectrophotometer at 450 nm.

5. Also add 5 microliters of the supernatant to 45 microliters of water to determine the concentration of "free" glucose in the mantle tissue. The remainder of the procedure for determining free glucose is identical to the procedure described above.

6. All values recorded in nanometers from the spectrophotometer were converted to milligrams glycogen per gram of mantle tissue using a standard curve. Pure glycogen from the blue mussel, *Mytilus edulis*, was used to construct the glycogen standard curve.

APPENDIX A.16

FIELD SAMPLING AND ANALYSIS PLAN FOR CRAYFISH

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APPENDIX A.16

FIELD SAMPLING AND ANALYSIS PLAN FOR CRAYFISH

1. INTRODUCTION

1.1 BACKGROUND

Crayfish, the largest and longest-lived freshwater crustaceans in North America, play an important role in aquatic ecosystems by processing large quantities of organic matter and by feeding directly on carrion (Thorp and Covich, 1991). Crayfish are omnivorous and feed on aquatic vegetation, mollusks, fish, and organic debris (Martin, 1997). Because they eat plants and animals occupying several trophic levels (Thorp and Covich, 1991), they are in a position to potentially bioaccumulate certain contaminants. Crayfish are also prey for large fish, wading birds, frogs, turtles, snakes, raccoons (*Procyon lotor*), river otters (*Luntra canadensis*), muskrats (*Ondatra zibethicus*), and mink (*Mustela vison*) (Martin, 1997).

The virile crayfish (*Orconectes virilis*) was the only species of crayfish captured and identified from the Housatonic River study area in 1999 during preliminary crayfish surveys. It was found in riverine habitat of varying depths (from 1 to 5 ft), substrates (fine sandy silts, organic enriched silts, and gravelly cobbles), and flow regimes (riffles, runs, and pools). Smith (1995) believes this species is likely the most common crayfish in Massachusetts, as it is found throughout the state except in the southeastern coastal drainage areas of Buzzards Bay, Cape Cod, and the islands. *Orconectes virilis* has also been reported to live in practically all types of permanent aquatic habitats (Smith, 1995).

Because of their life history characteristics, tissue residue levels of PCBs in crayfish will provide a useful indicator of the potential impacts of PCBs on the aquatic food web of the Housatonic River.

1.2 OBJECTIVES

The primary objectives of this study are to:

- Collect crayfish from areas of the Housatonic River representing a range of sediment PCB concentrations for tissue residue analysis for PCBs and (to a lesser extent) dioxins/furans and organochlorine pesticides.

- Provide data on the bioaccumulation of PCBs, dioxins/furans, and organochlorine pesticides in the aquatic food web for fate and effects and exposure models.

2. STUDY DESIGN

2.1 FIELD SAMPLING DESIGN

2.1.1 Sampling Locations

A total of six locations will be sampled for this study. To the extent possible, based on available habitat and presence of crayfish, sampling locations will be co-located with sediment toxicity and benthic macroinvertebrate sample areas (Appendix A.13, Figure A.13-1). These locations provide a range of PCB concentrations in sediments (i.e., nondetected to 213 ppm total PCBs). Of the six sampling locations, two sites will be reference locations. One reference location will be upstream of Center Pond in Dalton, and the other will be on the West Branch of the Housatonic River several hundred feet upstream of the confluence with the main stem. The four other sampling locations will be in the main stem of the river: 1¼ mile below Holmes Road, immediately above the Pittsfield Waste Water Treatment Plant discharge, 2 miles below New Lenox Road, and ½ mile above Woods Pond.

A maximum of 20 crayfish will be collected from each of the six sampling locations (corresponding approximately to sample locations H0-SEEC0011, HW-SE000398, H3-SD043702, H3-SE000428, H3-SEEC0031, and H3-SE000389 in Appendix A.13, Figure A.13-1). Additional crayfish will be collected from each sample area for duplicate and MS/MSD analyses if individual crayfish are not greater than approximately 30 g in weight. A power analysis, included as Attachment 1 to this study plan, was conducted to provide information to be considered in the determination of the number of samples to be analyzed.

2.2 ANALYTICAL REQUIREMENTS

2.2.1 Analyses

Currently, it is assumed that sufficient tissue mass (10 g) for the required analyses will be obtainable from each individual crayfish collected, and that up to 20 crayfish will be collected for whole body tissue analyses from each of six sites. A minimum of 10 crayfish samples from each site will be analyzed for PCBs (total, Aroclors, congeners, and homologs), percent moisture, and percent lipids. A subset of samples will be analyzed for organochlorine pesticides and dioxins/furans. The analytical methods and detection limits to be used follow those specified in Appendix C of the *Quality Assurance Project Plan* (QAPP) (WESTON, 2000).

2.2.2 Quality Assurance/Quality Control Samples

Duplicate analyses will be conducted for each parameter on approximately 1 of every 10 samples. An additional 10 g of tissue will be required for each set of analyses beyond the 10 g required for each set of original analyses.

In addition, a matrix spike/matrix spike duplicate (MS/MSD) sample is required for every 20 samples. An additional 20 g of tissue will be required for each set of MS/MSD analyses, beyond the 10 g required for the original analyses. Thus, the amount of tissue required for original and MS/MSD analyses on a single crayfish is about 30 g. If sufficient crayfish mass is not available from one individual for these samples, then the duplicate and MS/MSD analyses will consist of composites of two to three individual crayfish. Compositing samples will require additional crayfish beyond those required for the original analyses.

3. PROCEDURES

3.1 FIELD SAMPLING

Crayfish will be collected using several methods depending on water depth. In areas where the river is approximately less than 3 ft deep, a seine net or a hand-held net will be used to capture crayfish. In places deeper than 3 ft, baited crayfish traps will be deployed.

3.1.1 Seine Netting, Hand Netting, and Hand Captures

Two-person teams will travel to each sampling location and identify the boundaries of the site using project area maps, which will be marked with survey flagging. Water depths will be reviewed to identify places where there is less than 3 ft of water and seining and hand netting can take place. The seine net will be unfurled and the two-person team will deploy the net in the river, making sure that the bottom of the net skirts the bottom of the river. The sampling area will be systematically fished with the net until approximately 20 crayfish are captured or until no new crayfish are captured. Care will be taken during sampling to minimize habitat disturbance. A similar technique will be used to capture crayfish with the hand-held nets. Captured crayfish will be placed in labeled resealable plastic bags. Bags will be labeled with sampling location, date, time, and collector's initials. Crayfish will be kept in a cooler on wet ice until all locations have been checked and all individuals retrieved. Crayfish will then be transported back to the lab for processing. Repeat visits may be made to each sample area until approximately 20 crayfish are captured.

3.1.2 Trapping

In sample locations where water depth is greater than 3 ft, crayfish will be trapped using wire mesh crayfish traps measuring approximately 18 inches by 12 inches by 8 inches. At each location, three traps will be baited with fish collected from the river within 50 ft of the sampling location. Each trap will be baited with at least 2 ounces of baitfish. The traps will be anchored in the river and marked, and the shoreline at each location will be marked with a flag labeled with the location number. Traps will be deployed during late afternoon hours, and checked daily in morning hours until the sampling objective is achieved, or the decision is made to move the trap due to lack of success. Captured crayfish will be handled as described in Subsection 3.1.1.

3.2 PROCESSING

3.2.1 Initial Processing

Two-person teams will prepare a processing table with clean plastic sheeting and all processing equipment and supplies including aluminum foil rinsed with nitric acid/deionized water/hexane/isopropyl alcohol. Each crayfish will be identified to species and virile crayfish will be kept for processing. Other crayfish will be returned to the site where they were captured, and then released.

On the data sheet for each crayfish, the sample location, date and time of collection, initials of collector(s), individual crayfish identification number, WESTON identification number, species, sex, weight (g), total length (mm), and carapace length (mm) will be recorded. Length measurements will be collected according to EPA guidance (EPA, 1995). Each individual crayfish will also be inspected for abnormalities or deformities, which will be described on data forms.

3.2.2 Tissue Sample Processing

After morphometric information has been collected for a crayfish, the specimens will be individually wrapped in nitric acid/deionized water/hexane/isopropyl alcohol rinsed aluminum foil. Foil will be labeled with sample identification number, location, date, collector's initials, weight, and tissue type. The foil-wrapped sample will then be placed into a resealable plastic bag, similarly labeled, and then placed immediately into a –20 to –30 °C freezer. Sample attribute forms will be completed for each sample (whole body, duplicate, or MS/MSD samples). Duplicate samples (1 of every 10 samples) may require that at least two crayfish be composited. Individuals of comparable size should be composited (total minimum tissue mass of 20 g required). MS/MSD samples (1 of every 20 samples) may require that three individuals be composited (total tissue mass of 30 g). MS/MSD samples will be weighed and labeled in the same fashion as described above.

3.2.3 Sample Handling and Shipping

Samples will be kept in a –20 to –30 ^{o}C freezer until shipment to the laboratory. When ready to ship, the samples (wrapped in labeled foil and enclosed in labeled resealable plastic bags) will be placed in a large plastic bag and then into a cooler lined with vermiculite. Chain-of-custody forms, listing the contents of each cooler, will be completed and placed into a resealable plastic bag. The resealable plastic bag will be taped to the inside of the top lid of the cooler, or placed on top of the samples. The coolers will be sealed with two custody seals, and labeled with appropriate WESTON shipping labels, including the WESTON return address, and U.S. Fish and Wildlife Service (USFWS) laboratory address. Samples will be delivered by courier or overnight delivery to the analytical laboratory.

3.2.4 Sample Documentation

Field logbooks will be used to record the location, date and time, collector(s)' names, the number of crayfish collected, and any other pertinent information. Specimen data sheets for each crayfish will be completed to include: location; date and time of collection; method of collection; collector's initials; total weight; sex if known; total length; and analyses. Sample attribute forms will also be completed for each tissue sample, which will include the sample number for each sample and the date and processor's initials on the form. Copies of completed chain-of-custody records for each cooler of samples shipped to the USFWS laboratory will be maintained in the WESTON project files in Pittsfield, MA, and in the sample management files in West Chester, PA.

4. QUALITY ASSURANCE/QUALITY CONTROL

4.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

4.1.1 Data Quality Objectives

The two primary data quality objectives of the crayfish programs were outlined in Subsection 1.2 above. In addition, as part of the Housatonic River Project, the crayfish program must support and complement applicable data quality objectives established in Subsection 4.1 of the Final Quality Assurance Project Plan (WESTON, 2000) for the project. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Taxonomic identification of crayfish: All collected specimens must be identified to the species level whenever possible using the taxonomic keys for crayfish provided in Smith (1995).

- Biomass (wet weight) for each specimen: Biomass must be determined accurately and recorded to 0.01 g using a calibrated balance capable of accurately measuring weight to .001 g.

- Total body length for each specimen: Body length must be determined accurately and recorded to 1 mm using dial calipers capable of accurately measuring length to 0.1 mm. Body length will be measured from the tip of the rostrum to the end of telson.

- Total carapace length for each specimen: Carapace length must be determined and recorded to 1 mm using dial calipers capable of accurately measuring length to 0.1 mm. The carapace length will be measured from the tip of the rostrum to the end of the cephalothorax.

- Sex for each specimen: Sex must be determined and recorded for each specimen whenever possible. Sex will be determined by examining the morphology of the first pleopod, using a low-power microscope (10X) or hand lens.

- Presence of abnormalities/deformities: Each specimen collected must be examined for gross abnormalities and deformities. This morphological examination will be conducted under a low-power (10X) microscope or hand lens. The morphological examination is limited to determining the presence/absence of gross characteristics (i.e., whether body parts, such as pleopods, are complete, incomplete, or missing). The morphological evaluation will not include a specific examination for tumors and lesions, although these characteristics will be recorded if observed.

- Tissue residue concentrations for PCBs and other contaminants: Tissue residue analysis will be based on the whole body concentration for crayfish. Quality control considerations to ensure achievement of DQOs for PCBs and other contaminants will follow the QAPP (WESTON, 2000).

4.1.2 Data Quality Indicators

Data developed in the crayfish study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000). Each of these data quality indicators, some of which are not readily quantifiable for crayfish data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Rather than control and measure precision, the study design includes a goal for the number of samples to obtain sufficient statistical resolution. Precision may also be evaluated by assessing the degree to which sample collection procedures are consistent among the study sites. For the measurements that are not unique to the crayfish, such as sediment chemistry, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (species identification, biomass, total length, and carapace length) accuracy is defined as meaning that all specimens are correctly identified, correctly weighed, and correctly measured. Accuracy of identification is a function of each sample being processed carefully and consistently. The data generated by this study will be evaluated for accuracy via comparison with a preliminary crayfish study on the Housatonic River, as well as results from similar studies conducted in similar New England systems.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the crayfish program. To ensure achieving the planned statistical resolution, it would be desirable to achieve 100% completeness. However, other factors such as individual size, scarcity of crayfish in a study area, or analytical budget may preclude achieving the target sample number. A minimum of 10 individuals will be considered acceptable for the study. Completion of the tissue residue analyses may require that multiple crayfish be composited into a single sample or that alternative sample sites are selected. In cases where compositing is necessary to obtain sufficient tissue for analysis, crayfish of similar biomass will be combined.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. This data quality indicator is addressed

through implementation of the sampling design and sample processing methods and will be evaluated via comparison with known and/or expected results.

Comparability is a measure of the confidence with which the crayfish data may be compared to another similar data set. Comparability will be evaluated by examination of the intra-site and inter-site (particularly target sites versus reference sites) variability in key parameters as determined from the replicates to be collected at each location. Comparability will also be evaluated for this data set through comparison with previous crayfish work in the Lower Housatonic River, and with known characteristics of crayfish populations in similar aquatic systems in the biophysical region.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is largely not applicable to the biological parameters. Sensitivity of non-biological parameters is defined in the QAPP (WESTON, 2000).

4.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical and physical data are discussed in various sections of the project QAPP and will be applied whenever applicable in this study. For the biological data, usability will largely be determined by two factors: (1) the experience of the senior investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field; and (2) a direct comparison between the chemistry data and other data developed by the project from similar areas of the river.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

4.2 SAMPLING DESIGN

The rationale for selection of the six locations to be sampled in the crayfish study was presented in Subsection 2.1.1 above. The four “target” locations are not intended to be representative of the entire river but rather are intended to encompass the range of sediment PCB concentrations in the Lower River between the Confluence and Woods Pond. Two appropriate reference locations with near-background PCB levels will also be sampled.

Crayfish tissue residue concentration data, like other biological parameters, are variable in nature. To achieve acceptable statistical resolution, it is necessary to collect large numbers of samples. Data will be collected from a minimum of 10 samples at each of 6 stations. This number of samples was selected based in part on a power analysis (see Attachment 1) conducted on a similar data set from an Indiana study examining the relationship between sediment PCB concentration and crayfish tissue residues.

4.3 SAMPLING METHODOLOGY

4.3.1 Sampling Procedures

Sampling methods, as discussed in Section 3, have been selected to ensure that the objectives of this study are met. Note that the stated objectives do not include a characterization of the distribution of crayfish in the Lower Housatonic River. As a result, sampling methods for this study are biased toward collecting larger crayfish (i.e., crayfish with sufficient mass for tissue analyses); however, all crayfish captured will be processed.

All samples will be collected directly by the highly trained and experienced personnel on this subject to further promote comparability and reduce potential bias through the oversight and the use of the professional opinion of the expert. Subsamples for physical and chemical analyses will be collected following procedures documented in the project QAPP (WESTON, 2000) and will therefore be comparable with procedures followed for all other similar efforts throughout the Supplemental Investigation.

4.3.2 Quality Control Samples

Duplicate analyses will be conducted for each parameter on approximately 5% of samples. In addition, a matrix spike/matrix spike duplicate (MS/MSD) sample is required for approximately 5% of samples. Further discussion of duplicate and MS/MSD analyses, including tissue requirements, is presented in Subsection 2.2.2.

4.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all crayfish samples are provided in Section 3. Decontamination of the processing area between samples will follow procedures established in the project QAPP (WESTON, 2000). All samples will be held on wet ice and returned to the field laboratory twice daily and will be frozen at that time. Holding time for physical and chemical samples will follow procedures established in the project QAPP (WESTON, 2000).

4.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the collection of crayfish samples. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

4.4 SAMPLE ANALYSIS

4.4.1 Biological Samples

The collection of morphometric information for all samples will be conducted by experienced staff who have received specific training in the SOP and whose work is checked periodically by their supervisors and peers. Taxonomy, gross morphology, and sex will be evaluated under low-power (10X) microscope and hand lens; remaining measurements will be made without magnification.

Quality of taxonomic identification will be assured by maintaining voucher collections and requiring a consensus among all taxonomists at the processing laboratory prior to identification becoming accepted as a type for the voucher collection. In the event that the taxonomists are unable to agree on an identification, specimens will be sent to a third-party recognized authority for determination.

4.4.2 Physical/Chemical Samples

Samples for tissue chemistry will be processed following procedures and SOPs provided in the project QAPP (WESTON, 2000). These samples will be submitted in catalogs and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP.

4.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Subsection 2.2 above. The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations and will be prepared with specific reference to both the data quality objectives specific to the crayfish study (Subsection 4.1.1) and Subsection 4.1 of the project QAPP (WESTON, 2000).

5. EQUIPMENT LIST

5.1 FIELD

- First aid kit
- 18 crayfish traps and anchors for each trap
- Locator flags for traps
- Bait
- Polyethylene plastic sheets for work area at locations
- 2 boxes of Nitrile gloves
- 2 boxes of large gallon-size resealable plastic bags

- Waders, 1 pair per person
- Data sheets for each of 6 locations
- Field logbooks
- 100 ft of ¼-inch nylon rope

5.2 PROCESSING AREA

- Taxonomic keys for crayfish
- 2 folding tables
- Polyethylene plastic sheets
- 4 boxes of Nitrile gloves
- 4 boxes of gallon-sized resealable plastic bags
- Data sheets
- Pliers, probe, scissors, steel rod
- Weighing scale for up to 100 g
- 4 boxes of aluminum foil
- 2 large coolers for freezing samples
- 15 holding coolers, if necessary, with aerators
- Drill for putting hole in side of cooler
- 1 to 2 shipping coolers
- Ice to fill cooler, in plastic resealable plastic bags or free
- Dry ice for shipping
- Gloves for handling dry ice
- Indelible markers (fine and wide)
- Ballpoint pens
- Hexane in rinse bottle
- Nitric acid in rinse bottle
- Isopropyl alcohol in rinse bottle
- Distilled, deionized water in rinse bottle
- Large bucket for decontamination solutions
- Packaging tape
- Laboratory sample labels with unique sample numbers
- WESTON or U.S. Fish and Wildlife QA/QC labels

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4

ATTACHMENT 1

POWER ANALYSIS FOR CRAYFISH STUDY

ATTACHMENT 1

POWER ANALYSIS FOR CRAYFISH STUDY

Power analysis calculations to determine a desirable sample size for the crayfish study assume that regression analyses will be used on the data eventually collected. The data will consist of PCB concentrations in tissues from crayfish sampled from six sites having various levels of contamination. We are investigating the statistical significance of a relationship, if one exists, between concentrations in crayfish and concentrations in the surrounding sediment of their local environments.

To estimate power, we need *a priori* estimates of means and dispersion of the PCB concentrations in crayfish collected at a particular site. Although preliminary data about crayfish are not available for the Housatonic sites, we can use relationships gleaned from the literature that permit a forecast of the likely patterns in crayfish from the patterns seen in sediments from the six sites. Data from Richland Creek near Bloomington, Indiana (Figure 1), indicate most of the variation in crayfish tissue was explained by sediment concentrations (Ruelle, 1986). The depicted relationship was not explicitly described by Ruelle but it appears to be very good, with a coefficient of determination of 82%. These data represent composite samples with from 4 to 13 animals per composite. The nine plotted points yield a regression slope of 1.37, with standard error 0.153.

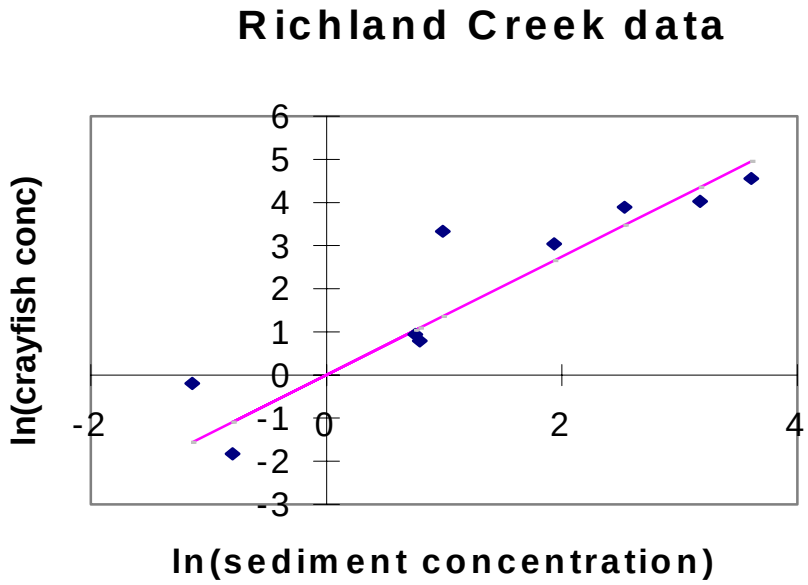

Figure 1 Linear Relationship Between Log-Concentrations of PCB in Crayfish Tissue and in Sediments at Sites in Indiana

Note: The original measurements were in ppm (i.e., mg/kg) dry weight.

Table 1 summarizes the observed concentrations of PCB in sediments in the Housatonic River, which happen to have roughly the same dynamic range as those seen in the Indiana data set.

Location	1	2	3	4	5	6
Mean	0.7	0.6	12.7	21.9	31.1	50.1
SD	2.3	0.46	14.4	9.6	29.9	58.5

Table 1 Averages and Standard Deviations (SD) for PCB Concentrations (mg/kg dry mass) in 0- to 6-inch Sediments at Locations 1–6 of the Housatonic River (WESTON Project Data)

Note: The data are presumed to have lognormal distributions.

Because such concentration data are routinely log-transformed to improve the fit to normal distributions and homoscedasticize dispersion, we modeled the log-concentration of PCB in crayfish tissue Y as a linear function of the log-concentration in sediments X with zero intercept

$$\ln Y = b \ln X + \varepsilon$$

where b is the coefficient that defines the slope of the regression line and ε represents normally distributed error terms with mean zero. The computer simulations used for the power analysis were thus based on regressions of log-concentrations of PCB in crayfish tissues against log-concentrations of PCB in sediments.

We estimated the power of a given sample size n by simulating n bivariate data pairs (X,Y) at each of six sites representing the putative relationship between sediment and crayfish concentrations. X values for the putative sediment concentrations were simulated for each site as random deviates from lognormal distributions having the means and standard deviations as reported in Table 1. A corresponding Y value for the crayfish concentration was then simulated for each X value according to the formula

$$Y = \exp(\delta \ln X + N(0,\sigma))$$

where δ represents a hypothetical value for the true slope in log-concentrations relating sediment and crayfish, and $N(0,\sigma)$ represents a normally distributed error term with zero mean and standard deviation σ. To estimate the dispersion σ of these little normal distributions of log-concentrations in crayfish we used the regression statistic

$$\sigma = s_{Y \cdot X} = s_b \sqrt{\sum x^2}$$

where s_b is the standard error of the fitted slope and x refers to the mean-centered value of the log-concentration in sediment. The computed value of σ was 0.714. Because the compositing in the Indiana data set almost surely reduces the apparent within-site variance in crayfish concentrations, we would therefore be justified in inflating the observed within-site dispersion of

crayfish concentrations before use in the power simulations. We assumed that the appropriate inflation factor is 5.

The bivariate pairs were then log-transformed and subjected to a regression analysis. A tally was kept of the number of times the resulting regression was statistically significant at the α=0.05 level. The number of significant regressions divided by the total number of regressions computed constituted an estimate of the power for that particular sample size (under the prevailing hypothesis about the true slope). A Pascal program was used to conduct the simulations. The results of this analysis are shown in Figure 2 giving the sample size required to achieve a power of no less than 80% as a function of the minimally detectable slope of the underlying log-log regression.

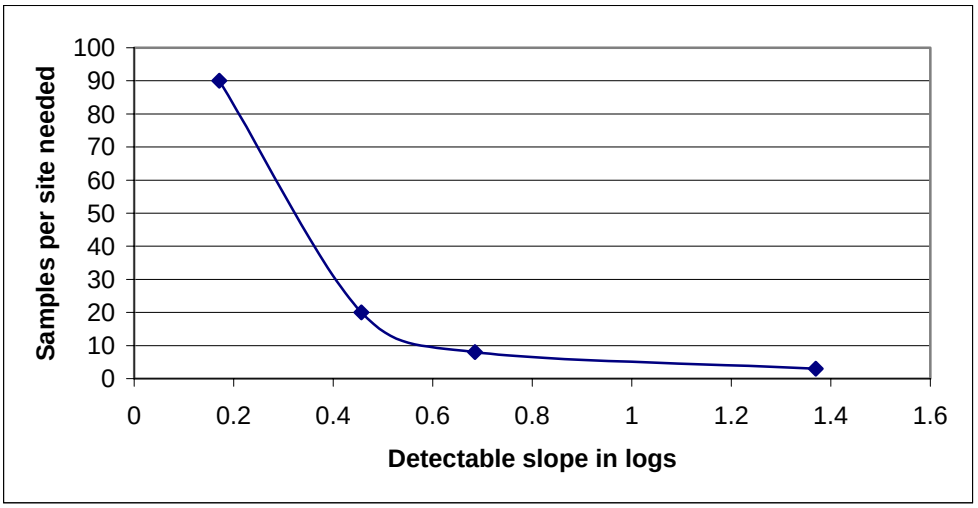

Figure 2 Estimated samples per site required to achieve 80% power at detecting a given slope in log-log concentration data assuming dispersion is 5 times σ.

Given that the example data set shown in Figures 3 and 4 are plausible scenarios for the patterns likely to be seen in the Housatonic crayfish study, we conclude that about 10 samples per site should be sufficient to detect, with 80% probability, a linear relationship in log concentrations having a slope as great as 0.6 (if such exists) under the hypothesis that a factor of 5 is appropriate for the inflation of σ seen in the Indiana data set. Increasing the sample size to 20 samples per site would increase the resolution of the analysis to a slope as great as about 0.5.

The effects of other possible values for the factor that inflates σ on the power results are shown in Figure 5. Obviously, for higher dispersions, the minimum detectable slope is larger.

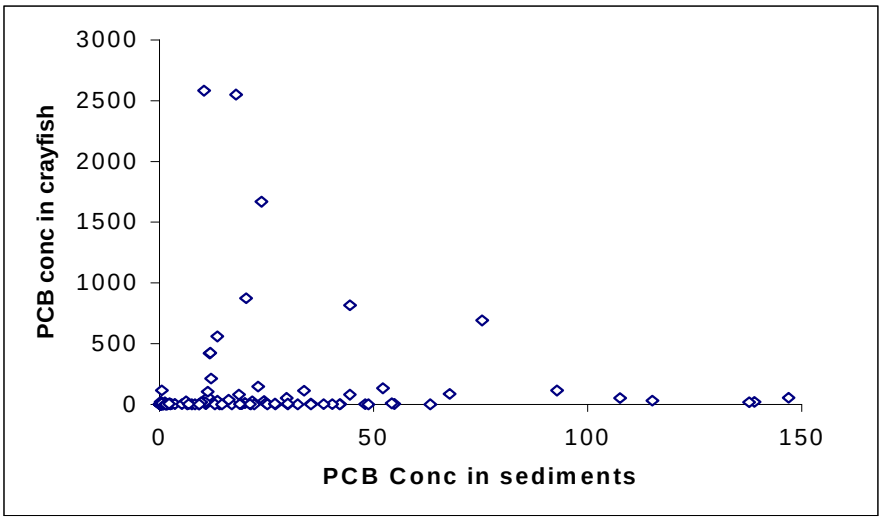

Figure 3 An example of one of the many simulated data sets used in the analysis. This data set has 20 samples per site and assumes a slope in logs of 0.5. The general character of this Scattergram is presumed to be comparable to that which might be collected in the crayfish study.

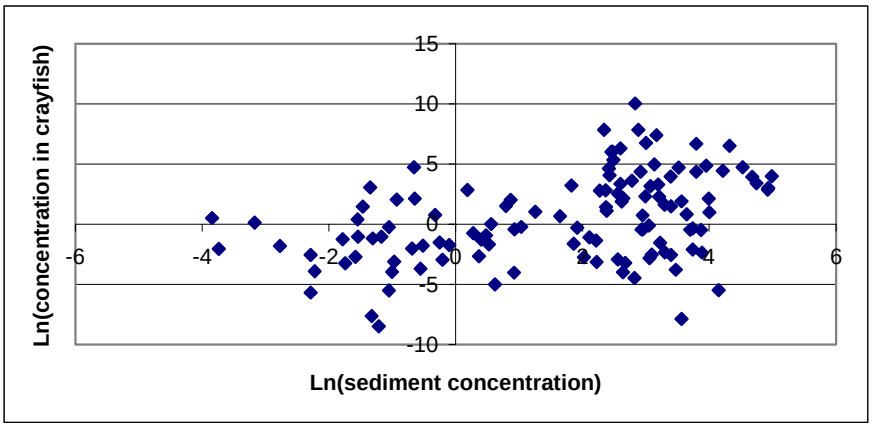

Figure 4 A log-log plot of the data depicted in Figure 3.

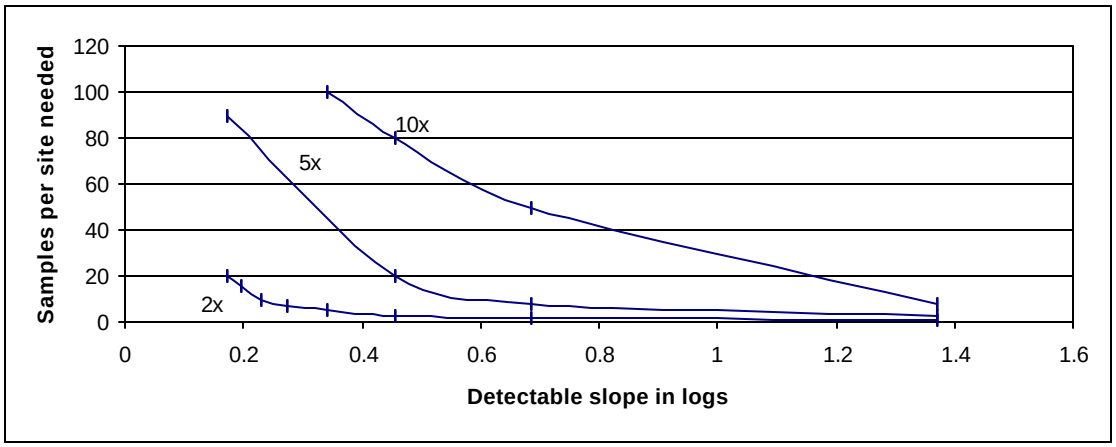

Figure 5 The effect of varying s on the estimated samples per site required to achieve 80% power at detecting a given slope in log-log concentration data. The 5x curve is the sample as in Figure 2.

LITERATURE CITED

Ruelle, R. 1986. “Indicator Organisms as Evaluators of PCB Migrations from a Superfund Site.” Pages 221–230, Hazardous Materials Spills Conference Proceedings.

APPENDIX A.17

FIELD SAMPLING AND ANALYSIS PLAN FOR BULLFROGS

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APPENDIX A.17

FIELD SAMPLING AND ANALYSIS PLAN FOR BULLFROGS

1. INTRODUCTION

1.1 BACKGROUND

Several life history characteristics make the bullfrog (*Rana catesbeiana*) a useful indicator of potential ecological effects of PCB contamination in Housatonic River sediments. The bullfrog is an aquatic species favoring permanent bodies of water (DeGraaf and Rudis, 1983; Smith, 1961), including river oxbows (DeGraaf and Rudis, 1983). In addition to being in frequent contact with water and sediment, it hibernates in the mud under water during winter months (Raney, 1940).

The home range of the bullfrog is small. In a study of a New York woodland lake, the average distance traveled in a day during the summer months ranged from 200 to 300 ft (Raney, 1940; Ingram and Raney, 1943). Male bullfrogs defend small territories during breeding season (DeGraaf and Rudis, 1983), which can extend into July in northern states (Smith, 1961). Thus, bullfrogs may act as indicators of localized contamination, should they be present.

In addition, the bulk of the bullfrog diet consists of aquatic insects and crustaceans (Smith, 1961). Bullfrogs are preyed upon by piscivorous birds such as bitterns and herons, by mammals such as raccoon (*Procyon lotor*) and mink (*Mustela vison*), and by aquatic reptiles such as snakes (Martin et al., 1951). Anecdotal information indicates that bullfrogs in the Housatonic River watershed are harvested for human consumption, posing potential human health risks that may require evaluation.

1.2 OBJECTIVES

There are two basic objectives of this field investigation:

- The whole-body frog tissue concentrations will be determined and used in the fate and effects model and the ecological risk assessment.

- To provide bullfrog leg muscle tissue for contaminant analysis that can be used to qualitatively evaluate the potential risk to human health from consumption of bullfrog leg muscle tissue, if warranted.

2. STUDY DESIGN

2.1 FIELD SAMPLING DESIGN

2.1.1 Number of Samples

Five to ten adult bullfrogs will be collected from each of four sampling areas. The number of bullfrogs sampled per area was determined on the basis of known differences in sediment PCB concentrations between areas within suitable bullfrog habitat. A power analysis was also conducted to determine an appropriate sample size based on literature data on PCB uptake in frogs (Attachment 1). However, the power analysis was not relied upon exclusively to determine sample size, given that the primary objective of the investigation is to collect tissue data for input into exposure models, as opposed to solely drawing statistical inferences between tissue data from different populations.

2.1.2 Sampling Locations

Sampling areas were selected by conducting a field reconnaissance to identify appropriate bullfrog habitat, followed by a review of available contaminant data to identify habitats both with and without detected PCB concentrations (for use as site-related and reference sampling areas, respectively). Within the Housatonic River study area, bullfrog habitat is primarily limited to Woods Pond and backwater areas within 1 mile north of Woods Pond. These two general areas have a lower water velocity, which allows deposition of contaminated sediments, as well as growth of submerged and emergent aquatic vegetation favored by bullfrogs. In general, historical data suggests that Woods Pond has higher sediment PCB concentrations than the backwater areas, which have relatively lower, but variable concentrations. The selected reference areas are Three-Mile Pond and Muddy Pond, in which PCBs have not been detected in sediment.

2.1.3 Supplemental Sediment Sampling

Because of the small home range of bullfrogs, existing sediment PCB data may not adequately characterize the range of sediment concentrations to which the frogs are most likely to be exposed. Additional sediment samples will be collected as necessary to obtain PCB concentrations in sediment where the frog is captured. Sediment sampling will occur after the frog sampling; frog capture locations will be marked with pin flags or flagging tape.

2.1.4 Collection Methods

Bullfrog sampling will occur primarily at night. From a boat, a portable spotlight will be shone along the shoreline in order to spot and blind the frogs. Frogs will be netted while blinded. If vegetation is too dense for netting, frogs may be speared using a long-handled gig or fork. Since this method could damage reproductive tissues, or potentially cause cross-contamination with

contaminated water or sediment, the gig or fork will be used only if necessary. If the gig or fork method is used, the gig or fork will be decontaminated between captures, and the captured frog will be immediately rinsed with deionized water.

Another method of collecting frogs consists of walking along the edge of the water wearing waders and capturing frogs with a large hand net either on the marsh surface or immediately after they jump in the water. This method is limited to daylight hours for health and safety reasons. As bullfrogs are often more vocal at night, this method will be used only if sufficient numbers of frogs cannot be obtained by spotlighting.

Intensive frog sampling will be conducted during a 1-week period in August (hereafter referred to as the "main sampling period").

Upon capture, bullfrogs will be given an identification number and returned to the central processing area. If possible, additional frogs at each site will be captured, so that the frogs to be retained for analysis will be of similar weight and sex distribution among the sampled areas. The frogs selected for analysis will be processed immediately.

Initial processing includes recording sex and other physical metrics (total body weight, age class, snout vent length, and leg length), followed by processing for tissue analysis. Leg muscle tissue samples will be prepared by skinning the frog, then removing each leg. The muscle tissue will then be removed from the bone, and the sample will be weighed, packaged, and labeled. The remaining carcass will be weighed, packaged, and labeled for separate analysis. After processing, each frog sample will be snap frozen with liquid nitrogen and held in a -20 to -30 ºC freezer.

2.2 ANALYTICAL REQUIREMENTS

Table 1 presents a summary of the number of frog tissue samples to be collected and the corresponding analyses to be undertaken. Each frog will be analyzed separately. Both leg muscle tissue and whole body tissue will be analyzed for the following parameters: PCBs (total, Aroclors, congeners, and homologs), percent moisture, and percent lipids. A subset of each group of samples will be analyzed for dioxins/furans and Appendix IX OC pesticides. The analytical methods to be used and the desired detection limits are specified in Appendix C of the *Quality Assurance Project Plan* (QAPP) (WESTON, 2000). A total of 10 g of tissue is required for each the whole body carcass and leg muscle tissue analyses. This tissue mass should be easily obtainable from individual adult bullfrogs, so that composite samples from different frogs will not be required.

Table 1
Analytical Summary for Bullfrogs
Housatonic River
Pittsfield, MA

Location	Number of Frogs	Number of Samples		Required Tissue Mass Per Sample (g)		Analytical Parameters				
		Leg Muscle Tissue	Whole Body Carcass	Leg Muscle Tissue[a]	Whole Body Carcass[a]	PCB	OC Pesticides[b]	Dioxins/ Furans[b]	Percent Moisture	Percent Lipids
Woods Pond	10	10	10	6	6	X	X	X	X	X
Backwater Areas Within 1 Mile North of Woods Pond	10	10	10	6	6	X	X	X	X	X
Three-Mile Pond Reference	5	5	5	6	6	X	X	X	X	X
Muddy Pond Reference	5	5	5	6	6	X	X	X	X	X
Total	30	30	30			30	12	12	30	30

Notes: [a] Required mass per sample is 10 g.
[b] Five frogs per target area, 1 frog per reference area

Actual whole-body tissue concentrations will be calculated after receipt of analytical results as the weighted average concentration in leg and carcass for each parameter. The weight of the leg muscle tissue will be multiplied by the concentration in muscle for each analytical parameter. The same will be done for the remainder of the carcass that is analyzed. The resulting sum (contaminant load in the leg muscle plus contaminant load in the carcass) will be divided by the total wet weight to obtain a wet-weight whole body concentration.

3. PROCEDURES

3.1 FIELD SAMPLING

1. Working in two-person teams, deploy boat with equipment.

2. At night a spotlight will be used to spot and blind frogs so they may be captured with a dip net, or if necessary, speared with a gig or fork.

3. Place the frogs captured at each location in a decontaminated 5-gallon polyethylene bucket filled with 2 to 3 inches of river water in the bottom. The lid of each container will be perforated to allow air exchange while the animals are held for processing.

4. After all frogs have been collected from a location, label the bucket lid and side with the location number, date/time, collector's initials, and method of collection.

5. Record a description of the location, date and time, method of collection, name(s) of collector(s), and the number of frogs collected in a field logbook.

6. Mark the exact location that each frog is collected with a pin flag or flagging, and record it on a map. Return later to record GPS coordinates at each flag point and to sample sediment.

7. Proceed to the next location and collect frogs as above. Return frogs to the central processing area.

8. If frogs are to be held for more than 3 hours, transfer them to coolers fitted with an aerator, and filled with 3 to 6 inches of river water from the same location.

3.2 PROCESSING

3.2.1 Initial Processing

1. Gather and set up equipment for two-person teams. Prepare processing table with clean plastic sheeting. One person records data, while the other processes the frog.

2. On the data sheet, record the location, date/time of collection, collector's initials, method of collection, and habitat description.

3. Decontaminate two to three pieces of aluminum foil with nitric acid/deionized water/hexane/isopropyl alcohol and air dry.

4. From the first location bucket, remove a frog while wearing Neoprene gloves and stun it with a sharp blow to the back of head with a decontaminated steel rod. The frog should then be double-pithed to ensure a humane death. This approach is deemed "conditionally acceptable" by the American Veterinary Medical Association Panel on Euthanasia. Sample processors will then tare the scale with the decontaminated aluminum foil, rinse the frog with deionized water, and weigh it.

5. Weigh the frog and record the data to the nearest 0.01g.

6. Measure frog leg length and total length (snout to vent) and record the data to the nearest 1.0 mm.

7. Identify and record the frog's age class (juvenile/adult). Male juveniles are generally less than 85 mm (females 89 mm) in length (Wright and Wright, 1949), and darker gray in color (Smith, 1961).

8. Identify the sex, and record on the data sheet. The following criteria may be used to ascertain sex:

   - Male bullfrogs have a tympanic membrane that is larger than the eye; in females it is as large or smaller than the eye.

   - Male bullfrogs may show stronger mottling near the vent.

   - Male bullfrogs are yellowish below the throat during breeding season, while females are whitish below the throat. This characteristic may not be as useful in August.

   - Males have enlarged thumbpads, which are larger and darker in color than in females.

9. Inspect the frog and note any abnormalities or deformities. Record the frog number on a resealable plastic bag.

3.2.2 Tissue Sample Processing

1. Complete a sample attribute form for each sample to be collected (leg muscle tissue and whole body carcass tissue, duplicate or MS/MSD samples) from the frog.

2. Make a small incision on lower back of frog with scissors or probe, and gently pull off skin from the legs with pliers.

3. Cut legs at the hind joint with scissors, and rinse with deionized water.

4. Remove the leg muscle tissue from the bone of each leg, and place the tissue on decontaminated foil and weigh it after first taring the scale with the foil. The weight should be at least 10 g and will be recorded to 0.01 g.

5. Label foil with: (1) location, (2) date and time, (3) collector's initials, (4) weight, and (5) tissue type (leg or body). The sample should be placed in a resealable plastic bag similarly labeled and immediately placed on ice in a cooler.

6. Place the leg bones and skin with the remainder of the carcass on a separate piece of foil and weigh it after first taring the scale. The weight should be at least 10 g and will be recorded to 0.01 g. The foil will be labeled as above and placed in a labeled resealable plastic bag on ice in a cooler.

7. Weigh and label duplicate and MS/MSD samples in the same fashion. These samples should also be labeled either "Duplicate" or "MS/MSD" as appropriate. These samples will require a minimum of 20 g and 30 g tissue mass, respectively.

8. Freeze the specimen and place it in a resealable plastic bag.

3.2.3 Sample Handling and Shipping

1. Keep samples in a -20 to -30 ºC freezer until shipment to the laboratory.

2. When ready to ship, place the samples (wrapped in labeled foil and enclosed in labeled resealable plastic bags) in a large plastic bag into a cooler lined with vermiculite.

3. Complete a chain-of-custody form listing the contents of each cooler, and place it into a resealable plastic bag. Tape the resealable plastic bag to the inside of the top lid of the cooler, or place it on top of the samples.

4. Seal the cooler with two custody seals, and label the cooler with appropriate WESTON shipping labels, including the WESTON return address, and U.S. Fish and Wildlife Service (USFWS) laboratory address.

5. Samples will be delivered by courier or overnight delivery to the USFWS. Samples sent to the USFWS should be shipped to:

Ken Carr/Ken Munney/Drew Major
USFWS
22 Bridge St., Unit 1 — Phone: 603-225-1411
Concord, NH 03301 — Federal Express Acct: 1510-1036-9

3.2.4 Sample Documentation

Use a field logbook to record the location, date and time, amount of time spent in collecting activities at each area, method of collection, name(s) of collector(s), the number of frogs collected, and any other pertinent information such as problems encountered.

Complete a specimen data sheet for each frog collected. Specimen data sheets should include: location; date and time of collection; method of collection; collector's initials; total weight, sex, total length, and leg length; sample type (whole body or legs); and analyses.

Complete a sample attribute form for each tissue sample. In most cases that will be two samples per frog (leg muscle tissue and whole body carcass). Put the sample number for each sample and the date and processor's initials on the form.

Complete a chain-of-custody form for each cooler of samples shipped to the USFWS laboratory. Provide copies to the task manager, who will retain them in the WESTON files.

4. QUALITY ASSURANCE/QUALITY CONTROL

4.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

4.1.1 Data Quality Objectives

The two primary data quality objectives of the bullfrog collection and tissue analysis were outlined in Subsection 1.2 above. In addition, as part of the larger Supplemental Investigation, the bullfrog program must support and complement applicable data quality objectives established in Subsection 4.1 of the Final Quality Assurance Project Plan (WESTON, 2000) for the project. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Total Biomass (wet weight) for each specimen: Biomass must be determined accurately and recorded to 0.01 g using a calibrated balance of 0.01 g sensitivity.

- Sex for each specimen: Sex must be determined and recorded for each specimen whenever possible. Sex will be determined by examining the morphological characteristics described in Subsection 3.2.1. The morphological characteristics that distinguish male and female bullfrogs are obvious to the naked eye; therefore, the use of a low-power microscope or hand lens is not necessary.

- Age class: The age class for each specimen must be determined accurately and recorded whenever possible. Age class will be determined by size and breeding characteristics.

- Total body length for each specimen: Body length must be determined accurately and recorded to the nearest 0.1 cm (1.0 mm) using a ruler capable of accurately measuring length to 0.1 cm. Total body length will be measured as snout to vent length for each specimen.

- Leg length for each specimen: Leg length must be determined accurately and recorded to the nearest 0.1 cm using dial calipers capable of accurately measuring length to 1 mm. Leg length will be measured on the right leg. (Note, to assure that the correct leg is measured, orient the frog on its ventral surface, head facing away from investigator.) Leg length will be measured from the top of the knee joint to the bottom of ankle joint.

- Presence of abnormalities/deformities: Each specimen collected must be examined for gross abnormalities and deformities, including tumors, scars, lesions, or other abnormalities. All observed abnormalities/deformities must be recorded. This morphological examination may be conducted by eye or using a low-power microscope or hand lens.

- Leg muscle tissue biomass (wet weight) for each specimen: The thigh and calf muscle tissue will be removed from both the left and right hind legs using the procedure outlined in Subsection 3.2.2. The combined leg muscle biomass must then be determined accurately and recorded to the nearest 0.01 g for each specimen, using a calibrated balance of 0.01 sensitivity.

- Offal biomass (wet weight) for each specimen: After the leg muscle tissue has been removed from the specimen, the remaining bullfrog tissue must be accurately determined and recorded to the nearest 0.01 g using a calibrated balance of 0.01 sensitivity.

- Tissue residue concentrations for PCBs and other contaminants: Tissue residue analysis will be conducted on 1) leg muscle tissue for each specimen, and 2) offal tissue for each specimen. Quality control considerations to ensure achievement of DQOs for PCBs and other contaminants will follow the QAPP (WESTON, 2000).

- Percent moisture and percent lipids: An analysis for percent moisture and percent lipids will be conducted on each tissue sample submitted for tissue residue analysis. Quality control considerations to ensure achievement of DQOs for these parameters will follow the QAPP (WESTON, 2000).

4.1.2 Data Quality Indicators

Data developed in the bullfrog study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability and sensitivity, as defined in Section 15 of the QAPP. Each of these data quality indicators, some of which are not readily quantifiable for the bullfrog data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Rather than control and measure precision, the study design includes an increase in the number of samples to obtain sufficient statistical resolution. For this study 10 samples per target site and 5 samples per reference site will be collected and processed. Precision may also be evaluated by an assessment of the degree to which sample collection procedures are able to ensure collection of consistent sample volumes. For the measurements that are not unique to the bullfrog study, such as tissue chemistry, precision is evaluated as defined in the QAPP (WESTON, 2000).

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (total biomass, leg tissue biomass, offal biomass, body length, leg length, sex determination, and age class determination) accuracy is defined as meaning that each specimen is correctly weighed, correctly measured, and correctly identified. The data generated by this study will be evaluated for accuracy via comparison with known and/or expected results from similar studies conducted in similar biophysical regions. Accuracy is as defined in the QAPP for abiotic parameters, such as sediment contaminants.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the bullfrog program. To ensure achieving the planned statistical resolution, it is important that completeness of 100% be achieved for all components of this study.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. This data quality indicator is addressed through implementation of the sampling design and sample processing methods and will be evaluated via comparison with known and/or expected results.

Comparability is a measure of the confidence with which the bullfrog data may be compared to another similar data set. Comparability will be evaluated by examination of the intra-site and inter-site (particularly target sites vs. reference sites) variability in key parameters as determined from the group of samples to be collected at each location. Comparability will also be evaluated for this data set through comparison with previous similar bullfrog studies (if located) and with known characteristics of bullfrog populations in similar stream systems in the biophysical region.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, will be assured for the biological parameters by using scales and measuring devices of appropriate resolution (see sensitivity discussions within each DQO above). The detection limits for chemical analysis specified in the QAPP will provide more than sufficient sensitivity for the purpose of providing insight into factors controlling abundance and distribution of the benthic taxa and populations.

4.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical and physical data are discussed in various sections of the project QAPP and will be used whenever applicable in this study. Usability will be largely be determined by two factors: (1) the experience of the senior investigator in establishing that the field sampling was conducted following the SOP and that accuracy and

precision were not compromised by an inability to control the sampling procedures in the field; (2) a direct comparison between the chemistry data and other data developed by the project from similar areas of the river.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

4.2 SAMPLING DESIGN

The rationale for selection of the four locations to be sampled in the bullfrog study was presented in Subsection 2.1.2 above. The locations are not intended to be representative of the entire river but rather are intended to encompass the range of sediment PCB concentrations in the Lower River between the Confluence and Woods Pond where bullfrog habitat occurs. Two appropriate reference locations with background PCB levels and two "target" sites will be sampled.

Bullfrog tissue residue concentration data are typically highly variable in nature. To achieve acceptable statistical resolution it is necessary to collect large numbers of samples. Data will be collected from 10 frogs at each of the target sites and from five frogs at each of two reference stations. This number of samples was selected based on a power analysis using data from bullfrog studies found in the literature and with consideration of what level of resolution would be needed to meet the objectives of this study (see Attachment 1).

4.3 SAMPLING METHODOLOGY

4.3.1 Sampling Procedures

Sampling methods, as discussed in Subsections 2.1.4 and 3.1, have been selected to ensure that the objectives of the study are met. Note that the stated objectives do not include a characterization of the distribution of frogs in the Lower Housatonic River. As a result, sampling for this study is limited to one species (bullfrogs) and sampling methodology is biased toward collecting bullfrogs with sufficient mass for tissue analysis.

All samples will be collected directly by the highly trained and experienced personnel on this subject to further promote comparability and reduce potential bias through the oversight and use of the professional opinion of the expert. Subsamples for physical and chemical analyses will be collected following procedures documented in the project QAPP, and will therefore be comparable with procedures followed for all other similar samples efforts throughout the Supplemental Investigation.

4.3.2 Quality Control Samples

Table 2 summarizes QA/QC requirements for tissue analyses.

Table 2
QA/QC Analytical Summary for Bullfrogs
Housatonic River
Pittsfield, MA

Location	Number of Frogs	Number of Samples[a]		MS/MSD Samples[b]		Duplicate Samples[c]	
		Leg Muscle Tissue	Whole Body Carcass	Leg Muscle Tissue	Whole Body Carcass	Leg Muscle Tissue	Whole Body Carcass
Woods Pond	10	10	10	0	0	1	1
Backwater Areas Within 1 Mile North of Woods Pond	10	10	10	1	1	1	1
Three-Mile Pond Reference	5	5	5	1	1	1	1
Lake Washington Reference	5	5	5	0	0	0	0
Total	30	30	30	2	2	3	3

Notes: [a] Required mass per sample is 10 g.

[b] Required total mass per MS/MSD sample is 20 g (Original sample 10 g, MS 10 g, MSD 10 g).

[c] Required total mass per duplicate sample is 20 g (Original sample 10 g, duplicate 10 g).

Duplicate analyses will be conducted for each parameter on approximately 5% of tissue samples (both whole body tissue and leg muscle tissue). An additional 10 g of tissue will be required for each set of analyses (whole body carcass and leg muscle tissue) beyond the 10 g required for each set of original analyses. Therefore, the amount of tissue required will be 20 g from the whole body carcass and 20 g from the leg muscle tissue. These samples will be submitted for analyses separately.

In addition, a matrix spike/matrix spike duplicate (MS/MSD) sample will be conducted on 5% of samples. These samples will also be collected from both leg muscle tissue and whole body tissues. An additional 20 g of tissue will be required for each set of MS/MSD analyses, beyond the 10 g required for the original analyses. Thus, the total amount of tissue required for original, duplicate, and MS/MSD analyses, if conducted on the same sample, is 40 g each from the whole body carcass and leg muscle tissue samples. This mass should still be obtainable from a single frog. The results of the analysis of these split samples will be compared for quality control purposes.

4.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all samples to be collected as part of the bullfrog study are provided in Subsection 3. Decontamination between samples will follow procedures established in the project QAPP (WESTON, 2000). All specimens will be held alive in site water and returned to the field laboratory twice daily. Biological samples will be frozen after processing; sediment samples will be frozen immediately. The holding time for physical and chemical samples will follow procedures established in the project QAPP.

4.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the collection of bullfrog samples. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

4.4 SAMPLE ANALYSIS

4.4.1 Biological Samples

The collection of morphometric information and dissection of all samples will be conducted by experienced staff who have received specific training in the SOP and whose work is checked periodically by their supervisors and peers. Biological samples will be processed following procedures and SOPs provided in Subsection 3.

4.4.2 Physical/Chemical Samples

Samples for sediment chemistry and tissue chemistry will be processed following procedures and SOPs provided in the project QAPP (WESTON, 2000). These samples will be submitted in catalogs and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP.

4.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Subsection 2.2 above. The findings will be included in the ecological risk assessment including all data, analyses, and interpretations and will be prepared with specific reference to both the data quality objectives specific to the bullfrog study (Subsection 4.1.1) and Subsection 4.1 of the project QAPP (WESTON, 2000).

5. EQUIPMENT LIST

5.1 FIELD

- First aid kit
- 4 headlamps, 16 AA batteries, extra set of 16 AA batteries
- Spotlights (2) 12-V battery
- 13 medium buckets for collection with lids and holes for ventilation
- 4 all-purpose nylon nets, 12-inch diameter and 5-ft extendable handle
- Indelible markers, duct/labeling tape
- Waders for each field technician
- Life vests
- Oars, anchor, rope for 2 jon boats, trolling motor
- Field logbook

5.2 PROCESSING AREA

- 2 folding tables
- Polyethylene plastic sheets
- 4 boxes of Nitrile gloves
- 4 boxes of gallon-sized resealable plastic bags
- Data sheets
- Pliers, probe, scissors, steel rod
- Knives/scalpels for incision or reproductive examination
- Weighing scale for up to 500 g
- 4 boxes of aluminum foil
- 2 large coolers for freezing samples

- 15 holding coolers, if necessary, with aerators
- Drill for putting hole in side of cooler
- 1 to 2 shipping coolers
- Ice to fill cooler, in plastic resealable plastic bags or free
- Dry ice for shipping
- Gloves for handling dry ice
- Indelible markers (fine and wide)
- Ballpoint pens
- Hexane in rinse bottle
- Nitric acid in rinse bottle
- Isopropyl alcohol in rinse bottle
- Distilled, deionized water in rinse bottle
- Large bucket for decontamination solutions
- Packaging tape
- Laboratory sample labels with unique sample numbers
- WESTON or USFWS QA/QC labels

6. REFERENCES

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ATTACHMENT 1

POWER ANALYSES FOR BULLFROGS

Regression – Analytical

The effects of releasing PCB in the environment on the concentrations of PCB in frog tissues may be tested by a regression of this variable against the concentrations of PCB in sediments. This statistical test is recommended because concentrations of PCB in frog tissues are expected to be a function of the concentration of these chemicals in the environment. Frogs frequently have small home ranges, and uptake PCBs via absorption through the skin (particularly when hibernating in sediments) and ingestion of contaminated water and food. For a desired power (1-β) and level of significance (α), the adequate sample size *n* is

$$n = ((Z_{\beta(1)} + Z_{\alpha})^2 / \zeta_0) + 3 \qquad \text{(Zar 1984)}$$

where β (1) is the one-tailed probability of the normal deviate, α is the level of significance, and $Z_{\beta(1)}$, Z_{α}, and ζ_0 are the Fisher *z* transformations at β (1), α, and ζ_o levels, respectively, and ρ_o is the specific correlation coefficient to be tested. For α = 0.05, $Z_{0.1(1)}$ = 1.2816, $Z_{0.05(2)}$ = 1.9600, power = 0.9, and β (1) = 0.1 the required sample sizes are shown in Figure 1.

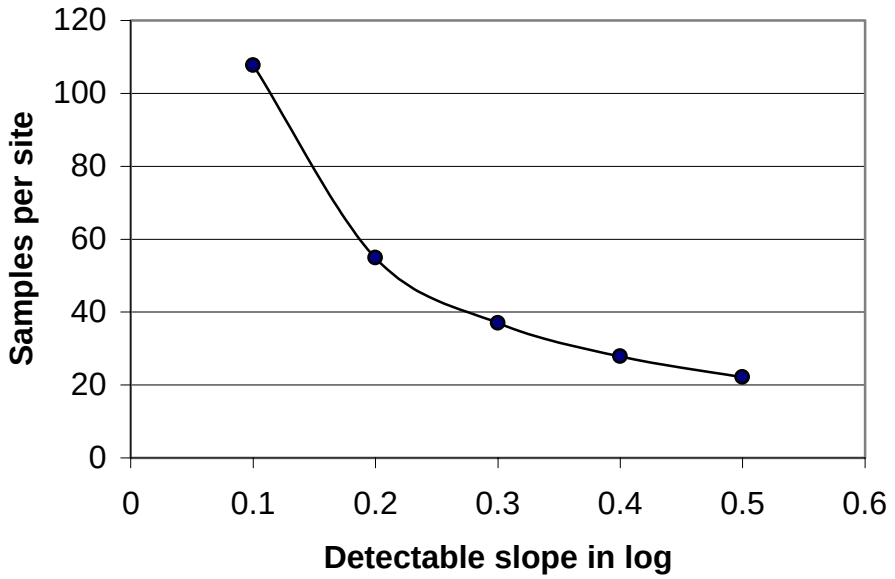

Figure 1. Estimated number of samples, for each site, required to detect a significant regression of log concentration of PCB in bullfrog tissues (wet mass) as a function of the log concentrations in sediments (dry mass). Power ≥ 0.9, α = 0.05.

Regression - Simulation

Computer simulations can also estimate the sample sizes required to achieve a desired power. In our model the log-transformed concentration of PCB in frog tissue (Y) was a linear function, with zero intercept, of the log-transformed PCB concentration in sediments (X):

$$\ln Y = \delta \ln X + \varepsilon(0,\sigma)$$

where δ represents a hypothetical value for the true slope of the regression line, and $\varepsilon(0,\sigma)$ represents a normally distributed error term with zero mean and standard deviation σ. The standard deviation σ of the error term was estimated from data in Huang (1999) on PCB concentrations in northern leopard frogs from the Green Bay, Wisconsin area (Figure 2).

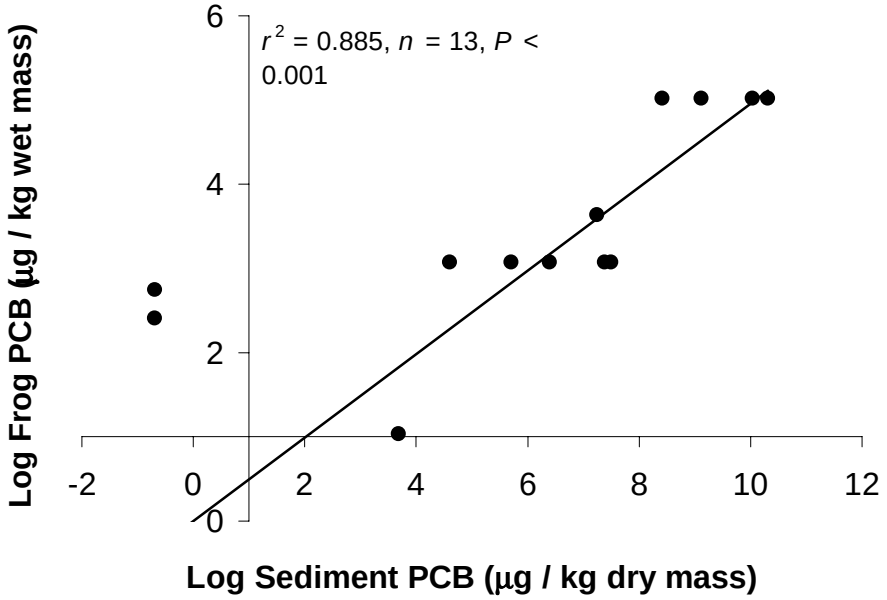

Figure 2. Linear relationship between PCB concentrations in sediments and tissues of northern leopard frogs. Data from Huang (1999).

σ is the root residual mean square of the regression line relating log concentrations of PCB in sediments and frog tissues

$$\sigma = \sqrt{\sum (Y - \overline{Y})^2 / (n-1)}.$$

In two of the sites where Huang (1999) sampled PCB concentrations in sediments and frog tissues, Deposit C and Strobe Island, there were multiple sediment samples corresponding to a single sample of frogs. Huang (1999) only reported their means and standard errors. This procedure reduces our estimate of the deviations in PCB concentrations in frog tissues in the values projected by the regression equation. We compensated for this reduction by arbitrarily generating values of PCB concentration in sediments that fit the reported mean and standard error. Based on the published data for the four sites without replicates, and the simulated data for Deposit C and Strobe Island,

$$\sigma = 0.643.$$

Sediment PCB concentrations (X values) were generated at random from lognormal distributions having the means and standard deviations given in Table 1.

Site	1	2	3	4	5	6
Mean	679	568	12,680	21,880	31,070	50,070
SD	2,312	458	14,370	9,592	29,890	58,540

Table 1. Arithmetic means and standard deviations (SD) of PCB concentrations (μg/kg dry mass) in 0–6-inch sediments at six sites of the Housatonic River (WESTON project data). The concentrations are presumed to be lognormally distributed, thus their log transformed values are likely to improve normality and homoscedasticity.

We also compensated for the reduced variance due to composite frog samples (4-8 animals per sample, Y. Huang, personal communication) by multiplying σ by a factor f, where $f = 1$, 2.5 or 5. Therefore, in the simulation, a corresponding Y value for the bullfrog concentration was then simulated for each X value according to the formula

$$Y = \exp(\delta \ln X + \varepsilon(0, f\sigma))$$

to generate 1, 2, 3, 4, 5, 7, 10, 15, 20, and 100 pairs of *X Y* values for each of the six sites in Table 1. The bivariate pairs were then log-transformed and subjected to a regression analysis. A tally was kept of the number of times the resulting regression was statistically significant at the α=0.05 level of significance. The number of significant regressions divided by the total number of regressions estimates the power for that particular sample size (under the prevailing hypothesis about the true slope). A Pascal program written for this analysis was used to conduct the simulations. The results of this analysis are shown in Figure 3, which displays the sample sizes required to achieve a power of no less than 80% as a function of the minimally detectable slope of the underlying log-log regression.

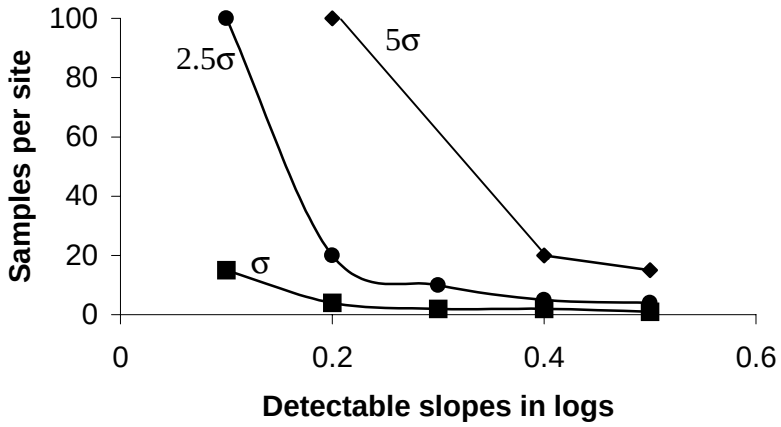

Figure 3. Estimated number of samples, for each of the six sites listed in Table 2, required to detect increasing slopes of the regression line relating log transformed concentrations of PCB in frog tissues (wet mass) and sediments (dry mass) with a power of > 0.8. Each curve has a distinct value for the standard deviation of the error term (σ = 0.643).

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Huang, Y. 1999. "Exposure of Northern Leopard Frogs in the Green Bay Ecosystem to PCBs, PCDDs, and PCDFs Is Measured by Direct Chemistry but not Hepatic EROD Activity." *Environmental Toxicology and Chemistry* 18:54-57.

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APPENDIX A.18

WORK PLAN FOR THE STUDY OF AMPHIBIAN REPRODUCTIVE SUCCESS WITHIN VERNAL POOLS ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (WOODLOT ALTERNATIVES, INC.)

APPENDIX A.18

WORK PLAN FOR THE STUDY OF AMPHIBIAN REPRODUCTIVE SUCCESS WITHIN VERNAL POOLS ASSOCIATED WITH THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

Roy F. Weston, Inc.
1400 Weston Way
West Chester, Pennsylvania 19380-1499

Submitted by:

Woodlot Alternatives, Inc.
122 Main Street
Topsham, Maine 04086

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1. INTRODUCTION

The United States Environmental Protection Agency (EPA) is characterizing the natural resources of the Housatonic River in portions of Pittsfield, Lenox, and Lee, Massachusetts. The study area is approximately 12 miles long and extends from Newell Street in Pittsfield to Woods Pond Dam in Lee. It includes riverine habitats, floodplain wetlands, and uplands associated with the main-stem of the river. Polychlorinated biphenyls (PCBs) that originated from the General Electric (GE) facility in Pittsfield have been found within the river and its adjacent floodplains (Blasland, Bouck, and Lee, 1996a and 1996b). This work plan is for a study of amphibian reproductive success within vernal pools that have varying levels of PCBs in their sediments.

The Housatonic River and its floodplains provide habitat for a wide variety of reptiles and amphibians (collectively called herps) and up to 40 species of snakes, turtles, frogs, toads, and salamanders potentially occur in the study area (TechLaw, 1998). Breeding amphibians (frogs, toads, and salamanders) use portions of the river and temporary (known as vernal pools) and permanent pools for courtship and egg-laying. These areas then support larval amphibians for periods ranging from several months to more than a year, depending on the species. Wood frogs (*Rana sylvatica*), for example, metamorphose into the adult form in 2 to 3 months, while green frogs (*Rana clamitans*) can take over a year (Hunter et al., 1992).

Documenting amphibian reproductive success within vernal pools is being conducted because these animals may be influenced by exposure to PCBs in contaminated sediments. They may also bioaccumulate PCBs, which can then be passed on to other animals in the food chain. In addition, several herps that could occur in the study area are listed as State-Endangered, Threatened, Special Concern, or Watch List species (Massachusetts Natural Heritage and Endangered Species Program, 1997). These include the Jefferson salamander (*Ambystoma jeffersonianum*), spotted salamander (*A. maculatum*), marbled salamander (*A. opacum*), spring salamander (*Gyrinophilus porphyriticus*), and four-toed salamander (*Hemidactylium scutatum*).

1.1 OBJECTIVE

The objective of this task is to determine if PCB contamination is potentially having an adverse effect on amphibian reproduction in vernal pools.

1.2 PROJECT APPROACH

Several steps will be taken to meet the task objectives, including: 1) literature review, 2) observing amphibian reproductive success in vernal pools that have varying levels of PCBs in their sediments, 3) data analysis, and 4) report preparation. The literature review will be used to locate information on the present and historic use of the study area by amphibians and to identify the potential effects of PCBs on amphibians.

As presented in Figure 1, vernal pools in the study area have been mapped and characterized using methods developed by Kenney (1995) for Massachusetts (TechLaw, 1998). Table 1 presents the estimated area and average depth for the vernal pools observed for the study area.

The concentration of PCBs in vernal pool sediments will be measured to identify a sample of pools exhibiting a range of contamination levels. Amphibian reproductive activity, including courtship and breeding, egg laying, hatching, larval growth and development, and metamorphosis, will then be observed in each of the sample pools to determine if PCBs may be influencing reproductive success. Carcasses of amphibians that succumb incidentally to the performance of this study will be analyzed to measure the level of PCBs in their tissues.

The data from these studies will be analyzed and presented in a report that includes results of the literature review and an evaluation of amphibian reproductive success in relation to observed PCB levels. Tasks will be performed in conjunction with EPA and Roy F. Weston, Inc. (WESTON®) scientists and other principal investigators, who will assist with study plan development and implementation, data analysis, and report preparation.

2. METHODS

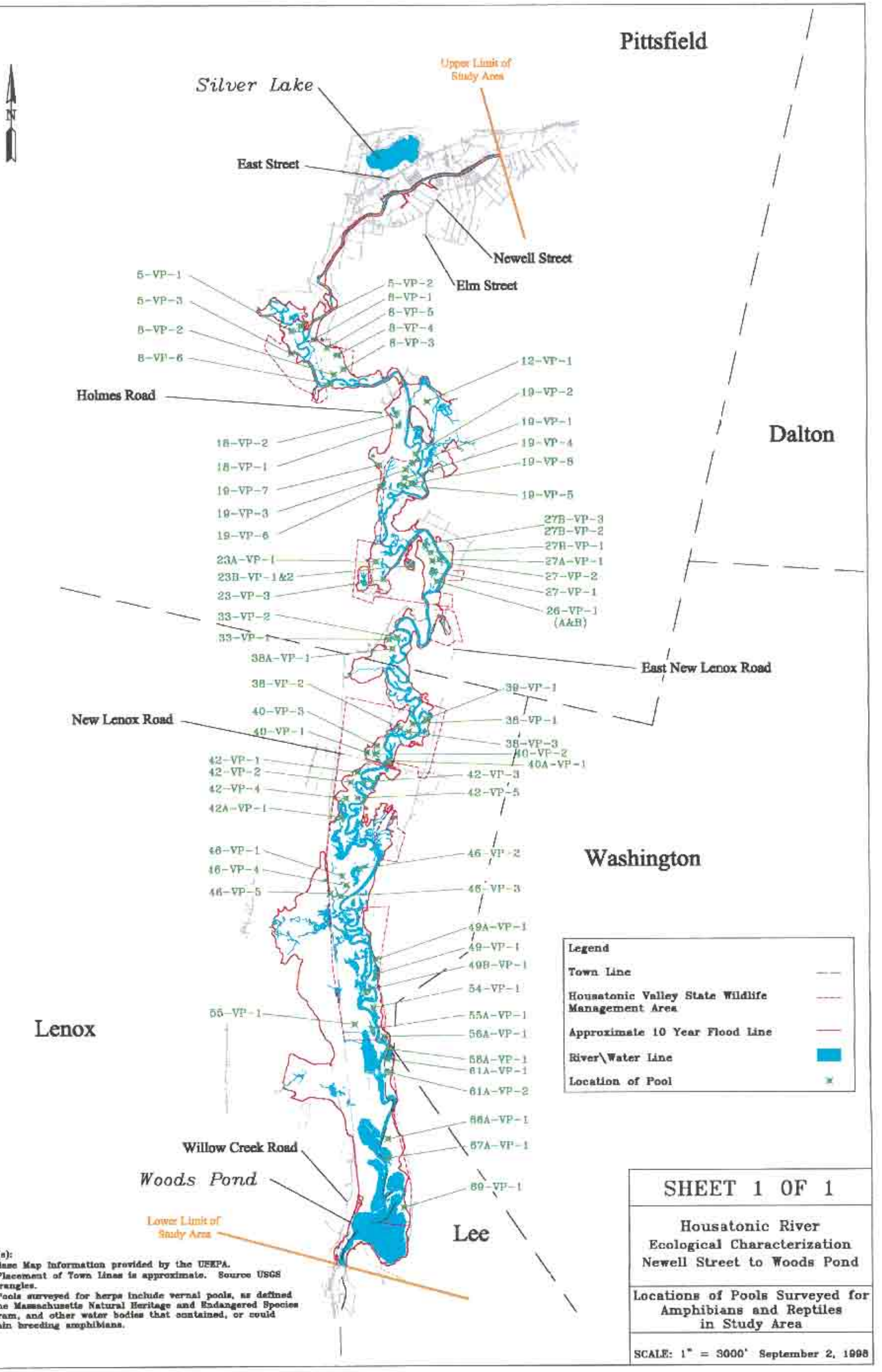
Prior to conducting field work, appropriate Scientific Collecting Permits for the studies described in this Work Plan will be obtained from the Commonwealth of Massachusetts, Division of Fisheries & Wildlife. If rare amphibians, invertebrates, or plants are encountered as part of this study, appropriate Division of Fisheries & Wildlife, Natural Heritage and Endangered Species Program Rare Animal and Rare Plant Observation Forms will be completed.

2.1 LITERATURE REVIEW

The literature review will be used to develop species identification protocols for amphibian eggs, larvae, recently metamorphosed juveniles, and adults. A list of scientific and technical articles related to the known and potential effects of PCBs on amphibians will also be developed as part of the literature review. These articles will be reviewed and references will be entered into an annotated Papyrus (version 7.0.14) database that includes: 1) author(s) name(s), 2) year of publication, 3) article title, 4) journal/source, 5) abstract, and 6) annotated comments.

2.2 AMPHIBIAN REPRODUCTIVE SUCCESS IN VERNAL POOLS

Sediment samples (0 to 6 inches from ground surface) will be collected from vernal pools in the study area and analyzed for total PCB concentration. Amphibian reproductive success within four pools exhibiting a range of PCB concentrations, from no contamination up to the highest observed concentration, will then be studied. The final selection of pools, including the number and location of sample pools, will be made in consultation with EPA scientists and will be based on PCB concentration, presence of target species, and similarity of physical and hydrologic characteristics. It is believed that at least three pools will be needed to represent pools with varying levels of PCBs, i.e., one low or non-detect, one moderate, and one high. A weather station w ill be established near the study pools to measure temperature, relative humidity,



Note(s):
1) Base Map information provided by the USEPA.
2) Placement of Town Lines is approximate. Source USGS Quadrangles.
3) Pools surveyed for herps include vernal pools, as defined by the Massachusetts Natural Heritage and Endangered Species Program, and other water bodies that contained, or could contain breeding amphibians.

SHEET 1 OF 1

Housatonic River Ecological Characterization Newell Street to Woods Pond

Locations of Pools Surveyed for Amphibians and Reptiles in Study Area

SCALE: 1" = 3000' September 2, 1998

Table 1

Housatonic River Floodplain Pool Data
Berkshire County, Massachusetts

Pool Designation	Estimated Area (ft^2)	Avg. Depth (Inches)	Date Observed
5-VP-1	1,275	5	4-20-98
5-VP-2	3,150	14	5-6-98
5-VP-3	10,000	12	5-7-98
8-VP-1	800	11	4-14-98
8-VP-2	250	14	4-14-98
8-VP-3	2,000	6	4-14-98
8-VP-4	6,000	18	4-14-98
8-VP-6	1,000	4	5-5-98
12-VP-1	6,000	20	4-20-98
18-VP-1	10,000	18	4-14-98
18-VP-2	10,500	18	4-20-98
19-VP-1	6,000	14	4-14-98
19-VP-2	400	12	4-14-98
19-VP-3	750	8	5-5-98
19-VP-4	2,700	6	5-5-98
19-VP-5	12,000	14	5-5-98
19-VP-6	16,000	10	5-5-98
19-VP-7	5,000	8	5-6-98
19-VP-8	675	4	5-6-98
23-VP-3	16,000	14	5-6-98
23A-VP-1	9,000	10	4-21-98
23B-VP-1	15,000	18	4-21-98
23B-VP-2	6,000	12	4-21-98
26-VP-1 (A+B)	2,560	14	4-21-98
27-VP-1	6,000	18	5-7-98
27-VP-2	8,400	10	5-7-98
27A-VP-1	2,000	15	4-21-98
27B-VP-1	2,200	14	4-21-98
27B-VP-2	5,625	15	4-21-98

Table 1

Housatonic River Floodplain Pool Data
Berkshire County, Massachusetts
(Continued)

Pool Designation	Estimated Area (ft^2)	Avg. Depth (Inches)	Date Observed
33-VP-1	3,750	11	4-21-98
33-VP-2	18,200	9	4-21-98
38-VP-1	3,400	14	4-22-98
38A-VP-1	2,450	10	4-22-98
38-VP-2	6,000	10	4-22-98
38-VP-3	8,000	14	4-22-98
39-VP-1	30,000	25	4-22-98
40-VP-3	9,000	18	4-22-98
42-VP-1	21,000	14	4-24-98
42-VP-2	12,000	10	4-24-98
42-VP-2	12,000	10	4-24-98
42-VP-3	4,225	>36	4-24-98
42-VP-4	50,000	>36	4-24-98
42-VP-5	28,900	15	4-24-98
42A-VP-1	175,000	>36	4-24-98
46-VP-1	23,800	>36	4-24-98
46-VP-2	180,000	21	4-28-98
46-VP-3	10,000	16	4-28-98
46-VP-4	300	4	4-28-98
46-VP-5	800	12	4-28-98
49-VP-1	44,000	>36	4-22-98
49A-VP-1	1,320	8	4-22-98
49B-VP-1	240	6	4-22-98
54-VP-1	40,000	>36	4-23-98
55-VP-1	30,000	18	4-29-98
55A-VP-1	56,000	18	4-29-98
56A-VP-1	30,000	>36	4-23-98
58A-VP-1	30,000	>36	4-23-98
61A-VP-1	26,250	>36	4-23-98
61A-VP-2	50,000	>36	4-23-98

Table 1

Housatonic River Floodplain Pool Data
Berkshire County, Massachusetts
(Continued)

Pool Designation	Estimated Area (ft^2)	Avg. Depth (Inches)	Date Observed
67A-VP-1	1,800	6	4-23-98
69-VP-1	4,900	7	4-23-98

Note: Pools surveyed for herps include vernal pools, as defined by the Massachusetts Natural Heritage and Endangered Species Program and other water bodies that contained or could contain breeding amphibians.

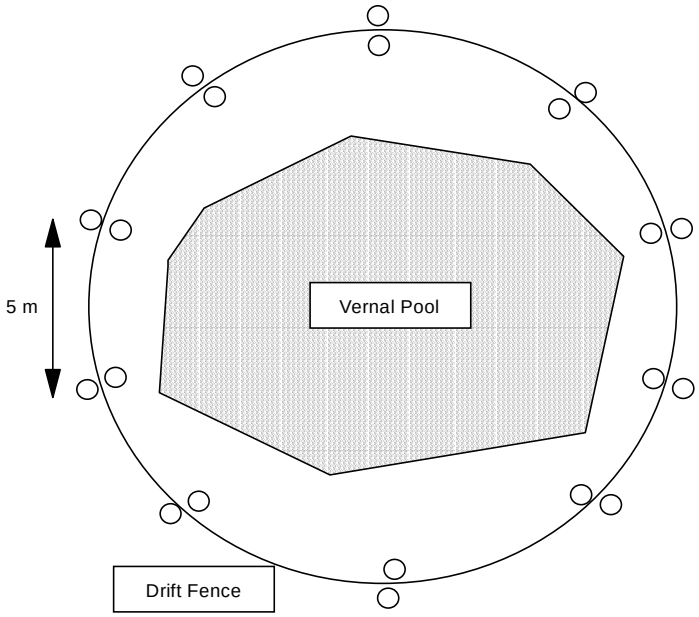
and rainfall throughout the study beginning on, or about, 1 April 1999.

2.2.1 AMPHIBIANS ENTERING VERNAL POOLS

To determine the relative abundance of amphibians entering vernal pools to breed, drift fences with pit traps (Corn and Bury, 1990; Corn, 1994) will be constructed in a circle around each of the study pools (Dodd and Scott, 1994). Pit traps will be constructed from two No. 10, or similar, metal cans (approximate diam. 61/8 in [15.6 cm], depth 14 in [35.6 cm]). Traps will be placed at approximately five meter intervals and will be paired inside and outside the fence (Figure 2). Each trap pair will be given a unique number and its position will be recorded using a Trimble ProXR™ GPS receiver. Drift fences and traps will be installed by 1 April 1999, or as soon as weather and ground conditions permit.

Figure 2

Drift Fence and Pit-Trap Configuration



Pit traps will be checked daily and captured individuals will be identified to the species level, sexed, measured, weighed (to nearest 0.1 g), marked, and released onto the pool-side of the drift fence. If large numbers of individuals are captured and marking all individuals becomes too time consuming, then a minimum random sample of 25 males and females per species will be marked. Body measurements (to nearest mm) will include head and body length (snout-vent length or SVL), tail length, total length, and the length of each hind and fore limb. Each individual will also be inspected for deformities, erosion, lesions, and tumors (DELTs) using procedures developed by the North American Reporting Center for Amphibian Malformations (Attachment 1). Individuals will be marked with fluorescent elastomers in colors and locations on the body that are unique to the individual animal, species, sex, and study pool (Donnelly et al., 1994). If necessary for identification, a collection of six voucher specimens (3 %:3 &) may be maintained

for captured species (Reynolds et al., 1994) using McDiarmid=s (1994) methods for specimen preservation. During the course of studies associated with the Housatonic River specimens will be held by Woodlot Alternatives, Inc. Following completion of work on the river, specimens will be offered to the Harvard Museum of Comparative Vertebrate Anatomy. If this museum does not accept the specimens, they will be offered to the University of Massachusetts.

2.2.2 Courtship and Breeding Behavior and Condition

Courtship activity and breeding behavior and condition within the study pools will be monitored as soon as the first amphibians are captured and placed on the pool-side of the fence. Audio surveys will be conducted to determine if male frogs in the vernal pools are calling to advertise their position to potential mates and rivals (Zimmerman, 1994). To conduct these surveys, observers will sit quietly beside a pool and note general presence/absence of frog chorus.

During audio surveys, observers will attempt to determine if frogs are engaged in breeding activities (e.g., observing amplectic embraces [Duellman and Trueb, 1986]). While searching for breeding activity in frogs, observers will also look for breeding salamanders. Salamanders do not vocalize during courtship, but instead engage in series of maneuvers designed to persuade the female to breed (i.e., a nuptial dance). With spotted salamanders, which occur in the study area, the nuptial dances of several individuals can Amake the water fairly boil@ (Bishop, 1941, *in* Hunter et al., 1992).

When marking individuals with fluorescent elastomers (see Subsection 2.2.1), unique combinations of colors and locations will be used to differentiate between species and sexes A blue light and amber goggles will be used to enhance the visibility of the elastomer marks. Adults captured in pit traps as they leave the vernal pools will also be inspected for external indicators of breeding activity. In addition to the reproductive organs and their associated tracts, external sexual differences exist in many amphibians. These can include body size, glandular development, skin texture, dermal ornamentation, vocal sacs, and coloration. Some persist throughout adult life, but others occur in response to gonadotropic hormones and can serve as indicators of reproductive activity (Duellman and Trueb, 1986). Secondary sexual characters also include nuptial excrescences (modified dermal and epidermal tissues) in salamanders and anurans (Duellman and Trueb, 1986).

2.2.3 Egg Laying, Hatching Success, and Larval Growth and Development

Each pool will be surveyed daily to locate amphibian egg masses. Within each study pool, five egg masses (if available) of wood frogs and spotted salamanders will be enclosed within a box sampler to monitor hatching success (Shaffer et al., 1994). Box samplers will be 50 cm long, 50 cm wide, and 50 cm deep and will be fitted with small mesh screens on the sides, bottom, and top to allow water to flow into the sampler while minimizing egg predation. Box samplers will be monitored daily to evaluate egg development. At this time, the temperature, dissolved oxygen (DO) concentration, pH, and conductivity of the water will also be measured from a station near the center and edge of the vernal pool. Temperature will be automatically recorded using a *HOBO7* data logger (Onset Computer Corp., North Falmouth, MA), and the remaining

parameters will be measured with hand-held instruments (*YSI*7 *85* salinity, conductivity, DO, and temperature system, and *Omega*7 *PHH-1X* pH meter).

Hatching normally occurs over a relatively short period of time; and once hatching is complete, the remaining egg gelatin and eggs (hatched and unhatched) will be collected and tested for PCBs (total and Aroclors). Eggs, both hatched and unhatched, will first be examined and necropsied by an expert in the field of amphibian larval development and then tested for PCBs. Following hatching, larval amphibians in the box sampler will be monitored daily for 10 days, a period sufficient to measure early growth, yet not detrimental to the young larvae, which feed on algae and microorganisms (Hunter et al., 1999) that are of limited supply in the samplers. Larvae will be captured with a sweep net, and then measured for body length (SVL), tail length, and total length. Each individual will also be inspected for DELTs. After 10 days, the larvae will be released into the vernal pool to be exposed to the normal conditions of the remainder of the pool. A random sample of 25 individuals/species, or 50% of the total if less than 50 are available, in each box sampler will be retained and examined by an expert in the field of amphibian larval development and then tested for PCBs (total and Aroclors) and if sufficient tissues mass is available, PCB congeners and homologs, dioxins/furans, and select OC pesticides.

At least 10 aquatic funnel traps will be placed in each pool selected for sampling to monitor larval growth and development on a weekly basis (Shaffer et al., 1994). Marbled salamanders lay their eggs in the fall and larval forms can be active even when ice covers the vernal pool (Kenney, 1995). Traps, therefore, will be deployed during the first week of April 1999, or as soon as site conditions allow. Traps will be placed in the pools in the evening and collected the following morning. If too many individuals are being captured, resulting in trap mortality, the amount of time the traps are operating will be reduced or the time of day during which the traps are operated may be changed. At each trap location, water depth and micro-habitat information will be recorded, as will the trap entry and exit date and time.

The total number of larval amphibians of each species will be recorded for each trap, and the SVL, tail, total length, and length of each limb of up to 25 individuals of each species will be recorded. For abundant species, such as wood frogs, five measured individuals from each trap will be weighed and examined by an expert in the field of amphibian larval development. This will result in a total sample of up to 50 individuals per pool per week (i.e., 10 traps and 5 individuals/ trap). If traps contain fewer than 10 individuals, then half of the individuals will be examined. For less common species, such as spotted salamanders, only one individual will be sacrificed from each trap per week (i.e., total sample of 10 individuals per pool per week). General notes on trap mortality, condition of larvae, associated invertebrates, and any other observations will also be recorded. Aquatic macroinvertebrates (some of which eat larval amphibians) from each trap will be collected and preserved in 80% ethanol for potential identification to the lowest practicable taxonomic unit. Dipnet sampling may also be used to supplement aquatic funnel trap sampling.

2.2.4 Metamorphosis and Exodus from Vernal Pools

Drift fences and pit traps will be monitored daily to record the exodus of metamorphosed juveniles from the pools. Captured individuals will be measured, weighed, and inspected for

DELTs using the methods described in Subsection 2.2.1. Following examination, these individuals will be placed on the outside of the drift fence.

2.3 TISSUE COLLECTION FOR PCB ANALYSIS

Amphibians that succumb incidentally to the performance of this study, or that are sacrificed, may be preserved and submitted for PCB (total and Aroclors) tissue analysis (Attachment 2). If sufficient tissue mass is available, additional analyses that may be conducted include PCB congeners and homologs, dioxins/furans, and select OC pesticides.

2.4 DATA ANALYSIS

2.4.1 Amphibians Entering Vernal Pools

The goals in analyzing data from this portion of the study are to: 1) compare the relative abundance of amphibian species using each of the study pools, 2) compare the time of arrival, by species and sex, between study pools, 3) compare the proportion of the population with DELTs between pools, and 4) compare the body sizes of amphibians, by species and sex, among pools. The primary purpose is to characterize the community of breeding amphibians entering each vernal pool. Identifying an effect of PCBs on amphibians entering the vernal pools is not a primary goal of this portion of the analysis because a variety of natural factors can influence species composition and condition within vernal pools. Significant differences in species composition, sex ratios, body size, and/or the proportion of the population with DELTs between pools with high and low concentrations of PCBs, however, will be interpreted as suggesting a potential effect. Such potential effects, however, will be investigated as part of the remainder of the study.

Species richness (i.e., the number of species) will be reported for each study pool and qualitatively compared to species richness within similar pools in the region based on existing pool descriptions compiled by the Massachusetts Division of Fisheries & Wildlife, Natural Heritage and Endangered Species Program. The relative abundance of amphibian species using each of the study pools will be compared using a contingency table and chi-square (χ^2) analysis (Fienberg, 1983; Zar, 1984).

The hypotheses for this analysis can be stated as:

H_O: The species composition is similar in each of the study pools.
H_A: The species composition differs between one or more of the study pools.

Due to the inherently variable nature of species composition in vernal pools, a significance level of 10% ($\alpha = 0.10$) will be used in determining whether to accept or reject the null hypothesis (H_O) for this analysis. The actual significance level (α) and test statistic (χ^2) of the analysis, however, will be reported. All statistical tests will be run using the *Statistica* J software package (StatSoft7, Tulsa, OK). The statistical power of this test (i.e., the probability of rejecting the null hypothesis when it is in fact false and should be rejected) will be reported using the *Power and*

Precision J software package (Biostat7, Teaneck, NJ) and a power value of 0.70 will be considered acceptable for this analysis.

Since the number of amphibians entering each pool cannot be predicted, the power of this test cannot be determined before implementing the study (a priori). A likely scenario, however, is that one or two species, particularly wood frogs, will be common in each pool and one or two species will either occur in low numbers or perhaps be absent from some pools. The absence of some species, such as the blue-spotted salamander, could conceivably be due to natural causes and/or the effects of PCB contamination. In the example in Table 2, 50 individuals were captured in each of the five sample pools and four species were observed. Two species, wood frog (RASY) and American toads (BUAM), were common and spotted salamanders (AMMA) and blue-spotted salamanders (AMLA) were absent from Pool 5. In this example, with $\alpha = 0.10$, the power of the χ^2 test is 0.76 (Borenstein et al., 1997), and it can be concluded that the observed difference in species composition was statistically significant. The biological significance of this observation, however, would depend upon the results observed in the remainder of the study.

This analysis is sensitive to small changes in the proportion of rare species in the sample. Changing the proportion of spotted salamanders in Pool 5 to 5%, and decreasing the proportion of wood frogs to 65%, for example, lowers the power value to 0.49, and it could be concluded that the difference in species composition was not statistically significant. In this example, however, the test is relatively weak (power=0.37). Even collapsing the table to combine Pools 1-4 in a comparison with Pool 5, which would be done if Pools 1-4 were not statistically different, would only increase the power of the test to 0.56. Ultimately, the power of this test will depend upon the number of individuals captured for each species, the observed sex ratios, and the number of pools sampled. The number of pools to be sampled will depend upon the results of the sediment PCB sampling. Once these data are available, a power analysis for this test will be run to assist in selecting the number of study pools.

Analyzing the proportion of the sample exhibiting DELTs would be accomplished using a three-dimensional contingency table (i.e., pools *x* species *x* with/without DELTs). Multiple models are possible with this type of analysis and are too complex to explain here (see Fienberg, 1983 and Zar, 1984 for a detailed discussion). The goal of the analysis, however, is to determine if there is a difference in the occurrence of DELTs between pools, between species, or some combination of pools and species (i.e., an interaction effect). The *Power and Precision* J software package does not calculate power values for multidimensional contingency tables. As with the preceding tests, however, an α of 0.10 will be considered statistically significant for this analysis. An assumption of this test is that animals coming back to the pool were either born in the pool and/or have come to breed in it in previous years. This assumption cannot be tested as part of this study, but is, nonetheless, reasonable based on previous studies. Efts (*Notophthalmus viridescens*), for example, usually return to the pond where they hatched, even when other pools are available (Hurlbert, 1969, *in* Duellman and Trueb, 1986). Individual amphibians, in fact, even frequently enter and leave vernal pools by the same path year after year (Shoop, 1965 and 1968; Hardy and Raymond, 1980, *in* Duellman and Trueb 1986).

Table 2

Sample Power Analysis for Amphibian Captures

	Proportion falling in column				Proportion in Row
	AMMA	AMLA	RASY	BUAM	
Pool 1	15	5	50	30	0.20
Pool 2	15	5	50	30	0.20
Pool 3	15	5	50	30	0.20
Pool 4	15	5	50	30	0.20
Pool 5	0	0	70	30	0.20

Number of cases = 250

Alpha (2-tailed) = 0.10, Power=0.76

Power computation: Normal approximation

The observed sex ratio for each species will also be compared between pools using chi-square analysis. With many amphibians, males can outnumber females in vernal pools even under natural conditions. Detecting a difference in sex ratios between pools, therefore, may involve comparing already low proportions (see Table 3 for an example).

Table 3

Power Analysis for Hypothetical Sex Ratio of an Amphibian Species

Borenstein et al., 1997

	Proportion falling in column		Proportion in Row
	Female	Male	
Pool 1	20	80	0.20
Pool 2	20	80	0.20
Pool 3	20	80	0.20
Pool 4	20	80	0.20
Pool 5	5	95	0.20

Number of cases = 125

Alpha (2-tailed) = 0.10, Power=0.37

Power computation: Non-central chi-square

Body weight is undoubtedly related to body size within each species. In addition, length/weight relationships can be used as a general indicator of the health of amphibians. A salamander with a normal SVL, but low body weight, for example, might be undernourished or have other health problems. The relationship between the body length (SVL) and weight measurements, therefore, will be analyzed using simple linear regression (Zar,1984). Weight will be used as the dependent variable and SVL as the independent variable. For these tests, an α of 0.10 and a power value of 0.70 will be used to test the significance of each regression. Since the number of animals captured can=t be predetermined or influenced by the design of the experiment, an *a priori* power analysis is not reported here. The power of each regression, however, will be reported when presenting the results of the analysis.

Assuming that the regressions of body size and weight are statistically significant, the slope of the regression lines (β) for each species will then be compared using analysis of covariance (ANCOVA) to determine if there is a significant difference in the length/weight relationship between pools (Zar 1984). The hypotheses for this analysis can be stated as:

$$H_O: \quad \beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta_5$$

$$H_A: \quad \beta_1 \neq \beta_2 \neq \beta_3 \neq \beta_4 \neq \beta_5$$

If the null hypothesis is rejected, multiple comparisons among slopes will be used to determine which βs are significantly different (Zar, 1984). From a biological standpoint, the analysis will be used to determine if one or more of the pools has a subpar length/weight relationship for adults as they enter the pool.

If the null hypothesis $\beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta_5$ is not rejected, it may still be possible that body size is significantly different among study pools (i.e., the length/weight relationship is similar, but animals are smaller in one or more of the pools). This difference will be tested by comparing the elevation of the regression lines using ANCOVA and multiple comparison procedures (Zar, 1984). According to Cohen (1977, *in* Zar, 1984), the power of this analysis can=t be determined, except in very unusual cases. In addition, the *Power and Precision* J software package does not calculate power values for this analysis. As part of this study, however, we will determine if other methods of determining the power of this test have been recently developed. If they have, the power of the test will be reported along with the observed significance level (α).

2.4.2 Courtship and Breeding Behavior and Condition

Data to be analyzed related to courtship and breeding behavior and condition include: 1) surveys for chorusing frogs followed by searches for visual evidence of breeding activity (e.g., amplexus in frogs), and 2) inspecting adults entering and leaving the pool for external indications of reproductive activity.

Amphibians are attracted to breed in vernal pools in response to a variety of endogenous and extrinsic factors (Duellman and Trueb, 1986). Endogenous factors include the seasonal development and activity of male and female gonads and the development of secondary sexual characteristics in males in response to secretions from the pituitary gland. These biochemical

processes are potentially influenced by PCB contamination, but are not the subject of this study. The study, instead, is focused on outward evidence that these processes are functioning normally. This evidence includes: 1) males and females of all species are coming to vernal pools at the appropriate times, 2) secondary external sexual characteristics are present, 3) males are calling or otherwise seeking out mates, and 4) breeding is taking place.

Audio chorus and visual evidence of breeding (e.g., amplexus in frogs, nuptial dance in salamanders) can be difficult to observe because: 1) amphibians may avoid human observers, 2) many amphibians, and salamanders in particular, are difficult to observe, and 3) for the previous two reasons the data acquired through direct observation may not be random. In addition, being in the pool for the length of time needed to collect quantitative data may unnecessarily disturb activity within the pool. Therefore, reproductive evidence will be qualitatively described for the pools.

Amphibians leaving the pool that are captured in pit traps will be weighed and inspected for DELTs and external evidence of breeding activity. Individuals may gain or lose weight while in the vernal pool, and excessive weight loss may be an indication of compromised health. Change in weight, therefore, will be compared between pools for each species. Data for this analysis will come from the weight of the individual (i.e., individuals will receive unique color markings) when it leaves the pool compared to its weight when it entered the pool expressed as a percent increase or decrease. Change in weight (the dependent variable) is potentially related to the length of time the animal spends in the pool. It is also expected to be influenced by species and sex and to potentially vary from pool to pool. Multiple linear regression, therefore, will be used to evaluate weight change in relation to: 1) pool, 2) species, 3) sex, and 4) length of time in the pool (Zar, 1984 et seq.). As with other analyses, a significance level (α) of 0.10 and a power value of 0.70 will be used to determine the statistical significance of these tests.

Methods for comparing the proportion of the population in each pool exhibiting DELTs has been described in Section 2.4.1 and will be repeated for animals captured leaving the pool. Similar methods will also be used to compare the proportion of the population, by species and sex, in each pool exhibiting external evidence of breeding activity.

Since animals will be marked as they enter the pools and counted again as they leave the pools, the proportion of the population surviving within each pool can be estimated as $N_{leaving}/N_{entering}$. Adult amphibians that come to vernal pools to breed generally do not remain in the pool after breeding and egg-laying. Animals that are not recaptured in the pit-traps therefore, will be assumed to have died in the pool. The number of individuals alive and dead at the end of the study will be compared between pools using contingency tables and chi-square analysis. Since the number of animals entering the pool cannot be manipulated, an a priori power analysis is not presented here. The significance level (α), power value, and test statistic, however, will be reported and an α of 0.10 and a power value of 0.70 will be considered statistically significant.

2.4.3 Egg Laying, Hatching Success, and Larval Growth and Development

Data related to egg laying, hatching success, and larval growth and development includes: 1) numbers of egg masses per species in each pool; 2) growth and development of larval forms in

box samplers; 3) survival of larval forms in box samplers over a 10-day period; 4) PCB concentrations in larval forms; and 5) growth and development of larval forms throughout the pools.

A complete count of all egg masses in each pool is likely not possible without significantly disturbing the pool. In addition, some species' egg masses are near the bottom of the pool or hidden among plants within the pool. The number of egg masses observed in each pool, therefore, will only be reported and qualitatively compared between pools in relation to the number of individual females of each species known to be in the pool.

Within each box sampler, the number of hatched and living larvae will be counted on a daily basis. The number of larvae will be determined by sweep netting the box sampler to retrieve all live larval forms. Enumeration of larvae in the box samplers may be estimated if total numbers are excessive and holding time required for counting is thought to adversely affect the larvae. The egg mass will be retained for PCB testing, and an expert in larval amphibian development will inspect the hatched and unhatched eggs.

Larval growth and development data include the SVL, tail length, and total length as well as the environmental covariates DO, temperature, conductivity, and pH. These data will be analyzed by multiple regression techniques using SVL as the dependent variable and age (days since hatching), DO, temperature, conductivity, and pH as independent variables. The multiple regression equations for each pool will then be compared using the techniques described by Zar (1984). The hypotheses for this analysis can be stated as:

H_O: The regression functions for each pool box sampler estimate the same population regression.

H_A: The regression functions for each pool box sampler do not estimate the same population regression.

The same analysis will be used to compare growth and development throughout the pools (i.e., the data from larval forms captured with aquatic funnel traps).

Survival of the larval forms within the box samplers will be examined and compared between pools using a 10-day daily survival analysis (Caughley, 1977 and *StatSoft*7 1995). The final models to be used in this analysis, however, will be determined in association with EPA scientists.

2.4.4 Metamorphosis and Exodus from Vernal Pools

Data related to metamorphosis and exodus from the pools will include: 1) numbers per species, 2) SVL, 3) weight, and 4) proportion of the population with DELTs. The number per species in relation to the number of adult females entering the pools will be compared between pools, by species, using chi-square analysis (i.e., a 2-dimensional contingency table). These techniques and the associated hypotheses have been described in previous sections. The length/weight relationship will be compared between pools using the regression techniques described in previous sections. In addition, the proportion of the population with DELTs will be compared using the contingency table and chi-square analysis described earlier.

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The objectives of the amphibian reproduction study were discussed in Subsection 1.1. To achieve these objectives, the following types of data will be required:

Taxonomic identification of amphibian organisms: taxa will be identified to specific level based on morphological, habitat, and distributional information. Adult individuals are generally unproblematic compared to larval stages. Knowledge of which adult species has entered the pools (i.e., distributional information) will be used in conjunction with time of appearance to assist species determinations.

Gender identification of amphibian organisms: the gender of adult individuals of each species will be determined on the basis on gender specific reproductive traits (e.g., swollen thumbs in males frogs, size of tympanum in bull frogs and green frogs, swollen venter in male salamanders).

Amphibian age: determination of age (adult, juvenile, metamorph) will be primarily performed though use of published size data (length and weight). Demonstration of sexual characteristics (as for gender determination) and date (metamorphosed individuals will not be present until early summer) will also be used as evidence for classifying the age of amphibian individuals.

Morphometrics for each individual: detailed length and weight measurements for individual amphibians will be recorded. Length measurements will be obtained to the nearest 0.1 mm using hand-held dial calipers. Weight measurements will be obtained to the nearest 0.01 grams using a calibrated balance designed to be capable of accurately measuring masses of this magnitude.

Visual inspection for deformities, erosions, lesions, and tumors (DELTs): each individual amphibian will be examined for DELTs as outlined in Subsection 2.2.1.

Unique individual indentifiers: individual amphibians will be marked through use of fluorescent elastomers that are injected under the skin, as described in Subsection 2.2.1. Elastomers are viewed through use of blue light and amber goggles. To determine the effectiveness of ingredient mixing (elastomer and curing agent), and stability and visibility of marks, captured amphibians will be cross-referenced with marking code charts to ensure that unique marking codes are applied to each individual.

Water chemistry and description data: each day, vernal pools will be measured for temperature, pH, dissolved oxygen, conductivity, and depth. Information will be acquired for the shallow edge and deeper center of the pools. Devices used to acquire the measurements will be the Hobo Data Logger, YSI 85 salinity, conductivity, dissolved oxygen, and temperature system, Omega PHHH-1X pH Meter, and staff gauges, as described in Subsection 2.2.3. Except for the Hobo

Data Logger and staff gauges, which remain in the pool for the length of the study, the remainder of the devices are hand-held units and are calibrated daily. Temperature will be measured to 0.1 Celsius, pH to 0.1, dissolved oxygen to 0.1 mg/l, conductivity to 0.1 Fs, and depth to 0.1 feet.

Weather data: each day, in the close vicinity of the vernal pool, current, daily minimum, and daily maximum temperature, rainfall, and humidity information will be recorded. Temperature will be measured to the nearest degree Celsius, rainfall to 0.1 mm, and humidity to 0.1%.

3.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. Each of these data quality indicators that are applicable to this study are discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Due to the type of organism involved (living organisms subject to stress from researcher handling) and its characteristics (though a plant may have multiple flowers per stem, an individual frog only has one right forelimb), repeated measurements will not be performed for many types of data collected. Rather than control and measure precision, the study design includes a large number of samples (25 or more) for each species involved in the study, when possible, to provide statistical resolution.

Accuracy is defined as the agreement of a measurement with its true value. For parameters of concern to this study, accuracy is defined as meaning individual amphibians are correctly identified (species, sex, and age) and measured (length and weight), and that physical and chemical data was collected appropriately. Accuracy will be controlled through training and careful supervision by the project manager and daily calibration of hand-held instruments.

Completeness is defined as the percentage of the planned samples actually collected and processed. For some aspects of this study, completeness is a difficult indicator to achieve. For example, during the aquatic funnel trapping effort (Subsection 2.2.3), traps will be deployed early in the season to determine the presence of marbled salamanders. During this time, other species of larval amphibians are not active. Due to the rarity of marbled salamanders, it is likely that few or no captures of amphibian larvae will result during the early trapping effort. The objective, however, is to collect 50 individuals per pool per week of trapping. Therefore, completeness must be a flexible indicator and samples will be processed as possible, given the limitations of season, pool size, species present, etc.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved sampling design and through careful implementation of the sample processing and analytical methods. Additionally, results from the amphibian reproduction study can be compared to similar studies for the region to evaluate the representativeness of the data collected.

Comparability is defined as the measure of confidence with which the results from this study may be compared to data from a similar study. Comparability of this study will be limited due to potential interaction of the results and PCB concentrations. However, a thorough literature survey may reveal similar studies from the region on which to make comparisons of study results.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For the amphibian reproduction study, chosen instruments are capable of measuring at desired levels of sensitivity. This data quality indicator also applies to identification of species, age, and sex of individual amphibians. Techniques for assessing these data will be important in order to limit the number of unknown determinations. As the number of unknown diagnoses increases, the ability of analyses to determine differences in sex and age ratios declines. This data quality indicator can be evaluated by comparing the number of unknown to potential known identifiers (the sex of some species as juveniles cannot be determined in the field due to lack of gender specific differences at that life stage, and therefore sex was not potentially known).

3.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical and physical data (e.g., sediment samples) are discussed in various sections of the project QAPP and will be used whenever applicable to the study. Usability of information gathered during this study will be based on: (1) the experience of the senior investigator to competently oversee field investigations and ensure that field surveys were conducted following the established plan and to accurately identify specimens collected at survey locations; and (2) an evaluation of the taxonomic data collected in the study area compared to previous studies from the biophysical region.

3.2 SAMPLING DESIGN

Survey locations for the amphibian reproduction study will be based on rationale presented in Subsection 1.2. The locations are not intended to be characteristic of the vernal pools in the study area, but rather represent a range of sediment PCB concentrations. To facilitate comparisons for statistical analyses, pools will be selected on the criterion of species observed during 1998 field surveys.

Herpetofauna communities are typically highly variable in nature. To achieve acceptable resolution for analyses it is important that sample size be sufficiently large to capture population variability and provide necessary power. When possible, twenty-five or more samples will be collected for each parameter (e.g., tail length for larval frogs, weight of adult males).

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Sampling methods, as described in Subsections 2.2 and 2.3, were chosen to insure the study objectives will be obtained. Sampling procedures were developed to encompass each stage of the amphibian life cycle (egg, larvae, metamorph, juvenile, adult). Project oversight and researcher training will serve to facilitate accurate and unbiased sampling.

3.3.2 Quality Control Samples

The nature of ecological studies and the type of sample involved (i.e., living organism) does not allow the incorporation of typical duplicate and blank samples as part of the study design. As well, weather and vernal pool chemistry (e.g., dissolved oxygen, pH, conductivity) data does not possess acceptable methods for obtaining samples in a manner analogous to duplicates and blanks collected for soil chemistry analysis. Quality control will be performed though use of project oversight and calibration of instruments.

3.3.3 Sample Processing and Preservation

Description of the types of samples to be collected for this study can be found in Subsections 2.2 and 2.3. Samples can be classified in the manner they will be processed: egg masses; larval frogs; and dead amphibians. Egg masses will be collected in the field in chemically clean jars with water from the site. At the field office, jars containing the egg masses will be placed in large coolers packed with vermiculite to protect contents during shipping. Each jar, as well as the cooler, will possess custody seals and chain of custody forms will accompany the shipment.

Larval frogs (i.e., tadpoles) will be collected in the field in chemically clean jars with water from the site. At the field office, jars containing living larval frogs will be placed in large coolers packed with vermiculite to protect contents during shipping. A small volume of wet ice (ca. 2 liters) will be placed in a resealable plastic bag and located in the cooler to insure the contents remain at a suitable temperature. Each jar, as well as the cooler, will possess custody seals and chain of custody forms will accompany the shipment.

Individual amphibians, regardless of the size or age, that have succumbed to incidental mortality will be collected in the field in labeled, resealable plastic bags and stored in a cooler on wet ice (enclosed in plastic containers), to prevent decay, until delivery to the field office. Amphibians will be removed from the plastic bags at the office, rinsed in distilled water, and snap frozen in liquid nitrogen in a decontaminated mortor. Samples will then be placed in labeled, chemically clean jars and stored in a freezer (maximum temperature -20 Celsius) until shipment to the laboratory. Specimens will be shipped in large coolers on dry ice with appropriate custody seals and chain of custody forms. Holding time for all samples will follow the procedures established in the QAPP.

3.3.4 Training

Sampling will be directed in the field by senior scientists with academic training and experience in the collection of amphibians and related data. Support staff will receive training from the senior scientists in the goals and techniques to be employed during the study to insure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Biological Samples

The collection of taxonomic and morphometric information for samples will be processed by experienced staff who have received specific training in the SOP and whose work is directly overseen by the project manager. Determination of species, sex, age, and collection of morphometric data does not require magnification, with the exception of larval amphibians. Magnification, in the form of a 10H hand lens, will be required for identification of these samples. Collection of samples for PCB analysis and laboratory study will follow procedures outlined in Subsection 3.3.3. Analysis of biological samples for PCB concentrations will follow standards established in the QAPP.

3.4.2 Physical/Chemical Samples

Sediment and water samples will follow procedures and SOPs provided in the QAPP. Weather and water chemistry data recorded daily for the amphibian reproduction study will assessed in the field rather than through collection of physical samples.

3.5 DATA ANALYSIS AND REPORTING

The overall analytical approach to collection, analysis, and reporting is detailed in Section 2. Numerical analyses will be performed using Statistica for Windows™ (Statsoft, 1999). The study findings will be included in the ecological risk assessment, including data, analyses, and interpretations, and will be prepared with specific reference to both the data quality and objectives specific to the amphibian reproduction study.

4. EQUIPMENT LIST

Equipment that will be needed as part of the field component of the study includes:

- Camera
- Binoculars
- Field notebooks
- Rubber knee- and hip-boots and chest waders

- Heavy-duty rain gear
- Eye protection
- Rubber gloves
- GPS receiver
- D-ring dipnet
- Dissecting tray
- Funnel traps with small rope
- Box samplers
- Fluorescent pigments
- Scale
- Sample jars, both chemically and non-chemically clean
- Plastic sheeting
- Hammer
- No. 10 (or similar) cans with covers
- Duct tape
- Wooden shingles
- Wooden stakes
- Heavy-duty stapler
- Ethanol
- Survey flagging
- Resealable bags
- Wet and dry ice
- Aluminum foil
- Coolers for shipping samples
- Conductivity, DO, pH meters
- Thermometer

5. RESULTS

The results of this study will be provided in a report that includes: 1) a physical description of each study pool using methods described in Work Plan Appendix A.9; 2) PCB concentrations within the sediments of each pool; 3) a summary of the potential effects of PCBs on amphibians based on the literature review; 4) summary statistics for data collection activities (e.g., mean, standard deviation, sample size); 5) results of statistical tests (e.g., final hypotheses, test statistics, significance levels, and power values where applicable); and 6) an interpretation of the results.

6. LITERATURE CITED

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ATTACHMENT 1

NARCAM PROCEDURES FOR CHARACTERIZING DELTs

Standard Field Forms

Included in this section are field forms designed by a working group of scientists researching the amphibian malformation issue. We are awaiting standard protocols as well. In the meantime, we suggest following the protocol designed for surveys on National Wildlife Refuges.

We are making these forms available to verifiers who are interested in collecting detailed data for their own purposes. If so, completing the forms ensures that data is collected in a standardized manner and is comparable to other efforts in North America.

General Survey Information

Reporter Information

Reporter's Name:_____
E-Mail Address:_____
Postal Address:_____
City:_____
State/Province:_____
Phone Number:_____

Survey Site Information

State/Province:_____
County (if in US):_____
Town/City:_____

Date

Date of Observation:_____/_____/_____

Species

Species Name:_____

Amphibian Numbers

Number of Normal Individuals:_____ Number of Malformed Individuals:_____

Malformation Description

Please complete this form for each malformed individual

*These categories are for use with metamorphosing (4 legs) or adult frogs and toads.
*Start exam from the head and work toward the hind legs, noting any abnormalities seen by checking the boxes below.
*If a deformity is seen that does not fit into one of the categories below, please describe the deformity in the sections marked "other".
*Deformities that are difficult to describe can be drawn in on the frog diagram below.
*NOTE: "Left" and "Right" refer to viewing the frog from above as it would rest normally.

Eyes:

L	R	
___	___	eye absent
___	___	eye smaller than normal
___	___	pupil abnormally shaped
___	___	eye in unusual position(describe):_____
___	___	extra eye(s) (describe):_____
___	___	other (describe):_____

Jaws:

L	R	
___	___	lower jaw shortened
___	___	upper jaw shortened
___	___	other deformity (describe):_____

Front limbs:

L	R	
___	___	entire limb missing at shoulder
___	___	limb partially missing:(describe):_____
___	___	foot missing
___	___	complete calf (tibiofibula) present, abnormal musculature
___	___	enlarged
___	___	small (atrophied)
___	___	digits missing from foot (specify digits):_____
___	___	digits fused or clubbed
___	___	other (describe):_____

Spine:

L	R	
___	___	curved to the left or right (scoliosis)
___	___	other (describe):_____

Webbing (cutaneous fusion):

L	R

___ ___ between thigh and calf (femur and tibiofibula)
___ ___ other (describe degree):_____

Hind Limb:

L R

___ ___ entire limb present, unusual angle (twisted, rotated, etc.)
(describe):_____
___ ___ entire limb present, abnormal size (atrophied, enlarged)
(describe):_____
___ ___ digits missing from foot (specify digits):_____
___ ___ digits shortened, fused or clubbed:_____
___ ___ digits in abnormal location (describe):_____
___ ___ extra digits:(describe):_____
___ ___ foot missing (tarsal bones)
___ ___ complete calf (tibiofibula) present, abnormal musculature
___ enlarged
___ small (atrophied)
___ ___ portion of calf (tibiofibula) missing:
(estimate length of calf present):_____
___ ___ entire calf (tibiofibula) missing
___ ___ complete thigh (femur) present, abnormal musculature
___ enlarged
___ small (atrophied)
___ ___ portion of thigh (femur) missing
(estimate length of thigh present):_____
___ ___ entire limb missing
___ ___ other (describe):_____

___ **Abnormal color or pattern:**

(describe and locate:)_____

Extra Limbs:

How many extra limbs are present?_____

For each extra limb, describe location of origin (left or right, hip, knee, spine, etc.). Also specify musculature (larger or smaller than normal limb), and completeness (entire limb present, or portion of limb). If only part of an extra limb is present, try to specify which part is present (thigh, thigh and calf, foot, etc.). Draw the extra limbs on the frog diagram below.

extra limb #1
location_____

musculature_____

completeness_____

extra limb #2
location_____

musculature_____

completeness_____

___ **Retained tail**

Fully:(length)_____
Partially (length):_____

___ **Any bleeding or fresh injuries?:**
(describe):_____

___ **Other abnormalities:**
(please describe):_____

Site Characterization Form

Field Crew:_____ **Date:** ___/___/___ **Site #:**_____

Weather: Clear/Sunny:___ **Some Clouds:**___ **Overcast:** ___ **Rain:**___ **Temp.(°F):**___

Location: Map Name:_____ **Map Scale:**_____

GPS Coordinates: Latitude:_____ **Longitude:**_____

GPS Sampling Time:_____ **GPS Post-Processing?: YES / NO**

Address nearest building/residence:_____

Street/Highway address:_____

Town:_____ **County:**_____ **State:**_____

Describe how to find the site (e.g. closest intersecting roads, mileage marker, etc.)
_____
_____
_____

Stream/River Name:_____ **Pond/Lake Name:**_____

Federal/State/Reserve/Park Name:_____

LAND USE/LAND COVER:
Describe Surrounding Land Uses:_____

Describe Major Land Uses:_____

Upland

___Urban/Roads/Commercial/Industrial ___Residential/Roads ___Cropland
___Barren Land ___Deciduous Forest ___Shrub/Brush ___Orchard
___Mixed Forest ___Coniferous Forest ___Plantation ___Pasture

Wetland/Water

___Upper perennial stream ___Lower perennial stream ___Intermittent Stream
___Open Water (Describe depth and extent, if known)_____
___Emergent ___Vegetated Shallows ___Wet Meadow/Agricultural ___Scrub/Shrub
___PFO1(broad-leaved deciduous e.g. red maple swamp)
___PFO2(needle-leaved deciduous e.g., tamarack swamp)

___PFO4(needle-leaved evergreen e.g., coniferous swamp)
___PFO5(e.g., dead snags)

Comments on Classification (e.g. dominant, sub-dominant, and minor cover types)
_____
_____
_____

Wetland/Hydrology Indicators (e.g. mottled leaves, ring around trees, adventitious sprouting):
_____
_____
_____

Soil Type:
_____
_____

DOMINANT PLANT SPECIES (note **dominant** species in each strata with % cover or class):

Trees (> 20 feet in height and > 5 inches d.b.h.):

Species_____ % Cover____ Species_____ % Cover____
_____
_____

Shrubs/Saplings:(typically < 20 feet in height < 5 inches d.b.h. for saplings):

Species_____ % Cover____ Species_____ % Cover____
_____
_____

Forbs/Herbs/Emergents/Graminoids:

Species_____ % Cover____ Species_____ % Cover____
_____
_____

Ferns/Fern Allies:

Species_____ % Cover____ Species_____ % Cover____
_____
_____

Organic Litter (e.g. leaf litter, deadfall water, etc.):

% Cover_____
_____
_____

Surface Substrate (e.g. soil, bedrock cobbles, etc.):

% Cover_____
_____
_____

Site General Description (e.g., describe variability in the site):
_____
_____
_____
_____

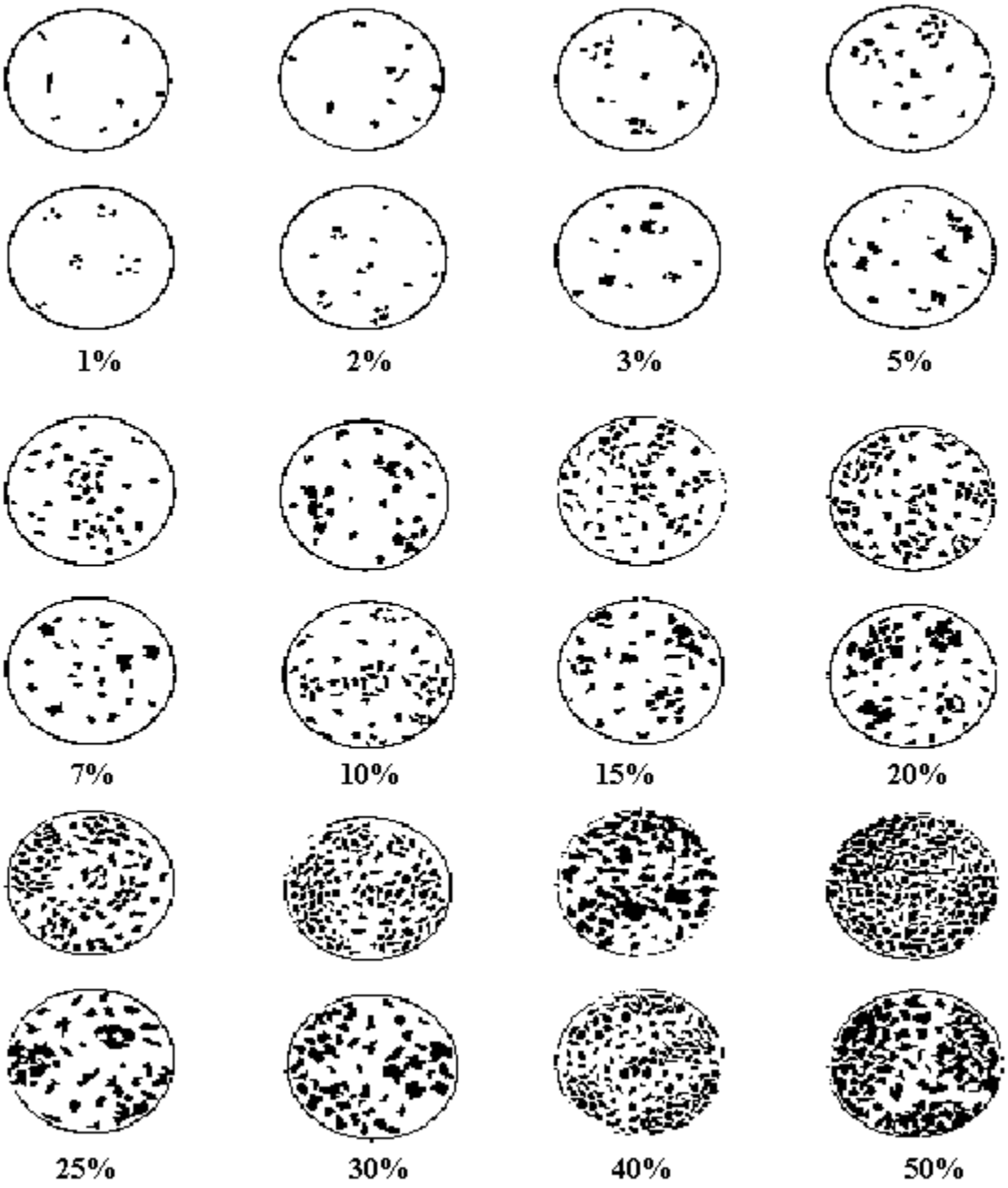
Photographs (Looking out from the center at each of the four cardinal directions. Use the chalk board for site I.D. For homogeneous sites two pictures may suffice):

Roll-Frame #	Compass Direction	Comments

Site Map: Diagram any distinct features or boundaries present within the verification site (indicate the position and compass direction you are facing in photos taken):

NW	NC	NE
WC	C	EC
SW	SC	SE

Comparison Chart for Visual Estimation of Foliage



ATTACHMENT 2

PCB TISSUE SAMPLE COLLECTION STANDARD OPERATING PROCEDURES

Sample Handling for Herpetofauna - Housatonic River. Pittsfield, MA Spring/Summer 1998

1. Mortality specimens (samples) from pitfall traps should be collected on a daily basis, preferably early each morning.. The date and time of collection should be noted.

2. Samples should be retained in the field in chemically clean jars. Each jar (8, 16 or 32 ounce dependent on biomass) should be representative of one trapline area or one pitfall. Jars should be labeled with the site name, trap line/pitfall number, date and time of collection. Samples should be kept on wet ice in the field. The wet ice should be retained in double ziploc bags to avoid contact with samples. If sample biomass is too large for a 32 ounce jar then dedicated decontaminated stainless steel or aluminum buckets should be used for transport.

3. Processing of samples should be as follows: individual specimens should first be thoroughly rinsed with dëionized water for removal of all external organic matter, identified to species, weighed (to the nearest 0.1 gram), measured (nose to vent, to the nearest millimeter), and examined for gross external pathology. All information should be recorded on a separate data sheet for each sample.

4. Each individual should be assigned a specific sample number, based on species, trap line and pitfall number. If samples are composited, the composite should consist of the same species and preferably the same size class. To the extent possible, the number of individuals per composite should remain consistent and be recorded.

5. Individuals should be wrapped in aluminum foil (shiny side out, dull side next to sample). Aluminum foil can be hexane-rinsed or a field blank of representative unrinsed aluminum foil from each roll used should be submitted for analysis. The field blank should be prepared in the same method as biota samples.

6. A sample label should be placed on the exterior of each individually wrapped sample or sample composite. The sample label should be securely taped with clear tape. The sample label should contain the sample number, sample type, analysis to be performed, site name, and date.

7. Aluminum-wrapped, labeled samples should be placed in a ziploc bag and sealed. That bag should be placed in another ziploc bag containing a separate sample tag with the sample number clearly showing.

8. Samples should be kept on dry ice until they are shipped (with dry ice) to the analytical laboratory (or the USFWS field office for freezer storage). For large cooler/numerous sample shipments, layering of samples with dry ice and newspapers allows for better sample preservation and longer dry ice persistence.

9. All pertinent information regarding each sample (sample number, sample type, date collected, etc.) should appear on a standard EPA chain-of-custody form and be included

in a sealed ziploc bag, taped to the lid, inside each sample cooler.

10. Samples should be sent Federal Express Overnight (Next morning delivery). Samples sent to the USFWS should be shipped to:

Ken Carr/Ken Munney
USFWS — Phone: 603-225-1411
22 Bridge St., Unit 1
Concord, NH 03301 — Fed Ex Acct #: 1510-1036-9

Shippers should call ahead to the receiving laboratory or the USFWS and notify that samples are being sent for next day delivery. Samples should not be sent to USFWS if Ken Munney, Ken Carr, or Drew Major are not available for receipt of the shipment. Samples need to be sent for arrival on a weekday only. Therefore, Thursday is the last day of the week to ship samples. Shippers should also call the receiving laboratory or USFWS the day of delivery to verify receipt of samples.

APPENDIX A.19

PROTOCOL FOR FROG REPRODUCTION AND DEVELOPMENT STUDY (FORT)

PROTOCOL FOR FROG REPRODUCTION AND DEVELOPMENT STUDY

STUDY PROTOCOL NO.: WESR01 – RSTS01 - 1

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Preface

The following protocol has been prepared for the "Supplemental Investigation Work Plan for the Lower Housatonic River" under the Technical Support Services, General Electric (GE) Housatonic Project, Pittsfield, Massachusetts, and is intended only for this specific project. The methods used to develop this protocol are available in public scientific literature and are thus non-proprietary. Potential risk associated with the use or misuse of this protocol outside the scope of this project will be assumed by future investigators.

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1. INTRODUCTION

This protocol describes a study design to evaluate reproductive performance and developmental effects in frogs potentially exposed to polychlorinated biphenyls (PCBs) in the Housatonic River study area. Frogs have been chosen as the representative amphibian species due to their presence in the Housatonic River study area, reported sensitivity to PCBs, high potential for exposure due to both aquatic and terrestrial life stages, and capacity to be evaluated for reproductive and developmental metrics in the field and laboratory, and are considered sentinel species in the environment.

The overall objective of this study is to assess the impact of potential PCB exposure on frog populations in the Lower Housatonic River area between the confluence of the East and West branches and Woods Pond Dam (target area). More specifically, the objectives of this study will focus on the potential impact that PCB contamination may have on reproduction, early development, and maturation (metamorphosis) in Northern Leopard frogs (*Rana pipiens*). These events represent critical stages in the life cycle of an amphibian and the evaluation of the effect of PCBs and potentially other organic compounds at these stages will determine the capacity of these contaminants to disrupt the life cycle of amphibians.

This study is designed to determine the effect of PCB exposure to sexually mature adult frogs on reproductive capacity and developmental fitness in their progeny using both target and reference sites. The routes of exposure and generational transport of the PCBs will also be assessed to determine the extent of maternal transfer to the oocytes and developing progeny, as well as the extent of bioaccumulation during early and later development. Reproductive performance and early developmental effects will be assessed by comparing the following endpoints:

- gravidity;
- numbers of eggs produced;
- necrosis;
- oocyte maturity (stage);
- sperm count;
- sperm morphology and viability;
- fertilization;
- early embryogenesis;
- hatching success;
- mortality; and
- morphological development (teratogenesis)

for frogs obtained from the target area, with the same endpoints in frogs originating from a uncontaminated reference area. To document potential impact on longer-term developmental processes, exposure studies will be conducted in the laboratory throughout metamorphosis. Metamorphosis, because of the array of biochemical processes occurring simultaneously, is a sensitive stage in the life cycle of amphibians and a stage that is sensitive to endocrine disruption. Since PCBs, along with organochlorine pesticides, have been shown to alter thyroid function in metamorphosing frogs (Fort et al., 1999a and 1999b), this phase of the study is of great importance.

An overview of this program is provided in Figure 1. In summary, male and female frogs will be collected from the target and reference areas and transported to the amphibian toxicology laboratory. The gravidity of the females will be recorded and the gravid females will be hormonally induced to superovulate egg masses, which will then be fertilized *in vitro*. The number of eggs produced per female will be estimated on a volumetric basis and rates of necrosis and stage determined. Sperm counts, morphology, and overall viability will also be assessed. The eggs will be monitored in the laboratory for fertilization, morphology, and coloration, while the embryos will be monitored for mortality, hatching success (including time to hatch), and morphological abnormalities. Deformities, particularly those that could directly affect juvenile survival and, therefore, the population, will be specifically documented by type of terata induced and number responding. Exposure studies will be conducted throughout metamorphosis of cultured progeny. The rate of metamorphosis, which will include an evaluation of the rate and morphology of limb development, rate and morphology of tail resorption, and development of secondary morphological characteristics, including mature skin, will be evaluated. In addition, a portion of each egg mass, as well as the ovaries of females from which egg masses are obtained, testes from the males, and whole bodies of developing embryos and larvae will undergo PCB and congener-specific analysis to allow determination of a concentration-response relationship between observed effects and PCBs. Although the number of parameters to be evaluated during this study will likely increase the experiment-wide error rate, the utility of measuring multiple parameters (endpoints) will also decrease the likelihood of overlooking a potential effect from PCB exposure.

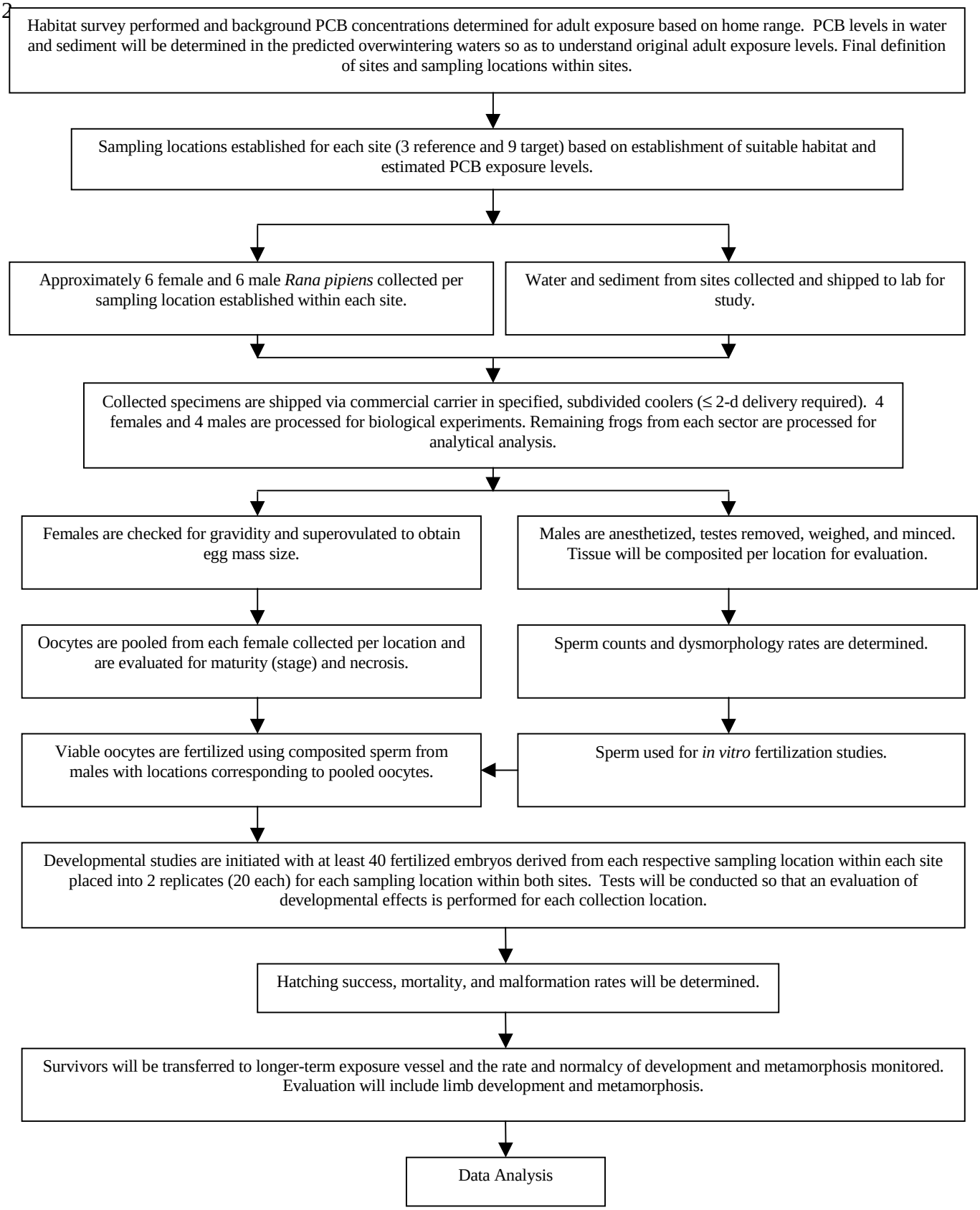
2. DEFINITION OF STUDY METRICS AND ENDPOINTS

Egg mass	Group of eggs laid by a single female
Gravid	Containing a mass of eggs in ovary
Reference area	An uncontaminated area with generally comparable flow regime, habitat, alkalinity, and hardness to the target area
Target area	The reach of the Housatonic River between the confluence and Woods Pond Dam
Viability	Capable of surviving and developing normally (in the case of embryos) or fertilizing eggs (in the case of sperm)

3. SELECTION OF TEST SPECIES

The species selected for this study is the Northern Leopard frog (*Rana pipiens*). Previous field surveys have indicated that Northern Leopard frogs are abundant in the Housatonic River study area and constitute an important component of the Housatonic River ecosystem. They have a limited home range, spending a good proportion of their life spans in aquatic environments; thus, their PCB body burdens should reflect the diet, sediment, and water column concentration in the areas from which they are collected. Also, because Northern Leopard frogs lay thousands of eggs, it should be possible to collect a sufficient number of eggs to ensure statistical power and confidence in study results. Finally, there is an established peer-reviewed methodology for *in vitro* fertilization of Northern Leopard frogs and culturing of their embryos in the laboratory

Figure 1 Overview of Frog Reproduction and Development Study



(Dickerson, 1969; Nussbaum et al., 1983; Carolina Biological Supply Company, 1993; Fort et al., 1996a; ASTM, 1998; Bantle et al., 1998).

Temporal applicability of the study is directed toward the peak breeding season for the Northern Leopard frog (Stebbins, 1995). Sample collection will ideally occur during the last week of April and the first two weeks of May.

The spatial applicability of the target area of the study is limited to the reach of the Housatonic River between the confluence and the Woods Pond Dam. This reach represents a range of PCB concentrations in sediment such that development of exposure-response relationships may be feasible. Additionally, Northern Leopard frog habitat is present in Woods Pond, and other backwater regions. Suitable locations that are representative of a reference area within the Housatonic River watershed will also be identified.

4. REQUIRED EQUIPMENT AND SUPPLIES

Equipment to be used during the field collection phase includes the following items:

- 3 ambient air thermometers
- 15 collection nets, drift fences, and funnel traps
- 2 digital GPS locators
- 45 cubic feet sphagnum moss, cured and sanitized
- 300 data forms
- 4 medium seines
- 50 fiberboard boxes
- field maps
- field notebooks and clipboards
- 25 field marking pencils
- 10 flashlights
- 150 ft bubble wrap
- 10 gallons distilled water
- 1,000 live crickets
- plastic sheeting
- 12 pairs of heavy work gloves
- 12 pairs of steel-toed boots
- polyethylene resealable plastic bags for shipping
- 12 portable cassette tape recorders and tapes
- 5 cellular 900 MHz telephones
- 250 preprinted sample labels
- 15 reusable ice packs
- liquid nitrogen
- dry ice
- 2 rolls of duct tape
- 100 sample containers for residual egg masses
- 50 sample bags for sacrificed female frogs
- 15 permanent magic markers

- Site Health and Safety Plan (HASP)
- 100 6-gallon Styrofoam coolers
- vermiculite
- 10-L 3% (w/v) MS-222 anaesthetic

5. QUALITY ASSURANCE/QUALITY CONTROL

5.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

As indicated in Section 1, the primary objective of this study is to assess the impact of PCB exposure on frog reproduction, development and maturation. Overall, this study will attempt to determine the effect of PCB exposure to sexually mature adult Northern Leopard frogs on reproductive capacity and developmental fitness in their progeny by comparing a series of biological and toxicological indicators in specimen collected from uncontaminated areas (reference sites) and contaminated areas (target sites). In order to achieve this objective, the following data and specific quality assurance criteria will be needed.

Gravidity – Reproductive capacity in female frogs will be evaluated initially by determining if egg masses are present within the specimen collected. Although gravidity is a binary response, gravidity will be determined immediately upon specimen arrival at the laboratory and will be recorded with accompanying chain-of-custody information (sample identification), and health characteristics upon arrival (general appearance and weight). Gravidity will also be used to confirm the sex of the specimen.

Numbers of eggs produced (egg mass) – Total egg counts must be determined and recorded accurately for each specimen. Volumetric determination of egg mass is not accurate or precise enough to use in this case. Therefore, manual counting of the egg masses will be required. Egg masses will be counted at least twice unless the values exceed 10% of one another. In the case of excessive variability, the process of counting will be repeated. In addition, the counts will be verified by a separate analyst using the same criteria described above.

Necrosis and oocyte stage profile – The number of necrotic eggs will be determined using the same approach and quality control measures as described for egg mass determination. The oocyte stage of development profile is one of the best indicators of reproductive status in frogs. The laborious nature of this process requires significant attention to consistency to be accurate and will require independent peer verification. Data verification using the approach described above for egg mass counting will be used to verify the results.

Sperm count, viability, and morphology – The assessment of male reproductive fitness on a gametogenesis level depends on the accurate collection and recording of the data. Total sperm cells and abnormal sperm will be counted at least three times unless the values exceed 10% of one another. In the case of excessive variability, the process of counting will be repeated. In addition, the counts will be verified by a separate analyst using the same criteria described above.

Fertilization – Fertilization will be microscopically determined based initially on the formation of a gray crescent region immediately dorsal to the animal-vegetal pole axis. The formation of the gray crescent occurs on the opposite side of sperm penetration into the egg and is the first sign of fertilization. Subsequent formation of a cleavage plane in the egg will be the unambiguous sign of fertilization and will be used to verify the number of eggs fertilized. The same quality control measures described for the previous metrics will be used to ensure the quality of the data collected and reported.

Early embryogenesis, hatching success, mortality, and morphological development – To determine the effect of PCB exposure on frog development, early embryonic development, hatching, and more advanced morphological development will be monitored. Embryolethal effects will also be recorded throughout development. As with the other metrics, close attention to accurate counting will be imperative. Counts will be verified by a separate analyst using the criteria described above.

Metamorphosis – The effect of PCB exposure on maturation of the larval frogs will be monitored since this life phase is often a sensitive indicator of potential stress. Detailed records of developmental stage, types and incidences of mal-development and the rate of limb development and tail resorption will be required. Digital photographic documentation of metamorphic events will represent an important record of maturation. Peer review by a separate analyst will be used to verify the data collected and authenticate the results.

Water and sediment PCB and other contaminant analyses – Analysis of water and sediment for the various contaminants identified in this Work Plan must result in data which is consistent with data for water and sediments collected for the other components of the entire project. Satisfactory results will be ensured by submitting samples to the same laboratories that are responsible for analyzing samples from the other studies associated with the program. Quality control specifications for these data are identified in the project QAPP (WESTON, 2000).

Tissue residue analysis – QC considerations to ensure achievement of the data quality objectives (DQOs) for this parameter will follow the QAPP (WESTON, 2000).

5.2 DATA QUALITY INDICATORS

Data developed in the frog reproduction and development study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000). Each of these data quality indicators, some of which are not readily quantifiable for data associated with this study, are discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Because of the biological heterogeneity inherent in Northern Leopard frog communities, it is not possible to take repeated independent measurements of the biological parameters. Rather than control and measure precision, the study design includes a total sample number and a number of replicates to obtain sufficient statistical resolution, as is defined in the subsequent section. Precision may also be evaluated by the assessment of the degree to which sample collection procedures are able to ensure collection of a consistent number of samples.

Endpoint or test metric precision within a given sample (frog) will be measured by ensuring an adequate number of replicates and are used to ensure adequate statistical measure of precision and significance. For measurements that are not unique to the frog reproduction and development study, such as water and sediment chemistry and tissue residues, precision is evaluated as defined in the QAPP (WESTON, 2000).

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy is defined as meaning that the test metrics are correctly determined in each sample, correctly enumerated, and correctly recorded. Accuracy of each test metric is a function of each sample being processed, reviewed, and recorded and of consistent field sampling techniques. The data generated by this study may be evaluated for accuracy via comparison with known and/or expected results from similar studies conducted in the Housatonic River or in similar New England ecosystems, although a limited number of comparable studies are currently available. For parameters such as water and tissue residue and sediment contaminants, accuracy is as defined in the QAPP (WESTON, 2000). With respect to the study design, a rough measure of precision and accuracy may be determined by the relation or degree of fit obtained between the biological effects observed (represented by each test metric) and the body burdens measured.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the frog reproduction and development study. To ensure achieving the planned statistical resolution, it is important that completeness reasonably near 100% be achieved for all components of this study, with the exception of the tissue residue analyses. The minimum sample size required to complete this study, based on the anticipated test metric variance, is approximately 30 males and 30 females for the study. Thus, approximately 3 male and 3 female frogs will need to be collected per sampling location. For the tissue analysis study component, the number of analyses will be determined by the material available for collection, and establishment of an *a priori* completeness goal is not possible.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. This data quality indicator is addressed through implementation of proper sampling design, sample processing methods, and sample analysis may be evaluated via comparison with known and/or expected results.

Comparability is a measure of the confidence with which the frog reproduction and development data may be compared to another similar data set. Comparability may be evaluated for this data set through comparison with previous amphibian studies in the Lower Housatonic River, if they exist, and with known characteristics of Northern Leopard frog communities in similar ecosystems in the Northeast. Comparability may also be evaluated by examination of the sample location variability in key parameters as determined from the large numbers of replicates to be collected at each location.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is difficult to apply to the biological parameters associated with this study. Frog reproduction, development, and maturation represent sensitive indicators of frog health and fecundity. The ability of the test metrics designed in this study to determine

potential changes in reproductive capacity or developmental fitness relative to corresponding tissue PCB residues or sediment PCB levels will be the primary determinant of the sensitivity of this model system. Sensitivity of analytical analyses alone is described in the QAPP (WESTON, 2000).

5.3 DATA VALIDATION, VERIFICATION, AND USABILITY

Procedures for data validation for the chemical and physical data are discussed in various sections of the project QAPP (WESTON, 2000) and will be used whenever applicable in this study. For the biological data, usability will be largely determined by three factors: (1) the experience of the principal investigators in establishing that the field sampling was conducted using appropriate techniques and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field; (2) an evaluation of the toxicological data as compared with previous studies; and (3) a direct comparison between the analytical chemistry and tissue residue data and similar data collected by other studies from similar areas of the river. The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

5.4 SAMPLING DESIGN

The rationale for selection of three reference site sampling locations with non-detectable sediment PCB levels, and nine target site sampling locations with varying degrees of sediment PCB contaminant levels to be sampled is presented in Subsection 6.1. The locations are not intended to be completely representative of the entire river, but rather are intended to encompass the range of sediment PCB levels in the Lower River between the Confluence and Woods Pond. The nine target sampling locations are sufficient to achieve the study DQOs consistent with the resources available for the study.

Frog reproduction and development data may be variable. To achieve acceptable statistical resolution, it is necessary to collect an adequate number of samples from each location. Ideally, six female and six male frogs will be collected from each of the 12 (three reference and nine target) sampling locations. This number of samples was selected based on power analyses.

5.4.1 Sampling Methodology

Sampling Procedures. Sampling methods, as discussed in Subsection 6.3, have been chosen to ensure an adequate collection of specimen that will permit comparisons between target and reference sites and determine the relationship between PCB body burden and toxicological effects on reproduction, development and maturation. Frogs will be collected personally by trained WESTON and Woodlot Alternatives personnel. Samples for physical and chemical analyses will be collected following procedures documented in the project QAPP (WESTON, 2000) and will therefore be comparable with procedures followed for all other similar efforts throughout the river.

Quality Control Samples. The nature of frog specimen sampling does not allow the incorporation of blank samples as part of the study design. Although it should not be considered directly analogous to a duplicate abiotic sample (i.e., water or sediment) collected for analytic chemistry analysis, additional frogs beyond those required for statistical evaluation will be collected from each sample location if available. As a general guideline, one additional female and one additional male frog will be collected for QC analysis. The QC specimen will be processed for biological examination and whole body or tissue residue analysis in a similar manner to the other samples collected. QC sample collection and analysis of sediment and water samples are described in the QAPP (WESTON, 2000).

Sample Documentation, Preservation, and Shipping. Detailed procedures for the documentation, preservation, and shipment of all samples associated with the frog reproduction and development study are described in Subsection 6.4 This study presents no unusual issues with regard to sample documentation, preservation, or shipment. Subsampling, homogenization (water and sediment samples), and decontamination between samples will follow procedures established in the project QAPP (WESTON, 2000). Holding time for physical and chemical samples will follow procedures established in the project-wide QAPP (WESTON, 2000). Since live frogs will be shipped, detailed methods of ensuring safe delivery to the laboratory will be used and are described in Subsection 6.4.2 and Attachment 2 of this document. Holding times for preserved biological samples for residue analysis and water and sediment samples will be strictly followed and are defined in the project-wide QAPP (WESTON, 2000).

Training. All sampling will be directed in the field by senior scientists at Woodlot Alternatives and WESTON with experience in the collection of Northern Leopard frogs in the field. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

5.4.2 Sample Analysis

Laboratory Studies. Processing of the frogs for *in vitro* fertilization, early developmental monitoring and evaluation of metamorphosis will follow procedures established in Subsection 7.1. All samples will be processed by experienced staff who have received specific training in this area and whose work is checked periodically by their supervisors and peers. Methods of QC for each metric evaluated were addressed in the DQOs. Each analysis will be repeated until consistent results are obtained (i.e., two separate egg counts within a given specimen should fall within 10% of one another). Verification by a separate analyst will also be used to authenticate the results. Corrective action, including reprocessing of samples and retraining of staff, will be instituted if these QC checks produce unsatisfactory results.

Physical/Chemical Samples. Samples for water and sediment chemistry and tissue residue analysis will be processed following procedures and SOPs provided in the project-wide QAPP (WESTON, 2000). These samples will be submitted in catalogs and batches with other samples from the larger project, and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP (WESTON, 2000).

5.5 DATA ANALYSIS AND REPORTING

Data collection, statistical analysis, and reporting for this study are described in Section 8. The study will produce a final report that will include all data, analyses, and interpretations and will be prepared with specific reference to both the DQOs provided in Subsection 5.1 of this specific protocol for the frog reproduction and development study and Subsection 4.1 of the project QAPP (WESTON, 2000).

6. FIELD PROCEDURES

Field procedures require the predetermination of sampling statistics, collection of frogs from the target and reference areas, collection of water and sediment samples for the bioassays, and all procedures associated with documentation, packing, and shipping samples. Each of these procedures is discussed in greater detail below. Target and reference area(s) will be verified Northern Leopard frog habitats, and an effort will be made to select reference area(s) that are as similar as possible to the target reach in relevant biotic and abiotic factors (apart from the presence of PCBs or other significant non-background chemical contamination). Such factors include, but are not limited to:

- topography;
- extent of industrial and residential development and habitat fragmentation;
- substrate type;
- soil type;
- level of dissolved oxygen in water;
- alkalinity;
- hardness of water;
- temperature; and
- total organic carbon (soil and water).

As noted previously, Northern Leopard frogs typically emerge from hibernation in mid-April and early May and begin breeding soon after. Unseasonably warm weather may accelerate this process such that migration of gravid females could occur prior to the planned field collection period. However, unseasonably cold weather or dry conditions may postpone breeding for several weeks. Weather conditions prior to field collection will be closely monitored to determine when to initiate collection efforts.

In the final stage of mobilization, all equipment specified previously will be assembled and packed. If any items need to be purchased, they will be ordered well in advance to ensure that the schedule is not impacted by equipment needs.

6.1 SAMPLING DESIGN

An overview of the sampling program and the anticipated number of samples to be collected for this study are provided in Figure 2 and Tables 1a and 1b. Again, the objective of this study is to evaluate frog reproductive capacity and developmental fitness by assessing gravidity, number of

Figure 2 Overview of Specimen Sampling for Frog Reproduction and Development Study

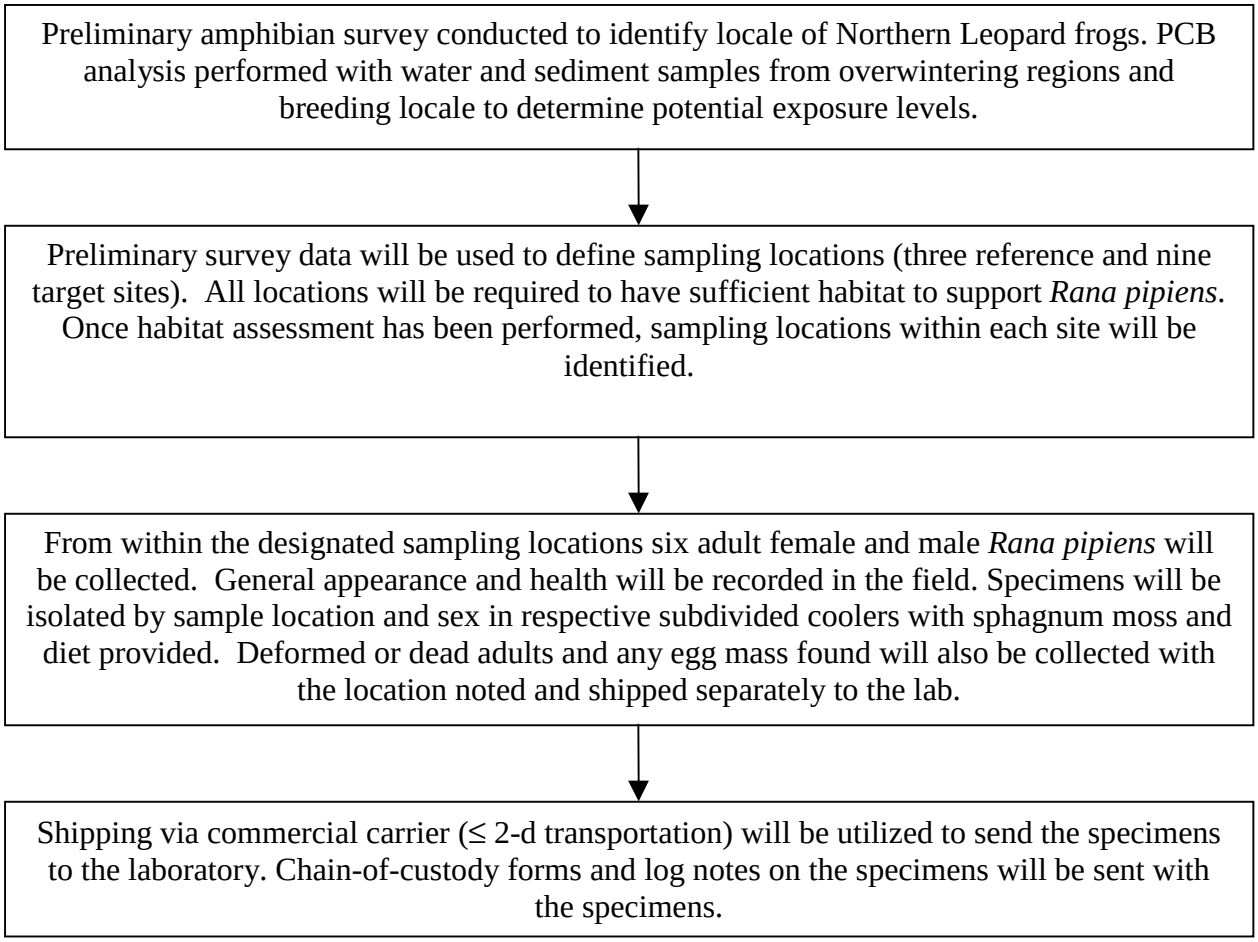


Table 1A and 1B

Proposed Sampling for Frog Reproduction and Development Study[1]

Sample	Sample Type	Grab Samples/ Sampling Location[2]	Estimated Sampling Locations/ Reference Site[3]	Estimated Sampling Locations/ Target Site	Total Samples/ Study
Water	Composite	4	3	9	12
Sediment	Composite	4	3	9	12

Sample	Sample Type	Samples/Sites	Estimated Sampling Locations/ Reference Site	Estimated Sampling Locations/ Target Site	Total Specimens or Samples/ Study
Adults (whole body)[4,5]	Individual	6 male/6 female	3	9	144
Ovary[4]	Composite	1	3	9	12
Testes[4]	Composite	1	3	9	12
Egg mass[4]	Composite	1	3	9	12
Larvae[4]	Composite	1	3	9	12

[1]One reference and one target site.

[2]Composite sample will be prepared from four grab samples collected.

[3]Anticipated 3 reference and 9 target site sampling locations.

[4]Based on availability. Does not include QA/QC specimens collected at rate of 1 male and female per sampling location.

[5]Based on individual specimen. Six adult males and six adult females will be collected per sample location within each site.

oocytes, oocyte viability, sperm viability and fertilization, mortality, developmental anomalies, growth, and maturation of embryos obtained from target area frogs as compared to results from frogs collected from paired reference sites. The study will evaluate data from sampling locations representative of a relatively uncontaminated site (referred to as a reference site) and sampling locations representative of the conditions of the target site, based on preliminary reconnaissance and a thorough evaluation of the habit and PCB concentrations. Sample locations such as those provided in Figure 3 will be considered in this study. Only regions containing suitable habitat will be used in this study. Statistical evaluation will be based on the adult frogs collected, resulting egg masses produced, and embryos produced from nine separate locations within the target area, and an additional three locations within the reference area designation. These locations will represent the sampling units for each site. In this design, three selected sampling locations within the reference area and nine selected sampling locations within the target area will be used for sample collection. These locations have been identified from habitat maps with potential PCB exposure information provided by WESTON (Figure 3). Six female and six male frogs will be collected from each sampling location. Approximately 36 frogs will be collected from within the reference area and 108 frogs from the target area. The three reference sampling locations will contain sediment PCB concentrations of <1.0 mg/Kg. Of the anticipated nine target site sampling locations, three will contain sediment PCB concentrations of <1 mg/Kg, 3 with sediment containing 1-30 mg/Kg, and 3 with sediment containing >30 mg/Kg. Additional QC specimens will be collected at each site as discussed in Subsection 5.4. Of the six frogs per sex collected per site, four frogs will be used for reproduction and development study, one for whole-body total PCB (tPCB) analysis, and one for ovary and oocyte, or testis tPCB analysis. This approach will provide a means of directly correlating a response within the sampling location to range of PCB concentrations (Steel and Torrie, 1980; Hicks, 1982; Thompson, 1992). This design will also allow statistical comparison between the reference and target areas.

A key variable with respect to ensuring adequate statistical power is the selection of appropriate sample sizes. For this study, sample size is defined as the total number of adult frogs evaluated from the target and the reference areas. A power analysis to determine minimal sample size requirements for the frog reproductive study was conducted to determine the total number of adult frogs needed per area, as well as the number of oocytes required to relate body PCB concentrations to the reproductive and developmental endpoints previously mentioned (Steel and Torrie, 1980; Hicks, 1982; Thompson, 1992). A summary of these statistical design analyses is provided in Tables 2 and 3.

Each endpoint has been evaluated to determine the sample sizes required (Tables 2 and 3). The number of organisms to be evaluated in development as recommended by power analysis is consistent with current organism loading practices used in the laboratory for each of the endpoints to be monitored (Fort et al., 1995; Fort and Stover, 1996a and 1996b, 1997a and 1997b). Based on this analysis, 6 adult female and 6 adult male frogs from each sample location will be required for the current study design and additional analytical analyses. Thus, at least six adult frogs of each sex will be collected per sampling location per site.

Table 2

Detectability, Sample Size, and Power Analysis of Respective Endpoints Associated with Reproduction and Development in *Rana pipiens* Using Hypothesis Testing[1]

Endpoint	Minimal Detectable Difference[2]	Sample Size (n/2)[3]	Power
Egg mass[4]	700	20	0.90
Egg necrosis[4,5]	20	20	0.90
Oocyte maturity[5,6]	25	10	0.90
Sperm count[4]	200,000	20	0.90
Sperm Dysmorphology[5]	5	20	0.90
Fertilization[6]	20	10	0.90
Teratogenesis[6]	20	20	0.90
Hatching Success[6]	20	20	0.90
Mortality[6]	20	15	0.90
Rate of Metamorphosis[6]	10	20	0.90
Gravidity[7]	30	15	0.90

[1]Analysis based on assumption that a t-test with normally distributed data and equal variances will be used to compare data sets.

[2]Minimal detectable difference based on anticipated standard deviation.

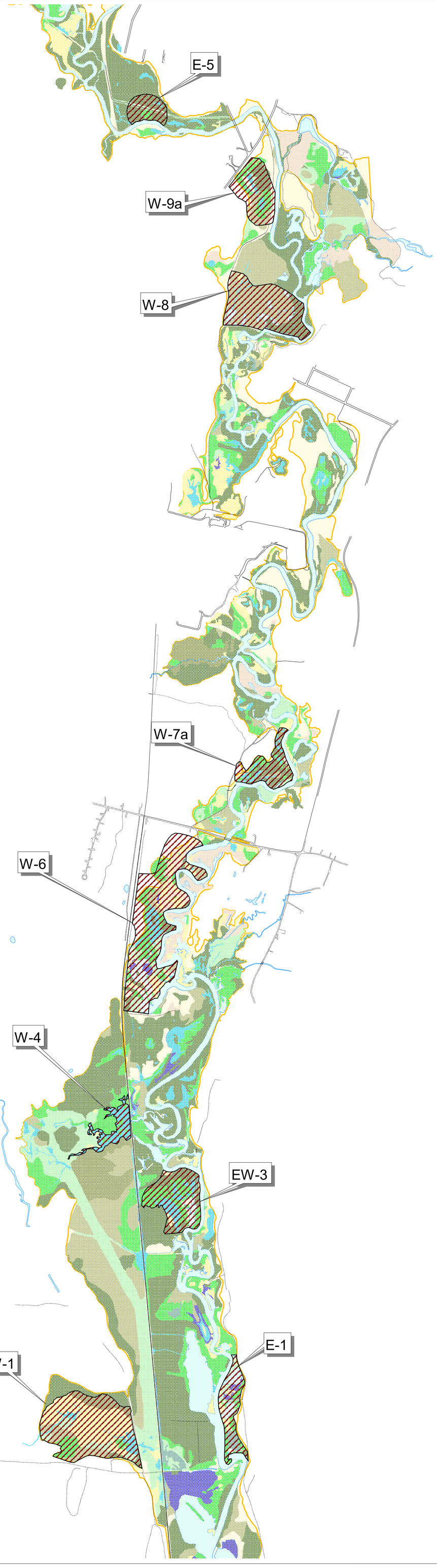
[3]Values reported as total n, but split into two equal replicates in order to evaluate variability.

[4]Expressed as total egg or sperm count, respectively.

[5]Expressed as a transformed proportion for statistical evaluation.

[6]Based on proportion of oocytes reaching advanced maturity relative to viable egg mass.

[7]Binary variable. Estimates based on assumption provided above.



LEGEND:

- Survey Locations
- Roads
- Hydrology
- 10-year Floodplain

Wetland Habitat

- WAI_PAB
- WAI_PABUB
- WAI_PEM
- WAI_PEMAB
- WAI_PFO
- WAI_PFOEM
- WAI_PFOSS
- WAI_PSS
- WAI_PSSEM
- WAI_PUB
- WAI_RIV_HLNE
- WAI_SAND
- WAI_WMEAD

N

Scale in Feet

1000 0 1000 2000 3000

SI Work Plan
Lower Housatonic River
Massachusetts

Appendix A-20
Figure 3
Leopard Frog
Survey Locations

| O:\gepitt\aprs\frog_locs.apr | layout - 1 | o:\gepitt\epsfiles\portrait24x36\in\frog_locs.eps | 4:54 PM, 2/2/2000 |

Table 3

Detectability, Sample Size, and Power Analysis of Respective Endpoints Associated with Reproduction and Development in *Rana pipiens* Using Simple Linear Regression Modeling[1]

Endpoint	Predicted Distribution (N or LN)[2]	Minimal Detectable Difference[3]	Sample Size (n)[4]	Power
Egg mass[5]	N	700	40	0.8
Egg necrosis[4,6]	LN	20	40	0.95
Oocyte maturity[5,7]	LN	25	20	0.95
Sperm count[4]	N	200,000	40	1.0
Sperm Dysmorphology[5]	LN	5	40	0.85
Fertilization[6]	LN	20	20	0.95
Teratogenesis[6]	LN	20	40	0.85
Hatching Success[6]	LN	20	40	0.85
Mortality[6]	LN	20	30	0.9
Rate of Metamorphosis[6]	N	10	40	1.0
Gravidity[8]	---	30	30	---

[1]Analysis based on normally distributed data or log normally distributed date based on anticipated data set. Paired data will be based on the reproductive effect regressed against either tissue PCB levels or sediment/soil levels.

[2]N = normal distribution; LN = log normal distribution

[3]Minimal detectable difference based on anticipated standard deviation.

[4]Values reported as total n.

[5]Expressed as total egg or sperm count, respectively.

[6]Expressed as a transformed proportion for statistical evaluation.

[7]Based on proportion of oocytes reaching advanced maturity relative to viable egg mass.

[8]Binary variable. Modeled as a Bernoulli variable.

The current sampling requirement of 144 adult frogs (72 male and 72 female), plus an additional 24 (12 male and 12 female) for QA/QC should provide a suitable cushion to allow for the following contingencies:

- all of the females collected may not be gravid;
- injury or death of frogs during transport;
- QA/QC measures.

Eggs will be collected from a minimum of 20 female frogs per site in order to allow subsequent statistical analyses. As a standard procedure, 40 eggs/embryos are typically taken from each egg mass (20 per replicate) when evaluating chemical effects on frog embryos (Fort and Stover, 1996a; Bantle et al., 1998). Thus, the sample size for each area will be 800 fertilized eggs, corresponding to 40 eggs collected from each of 20 females from each area. A larger sampling size from each egg mass will be used if practicable.

In summary, 54 female and 54 male frogs should be collected from the target area and 18 female and 18 male frogs from the reference areas, for a total of 144 frogs.

6.2 SAMPLING APPROACH

Following authorization, WESTON or its contractors will collect frogs from the target and reference areas over one or more three-day periods at the end of April or beginning of May, unless alerted earlier to the presence of migrating female Northern Leopard frogs. As previously indicated, nine sampling locations within the target area and three sampling locations within designated reference areas will be used to ensure systematic searching of the areas and, in the case of the target area, a broad distribution of PCB concentrations (including areas with elevated concentrations). As a requirement of this study, both sites to be used in this study must have sufficient habitat to support Northern Leopard frogs. The search for specimens will focus on areas known to be favored by Northern Leopard frogs, such as shallow areas on the edges of relatively deep standing waters that are sheltered by overhanging trees and brush. Optimal times to search for gravid females will be evenings in light rain. Because gravid females tend to go to areas beyond their typical daily habitat to lay eggs, roadsides and open areas adjoining favored aquatic habitats will also be searched. A key element in the search for females will be to listen for the calls of male Northern Leopard frogs, which tend to reside in the shallow regions of their aquatic environment. While females typically prefer deeper waters, they will come to the shoreline in response to male vocalization during the breeding season. Depending on the difficulty of collecting frogs, male vocalizations may be recorded and played back in an effort to lure female frogs.

The order in which the sampling locations in the target area are sampled will be flexible, yet systematic, with the objective of collecting frogs from all sampling locations. The goal in collecting frogs from multiple sampling locations is to ensure that target frogs represent a range of exposures, thus supporting the subsequent regression analysis for potential dose-response relationships. The anticipated sampling locations range in tPCB concentrations of <1 mg/Kg to > 30 mg/Kg sediment.

Ideally, all frogs will be collected within as short a time frame as possible in order to reduce stress to the frogs that are caught early in the collection phase, and to minimize the possibility of premature release of eggs while in captivity. While females can be held in captivity for a period of up to four days, it is not advisable to extend this time frame.

Since shipment of specimens to the lab will be staggered, the sampling team will continue with field collection efforts (if necessary) while the laboratory proceeds with evaluation of gravidity, sperm viability, and *in vitro* fertilization. The laboratory will then contact the project manager and advise as to the number of gravid females and the egg masses collected.

6.3 METHODS OF FROG COLLECTION

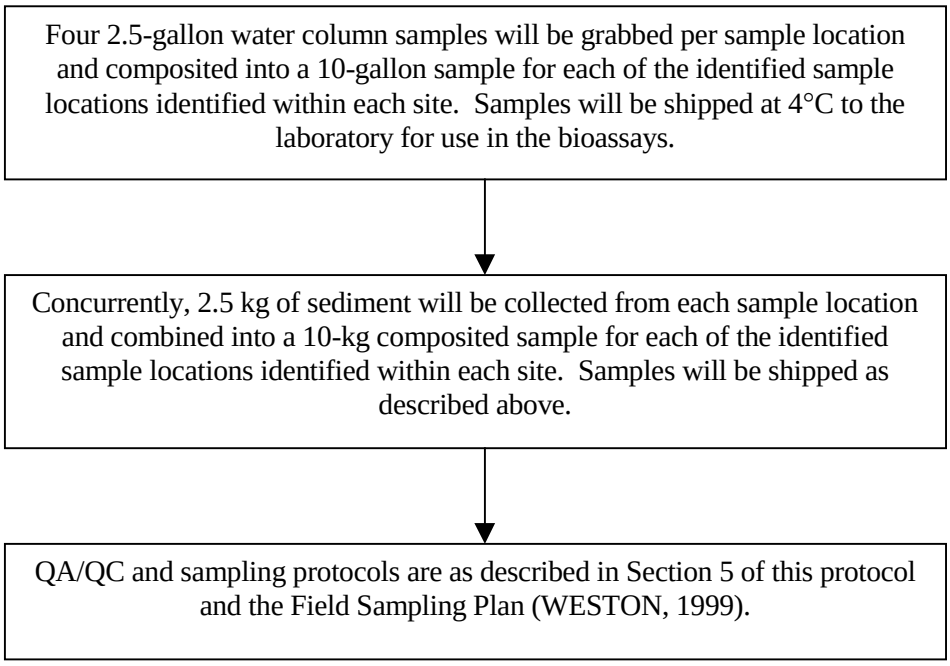
The sampling team will capture frogs using several techniques, including drift fences and pitfall traps. The frogs will be delivered to the processing area in separate containers labeled with location, sex, and date of collection. Each frog will then be placed into its own compartment into a six-gallon Styrofoam cooler lined with moist sphagnum moss for shipping to the laboratory. Perforated lids will be securely affixed to the coolers with duct tape to prevent escape and the coolers will be labeled.

Prior to delivery to the laboratory, the coolers containing the frogs will be kept in air-conditioned rooms with temperatures ranging from 10 to 15 °C. The frogs will be fed a daily diet of live crickets and water. The water used for maintaining the frogs during transportation will be collected from the locations in which the frogs are collected. Additionally, the sphagnum moss will be changed as needed and kept moist. Representatives of the laboratory will be present in the field during the collection to assist in making decisions on sampling if additional sampling locations are needed or the number of frogs requested cannot be achieved for any reason. The specimens should be shipped by a priority overnight (24-h) service which offers a ground service, or by a commercial carrier offering air transit of live specimens. Packaging of the specimens with moss, food (excess live crickets), and water will be adequate to ensure successful arrival. Laboratory representatives will provide instructions and assist in packaging for shipment.

6.4 COLLECTION OF SEDIMENT AND WATER SAMPLES

Sediment and water column samples will be collected at each of the sampling locations within both areas. At each location, four grab samples of sediment from inundated areas will be collected by WESTON in accordance with the methods specified in the Field Sampling Plan (WESTON, 1999). The four grab samples will then be composited into one sample for each of the five locations within the test areas. A similar approach will be used to collect water column samples. The water and sediment samples will be used in the embryo-larval and metamorphosis bioassay studies. Thus, for both areas composited samples of sediment and water will be collected. Duplicate samples will be collected for analytical analysis as necessary. Sample size and volume requirements are further defined in Figure 4.

Figure 4 Overview of Water/Sediment Sampling for Frog Reproduction and Development Study



6.4.1 Sample Documentation and Labeling

Field notes will be recorded in a logbook in accordance with the Field Sampling Plan (WESTON, 1999). Each frog will be identified in the logbook using a unique 16-digit sample identification number. Sample nomenclature methodology is specifically described in the QAPP (WESTON, 2000). The label coding system will not be explained to biological laboratory personnel to ensure that they remain blind as to the origin of a given animal. Global positioning system (GPS) data will be collected so that the geographical coordinates of the sampling locations are identified. Specific documentation of habitat at each location will be provided using digitally collected images and written field observations. In addition, analytical samples will be recorded in a logbook using labeling consistent with that specified in the QAPP (WESTON, 2000).

6.4.2 Sample Preservation and Shipping

Live female and male frogs will be transported in Styrofoam coolers lined with moist sphagnum moss and an excess of live crickets in accordance with the standard operating procedure, included as Attachment 2. The coolers will be labeled and sealed with perforated sides and lids. Two signed and dated custody seals will be placed on two sides of the cooler to ensure the specimens are not tampered with during shipment.

A member of the project team will transport them to the biological laboratory. Following *in vitro* fertilization, all females that had been gravid will be euthanized, frozen, and packaged for shipment to the laboratory for whole-body PCB and congener-specific analysis. Additionally, residual portions of the egg masses from which embryo groups are obtained for the bioassay will be prepared for PCB analysis as well.

Frogs, residual egg masses, and water and sediment samples will be shipped to the analytical laboratory in dry, clean, perforated sample containers (Styrofoam ice chests) that are labeled in accordance with ERT/REAC SOP #2002 (EPA, 1994). The Styrofoam ice chests will be placed into polyethylene bags (one sample per bag), which will then be sealed and placed into U.S. Department of Transportation (DOT) approved fiberboard boxes lined with plastic sheeting, bubble wrap, and sufficient vermiculite to absorb any potentially leaking material. All outer packing materials will also be perforated to allow gas exchange. One chain-of-custody form (in triplicate) will be placed into a watertight bag and taped to the inside of the lid of each cooler. Specimens to be analyzed for analytical parameters, including PCBs, will be packaged as described above following snap freezing in liquid nitrogen and inclusion of dry ice. In accordance with DOT regulations, the lids will be slightly perforated to allow for release of carbon dioxide gas as the dry ice melts. In this case, the Styrofoam coolers will then be placed into cardboard boxes that have also been perforated to allow gas release. The boxes will be securely taped and appropriately labeled according to the courier's protocols. International Civil Aviation Organization regulations stipulate that any volume of dry ice is a Class 9 Miscellaneous Hazardous Good (IATA, 1993). In order to provide a means by which the entire path of a sample can be traced, a chain-of-custody record will be maintained from the time a sample is collected through analysis or hatching, as specified in the QAPP (WESTON, 2000).

7. LABORATORY METHODS

It is anticipated that *in vitro* fertilization and monitoring of developing embryos will be performed by THE STOVER GROUP in Stillwater, Oklahoma. Tissue, surface water, and sediment chemical analyses will be conducted by laboratories under contract to WESTON or USFWS. The laboratory procedures are described below.

7.1 IN VITRO FERTILIZATION, EARLY DEVELOPMENTAL MONITORING, AND EVALUATION OF METAMORPHOSIS

After allowing female frogs to acclimate for 24 hours following receipt by THE STOVER GROUP, female frogs will be induced to ovulate and the egg masses will be stripped and fertilized *in vitro.* It is only upon stripping the eggs that a definitive determination of gravidity can be made. The number and identity of gravid versus non-gravid females will be recorded for a subsequent analysis of the differences in percent gravidity in the target and reference areas. In addition, the number of eggs produced by each female will be specifically counted, rather than using volumetric techniques.

Superovulation will be induced by injection of 100 IU (international standard units) of luteinizing hormone releasing hormone (LHRH) or pituitary extract in Amphibian Ringers solution using tuberculin syringes with ½-inch-long 26 gauge needles, in accordance with standard methods cited in Bantle et al. (1998), Fort and Stover (1995 and 1996a), and ASTM (1998). The females will be carefully immobilized prior to injection by holding them underneath an aquarium net. The solution will be injected into the dorsal lymph sac, which is bound by the lateral line that runs along the side of the frog and appears as stitching on the skin. Care will be taken to inject the frog subcutaneously by wrinkling the skin.

Approximately 24 to 36 hours after the females have been superovulated, male frogs from each location will be sacrificed and the testes will be removed. Testes from each male will be gently mashed together in a Petri dish containing 9 mL of dechlorinated tap water and 1 mL of Ringers solution that has been left standing for 48 hours. A 1-mL aliquot of the resultant sperm solution will be checked for sperm count, motility, and dysmorphology under a microscope (Fort et al., 1999c).

The concentrated sperm solution from males collected from each sample location within a site will then be divided into Petri dishes. Sperm solutions prepared from the combined testes of these males will be used to fertilize eggs from each respective location within the sample site. This approach will be important to determine effects within each site due to differing levels of PCBs within the site itself. Care will be taken to ensure that target area sperm fertilize target area eggs and that reference area sperm fertilize reference area eggs. Egg masses will be squeezed out of the females into the concentrated sperm solution by firmly grasping the frog, extending the legs back and close to each other, and applying gentle pressure on the abdomen. After standing in the sperm solution for 30 minutes, the eggs will be flushed with dechlorinated tap water and will be loosely separated to ensure adequate oxygen viability. After two hours the eggs will be checked for fertility and quality. Grey crescents that form on the opposite side of

sperm entry immediately above the equator, dividing the animal pole from the vegetal pole, will be the first sign of fertilization. Normal cleavage (indicating successful fertilization) will be determined based upon the general technique of Nieuwkoop and Faber (1994) and Dickerson (1969), in which the embryos are characterized by vegetal hemisphere cells that are creamy white in color and are always larger than the pigmented animal hemisphere cells; the pigmentation line should be at the equator of the blastula. However, because cellular development can be observed in the Northern Leopard frog, early embryogenesis will also be monitored. Egg masses characterized by significant infertility will be recorded relative to the female of origin, so that differences in fertility rates between target and reference area females can be subsequently evaluated.

Groups of 40 embryos will be separated from each fertilized egg mass (each female within each sampling location), keeping the jelly coat intact, and placed in each of two replicate dishes containing 20 embryos each. Embryos that are not perfectly round, blastula with abnormal pigmentation, or gastrula that have bleeding yolks will be excluded from the remainder of the bioassay, although such abnormalities will be recorded relative to the female of origin so that differences in viability can be subsequently evaluated.

Each group of 20 embryos from each female will be placed in specimen jars for monitoring through the seven-day post-hatch observation period in each of two replicates per female. Because we cannot assume that the primary source of PCBs to the developing embryos is via maternal transfer during egg formation, it will be necessary to add target site or reference site water and sediments to the test vessels. Furthermore, based on its composition, it is likely that the jelly coat surrounding the embryo will not completely prevent passage of PCBs to the developing embryos. Thus, testing of pre-hatch embryos will be performed in 9-oz specimen bottles equipped with a glass tube/Teflon® mesh insert as the exposure chamber. Thirty-five g of sediment (wet weight) will be placed in the bottom of the specimen jar, the exposure insert added, and the jar filled with 140 mL of dilution water (FETAX solution, reference site water, or test site water). This represents a 1:4 dilution of sediment to dilution water. Early embryos will be placed on the Teflon® mesh insert that will rest over the top of the sediment in the sediment/water interface region. This represents a fairly realistic exposure scenario in the laboratory (Fort and Stover, 1997b). Vessels containing embryos from reference and target areas will be discretely labeled and then randomly distributed within an incubator. Laboratory personnel will be blind to the origin of the embryos in each beaker. Since embryos from at least 20 females will be used per site, each site, including designated reference sites, will have a total of 40 vessels containing 20 embryos, for a total of 800 embryos evaluated per site (1,600 embryos for the study). In addition, a separate set of 40 embryos collected from 20 females (for a total of 800 embryos) obtained from a commercial source (Carolina Biological Supply, Burlington, NC) and confirmed to be devoid of PCB or dioxin contamination will be placed in each of two replicates (20 per replicate) containing clean sand and laboratory culture water. These uncontaminated embryos will be designated as laboratory controls. The purpose of including these controls in the study will be to ensure that the laboratory culture conditions are adequate to culture *R. pipiens* successfully.

In order to determine what extent of developmental effects induced in target site embryos/larvae are due to maternal PCB transfer, a separate set of experiments will be performed concurrently. In these studies, an additional set of 120 embryos from reference site females will be exposed to

water and sediment from three selected locations within each target site containing representative levels of PCBs, and developmental effects (hatching and metamorphosis) monitored as described in this section. Thus, 40 embryos will be collected from each of three randomly selected reference site females and exposed in sets of 40 (20 per replicate) to the three target site samples. The reverse experiment will also be conducted to help confirm these findings by exposing target site embryos (120 total, 40 per female) from adults collected from a representatively contaminated test site location to reference site water and sediment collected from three selected locations as described above.

A pH of 7.5 in the culture solution will be maintained at all times, as well as a temperature of 24 ±1° C. Dissolved oxygen (DO) will be monitored on a daily basis and will not be allowed to drop below 6.0 mg/L. Prior to hatching, the test chambers will be maintained on a 12-hour day/12-hour night cycle, and test solutions and sediment will be changed every 4 days using a Pasteur pipette with an enlarged fire-polished orifice. Dead embryos will be removed, counted, and recorded every 24 hours.

Time to hatch will be monitored. The embryos are expected to hatch within seven to ten days. Juvenile frogs will not be fed during the seven-day pre-hatch observation period, since the yolk sac that remains following hatching provides sufficient nourishment for the first seven to eight days. If overall mortality in the laboratory culture water controls exceeds 10 percent in the embryo-larval evaluation, results will be considered conditionally acceptable pending further review. In the case that the data is considered unacceptable, evaluation of study results would be limited to the gravidity, sperm viability, and fertilization rates.

Morphological evaluation of the embryos and juveniles will be conducted either at the end of the observation period or upon the death of the juvenile or embryo. The following specific abnormalities will be recorded:

- gut;
- hemorrhaging;
- axial malformations;
- blistering and edema;
- head, face and eye;
- heart; and
- brain.

Following hatching (longer-term evaluation), larvae will be fed Salmon Starter fish food, which has been successfully used to culture *Rana* tadpoles in the laboratory (Carolina Biological Supply Company, 1993). Post-hatch larvae will be cultured as described above in 5-L glass aquaria with underlying sediment. Five hundred g of sediment or blasting sand will be placed on the bottom of the aquaria and filled with 3 L of site water, reference site water, or FETAX solution. Mortality, limb development, and other morphological markers of metamorphosis (skin maturation and tail resorption) will be morphologically evaluated through this process. We anticipate that metamorphosis and limb development (hind limbs) will be complete within 3 months of hatch. Renewal of test solutions and sediments during this longer-term development phase will be performed every seven days (weekly).

During this phase, digital images of the developing larvae will be recorded during the renewal process both for observation of the developing limbs and resorbing tail, as well as photographic documentation of the results, in accordance with the methods for Fort and Stover (1996b, 1997a; Fort et al. 1999a).

7.2 ANALYTICAL ANALYSES

It is anticipated that the Texas A&M University Geotechnical and Environmental Research Group (GERG) will conduct PCB and lipid analyses on adult frogs, residual egg samples and testes, sediments, and water samples within ten days of receipt. A summary of the sample matrix and analytical requirements are identified in Table 4 and Figure 5. Other analyses will include, but are not limited to, Aroclor-specific PCBs, congener homologs, dioxins, dibenzofurans, and organochlorine pesticides. As discussed in the introduction to this protocol, there is a need to perform a PCB and congener-specific analysis on the male frogs, even if the majority of PCBs found in offspring may be derived from the female. This analysis will complement the male reproductive assessment. Tissues will be analyzed from at least one female and male frog per sampling location per site. Each composite and random grab sample of water and sediment will be analyzed for the parameters indicated above.

8. DATA ANALYSIS

8.1 DATA COLLECTION

For embryo-larval and limb development, mortality and malformation rates will be determined for the test and reference site using a dissecting microscope (Fort et al., 1995, 1996b and 1997a; ASTM, 1998). For monitoring the rate and extent of tail resorption (metamorphosis), video images will be captured using a Sony CCD-iris high-resolution color digital video camera. A Pentium 233 MHz computer with image processing software and a FlashPoint 128 (Integral Technologies, Inc., Indianapolis, IN) video frame grabber will be used to digitize tail lengths throughout the study. A ruler videotaped with the larvae will be used to correct for image distortion and calibrate the length-measuring program to ensure accurate measurements of the larvae. Tail lengths will be measured using Sigma Scan (SPSS, Corte Madera, CA).

Overall, data evaluation will entail:

- preparation of a database that includes chemical concentrations, gravidity, number of eggs produced, number of necrotic eggs, portion of eggs in each respective stage of development, sperm count, sperm morphology/dysmorphology, egg viability, fertilization rate, time to hatch, hatching success, incidences of mortality and abnormality for target and reference area embryos and/or frogs, rate of limb mal-development, rate and extent of the tail resorption, and morphological observations during metamorphosis;

Table 4

Proposed Analytical Analysis for Frog Reproduction and Development Study Reference Site[1]

Sample	Sample Type	Grabs or Individual Samples/ Location	Anticipated Sample Locations/ Study	Analytical Analyses				
				tPCBs	Congeners[2]	Dioxin[3]	Dibenzo-furan[3]	Organo-chlorine Pesticides[3]
Water	Composite	4/location	12	12	12	12	12	12
Sediment	Composite	4/location	12	12	12	12	12	12
Adult whole body[4,5]	---	1male/ 1 female	24	24	24	24	24	24
Ovary[4]	---	1	12	12	12	4	4	4
Testes[4]	---	1	12	12	12	4	4	4
Egg mass[4]	---	1	12	12	12	4	4	4
Larvae[4]	---	1	12	12	12	4	4	4

[1] One reference and one target area with nine sampling locations within the target area and three sampling locations within the reference area.

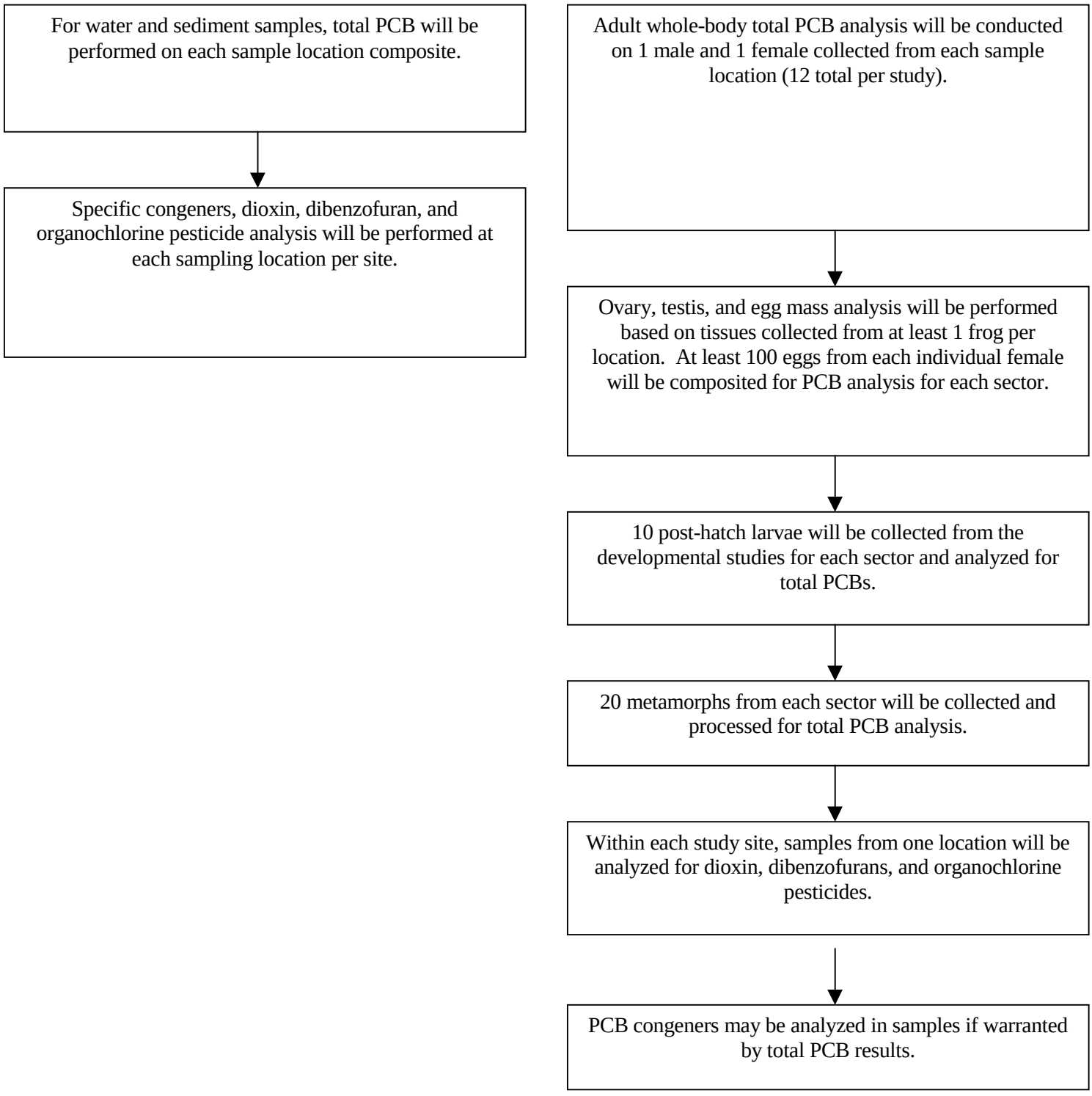
[2] Collected but only analyzed if tPCB analyses results warrant.

[3] Two randomly selected composite samples per site.

[4] Based on availability.

[5] Based on individual specimen.

Figure 5 Overview of Analytical Analysis for Frog Reproduction and Development Study



- statistical analysis of the data comparing endpoints between target and reference area frogs;
- statistical analyses relating endpoints to PCB concentrations (congener-specific, as practicable) in female frogs and residual eggs; and
- documentation of study results and supporting QA/QC data.

As an initial step in the evaluation, THE STOVER GROUP will develop a database for the target and reference areas. This database will be developed in spreadsheet format and sorted by area and by embryo groups derived from each female. The database will include the following information:

- identification number of the maternal and paternal frogs;
- gravidity;
- egg mass;
- necrosis;
- portion in respective oocyte stages;
- number of eggs;
- sperm counts;
- sperm morphology;
- fertilization rate;
- time to hatch;
- mortality incidence;
- abnormality incidence by type of deformity and total number;
- limb mal-development;
- rate of and abnormalities occurring during metamorphosis; and
- PCB concentrations in the associated fertilized egg mass and adult tissues.

Because the concentration of PCBs is often correlated with the lipid content of a tissue sample, tissue PCB data will be normalized to the lipid concentrations before statistical analyses are performed. THE STOVER GROUP will verify the accuracy of the data entry prior to statistical evaluation.

8.2 STATISTICAL ANALYSIS

8.2.1 Hypothesis Testing

Statistically significant differences in outcomes between target and reference areas will be evaluated based on a homoscedastic t-test (two-sample comparison of the means) providing the data sets are found to be normally distributed. If the homoscedasticity assumption is violated, a heteroscedastic t-test will be used to compare the data sets. If the data sets from either site do not meet normality assumptions, transformation of the data (arc sine) will be used to normalize the results. If no transformation proves useful in normalizing the data sets, non-parametric tests, such as the Mann-Whitney or Wilcoxen tests, will be used. In addition to evaluating the means of the two data sets, we will also use the Kolmogorov-Smirnov (KS) two-sample test to compare

the overall distribution of the variability in the two sites. Abnormalities will be evaluated individually, as well as cumulatively.

8.2.2 Regression Analysis

In an effort to establish a concentration-response relationship between PCB concentration and biological effects observed, adult female or male tissue and egg mass PCB concentrations and water and sediment PCB concentrations will be regressed separately against the biological endpoints. The proportions themselves can be thought of as comprising a continuous distribution in which each proportion represents a numerical data point that is matched in the regression to the PCB concentration of the group. Regression analysis will be used to determine if a relationship exists between rank (extent of biological efforts) and deviation from monotonicity. In this analysis, monotonicity will be measured by the difference in the slopes of the ranks. Although this sample size may fluctuate slightly, the approach will remain statistically powerful (Table 3) and should determine if a correlation exists between PCB levels and biological effects observed in the laboratory. As with the hypothesis test, data sets will be evaluated for normality or, in the event that the data is not normally distributed, log normality. We anticipate that some of the data will require transformation prior to simple linear regression analysis. Because records of habitat will be collected during sampling, we will also be able to determine if biological responses may be attributed to habitat or lack of habitat. Following analysis, an exposure-response model will then be fit to the data, if possible. As indicated above, standard curve fitting approaches for dose-response data will be used and may include log or arc sine transformations. Because gravidity is a binary variable, it will be evaluated as a Bernoulli variable rather than using the aforementioned methods. If the data analysis indicates statistically significant differences between target and reference areas that do not appear to be consistent with a PCB exposure-response relationship, the archived frog tissue and egg samples may be analyzed for selected other chemical constituents, and the results evaluated in order to help in understanding the differences.

The last step will be to document the methods, results, and conclusions in both text and tabular form. The methods section will state any assumptions made and any changes made to this protocol. The results will be explained in terms of the data analyses and statistical procedures described above. Uncertainties associated with the analysis, as well as their potential impacts on the results, will be incorporated into these discussions.

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ATTACHMENT 1

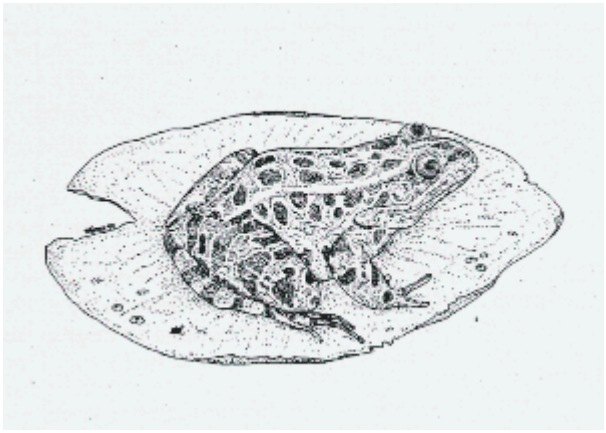
HOME RANGE OF *RANA PIPIENS*

Environment Canada Environnement Canada

Français

Representative Species - Canadian Great Lakes Frogs and Toads

Leopard Frog - *Rana Pipiens*



Distribution

- Found throughout Ontario but more abundant in southern and central areas.

Home Range

- Adults maintain small home ranges (up to 500 m^2) in fields or open forest during the summer. Where terrestrial habitats are quite dry, home ranges include some shoreline.
- A minimum of 4 ha of terrestrial habitat is recommended for the vicinity of breeding sites, however, individual adults may move several kilometres away.
- Most recently metamorphosed froglets stay within 20 m of shoreline although some froglets begin dispersal before metamorphosis is entirely complete.

Food

- Larvae eat algae, phytoplankton, periphyton and detritus.
- Adults eat mainly invertebrates but will also take tadpoles or very small froglets.

Reproduction

- Successful breeding sites are permanent ponds, marshes, or pools or backwaters of streams.
- Breeding occurs from mid-March to mid-May in southern Ontario, and a few weeks later further north.
- Metamorphosis occurs in 2-3 months.
- Tadpoles require minimum oxygen concentrations of 3 ppm.

Cover/Habitat

- Relative to bullfrogs and green frogs, leopard frogs use open fields more and prefer denser terrestrial vegetation.
- In aquatic habitats, submerged vegetation, detritus and soft mud are used for cover.

Lookout/Sunning

- Eggs and tadpoles require warm (prefer 18 - 28 C), shallow, sunny areas.
- Froglets require muddy shorelines, lily pads, rocks, logs or beaver dams with clear access to deeper water.
- Adults prefer unmowed fields (15 - 30 cm high, no more than 1 m high vegetation) or open forest in the vicinity of shallow open marshes.

Connectivity/Corridors

- Corridors may be required among breeding, hibernation and summeringhabitats, within 2 km. These may be either aquatic (streams or rivers) or terrestrial (field or forest, usually not cropland except

during periods of irrigation).

Hibernation

- Hibernate in deep or running water that will not freeze solid or become anoxic. Are found hibernating on muddy substrate or under rocks, sunken logs, leaf litter or vegetation.
- Oxygen levels at one known successful hibernation site were 7 ppm.
- Tadpoles metamorphose in the year of hatching.

Hydrology

- Permanent wetlands with fishless areas or near fishless (temporary) wetlands.
- Breeding requires sufficient water for metamorphosis to be completed (mid-late August).

Soils/Substrate

- In water prefer muddy bottom.
- On land prefer moist soil, leaf litter or moss.

Design Criteria

Vegetation

- Prefer egg-laying sites with emergent vegetation on about 2/3 of edge and submergent vegetation in 1/2 of surface area in May.

Structures

- Rocks, logs, floating vegetation or dams to sun on, with access to deep water.
- Submerged vegetation, logs or rocks to hide in.

Soils, Slope, & Substrate

- Prefer wetlands with gradual slope at edge.

Hydrology

- Hibernate in streams with minimum depth 90 cm, moderate mid-depth water velocity, minimal sedimentation, and rocks with average diameter of 20 cm.

Critical Periods

- Breed April-June, metamorphose July-September

Other Considerations

- Froglets are used as bait for fishing.
- Has declined in much of its western range and apparently in northern Ontario.
- Tadpoles and froglets are vulnerable to predation by large Bullfrogs and fish.

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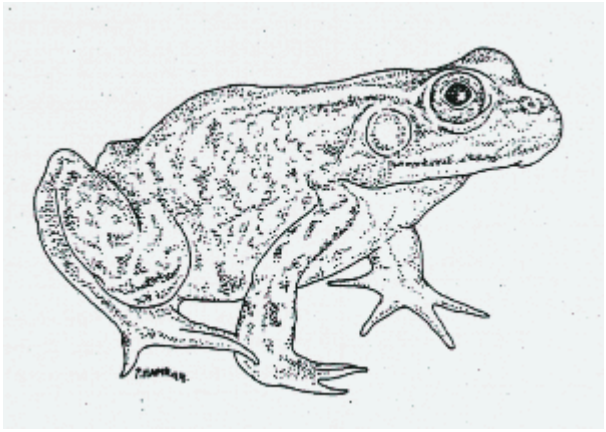
Green Frog - *Rana clamitans*



Distribution
- Widespread and abundant in Ontario.

Home Range
- Shoreline species, using the area closer to shore than bullfrogs.

Food
- Adults are carnivorous, stalking spiders, insects, snails, slugs, and aquatic crustaceans like crayfish.
- Tadpoles feed continuously on phytoplankton.

Reproduction
- Breed from June-August.

Cover/Habitat
- Edges of slow streams and rivers, ponds, lakes, reservoirs, marshes, swamps, and bogs.

Hibernation
- Tadpoles that hatch late - in small crevices between rocks, fallen logs, and branches.
- Adults - on land in leaf litter and soil pockets.

Hydrology
- Prefer a greater water depth immediately offshore and cooler water than bullfrogs.

Soils/Substrate
- In water - muddy bottom.
- On land - loose soil.

Design Criteria

Vegetation

- Prefer egg-laying sites with lots of underwater plants such as *Elodea* which make up mats on which the eggs rest.
- In water - prefer greater submergent vegetation canopy, with greater stem diameters than bullfrog.
- On land - prefer less dense vegetation than leopard frog.

Structures

- Rocks and logs to sit on. Rocks away from the shoreline are safer for froglets.

Soils, Slope, & Substrate

- Prefer a muddy bottom.
- >On land - loose soil.

Hydrology

- Slow streams and rivers, ponds, lakes, reservoirs, marshes, swamps, and bogs. Prefer greater water depth immediately offshore and cooler water than bullfrogs.

Critical Periods

- Breed June-August.

Other Considerations

- The leopard frog has similar habitat requirements, but breeds earlier.

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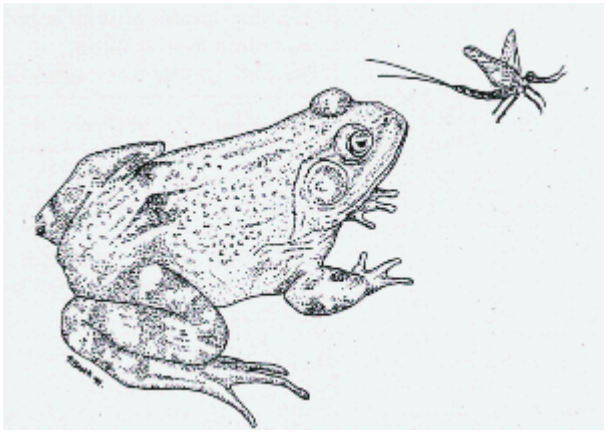
Bullfrog - *Rana catesbeiana*



Distribution

- Southern Ontario as far north as Nipissing District.

Home Range

- Adult males aggressively defend territories. Boundaries are not stationary, but males defend 3-25 m of shoreline.

Food

- Tadpoles - aquatic plant material, invertebrates, and dead fish or tadpoles.
- Adults - voracious 'sit and wait' predators - fish, mice, moles, bats, snakes, ducklings, birds, and other bullfrogs.

Reproduction

- Breed May-July.

Cover/Habitat

- Vegetated shoals, sluggish river backwaters and oxbows, farm ponds, reservoirs, marshes, and still waters with tules, dead trees, snags, and twisted roots.

Hibernation

- Bury themselves in surface mud and construct protective pits or cave-like holes underwater.
- Adults may disappear before frost begins. Bullfrogs are last ranids to emerge in spring.

Hydrology

- Permanent water bodies, and prefer warm, still, shallow waters.

Soils/Substrate

- Muddy bottom to water body.

Design Criteria

Vegetation

- May be dense - pickerel weed, lily pads, cattails, sedges, berry vines, and willows.

Structures

- Still waters with tules, dead trees, snags, and twisted roots.

Soils, Slope, & Substrate

- Muddy bottom to shallow water body with gently sloping sides.

Hydrology

- Permanent water bodies - prefer warm, still, shallow waters.
- In potentially hot water bodies, bullfrogs must find cooler areas to keep eggs from dying.

Critical Periods

- Breed May-July.

Other Considerations

- Abundant in habitats modified by humans.

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GLIMR

Date: September 5, 1995 by: Scott Monds

ATTACHMENT 2

SAMPLE SHIPPING PROCEDURE

FINAL

ATTACHMENT 2
SAMPLE SHIPPING PROCEDURE
Rana pipiens REPRODUCTION AND DEVELOPMENT STUDY
Effective 1/19/2000
Revision No. 0

1. INTRODUCTION

1.1. ROY F. WESTON, INC. has contracted THE STOVER GROUP to perform a *Rana pipiens* Reproduction and Development Study. Live biological samples and environmental water and sediment samples will be collected in the field and shipped from the WESTON Pittsfield, MA Office to THE STOVER GROUP in Stillwater, OK.

2. APPARATUS

2.1. 1-gallon amber glass containers (100 count) with Teflon® caps

2.2. 1-liter wide-mouth amber glass containers (100 count) with Teflon® caps

2.3. Wide-mouth glass specimen jars

2.4. Cardboard boxes for shipping glass bottles

2.5. Styrofoam coolers encased in cardboard boxes (20 count)

2.6. Ice chests

2.7. Sphagnum moss

2.8. Live crickets

2.9. Packing tape

3. SHIPPING ADDRESSES

3.1. THE STOVER GROUP, Attention R&D Laboratory, 5302 W. Sixth St., Stillwater, OK 74074. Telephone number is 405-743-1435, Fax number is 405-743-1489, and e-mail address is rlrogers@stovergroup.com.

3.2. ROY F. WESTON, INC., Pittsfield Field Office, Attention Scott Campbell, 10 Lyman St., Pittsfield, MA 01201. Telephone number is 413-442-4224 and Fax number is 413-442-4447.

4. WATER/SEDIMENT ENVIRONMENTAL SAMPLE SHIPPING PROCEDURE

4.1. Randomly pull 4 – 2.5-gallon water column samples from each sampling location.

4.2. Composite the 4 water samples into a single, homogenous, 10-gallon sample.

4.3. Transfer the composited 10-gallon water sample to 1-gallon glass amber containers and cap with Teflon® caps.

4.4. Concurrent to the water sampling, randomly take 4 – 2.5-kilogram sediment samples from each of the sampling location.

4.5. Composite the 4 sediment samples into a single, homogenous, 10-kilogram sample.

4.6. Transfer the composited 10-kilogram sediment sample to 1-liter, wide-mouth, glass amber containers with Teflon® lids.

4.7. Label all sample containers appropriately with the sample date, sample description, site and location designation, WESTON sample number, and container number (i.e. 1 of 10, 2 of 10, etc.).

4.8. Individually wrap each glass container with bubble wrap or equivalent packaging material to prevent breakage.

4.9. Place wrapped containers in ice chests.

4.10. Add blue ice or zip locked bags of ice to maintain a temperature of 4°C during shipping.

4.11. Ship ice chests to THE STOVER GROUP.

5. ADULT *Rana pipiens* SAMPLE SHIPPING PROCEDURE

5.1. Adult *Rana pipiens* will be collected live from each sample location.

5.2. During a collection period, store frogs in cardboard boxes lined with damped sphagnum moss. Keep specimen separated by sex and location.

5.3. Feed frogs live crickets daily (2-4 crickets per frog).

5.4. Live specimen holding times for a collection period should not exceed 48-hours before frogs are packaged for shipment.

FINAL

5.5. Shipping containers for the live biological samples are Styrofoam coolers with each cooler packaged inside its own cardboard box. Each cooler/box set will need approximately 8 – ½-inch holes drilled into the top and sides for ventilation.

5.6. Line each Styrofoam cooler with damped sphagnum moss and add live frogs separated by sample location.

5.7. A chain of custody will be attached to each cooler, documenting the sample date, sample description, sample site and location, WESTON sample number, sampler's signature, and packager's signature. Place the chain of custody in a zip lock baggy to prevent damage and tape to Styrofoam cooler inside the cardboard box.

5.8. Clearly label the outside of each cooler/box as "LIVE ANIMALS".

5.9. Ship cooler/boxes by express carrier, with a no longer than 2-day delivery time, to THE STOVER GROUP.

5.10. Any miscellaneous live biological aqueous samples, other than adult frogs (i.e. larvae, egg masses, etc.), should be collected in wide-mouth glass specimen jars, bubble wrapped and shipped to THE STOVER GROUP using an overnight express carrier. Do not lower sample temperatures for shipping.

5.11. Any miscellaneous dead biological samples should be shipped on dry ice in ice chests to THE STOVER GROUP using an overnight express carrier.

APPENDIX A.20

FIELD SAMPLING AND ANALYSIS PLAN FOR FISH COLLECTION AND PROCESSING

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APPENDIX A.20

FIELD SAMPLING AND ANALYSIS PLAN FOR FISH COLLECTION AND PROCESSING

1. INTRODUCTION

1.1 BACKGROUND

Fish are sensitive to both long-term and short-term changes in habitat, sediment, and water quality. While fish are mobile, they frequently spend most of their lives in a single location, due to their territorial behavior or when the location is subjected to impounding structures that limit fish movement. Thus fish can serve as effective indicators of environmental conditions in that location. The fish community in streams, rivers, and ponds is an important food source for instream consumers, as well as for some bird and mammal species, including man. Fish generally depend on other aquatic sources for their various life functions and are a principal component of the aquatic food chain sequence from contaminated sediments and water through benthic macroinvertebrates, to smaller consumers, such as the cyprinids; intermediate consumers, such as the yellow perch and larger sunfish; larger consumers, such as the largemouth bass; and scavengers, such as the bullhead. Each of these may itself be the prey of a higher level consumer, such as an otter, a heron or kingfisher, and ultimately, man. Predation upon fish represents an important transport mechanism for the movement of contaminants from in-stream sediment to terrestrial sources. Because of PCB contamination in fish, a fish consumption advisory in the Housatonic River has been in place since 1988.

Fish community structure and function have been used extensively to evaluate the quality of water resources and characterize causes and sources of impacts in lotic (flowing water) and lentic (standing water) freshwater ecosystems. The individual organisms that make up fish communities respond to both biotic and abiotic environmental variables; therefore, the structure of these communities reflects the integration of the influence of these variables. Biotic variables may include competition, predation, and food availability, whereas abiotic variables may include stream temperature, dissolved oxygen, flow characteristics, and pollutants.

Because of the long-recognized importance of fish community structure in evaluating the health and condition of aquatic habitats, and the importance of fish tissue as a measure of the transport of contaminants through the ecosystem, collection and assessment of fish representing the community in this system is an essential element of the ecological characterization. Fish tissue sampling and community assessment will be conducted to determine if PCB contamination from the GE facility is adversely affecting fish in the study area and accumulating in fish tissue at concentrations detrimental to human and ecological consumers, fish tissue sampling and community assessment is to be conducted.

1.2 OBJECTIVES

The principal objective of the fish collection effort is to determine the PCB and other organic contaminant concentrations in tissue for use in both human health and ecological risk assessments, to evaluate congener patterns by species for use in the fish and mink reproduction studies, and in the PCB fate and effects model. In addition, the fisheries community will be qualitatively assessed (see Attachment1) for use in the ecological characterization of the river system.

Fish tissue, whole body samples, and fillet and offal samples will be analyzed for PCBs (Aroclor and congener/homologue analyses) and for dioxin/furans and organochlorine pesticides. Fish tissue sample collection and analysis will be used to evaluate both ecological and human health endpoints. Fish tissue concentrations will be used to determine potential risks to individuals who may be catching and eating fish in violation of the fish consumption ban, as well as to determine risk to subsistence and recreational anglers in the absence of administrative or institutional controls. Ecological measurement endpoints are the comparison of tissue concentrations to Maximum Allowable Tissue Concentrations (MATCs) from literature and reference area concentrations, and incorporation in food chain models for piscivorous receptors. Tissue analyses will be conducted in accordance with the QAPP (WESTON, 2000).

All fish collection and capture will be performed by personnel from the U.S. Fish and Wildlife Service (USFWS), Office of Fishery Assistance, Laconia, New Hampshire, and Office of Fishery Assistance, Sunderland, Massachusetts. Fish capture techniques and protocols will be conducted using the USFWS standard operating procedures (SOPs) and safety requirements (Attachment 2).

2. STUDY DESIGN

2.1 FIELD SAMPLING

2.1.1 Sample Locations

Fish will be collected from the following seven locations:

Upper East Branch Housatonic River (Dalton)—This reach will serve as the non-impacted flowing reference reach. A dam separates this reach from the lower river system, prohibiting fish passage from the contaminated portion of the system into this reach; however, it does not have the extreme “impoundment” characteristics of the slower, lower river system. The upper portion of this reach is similar to the “shallow” river reach.

Housatonic River (Confluence to WWTP) “Shallow Reach”—This contaminated reach has shallow water of moderate velocity, larger sediment grain size, less total organic carbon (TOC) in the substrate, and less in-stream cover. It is also free from enrichment from the wastewater treatment plant (WWTP).

Housatonic River (WWTP to Contaminated Backwaters) "Deep Reach"—This contaminated reach is characterized by deeper, slower water and more in-stream cover. It is also downstream of the WWTP outfall.

Housatonic River - Impoundment (Contaminated Backwaters and Woods Pond)—This reach includes areas of the river where contaminated sediments have accumulated from upstream areas. This area is characterized by large impoundment areas with associated shallow and deep water habitat.

Rising Pond—This impoundment in the Housatonic River is located approximately 17 miles downstream of Woods Pond. PCBs have been detected in the sediment (although at lower concentrations than Woods Pond).

Goodrich Pond—This is a small pond located within Pittsfield adjacent to some residential properties known to contain fill from the GE facility, but is currently not posted against fish consumption. This pond was included in this study to determine if fish are contaminated with PCBs at levels adverse to human health. A tributary from this pond discharges into the East Branch of the Housatonic River, and sediments from this tributary have been found to be contaminated with PCBs.

Three-Mile Pond—This impoundment serves as the reference impoundment within the Housatonic River watershed outside of site influence. This pond reportedly has most or all of the target species selected for this investigation.

2.1.2 Target Species

- Largemouth Bass (*Micropterus salmoides*).
- Yellow Perch (*Perca flavescens*).
- Sunfish: pumpkinseed (*Lepomis gibbosus*), bluegill (*Lepomis macrochirus*) or other.
- Cyprinids: golden shiner (*Notemigonus crysoleucas*), common shiner (*Luxilus cornutus*), or other.
- Brown Bullhead (*Ictalurus nebulosus*).
- Goldfish (*Carassius auratus*).

2.2 QUALITATIVE FISH SAMPLING

Fish in the Housatonic River study area and in reference areas will be qualitatively sampled to characterize fish communities in terms of species presence and relative abundance. In each area fish will be captured or observed by electrofishing using electroshocking boats or backpacks. All fish sampling will be performed by U.S. Fish and Wildlife Service personnel in compliance with their standard operating procedures and safety requirements detailed in Attachment 2.

Timed electrofishing surveys for qualitatively characterizing fish communities will occur over 30 minute periods within representative areas in each of the above mentioned sampling sites. Starting and ending locations of each timed survey will be plotted on data sheets or located in the field using GPS equipment. During each 30-minute survey all fish that are shocked will be identified to species and enumerated by personnel in the boat. Certain fish may be netted and put into live wells, or buckets, to verify species identification. Additional information collected during each timed survey will include date, location, capture method, weather, crew members, and miscellaneous comments. Data forms will be completed during each timed survey.

2.3 FISH PREPARATION FOR TISSUE ANALYSIS

Fish will be collected in accordance with the methods identified in Attachment 2 by location and retained in live wells containing location-specific water until sample processing is initiated. Fish containers (e.g., live wells) will be labeled with capture location information and aerated to minimize fish mortality before fish processing. All fish retained for potential sample analysis will be enumerated and separated by species and size class. This information will subsequently be used to determine the number of samples and associated IDs. Fish will be sacrificed by cervical separation or sharp blow to the head with a stunning rod. All fish not retained for analysis will be released at their approximate capture location unharmed after processing.

The following metrics will be recorded for each individual fish included in any sample:

- Total Length (cm) — The greatest dimension of a fish from its anterior-most extremity to the end of the tail fin. For fish with a forked tail, the two lobes should be pressed together, and length of the longest lobe should be recorded.
- Total Weight (g) — Fish will be placed in a pre-weighed decontaminated tray and weighed to the nearest gram.
- Fillet Weight (g) — For appropriate samples (same procedures as total weight).
- Offal Weight (g) — For appropriate samples (same procedures as total weight).
- Sex (M/F) — When possible (i.e., bass), fish sex will be identified by external morphological characteristics or internal reproductive examination.
- Age — Otoliths and scale samples will be collected to determine the age of largemouth bass. Age will be determined in a laboratory setting at a later date by USFWS or designated EPA contractor.
- Physical Exam — Gross pathological examination of all fish will be conducted and documented. Special consideration will be given to gross pathological conditions on largemouth bass.

Upon completion of collection of metrics, fish samples will be either submitted for whole body, or fillet and offal analysis.

2.3.1 Whole Body Sample Processing

Fish samples for whole body analysis will be rinsed of all debris with deionized water and placed in decontaminated aluminum foil (dull side toward the fish). The sample ID labels will be placed on the outside of the aluminum foil and secured with clear tape. If more than one fish is used for a sample (composite), all fish used for the sample will be placed on one piece of aluminum foil, wrapped and labeled with the appropriate sample ID. To preserve sample integrity, samples will be placed in double resealable plastic bags with a second ID label and placed in either a cooler with dry ice or a suitable freezer until analyzed.

2.3.2 Fillet and Offal Sample Processing

Procedures for filleting fish are described below.

An initial cut should be made from the dorsal fin to the pelvic fin, just behind the opercular flap. Run the tip of the knife along the dorsal side of the fish, from the initial cut to the caudal fin. Continue making successively deeper cuts, running the knife blade as close to the neural spines and ribs as possible. After the fillet is obtained, remove the skin. Place the skin side of the fillet down on the dissecting tray, hold on to the tail portion of the fillet, and run the knife between the skin and the muscle tissue. Remove any debris from the skinless fillet by rinsing with deionized water.

After a fillet is cleaned, place the sample in a pre-weighed decontaminated tray and record the weight to the nearest gram. For composite samples, obtain all the fillets for the composite and weigh to the nearest gram. Fillet samples will be placed in decontaminated aluminum foil (dull side toward the fish). Offal samples (fish tissue remaining after fillets have been removed) will also be placed on decontaminated aluminum foil in the same manner. The sample ID label will be placed on the outside of the aluminum foil and secured with clear tape. The samples will be placed in double resealable plastic bags with a second ID label and stored on dry ice or suitable freezer until submitted to a designated laboratory.

2.3.3 Tissue Analysis

Fish tissue, whole body samples, and fillet and offal samples will be analyzed for PCBs (Aroclor and congener/homolog analyses) and for dioxin/furans and organochlorine pesticides. Tissue analyses will be conducted in accordance with the QAPP.

2.4 SAMPLE SIZE

Individual and composite fish samples will be collected for the aforementioned sample reaches and impoundments. The following provides an outline of the types of samples targeted for collection (Table 1).

- For yellow perch, bullhead, and sunfish, 10 to 25 edible size fish will be submitted for analysis as fillet and offal samples. Both sides of the fish will be filleted to obtain the minimum sample weight of 30 grams. All fillet samples will have the skin removed.

- For yellow perch, bullhead, sunfish, and cyprinids, five composite samples of five fish each, of forage size (5 to 9 cm), will be submitted. Each composite will contain fish within 75% of the total length between the largest and smallest fish of each composite.

- One size range of goldfish (23 to 28 cm) will be submitted as a whole body sample.

- A total of 25 largemouth bass of all size ranges observed, and an additional five composites of forage size fish will be submitted for analysis. Bass will be broken up into three size ranges:

    1) Bass greater than 12 inches (28.5 cm) MA legal limit.
    2) Bass less than 12 inches (28.5 cm).
    3) Bass 5 to 9 cm.

Bass greater than 12 inches will be submitted as fillet samples with the skin removed. Bass less than 12 inches will be submitted as whole body samples.

Table 1. Sample Grouping Strategy, Housatonic River, MA

Species	Largemouth Bass	Yellow Perch	Brown Bullhead	Sunfish	Cyprinid	Goldfish
Fillet	X	X	X	X*		
Offal	X	X	X	X*		
Whole Body	X					X
Forage size Whole Body	X	X	X	X	X	

* Sunfish will be sampled as fillets/offal if determined to be large enough for human consumption.

2.5 DOCUMENTATION

All sample documentation will follow project specific SOPs for field sample ID, data sheet, chain-of-custody, and custody seal procedures.

2.6 DECONTAMINATION

All dissection equipment will be decontaminated following the project-specific SOP for equipment decontamination including detergent/water wash, potable water rinse, hexane rinse,

isopropyl alcohol rinse, and deionized water rinse. All aluminum foil will be hexane rinsed prior to use.

2.7 FIELD QUALITY CONTROL SAMPLES

One field rinsate blank sample will be submitted during each day of processing. One duplicate sample (left side fillet) will be collected every 20 samples for samples large enough to produce the minimum required sample mass (approximately 30 grams) per fillet. One MS/MSD sample will be collected for every 20 samples large enough to provide triple the required sample mass.

2.8 SAMPLE SHIPPING

Samples should be sent by overnight delivery service (next morning delivery) or hand delivered. Samples sent to the USFWS should be shipped to:

Ken Carr/Ken Munney
USFWS
22 Bridge St., Unit 1 Phone 603-225-1411
Concord, NH 03301 Fed. Ex Acc# 1510-1036-9

Shippers will notify the receiving laboratory or the USFWS and notify that samples are being sent for next-day delivery. Samples should not be sent to USFWS if Ken Munney, Ken Carr, or Drew Major are not available for receipt of the shipment. Samples need to be sent for arrival on a weekday only. Therefore, Thursday is the last day of the week to ship samples. Shippers should also call the receiving laboratory of USFWS the day of delivery to verify the receipt of samples.

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 Data Quality Objectives

The three data quality objectives of the fish collection and evaluation are outlined in Subsection 1.2. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Taxonomic identification of fish to LPIL (lowest practical identification level)—Fish must be identified to the species level whenever possible. When identification to the species level is not possible, the LPIL will be consistent with standard practice for fish. The six target species must be identified to species. Fish collected as part of an incidental take should be identified to the species level where possible.

- Enumeration (counts) for each species in each replicate sample—Counts must be made and recorded accurately. Accurate counts are readily achievable in the field.

- Total length (cm) for each fish in each of the target species collected—Total length must be measured accurately in the field using a fish board and recorded accurately. Procedures have been established (Subsection 2.2, above) to ensure that consistent length measurements are taken and recorded.

- Biomass (total weight) for each fish—Total weight must be determined accurately and recorded to 1 g using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude.

- Fillet weight (total fillet weight) for each fish—Fillet weight must be determined accurately and recorded to 1 g using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude. Adherence to the fillet sample processing procedure described in Subsection 2.2.2 is essential.

- Offal weight (total offal weight) for each fish—Offal weight must be determined accurately and recorded to 1 g using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude. Adherence to the offal sample processing procedure described in Subsection 2.2.2 is essential.

- Age for largemouth bass—Collection of otoliths and scale samples using the accepted procedures is essential. Age determinations will be made in a laboratory setting at a later date by the USFWS or a designated EPA contractor.

- Physical exam of all fish—Gross pathologies for each fish collected must be accurately recorded.

- Tissue chemistry for PCBs and selected other contaminants—Analysis of tissues (whole body, fillet, or offal samples) for chemical constituents must result in data that are consistent in all respects with other contaminant data collected as part of the larger project. Satisfactory results will be ensured by following the quality control specifications for these data as delineated in the project QAPP (WESTON, 2000).

- Qualitative fish community data including number of fish per species observed per unit effort.

3.1.2 Data Quality Indicators

Data developed in the fish community and tissue study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000) that are appropriate to the data quality objectives. Each of these data quality indicators, some of which are not readily quantifiable for fish community data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. The study design includes an increase in the number of replicates to increase the statistical resolution; for this study the number of replicates (up to 25 largemouth bass

samples, for example) is used in this manner. Precision during the fish community evaluation is defined as agreement on species identification and enumeration by multiple personnel involved with collection efforts.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (fish taxonomy and biomass), accuracy is defined as meaning that the fish are correctly identified in each sample, correctly enumerated, and correctly measured for length and weight. Accuracy of these parameters is a function of each fish being processed by eye, and of consistent field sampling techniques. The data generated by this study will also be evaluated for accuracy via comparison with known and/or expected results from similar studies conducted in the Housatonic River or in similar New England systems. For parameters such as tissue contaminants, accuracy is as defined in the QAPP. For the qualitative fish survey, accuracy is defined as the ability to identify the fish species observed by eye, and to generate a reasonable estimate of number of individuals observed in the water during electrofishing. This is constrained by a number of factors including the selective nature of the likelihood of the electrofishing to stun different species, and the ability to accurately estimate number of individuals observed for either small fish or fish that are observed in large numbers instantaneously.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the fish program. To ensure achieving the planned statistical resolution, it is important that completeness of 100% be achieved for all components of this study with the exception of the tissue residue analyses. For this latter study component, the number of analyses will be determined by the material available for collection; therefore, establishment of an *a priori* completeness goal is not possible. For the qualitative fish survey, completeness will not be 100% because of the known fact that electrofishing will not result in an observation of all fish at a given location. It is expected that there will be a more complete response in the shallow water areas and that completeness will decline with water depth.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved, thorough sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of representativeness will also be evaluated via comparison with known and/or expected results based on previous investigations of the Lower Housatonic River and other similar systems. Representativeness of the qualitative fish survey will be constrained by the differential response of species to the electrofishing technique.

Comparability is a measure of the confidence with which the fish data may be compared to another similar data set. Comparability will be evaluated by examination of the in-station variability in key parameters as determined from the large numbers of replicates to be collected at each location and fish observations to be made. Comparability will also be evaluated for this data set through comparison with previous fisheries work in the Lower Housatonic River and with known characteristics of fish populations in similar stream systems in the Northeast.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is related for fisheries investigations to the ability of the taxonomic analysis to resolve the various fishes into individual species. This data quality

indicator will be evaluated by comparing the number of species-specific separations against the number of unresolved larger taxonomic groups. As the number of unresolved groups increase, the community metrics such as species richness and diversity are less able to resolve differences between samples. Sensitivity is applicable and important for the chemistry parameters that will be analyzed as part of the tissue study. For these parameters, the detection limits for chemistry specified in the QAPP will provide appropriate sensitivity for the purpose of providing insight in to factors controlling abundance and distribution of the fish populations.

3.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical and physical data are discussed in various sections of the project QAPP and will be used whenever applicable in this study. For the biological data, usability will be largely determined by three factors: (1) the experience of the senior investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field; (2) an evaluation of the taxonomic data both within the study and compared with previous studies in the river and in the New England area; and (3) a direct comparison between the chemistry and similar data developed from co-located samples that have been collected as part of other project components.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

3.2 SAMPLING DESIGN

The rationale for selection of the seven locations to be sampled in the fish study is presented in Subsection 2.1.1. The locations are not intended to be representative of the entire river but rather are intended to encompass the range of sediment PCB concentrations, and the associated fish tissue concentrations, in the Lower River between the Confluence and Woods Pond; two appropriate reference locations with background PCB levels will also be sampled.

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Sampling methods, as discussed in Subsections 2.2 and 2.3 and Attachment 2, were chosen to ensure unbiased (i.e., accurate) samples that will facilitate comparisons with other fish data, both from the Housatonic River and from other areas. All samples will be collected by trained and experienced personnel; senior oversight of all aspects of the sampling and sample processing will further promote comparability and reduce potential bias. Subsamples for tissue chemical analyses will be collected following procedures documented in the project QAPP and will therefore be comparable with procedures followed for all other similar efforts throughout the Supplemental Investigation.

3.3.2 Quality Control Samples

The nature of fish sampling does not allow the incorporation of typical duplicate and blank samples as part of the study design. For community metrics, there is no acceptable method of obtaining such samples in a manner analogous to that for duplicates and blanks collected for chemistry analysis.

Duplicate samples for tissue chemistry will be collected in this study. Quality control of tissue chemistry analyses will be provided by the analysis of duplicate samples (including MS/MSD samples) at a rate of approximately 5% of samples collected. Duplicates will be processed in accordance with the QAPP and via comparisons with results from split samples provided to GE.

3.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all samples to be collected as part of the fish study are provided in Subsection 2.3. Subsampling, homogenization, and decontamination between samples will follow procedures established in the QAPP. All samples will be held on dry ice and returned to the field laboratory daily and will be either frozen (physical, chemical samples) or preserved (taxonomic samples) at that time. Holding time for physical and chemical samples will follow procedures established in the QAPP; there is no holding time for taxonomic samples.

3.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the collection of fish samples. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Taxonomy Samples

Processing of taxonomy samples will follow USFWS procedures as documented in Attachment 1. All samples will be processed by experienced staff who have received specific training in the SOP and whose work is checked periodically by their supervisors and peers. While performing the qualitative fish community survey, any individual for which the identification to species is in question will be captured and either identified and released, or if not definitively identified the individual will be retained for identification in the laboratory. Five percent of the fish will be re-checked by someone other than the original identifier. Corrective action, including reclassification of fish samples and retraining of staff, will be instituted if these QC checks produce unsatisfactory results.

Quality of taxonomic identification will be ensured by maintaining voucher collections and requiring a consensus among all taxonomists at the processing laboratory prior to an identification becoming accepted as a type for the voucher collection. In the event that the

taxonomists are unable to agree on an identification, specimens will be sent to a third party for determination.

3.4.2 Physical/Chemical Samples

Samples for tissue chemistry will be processed following procedures and SOPs provided in the QAPP. These samples will be submitted in catalogs (sample delivery groups) and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP.

3.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Subsection 2.4. The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations and will be prepared with specific reference to both the data quality objectives specific to the fish study (see Subsection 2.3.1 above and Subsection 4.1 of the QAPP).

4. PROCEDURES

4.1 FIELD SAMPLING

4.1.1 Collection of Taxonomy Samples

All fish collection and sampling will be conducted by personnel from the USFWS, Office of Fisheries Assistance, Laconia, New Hampshire and Office of Fishery Assistance, Sunderland, Massachusetts, following the SOP provided as Attachment 2.

4.1.2 Initial Processing of Fish for Tissue Residue Analysis

Fish preparation for tissue analysis, whole body analyses, and fillet and offal tissue samples will be conducted pursuant to the procedures outlined in Subsection 2.3.

5. REFERENCES

WESTON (Roy F. Weston, Inc.). 2000. *Final Quality Assurance Project Plan.*

ATTACHMENT 1

FISH COMMUNITY DATA SHEET

GENERAL ELECTRIC/HOUSATONIC RIVER
SUPERFUND SITE FISH CAPTURES

Date: _____ Pond/River Name: _____

Capture Method: _____ Weather/Temp: _____

Crew: _____ Photo/s: _____

_____ _____

River Section: _____ River Reach: _____

Pond Run: _____ Map Reference: _____

Time Start: _____ Time Finish: _____ Total Time: _____

Fish Species Observed	Estimated Numbers	Total

Comments: _____

_____

_____

_____

_____

_____

GENERAL ELECTRIC/HOUSATONIC RIVER
SUPERFUND SITE FISH CAPTURES

Date: 9/28/98 Pond/River Name: Housatonic River

Capture Method: Electroshocking, Boat Weather/Temp: Sunny, 70's

Crew: Ev, Joe, Johanna, Ken, Doug Photo/s: 2, 3, 4

River Section: Upper - EPA Desig? River Reach: Smokestack to ledge outcrop

Pond Run: If pond - Run # 1, 2, etc. Map Reference: Map 1 - "A"

Time Start: 5:30 PM Time Finish: 7:30 PM Total Time: 2 hours

Fish Species Observed	Estimated Numbers	Total
LMB	2, 6, 0, 3, 1	12
SMB	1, 0, 0, 2, 0	3
YP	10, 14, 18, 22, 3	67
CP	1, 0, 1, 2, 0	4
GF	4, 5, 3, 1	13

EXAMPLE

Comments:

ATTACHMENT 2

USFWS FISH COLLECTION PROTOCOLS

U.S FISH AND WILDLIFE SERVICE
STANDARD OPERATING PROCEDURES

General Electric/Housatonic River Superfund Site
Pittsfield, Massachusetts
September - October, 1998

All fish sampling will be performed by personnel from the U.S. Fish and Wildlife Service (USFWS), Office of Fishery Assistance, Laconia, New Hampshire and Office of Fishery Assistance, Sunderland, Massachusetts. Reference sites will be sampled before contaminated sites. Bass, perch, bullheads, sunfish and cyprinids are the species likely to be targeted for contaminant analyses. Fish will be captured using electroshocking boats, backpack electroshockers, gill nets, minnow traps, and trot lines. Electrofishing will be conducted in compliance with USFWS standard operating procedures and safety requirements per 24 AM 13 (attached).

SITES

Contaminated

Housatonic River - Pittsfield to Lee
Goodrich Pond - Pittsfield
Woods Pond - Lee
Rising Pond - Great Barrington

Reference Sites

East Branch Housatonic River - Pittsfield
Three Mile Pond - Sheffield

FISH CAPTURE PROTOCOL

Fish captured by boat electroshocking will be held in live wells on board the boat containing water from the site until transferred to appropriate EPA or contracted personnel. It is anticipated that each boat will have a minimum of one operator, two netters and one person to record data. In riverine situations, boat operators will select a specific reach within a river section and will designate the upper and lower boundaries of the section of the river to be electrofished. Data will be recorded for each reach. In pond situations, boat operators will electrofish in one half hour intervals, recording data for each interval until such time as appropriate samples of target species are collected. Approximate locations of electroshocking runs will be designated on a map for all sites.

Fish captured by backpack electrshocking will be held in buckets containing water from the site, or in live cars placed in the site until transfers can be made to appropriate personnel. A minimum of one operator, one netter and one data recorder is anticipated at each site chosen for backpack

electroshocking.

Trot lines will be baited only with bait from the site in which the trot line is deployed. Gill nets will be closely monitored to minimize mortalities. Data sheets will be completed for all capture sites, and will include site location, date, capture method, time required for collection, fish species observed, relative abundance of all species, and other pertinent data. (See attached sampling form.)

FISH HANDLING PROTOCOL

All fish captured for contaminant analyses will be held by the USFWS until transferred to EPA personnel or contractors working under EPA supervision. Fish will be held at all times in water from the collection site prior to processing. Containers of fish will be labeled with capture location and any other information deemed approriate by EPA personnel. Aeration will be provided until fish can be processed.

CHAPTER 13

Electrofishing

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Electrofishing

13.1 Purpose. To ensure the safe conduct of electrofishing operations by establishing Servicewide competency requirements for electrofishing operations. This chapter also provides guidelines for the safe construction, modification, and operation of electrofishing equipment.

13.2 Scope. The provisions of this chapter apply to all Service activities using electricity (produced by gasoline powered generators/alternators or batteries) to sample animals in aquatic habitats.

13.3 Policy. The Service recognizes the electrofishing operation as a hazardous activity for which skills training is required in accordance with 24 AM 1.7 B (2).

It is, therefore, Service policy that all personnel serving as electrofishing team leaders demonstrate knowledge of the principles and techniques of electrofishing. Team leaders will be considered knowledgeable of the principles and techniques of electrofishing upon satisfactory completion of the National Fisheries Academy course, Principles and Techniques of Electrofishing. In lieu of course completion, Service personnel may satisfactorily complete a certifying examination by the Superintendent, National Fisheries Academy.

13.4 Authority.

A. 29 CFR 1910 - General Industry Standards.

B. Federal Boat Safety Act of 1971 as amended (46 U.S.C. 1451-89).

C. National Fire Protection Association (NFPA) 70-1981, National Electric Code (NEC).

13.5 Definitions.

A. Anode. The positive electrode.

B. Bonding. The permanent joining of metallic parts to form an electrically conductive path which assures electrical continuity, with the capacity to safely conduct current.

C. Branch circuit. The circuit conductors between the final overcurrent device protecting the circuit and the electrical load(s).

D. Cathode. The negative electrode.

E. Circuit breakers. A device designed to open and close a circuit by a non-automatic means, and to open the circuit automatically on the predetermined overcurrent without damage to itself when properly applied within its rating.

F. Deadman switch. A switch which requires constant pressure to supply electrical current to the circuit.

G. Electrofishing. The use of electricity to provide a sufficient electrical stimulus in fish to permit easy capture by netting.

H. Electrofishing team leader. The individual in charge of the electrofishing operation. Only persons demonstrating knowledge of the principles and techniques of electrofishing in accordance with 13.6D can serve as electrofishing team leaders.

I. Ground. A conducting connection, whether intentional or accidental, between an electrical circuit or equipment and the earth, or to some conducting body that serves in place of the earth.

J. Isolation transformer. A transformer inserted into a system to separate one section of the system from undesired influences with other sections.

K. Netter. The individual who nets the captured fish during electrofishing operations.

L. Power control circuit. The circuit which interconnects and adjusts the power from the pulsator or generator to the electrodes.

M. Raintight. Constructed or protected so that exposure to a beating rain will not result in the entrance of water.

N. Variable voltage pulsator electroshocker. The device used to deliver the pulsed electric current.

O. Watertight. Constructed so that moisture will not enter the enclosure.

P. Weatherproof. Constructed or protected so that exposure to the weather will not interfere with successful operation.

13.6 Responsibilities. These responsibilities supplement those found in 24 AM 1.5.

A. Chief, Office of Safety and Security. Will maintain a current listing of all Service personnel possessing an electrofishing certificate of competency, and provide regional safety managers with such listing.

B. Regional directors. Regional directors will ensure that all persons serving as electrofishing team leaders have received from the Superintendent, National Fisheries Academy, a certificate of competency for electrofishing.

C. Superintendent, National Fisheries Academy.

(1) Prepares electrofishing certifying examination for persons desiring to demonstrate knowledge of the principles and techniques of electrofishing by satisfactory completion of a certifying examination in lieu of completion of the National Fisheries Academy course, Principles and Techniques of Electrofishing. The certifying examination may be taken 3 times, in intervals of at least 30 days. Persons failing to satisfactorily complete the certifying examination in 3 attempts will be required to complete the National Fisheries Academy course, Principles and Techniques of Electrofishing, prior to serving as a team leader.

(2) Ensures sufficient scheduling of the course, Principles and Techniques of Electrofishing.

(3) Issues certificates of competency for individuals either completing the course, Principles and Techniques of Electrofishing, or satisfactorily completing the certifying examination.

(4) Provides the Office of Safety and Security with a listing of all personnel possessing an electrofishing certificate of competency and update such listing as appropriate.

D. Electrofishing team leader. Only individuals demonstrating knowledge of electrofishing techniques can serve as electrofishing team leaders. Team leaders will be considered knowledgeable of the principles and techniques of electrofishing upon satisfactory completion of the National Fisheries Academy course, Principles and Techniques of Electrofishing. In lieu of course completion, Service personnel may satisfactorily complete a certifying examination prepared by the Superintendent, National Fisheries Academy. Training and education for electrofishing operations will otherwise be in accordance with section 13.7. As the individuals in charge of electrofishing operations, the team leaders will do the following:

(1) Identify hazardous conditions associated with proposed electrofishing operations, determine measures to protect electrofishing team members, and appropriately brief team members (see section 13.7B).

(2) Ensure that employees have and utilize the proper safety equipment.

(3) Ensure adequate warning is provided to the public to avoid public exposure to the potential hazards of electrofishing operations.

(4) Ensure precautions are taken to avoid harm to pets, domestic animals, or wildlife.

(5) Ensure that all electrofishing operations cease and all crew members go ashore in the event of a thunderstorm.

(6) Ensure that only those persons necessary to conduct a safe and efficient operation, and observers being trained, engage in each electrofishing operation.

(7) Ensure the availability of a well equipped, water-tight first aid kit. Questions concerning the contents of the first aid kit may be directed to the regional safety manager.

(8) The team leader should review the electrofishing considerations checklist found in Exhibit 1, and ensure the addition of specialized items to the checklist that pertain to his/her region or operation.

E. Project leaders. Ensure compliance with the provisions of this chapter.

F. Employee. Report all potential work hazards/accidents/incidents and job related illnesses/injuries to his/her supervisor immediately.

13.7 Training and education.

A. Team leader training and education will cover the areas identified below.

(1) The basic principles of electricity and transmission of current in water.

(2) The basic concept and design guidelines for electrofishing equipment.

(3) Electrofishing equipment and the equipment's capabilities, limitations, and safety features.

(4) The safety precautions to employ while using electrofishing equipment.

(5) The team leader must have a current certification in cardiopulmonary resuscitation (CPR) training and first aid.

Completion of the course, Principles and Techniques of Electrofishing, at the National Fisheries Academy or at a field location, or successful completion of the certifying examination, will serve to satisfy competency for factors 1, 2, 3, and 4. A certificate from the Red Cross or other recognized institution will certify CPR and first aid training.

B. All members of the electrofishing crew will be briefed in the following areas:

(1) Hazards involved in electrofishing.

(2) Safe operation of electrofishing equipment.

(3) Basic emergency procedures for drowning, unconsciousness, and electrical shock.

(4) All members of the electrofishing crew will also be knowledgeable of defensive driving techniques, including towing and backing of boat trailers if an electrofishing boat is used, and safe boating operations.

13.8 Electrical equipment: specifications and operation.

A. General.

(1) Isolation transformer. AC voltage from the generator will be isolated from ground either by removing the ground strap from the generator case or by adding an isolation transformer.

(2) Voltage. Rated voltages of insulation of conductors used to deliver output current from the pulsator to the electrodes must exceed the maximum potential voltage of the pulsator or generator by the next higher rating as follows:

Pulsator/generator	Minimum insulation rating of conductor
0 - 249 volts	250 volts
249 - 599 volts	600 volts
599 - 899 volts	900 volts
900 - 12,999 volts	13,000 volts

(3) Conductor size. Conductor size (i.e., current carrying wire) will be approved for rated amperage of equipment as follows:

Maximum amperage	Minimum conductor size
10	16 AWG
15	14 AWG
20	12 AWG
30	10 AWG

(4) Conductor type.

(a) Conductors will be of the stranded type for flexibility and be suitable for use in dampness.

(b) All conductors in the boat will be enclosed in conduit or liquid-tight, flexible conduit; however, appropriate heavy duty rubber cord can be used where flexibility is desired.

(c) Connectors used in association with flexible cords will be of the locking, waterproof type.

(5) Connections.

(a) Solices in wiring will not be permitted. If connections are necessary, the rating of the connector must be the same or greater than the wire.

(b) All equipment will be turned off before making any connections or replacing parts.

(6) Junction boxes. Junction boxes will be cast iron, cast aluminum, fiberglass, plastic, or rubber. All types must either be weatherproof or raintight depending on use. All junction boxes with switching equipment must be weatherproof. Junction boxes without switches may be raintight.

(7) Circuit breakers.

(a) Power output conductors from the generator or alternator will include a circuit breaker or fuse to provide branch circuit protection.

(b) Circuit breaker or fuses used for providing branch circuit protection will be enclosed in a weatherproof enclosure or cabinet that complies with National Electric Code, Article 373-2, which states the following:

"In damp or wet locations, cabinets and cutout boxes of the surface type will be so placed or equipped so as to prevent moisture or water from entering and accumulating within the cabinet or cutout box and will be mounted so that there is at least $1/4$-inch air space between the enclosure and the wall or other supporting surface. Cabinets or cutout boxes installed in wet locations will be weatherproof."

(8) Electrodes and net handles. Net handles will be constructed of a non-conductive material and will be of sufficient length to avoid hand contact with the water.

(9) Noise. Noise levels will be maintained within the acceptable exposure of 85 dba for 8-hour exposure. Personal protective measures, such as use of earplugs, are described in 24 AM 8. The purchase of sound powered headphones is authorized through station funding. This type of headphone shuts out generator and motor noise and provides clear communication between the netter and equipment operator.

(10) Exhaust from power source. The exhaust from gasoline powered engines and generator alternators will be directed away from the equipment operator. Exposed hot pipes will be enclosed in protective screening to reduce the potential of burn exposure to crew members. The use of galvanized pipe for exhaust is discouraged due to the potential release of toxic gases that are produced under extreme heating conditions.

(11) Fuel storage. Gasoline will be stored and transported in approved metal containers. Such containers when used for storage on metal hull boats will be grounded.

(12) Refueling. To refuel the generator/alternator, all equipment will be turned off. Hot surfaces will be allowed to cool. It is recommended that all tanks be filled prior to each operation to avoid the potential for explosion or fire while refueling hot gasoline engines.

(13) Instruction sheets. Instruction sheets for boat, equipment, and operational procedures will be enclosed in waterproof plastic and be readily available for reference at all times during the electrofishing operation.

(14) Preventive maintenance.

(a) All equipment used in electrofishing will be scheduled for an annual preventive maintenance inspection. In addition, all equipment will be inspected before each use.

(b) Any equipment deficiency which may present a safety hazard will be corrected before each field operation or when equipment damage occurs during actual use.

B. Portable electroshockers.

(1) Electrodes.

(a) Electrode handles will be constructed of a nonconductive material and be long enough to avoid hand contact with the water.

(b) The positive electrode (anode) used with portable electroshockers will be equipped with a pressure switch that breaks the electric current upon release.

(2) Netter position. Netters will work beside or behind the individual with the electrofishing equipment to ensure the electrical field is well in front of both workers.

(3) Standard safety equipment.

(a) All persons using portable electroshockers will wear rubber footwear which will insulate the wearer from electrical shock. All footwear will be equipped with nonslip soles.

(b) Rubber linesman gloves, rated above the voltage being used in the electrofishing operation, will be worn. These gloves will be inspected for punctures before each use and will be replaced at adequate intervals.

(c) Polaroid sunglasses will be worn when there is glare.

(4) Portable electrical power source.

(a) Batteries used as electrical power source for backpack shockers will be of the gel type that will not leak when tipped or overturned.

(b) Backpacks will be equipped with a quick release belt (hip) and shoulder straps.

(5) Power control.

(a) The operator will have a switch to the pulsator or power control unit so that the electricity can be turned off quickly in an emergency.

(b) All equipment purchased after October 1, 1985, must be equipped with a tilt switch that breaks the circuit if the operator falls. The switch must be a type that has to be manually reset after the operator has regained his/her footing.

(6) Personal flotation devices. All persons will wear U.S. Coast Guard approved personal flotation devices (Type II) (i.e., life jackets or float coats) when operating in waters that are deep, high velocity, or turbid, to prevent drowning.

Note: Flotation devices constructed of materials such as ensolite are not bulky and are light weight. This material used in float coats can provide some protection against loss of body heat if the person accidentally falls into cold water.

(7) Hazard awareness. All persons will be aware of the hazards involved in using portable electroshockers in running waters such as slippery surfaces, swift water currents, deep areas, and obstacles such as logs or similar objects.

C. Electrofishing boats.

(1) Design.

(a) Electrofishing boats will provide adequate flotation and freeboard clearance consistent with equipment, cargo, and passenger weight when being operated. The boat will be equipped to meet U.S. Coast Guard or State boating regulations.

(b) The boat deck will be painted with a nonslip or skid resistant coating.

(2) Clear working space. General boat housekeeping must provide adequate working space to conduct safe operations. Care will be exercised to prevent clutter that may result in safety hazards.

(3) Boat inspection before each use. The boat and equipment will be visually inspected for safety by the supervisor or operator in charge prior to each use. Significant deficiencies, which could result in employee injury, will be corrected prior to operation or use of the equipment.

(4) Controls for electrical equipment.

(a) Electrical amp-volt meters will be installed to provide adequate monitoring of boat electrical power equipment.

(b) The boat operator should be able to operate an electrical control or switch to cut the power in case of an accident.

(c) The netter will have a deadman switch connected to the power control circuit from the pulsator or generator source. This allows the current between the electrodes to be broken in case of an accident.

(d) Power control circuits will not exceed 24 volts.

(5) Grounding/bonding. All metal surfaces within a metal boat will be electrically connected, grounded, and bonded to the boat hull to eliminate differences in electrical potential that may result in electric shock. The metal boat hull may also be used as a cathode.

To avoid possible electrolysis problems when the metal hull is being used as a cathode, zinc strips should be attached to the hull as "sacrificial anodes." The electrolysis will occur on the zinc strips which will preserve the integrity of the hull.

(6) Battery enclosure. An acid proof, nonmetallic enclosure and holder will be provided for wet cell batteries.

(7) Conductor protection. All conductors may be installed in a common raceway (conduit) provided each conductor installed is continuous (without connectors, breaks, or splicing), is independently and correctly insulated. All low voltage (24 volts or less) circuits will be contained in separate raceways from those containing high voltage conductors.

(8) Auxiliary circuits. Lighting and other auxiliary circuits should not exceed 24 volts. Note: 110 volt lamps may be used if the lamp is shielded with a nonconductive cage.

(9) Lighting.

(a) When the boat is to be operated at night, adequate on-board lighting (12-24 volts) will be provided for working areas.

(b) Adequate lighting will also be provided while electrofishing to avoid safety hazards such as striking logs, rocks, and overhead tree branches.

(10) Safety rails. Safety rails will be provided around the outside of the netting area and will be at least 42 inches high and be constructed of at least 3/4-inch diameter heavy-walled steel pipe or 1 1/2-inch heavy wall aluminium pipe. Rails will be so designed to withstand a 200-pound side thrust. The work deck will be covered with nonskid material and sloped to allow drainage. The high gunnels of wooden draft boats are satisfactory as safety rails.

(11) Fire extinguisher. Each boat will be equipped with at least one 5-pound type ABC fire extinguisher mounted in a holder for easy access to the boat operator and away from high fire potential sources.

(12) Personal flotation devices. All occupants will wear U.S. Coast Guard approved personal flotation devices at all times. Life vests that meet the requirements of Type II are designed to turn an unconscious person in the water from a face downward position to a vertical or slightly backward position. Float coats may provide some protection against the loss of body heat if the person were to accidentally fall into the cold water.

(13) Standard safety equipment.

(a) Hip boots will be worn so they can be easily removed in case the boat capsizes.

(b) Rubber chest waders will also be worn when necessary in order to remain dry as protection against electrical shock.

(c) Rubber gloves will be worn that are rated above the voltage being used. These will be inspected before each use and replaced at adequate intervals.

(d) Polaroid-type sunglasses will be worn to reduce glare from the water.

(14) Color coding/labeling of significant hazards. To ensure visibility, the color red will be used to identify fire extinguishers, safety cans, and stop buttons for electrical equipment. The color fluorescent orange will be used to identify all other safety switches.

APPENDIX A.21

FISH HEALTH AND TOXICITY PROTOCOL

APPENDIX A.21

Fish reproductive health assessment in PCB contaminated regions of the Housatonic River, Massachusetts, USA: Investigations of causal linkages between PCBs and fish health

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APPENDIX A.21

Fish reproductive health assessment in PCB contaminated regions of the Housatonic River, Massachusetts, USA: Investigations of causal linkages between PCBs and fish health

1. INTRODUCTION

The Housatonic River is a valuable aquatic resource, both aesthetically and economically (Orciari and Leonard, 1990). Draining over 2,000 square miles, the Housatonic flows south through a series of impoundments in western Massachusetts and western Connecticut, terminating in Long Island Sound. During the past two decades there has been increasing concern regarding the threat posed to fish and wildlife inhabiting the river due to the presence of highly toxic environmental contaminants (Henning et al., 1997). The principal cause of this contamination is a polychlorinated biphenyl (PCB) point source located on the East Branch Housatonic River at Pittsfield, Massachusetts. Total PCB concentrations in both fish and surface sediments downstream of this source have been reported at levels as high as 200 ppm (Smith and Coles, 1997). Although PCBs are considered to be the major toxic input to the river, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) may also contribute to contamination of the river (Eitzer, 1993).

PCBs, PCDDs, and PCDFs are collectively referred to as planar halogenated hydrocarbons (PHHs). The toxic effects of PHHs and structurally similar compounds are thought to be mediated through contaminant binding to a cytosolic aryl hydrocarbon receptor (AhR). This ligand activated transcription factor binds with selected sites on DNA called dioxin responsive elements (DRE), which control the expression of genes that encode both Phase I and Phase II enzymes. The induction of these proteins are part of a response to dioxin-like chemicals that leads to alterations in cellular homeostasis (DeVito and Birnbaum, 1994). Binding of PHHs to the AhR has been linked to several molecular events including the production of electrophilic metabolites and oxygen radicals, reduced capacity for xenobiotic metabolism, and alteration in the rates of endogenous substrate metabolism (Stegeman and Hahn, 1994). In fish, early life stages appear to be particularly sensitive to the effects of AhR ligands (Mehrle et al., 1988; Walker and Peterson, 1991) and recent evidence indicates the involvement of cytochrome P450 enzymes, specifically in this embryotoxic response (Cantrell et al., 1996; 1998).

A detailed assessment of the toxicological impact of PHHs on fish from the Housatonic has not been conducted. Using a combination of validated field- and laboratory-based tools, this study will evaluate the potential for contaminants in the Housatonic River to elicit embryotoxic effects on fish that could alter population structure. The study would have the combined benefits of not only demonstrating any association between PCB exposure and changes in early fish development, but also would develop the foundation for the determination of causality, if such a relationship exists. The studies would provide dose-response relationships for fish embryotoxicity caused by the actual mixture of chemicals found in fish from the Housatonic River. The multiple levels of biological organization (biochemical, histological, organismal) investigated will provide various lines of evidence for the conclusions of the studies.

2. HYPOTHESES AND SPECIFIC OBJECTIVES

2.1 HYPOTHESES

1) PCBs present in fish from the Housatonic river elicit detrimental effects through an AhR-mediated mechanism of toxicity.

2) Early life stages of fish species endemic to the Housatonic River are sensitive to the amount and composition of the PCBs found in the fish.

2.2 SPECIFIC OBJECTIVES

1) Evaluate the survival and development of offspring of fish collected from the selected PCB-contaminated locations of the Housatonic River.

    a) Determine the appropriate rearing conditions for the embryos of the representative species in the model.
    b) Determine the dioxin-like effects present in embryos and early life stages of fish eggs collected from the areas of interest in the Housatonic River.
    c) Determine the concentration of PCBs and other organic contaminants present in the ovaries of fish used in the rearing studies.
    d) Determine the ability of an additive model of toxicity of dioxin-like chemicals to predict the effects observed in the embryos and fry in the rearing studies.

2) Determine the embryotoxic effects of PCBs found in fish from selected areas of the Housatonic River.

    a) Develop an organic extract of the fish from the four areas and characterize the PCBs and other hydrophobic organic chemicals in the extracts.
    b) Determine the embryo toxic effects of the extracts in a species of interest from the Housatonic River and a laboratory surrogate species.
    c) Determine the extent to which an additive model of dioxin-like toxicity accounts for the toxicity of the chemicals in the complex organic extracts taken from fish from the selected areas of the Housatonic River.

3. EXPERIMENTAL APPROACH

The first phase of the assessment of PCB impacts on fish health in the Housatonic River is the collection of brood fish from the study areas with subsequent rearing of the embryos in the laboratory. Stage-specific mortality, gross pathologies, histological examination, and biochemical measurements will be made on the developing embryos and resultant fry, and offspring of fish collected from the four study areas of the Housatonic River. This will be followed by a 15-day

growth and mortality study of the surviving swim-up fry . The information on survival, development, and growth will be augmented with exposure assessment. The brood fish for these animals and portions of the eggs collected for this phase of the study will be analyzed for organochlorine chemicals including pesticides, congener PCBs, planar PCBs, and dioxins and furans. These exposure data will be used in conjunction with the effects observed in the developing embryos to determine the ability of the laboratory models (Phase II) to predict dose-response relationships in fish eggs collected from the Housatonic River. Therefore, studies with eggs reared in the laboratory from field-collected fish will serve as a validation exercise for the laboratory generated dose-response curves.

The most direct measure of the embryo toxicity of contaminants present in fish from the Housatonic River is to use those exact chemical mixtures to develop dose-response relationships in fish eggs. This will aid in revealing the toxicological mechanisms and allow for quantitative dose-response relationships to be developed exclusive of other stressors. The studies proposed in this portion of the proposal are designed to allow controlled laboratory exposures of fish eggs and developing embryos to an extract of PCBs from the whole body of fish from the study areas. The specific objectives of these studies will be achieved through the use of egg injection techniques and subsequent monitoring of the developing embryos until a time of exogenous feeding occurs in the fry or juvenile stage of the fish. The egg injection procedures are an alternative to full-life cycle studies, in which the adults are reared on contaminated feed designed to mimic the environmental conditions (Walker et al. 1996). The egg injection procedures effectively mimic the maternal transfer of hydrophobic contaminants, such as PCBs, to developing oocytes. Moreover, the toxicity of the contaminants received from such injections has been similar to that observed in studies where the eggs obtained the contaminants through maternal deposition (Walker et al., 1994).

The extent and nature of contaminant exposure in fish from the areas of the Housatonic River will be assessed through analytical measurements (OCs, PCBs, PCDDs and PCDFs as in previous phases of this project). As additional lines of evidence, biological indicators of contaminant exposure including ethoxyresorufin-*O*-deethlyase induction (EROD, a measure of cytochrome P450 induction) will be assessed in adult fish. Physiological and biochemical measures of effect in these fish may be assessed through the measurement of plasma concentrations of estrogen and testosterone, observation of the ratios of these steroid hormones (E/T ratios), plasma concentrations of vitellogenin, or selected other indicators of effect based on observations in the laboratory studies. Again, the appropriate selection of endpoints in the field-laboratory studies will allow the characterization of any causal linkages among contaminant exposure and adverse effects through a comparison with the laboratory-based results of these studies.

The results of the field-laboratory (Phase I) and laboratory (Phase II) studies will elucidate the extent, if any, to which PCBs are adversely affecting the early life stages of fish in the study areas of the Housatonic River. The two phases of the study will support or controvert the findings of each other and allow for stronger conclusions regarding the contaminant species involved in observed embryotoxicity. The laboratory studies serve as a standard curve of the effects expected to occur in the field. The studies proposed in Phase I offer a bridge between the controlled laboratory studies and the realistic findings of the field studies.

3.1 FIELD COLLECTION OF BROOD FISH

The first phase of these studies calls for fish to be collected from the study locations on the Housatonic River during or near spawning. The fish are to be transported to the CERC in Columbia, MO, where they will be bred. The intent of this portion of the study is to observe the development of the offspring and determine if any effects are observed in the offspring of species of fish collected at the areas of concern in the Housatonic River. The same endpoints of dioxin-like toxicity will be measured and assessed in the developing embryos and fry of adult fish taken from the Housatonic River as will be assessed in the subsequent egg injection studies. The egg injection studies will, as such, serve as a standard curve for calibration of any effects observed in the field-collected fish. Additionally, the rearing of field-collected eggs will serve as a validation exercise of the model developed from the egg injection studies described above.

The collection of brood fish will be performed by U.S. Fish and Wildlife Service personnel under its collection protocols. CERC personnel will assist in the collections and transport as needed. Any fish not used as brood fish will be processed in accordance with the protocols set forth in the Biomonitoring of Environmental Status and Trends (BEST) program of the USGS (Schmitt et al. CERC SOP P.326). The carcasses will be logged in the centralized sample tracking system and stored at -20°C until extracted and analyzed.

3.2 EMBRYO REARING

Wild-caught largemouth bass will be stocked in ponds (six males and six females) for spawning (two ponds per Housatonic River collection site) supplied with spawning mats. After the largemouth bass have completed spawning, the largemouth bass will be removed from the ponds and the bluegill will be stocked in enclosures in the ponds for spawning. Ten enclosures for each Housatonic River collection site will contain two females and two males of the wild-caught bluegill. The 40 enclosures that contain fish from each of the four sites (10 enclosures/site) will be randomly distributed among four ponds. Eggs collected from each of the spawning mats will be handled separately and will be treated as a separate spawning event. Fertilization rates will be checked by clearing of the eggs in glacial acetic acid and observation of a germinal disc. Each spawning event will be split into six replicates in the laboratory and each replicate will have 50 to 100 eggs. An additional set of two batches of eggs will be designated for temporal sampling for histological examination and immunohistochemical analysis (see below).

Developing embryos will be incubated according to optimal procedures to be developed in the first phases of the study. The bluegill, bass, and surrogate warmwater fish will be held in incubation chambers and the embryos gently rolled during development (Piper et al., 1982). Cold water species, used as surrogates in the injection studies (e.g., rainbow trout), will be held in vertical incubator trays with a constant flow of chilled water (8-12°C). Water quality is maintained within acceptable limits for embryo development, and water quality parameters are monitored periodically during the experiment, including dissolved oxygen, pH, water hardness, ammonia, and alkalinity.

Offspring of Housatonic River collected fish will be reared and observed for PCB-related effects. The endpoints that are to be monitored in these studies and the subsequent injection studies are those

consistent with a Ah-R mediated mode of action (Peterson et al., 1993) and include: mortality (daily); time to hatch (daily); gross pathology (weekly measurements of edema, hemorrhage, and craniofacial anomalies); histopathology (weekly sampling); weight and length (end of study); and in selected samples, apoptosis (programmed cell death) and cytochrome P4501A induction. The incidence of gross physical abnormalities will be quantified weekly for all experiments.

3.3 EGG INJECTIONS

The treatments in the experimental design matrix call for fish collected from the study locations to be extracted with organic solvents (see below) and the extract be used in fish egg injection studies to determine the toxicity of the chemical mixtures found at each of the sites. The extracts will be injected in graded doses into freshly fertilized fish eggs (Wilson and Tillitt, 1996; Walker et al., 1996; Tillitt and Wright, 1997; Wright and Tillitt, 1999). The concentrations of the extracts employed will be based on an estimated percent of adult body burden predicted to be transferred maternally to the eggs naturally. The eggs will be incubated under appropriate conditions and various endpoints associated with dioxin-like toxicity will be measured in addition to stage-specific mortality (see above).

The egg injection procedure to be used in these studies allows the accurate injection of nano- to picoliter amounts of various liquids into fish eggs to determine early life stage toxicity. The injection equipment allows small volumes of liquid to be delivered precisely with pulled-glass micropipettes, a regulated gas pressure system, and a digital control device. Pressure is applied pneumatically (compressed nitrogen gas) to the micropipettes with digital control of the dwell time, which allows delivery of a range of volumes. Eggs of various sizes, such as Atlantic salmon (*Salmo salar*), fathead minnow (*Pimephales promelas*), sea trout (*Salmo trutta trutta*), and northern pike (*Esox lucius*) can be used with this method with only minor modifications (Wilson and Tillitt, 1996; Walker et al., 1996). This procedure may be applied to various life stages of fish embryos, but was developed for use with newly fertilized eggs prior to completion of epiboly.

The species that are to be used for injection include one representative of the areas of interest and another species that is a routine surrogate used in toxicity testing. The decision about which species will be used in the laboratory portions of these studies will depend on the ability to culture the organism in the laboratory or obtain their eggs from a hatchery. It is anticipated that largemouth bass will be the species representative of the study area. The fathead minnow (*Pimephales promelas*) is a possibility for the laboratory surrogate species. The laboratory surrogate species is important to use in addition to the representative warmwater species because it will allow the results to be directly compared with the results from other laboratory examinations of PCBs and their mixtures. Additionally, the use of fathead minnows ensures that there will be a ready supply of eggs for use in the injection tests. Inclusion of the laboratory surrogate species in the experimental design ensures results from the laboratory egg injection studies for assessment and comparison to the other portion of the overall fish health assessment in the circumstances that difficulties are encountered in culturing the "endemic" species.

The experimental design for these studies calls for dose-response curves to be developed in the representative warmwater species and the surrogate species. Additionally, rainbow trout will be used

in dose-response studies because of the large amount of PCB-related reference information available for this species (Walker and Peterson 1996) and the fact that while not a dominant species in the study areas, salmonid species are present in the Housatonic River. The chemicals that will be used as standards include 2,3,7,8-TCDD and PCB 126 (3,3',4,4',5-pentachlorobiphenyl). The dose-response of embryo toxicity produced by the organic extracts of Housatonic River fish will be compared to the response of the test species to the chemical standards. The relative potencies of the various extracts may then be calibrated against these two standards. TCDD was chosen because it is the hallmark chemical for Ah-receptor (Ah-R) related responses. PCB 126 was chosen as the other standard because it often accounts for the majority of the dioxin-like potency of PCB mixtures in the environment and has the greatest toxic equivalency factor (TEF) of all of the 209 PCB congeners in fish (Van den Berg et al., 1998).

The specific objectives of these studies will be achieved through the use of egg injection techniques and monitoring of the developing embryos until exogenous feeding begins. The treatments in the experimental design matrix are: fish species to be used as source of eggs (N=3; bass/bluegill, fathead minnow or medaka, and rainbow trout); type of chemical or mixture of chemicals (N=6: PCB 126, TCDD, and four environmental extracts of Housatonic River fish); and dose of the chemical or chemical mixture (N=6, control and five graded doses). The combinations of these treatments are described below (Table 1).

Table 1

Experimental Design Matrix for the Egg Injections

Chemical/Mixture	Bluegill/Bass	Fathead Minnow	Rainbow Trout
2,3,7,8-TCDD	5 doses and sham	5 doses and sham	5 doses and sham
3,3',4,4',5-PCB	5 doses and sham	5 doses and sham	5 doses and sham
Extract Source			
Three-Mile Pond (H9)	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group
H. River Deep (H3) (RM 7-11)	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group
Woods Pond (H4)	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group
Rising Pond (H5)	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group	5 doses, sham, & 2 histological group

A complete dose-response relationship will be developed for each of the treatment combinations as described in the matrix. There will be five doses and the control (sham-injected) in each of these treatment combinations. The actual amount to be injected will be defined to bracket the concentrations observed in fish from the study sites. The doses will be defined as gram-equivalents (g-EQ) in which one g-EQ will equal the amount of the extract that corresponds to one gram of fish and will be lipid normalized based on the lipid content of the species of interest (Tillitt and Wright,

1997). The dosing range will be defined as one-tenth to one hundred times the concentration in the field collected samples. Each dose will be replicated three times and there will be 20 eggs injected at each of the doses, in each of the dose-response curves. There will also be a separate set of doses (at or near the expected ED50 of each of the mixtures and a sham-injected group) that will be incubated and sampled temporally for histopathological and biochemical lesions. The dose to be used in each case will be defined to bracket the LD50 values generated for bluegills using 2,3,7,8-TCDD and PCB 126 standards. An estimate of the LD50 for the extracts will be based on the PCB content of each extract.

The details of the egg injection procedures, reagents, and preparatory step are given in Walker et al. (1996). Briefly, the injections are conducted on freshly fertilized eggs, prior to epiboly. Eggs are held in a petri dish by agarose and the injections are conducted with drawn glass needles (micropipettes). Injections of graded doses of the chemicals or extracts are delivered accurately and precisely through the use of a regulated gas pressure system, and a digital control device. Pressure is applied pneumatically (compressed nitrogen gas) to the micropipettes with digital control of the dwell time, which allows delivery of volumes of 0.1% of the egg volume or less (often 0.5-20 nL). Following injection, the eggs of the various species will be incubated according to the appropriate procedures for that species.

The exposure concentration of the graded doses of the extracts that are to be injected into the eggs will be assessed with the toxic equivalency approach. This approach assumes an additive model of toxicity for the dioxin-like congeners (see Van den Berg et al. 1998 for further details on this approach). From this approach, the contribution of the planar PCBs (non-ortho-chloro-substituted congeners) to the overall dioxin-like toxicity may be estimated. The toxic equivalency factors (TEFs) that we will use are those developed from fish embryo mortality (Van den Berg et al., 1998). The dioxin toxic equivalents (TEQs) estimated to be each dose of the extracts allows calibration of the dose-response relationship of the complex extracts against the model compound for this class of chemicals, TCDD. The slopes of the extract dose-response curves may then be compared with that of the standard, TCDD. If the prevailing mode of toxic action of the extract is through an Ah-R mediated pathway, then it would be expected that the slope of the dose-response curves of the extract and TCDD would be parallel. Additionally, comparison of the median values for mortality (LD_{50}) between the extract and TCDD dose-response curves will also allow us to evaluate if an additive model of toxicity is appropriate for the various complex mixtures that we will test. If the slopes of the extract dose-response curves are parallel to TCDD and the toxicity is largely additive, then the contribution of PCBs to the overall dioxin-like toxicity can be estimated from this set of data.

3.4 PREPARATION OF EXTRACTS

Whole body samples of fish collected from the Housatonic River will remain frozen until the start of the sample preparation process. An extract will be developed for each of the study areas from the same species of fish used in the survival and development study of Phase I. The organic extracts will be used for the egg injection studies and will represent the complex mixture of organochlorine chemicals found in the fish at each location. The extraction and cleanup procedures are described below in detail, but generally will follow the methods described in Meadows et al. (1993; 1996). The fish will be processed individually through the grinding process. Fish will be sliced in 2- to 3-cm

slices while frozen and passed twice through a Hobart meat grinder. Approximately 15 kg of the ground tissue from each site will be weighed and thoroughly homogenized, individually by site. An aliquot equal to 1% of the total weight of each fish composite will be removed and set aside for analytical characterization of the chemical composition of the fish used to make the extract from each study area. Additionally, 20 to 50 g of each individual fish used to make the composites for extraction will be archived frozen at -20°C.

The remaining 99% of each composite will be chemically dehydrated by mixing with four times the tissue weight of anhydrous sodium sulfate. After dehydration, the tissue will be extracted by column percolation with methylene chloride. A set of five glass columns, 6 cm i.d. x 80 cm, with 1L reservoirs, will be used for this purpose. Each column can accommodate approximately 400 g of tissue with its attendant sodium sulfate. The columns will be filled in a serial fashion with the dehydrated tissue. The tissue composite of a given site will be exhausted before proceeding to the homogenate from the next site. Each column will be extracted with 1800 mL of methylene chloride. The tissue extracts from each site will be pooled and the extraction solvent removed from the extracted lipids by rotary evaporation.

The environmentally incorporated contaminants will be separated from the lipids by large-scale dialysis with polyethylene membranes (PM). Aliquants of 50 mL volume will be placed in 32-inch by 2-inch layflat tubing (0.005-inch wall thickness), heat-sealed, and dialyzed with 1800 mL of 80% hexane/20% methylene chloride. Each extract-filled PM will be dialyzed twice. The first dialysis will be for 4 days, followed by a change of dialytic solvent and additional dialysis for 3 days. Two 50-mL aliquants of clean salmon oil containing ^{14}C-2,5,2',5'-tetrachlorobiphenyl recovery spikes will be processed with each wash of each group as QC samples to monitor recoveries. The dialysates of each wash of each group of PMs will be composited and a portion equal to 0.1% of each removed for gravimetric lipid determination. Residual lipid removal will be through reactive cleanup with acid- and base-treated silica gels (Meadows et al., 1996). The effluents of the columns will be combined and placed at a volume of 40 mL. The combined extract will then be passed through high-performance gel permeation chromatography (HP-GPC) in 1-mL aliquots. The separation system consists of a 50-mm by 7.8-mm Phenomenex Phenogel® guard column and a 250-mm by 21.5-mm Phenomenex Phenogel® HP-GPC column both with a pore size of 100 A and particle size of 10 microns.

The purified sample extracts from each site will be composited and placed at a volume of 2 mL and the relative concentration (g-equivalents/mL) determined. A gram-equivalent is the amount of the extract equivalent to 1 gram of the original fish composite on a lipid normalized basis, relative to the eggs that are being injected. Three portions of 5 nL (approximately 40-g-equivalents each) each will be removed for analysis. The triplicate samples will be each brought to a volume of 5 mL (~ 8 g-equivalent/mL) and a 1-mL portion of each (~8-g-equivalents) will be used for chemical analysis. Internal standards for quantification of recovery and determination of the chemical content are to be added to each of the triplicate 1-mL portions prior to further processing (carbon fractionation on PGC and alumina cleanup for the dioxin/furan fraction). Comparison of the results of these three aliquots with analytical data from the original tissue will be used to indicate the recovery efficiency through the procedure as well as verify that no contaminants have been added during the procedure.

3.5 CHEMICAL CHARACTERIZATION OF PCBs

PCB exposure assessment in fish from the Housatonic River is the initial step for the determination of toxicological risk associated with these chemicals. Therefore, our first objectives in chemical characterization will include determination of the PCB congener profiles and dioxin-like chemicals in the ovaries of the fish collected from the study areas on the Housatonic River and used in the first phase of this study. The purpose of this will be to characterize the relationship between exposure to PCBs and dioxin-like chemicals and any effects observed in the rearing and growth studies described above. The carcasses of the adult fish collected from the four study areas on or near the Housatonic River will be the source of fish for development of extracts that will be used in the egg injection portion of the study. Thus, the same fish collected from the Housatonic River as a source of embryos to study survival and development will be used to make the chemical extract for use in the egg injection studies. The organic extracts will be used for the egg injection studies and will be representative of the complex mixture of organochlorine chemicals found in the fish at each location. The toxicity of each organic extract of fish will be determined from their ability to cause embryo toxicity in the subsequent portions of these experiments. Therefore, the second objective of the chemical exposure assessment portion of this work will be to characterize the extracts for PCBs, other dioxin-like chemicals, and organochlorine pesticides. Characterization of the fish egg dosing solutions prepared from the organic extracts will include quantification of congener PCBs, planar PCBs, chlorinated dioxins and furans, and organochlorine pesticides. The chemical analytical procedures that will be used are briefly described below.

Congener-specific PCB analysis for determination of PCB profiles in the field-collected fish from the study locations will proceed as follows. Sample preparation and analysis will generally follow the methods described by Schwartz and Stalling (1991). A 5-g portion from each sample will be dried with 20 g of anhydrous sodium sulfate (Merck, USA, 99 %) and ground. The samples will be homogenized with sodium sulfate and column extracted with CH_2Cl_2. A portion of each sample will be used to gravimetrically determine the lipid content and the remainder of each extract will then be treated by two stages of reactive column cleanup, followed by high-performance gel permeation chromatography. PCB congeners will be analyzed with a Hewlett-Packard 5890A Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector (ECD) and a Hewlett-Packard 7673 autosampler. The detector temperature will be 330°C and the injector set to follow the oven temperature. Injections will be 1-nL cool on-column onto a 30-m by 0.25-mm by 0.25-μm DB-5 capillary column (J & W, Folsom, CA, USA) with a 1-m x 0.53-mm deactivated retention gap connected to the column, with H_2 carrier gas maintained at 12 psig, linear velocity 60 cm/s. The oven temperature program will be as follows: 60°C, 10°C/min to 120°C, 2°C/min to 240°C, and then 10°C/min to 320°C with a 5-minute hold. Data will be collected with PC-based PE Nelson chromatography software (Perkin Elmer, Norwalk, CT, USA). Quantitation of approximately 100 PCB congeners will be based on an internal standard calibration.

Characterization of the chemical composition of the organic extracts will include chemical measurements of the OC pesticides, PCBs, PCDFs, and PCDDs found in the extracts (Feltz et al., 1995; Peterman et al., 1996). The determination of the exact amounts of dioxin-like chemicals in the fish composites and dosing solutions will proceed as follows. The raw fish composites will be homogenized, dried with sodium sulfate, spiked with the appropriate standards and column-

extraction with methylene chloride (Feltz et al., 1995). All the concentrated extracts will then be treated by a two-stage reactive cleanup; using first a sulfuric acid silica gel/potassium silicate column, and second, a column of sulfuric acid silica gel/potassium silicate/silica gel. High-pressure gel permeation chromatography (HP-GPC) cleanup will follow to remove residual lipids (Feltz et al., 1995).

The fish tissue extracts and aliquants of the final extracts will be fractionated using high-performance porous graphitic carbon chromatography (HP-PGC) into fractions containing: 1) bulk through mono-*ortho* chlorine substituted PCB congeners, 2) non-*ortho* chlorine substituted congeners, and 3) 2,3,7,8-PCDDs and PCDFs according to the procedures in Echols et al. (1997). The instrumental analysis for the determination of the congener-specific PCBs (fraction 1) will be as described above by GC/ECD. Non-ortho PCBs (fraction 2) will be analyzed by gas chromatography/high-resolution mass spectrometry (GC/HRMS) (Peterman et al. 1996). Finally, PCDD/PCDFs (fraction 3) will be eluted through basic alumina (according to CERC SOP C5.152) for removal of potential co-contaminants such as polychlorinated diphenyl ethers (PCDEs) and residual polychlorinated naphthalenes (PCNs) and PCBs. The instrumental internal standard, ^{13}C-labeled 1,2,3,4-PCDD, will be added to each semiconical autosampler vial prior to transferring the PCDDs/PCDFs (fraction 3). PCDFs and PCDDs will be determined by GC/HRMS by monitoring five sequential mass windows of selected ions during the chromatographic separation (according to CERC SOP C5.183 and Peterman et al. 1996). GC/HRMS analysis will be performed using a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high-resolution mass spectrometer. An HP 7673 autosampler will be used to introduce 2 of 25 nL of the enriched extract from a conical vial through a spiral uniliner onto a 5-m by 320-µm deactivated fused silica retention gap via a heated (285 °C) direct inlet. The analytes of interest will be separated on a 50-m by 200-µm by 0.11 µm Ultra-2 (Hewlett Packard) capillary column with an initial hold of 1 min at 120 °C followed by a ramp to 200 °C at 20 °C/min, another ramp to 300 °C at 2.3 °C/min, and a final hold of 5 mins. The He carrier gas is maintained at 44 psig with an initial linear velocity of 25 cm/s. All column-to-column connections were made using fused silica press-tight connectors.

The VG GC/HRMS system is tuned to 10,000 R.P. and calibrated using perfluoro-tetradecahydro-phenanthrene, and mass windows are established for five ion groups to measure Cl_{4-8} PCDFs and PCDDs. These windows are monitored sequentially during the temperature program. Within each mass window, the two most abundant ions are measured for positive identification and quantitation of each analyte. The ion responses are quantitated and averaged, unless interferences occur. Within each mass window, additional ions monitor any responses from Cl_{5-9}_PCDEs, Cl_{5-7}_terphenyls, Cl_{6-7}_PCNs, Cl_{3-8} dibenzothiophenes, and Cl_{3-8} phenanthrene/anthracenes.

Determination of non-ortho PCBs (planar PCBs) in fraction 2 above is conducted by GC/HRMS analysis and performed with a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high resolution mass spectrometer. An HP 7673 autosampler is used to introduce 2 nL of the enriched extract from a conical vial onto a 2.5 m x 530 µm deactivated fused silica retention gap via a cool on-column injection technique. A 50-m by 200-µm by 0.11-µm Ultra-1 capillary column (Hewlett-Packard's equivalent to DB-1) is used to resolve most non-*ortho*-PCBs from interferences. The GC oven is held at 120°C for 1 min, programmed to 240°C at 2.2°C/min, then ramped to 310°C at 5°C/min, for a final hold of 5 mins. Helium carrier gas is maintained at 48 psig with an initial

linear velocity of 25 cm/s. The analytical column is put into the MS interface, heated to 310°C. All column-to-column connections are made using fused silica press-tight connectors.

The VG GC/HRMS system is tuned to 10,000 R.P. and calibrated using perfluorodecalin, and mass windows are established for two groups of non-*ortho*-PCBs. Group 1 from 23-48:00 min included ions for Cl_4-biphenyls 77 and 81 and Cl_5-biphenyl 126; Group 2 from 48:05-65 min included ions for Cl_6-biphenyl 169. Within each mass window, the two most abundant ions are measured for positive identification and quantitation of each analyte. The ion responses are quantitated and averaged, unless interferences occur. Within each mass window, additional ions monitor the responses of higher chlorinated, potential interfering PCB congeners, Cl_{4-8} naphthalenes (PCNs), Cl_{3-5} terphenyls (PCTs), Br_5- and Cl_6-diphenyl ethers, and Cl_4-PCDF (to ensure no breakthrough of PCDFs).

The amount of each analyte detected is inherently self-corrected for losses through the whole analysis (extraction, isolation of analytes, and instrumental analysis). A calibration curve describing the response of each native congener to that of a isotope-labeled congener is used directly in the calculations and its range of values is determined in the calibration procedure. Concentrations of the native PCB congeners in standards ranged from 0.25 to 2,500 pg/nL. Each calibration curve is specifically matched to the range of analyte responses in the sample set.

3.6 HISTOLOGICAL EXAMINATIONS

Preparation of embryos for histological analysis will be performed according to procedures in Cantrell et al. (1998). Developmental stages will be chosen based on the embryology of the selected surrogate and representative species. Key stages for analysis will include the development of the vitelline vasculature, gill and digestive organs, and hatching of embryos from the chorionic membrane. Embryos collected from the incubation chambers will be assessed for the presence of a heartbeat, an intact pericardial sac, and circulating blood to assess viability. Embryos not meeting all criteria will be scored as nonviable and will not be used for histological analysis. Eggs and fry will be collected and preserved in buffered neutral formalin, washed in 10 mM HEPES (pH 7.4), and dehydrated by immersion in solutions containing ethanol from 50 to 100%. This is followed by immersion in xylene and infiltration with paraffin. The paraffin-embedded samples will be placed into plastic molding cassettes, sectioned into 10-µm sections, placed onto silanized slides, and stored at room temperature until analysis. For histochemical staining, tissue sections will be dewaxed and rehydrated by immersion in solutions containing decreasing concentrations of ethanol (100 to 0%). Gross morphological alterations (e.g., deformities and hemorrhaging) will be determined by hematoxylin/eosin staining at each developmental stage.

3.7 BIOCHEMICAL ANALYSIS

3.7.1 Apoptotic Cell Death in Embryonic Fish

Previous work in our lab demonstrated DNA degradation and morphological changes characteristic of apoptosis in cells of the embryonic vasculature in Japanese medaka exposed to 2,3,7,8-TCDD

(Cantrell et al., 1996, 1998). To determine if PCBs in Housatonic fish extracts exert toxic effects through a similar mechanism, apoptosis will be examined in the paraffin-embedded tissue sections prepared above. Sections will be dewaxed and rehydrated by immersion in solutions containing decreasing concentrations of ethanol (100 to 0%). The rehydrated tissue sections will be analyzed for the presence of apoptotic cells by using terminal transferase-based assay, which tags 3´OH DNA strand breaks with a fluorescein-conjugated antibody. The presence of numerous 3´OH DNA strand breaks is a hallmark of apoptosis (Compton 1992). Apoptosis detection will be accomplished using a commercial apoptosis detection kit (Oncor, Gaithersburg, MD). The rehydrated tissue sections will be washed in phosphate-buffered saline (PBS) and digested with 20 µg/mL solution of proteinase K. Slides will be washed in PBS and incubated with a digoxigenin-conjugated nucleotide in the presence of terminal deoxynucleotidyl transferase (TdT) followed by incubation with a fluorescein conjugated anti-digoxigenin antibody (Compton, 1992; Cantrell et al., 1998).

Image analysis will be performed using a Nikon inverted Diaphot-TMD microscope along with a PT/LPS-210/250 DC power supply and Xenon arc lamp. The fluorescein-based DNA labeling assay and the low-light amplification system allows for sensitive quantitative analysis of individual cells in the tissue sections of the embryos. To quantitate apoptotic cell death, the total number of apoptotic cells in a defined area will be counted from each image. This number will be expressed as a percentage of the total cells in the same defined area of tissue. Three separate embryos from at least two independent dosing experiments will be used to obtain a mean ± SD for each extract/standard dose.

3.7.2 Cytochrome P450 1A Induction in Embryonic Fish

The presence of compounds, such as PCBs, that can exert effects through the aryl hydrocarbon receptor (AhR) is commonly inferred from the level of cytochrome P450 1A induced following contaminant exposure. Immunodetection of induced cytochrome P450 1A in the paraffin-embedded tissue sections will be accomplished using an indirect peroxidase-labeling method. The tissue sections will be deparaffinated and hydrated in 1% bovine serum albumin/PBS containing 1% bovine serum albumin. The hydrated slides will be incubated in 0.5% H_2O_2 in methanol for 45 min to block endogenous peroxidase. The hydrated tissue sections will be immunochemically stained using monoclonal antibody (mAb) 1-12-3 made against scup P450E as the primary antibody (Park et al., 1986). The tissue samples will be observed for peroxidase staining (red-brown deposit) using light microscopy. Companion sections will be incubated with non-specific monoclonal immunoglobulin G2 (Smolowitz et al., 1991). All sections will be counterstained with Mayer's hematoxylin.

3.7.3 Adult Fish

The extent and nature of contaminant exposure in fish from the areas of the Housatonic River will be assessed through analytical measurements (OCs, PCBs, PCDDs, and PCDFs as in previous phases of this project). As an additional line of evidence, biological indicators of contaminant exposure including ethoxyresorufin-*O*-deethlyase induction (EROD) will be assessed in adult fish. Physiological and biochemical measures of effect in these fish may be assessed through the measurement of plasma concentrations of estrogen and testosterone, observation of the ratios of these

steroid hormones (E/T ratios), and plasma concentrations of vitellogenin. The appropriate selection of endpoints in the field studies will allow the characterization of any causal linkages among contaminant exposure and adverse effects through a comparison with the laboratory-based results of these studies.

3.7.4 7-Ethoxyresorufin-O-deethylase Induction

Induction of cytochrome P450 1A in adult hepatic tissue will be inferred from the catalytic activity of 7-ethoxyresorufin-*O*-deethylase (EROD). This assay is based on the work of Pohl and Fouts (1980). Preparation of hepatic microsomes and EROD analysis will be performed according to CERC SOPs P.123 and P.124. Microsomal preparation involves homogenization of livers in cold phosphate buffer (pH 7.4) with two passes of a hand-held tissuemizer (Omni International, Warrenton, VA). After centrifugation for 25 min at 9000 g and 4°C, the supernatant fraction will be centrifuged at 105,000 g for 50 min at 4°C. Microsomal pellets will be resuspended in phosphate buffer and then recentrifuged at 105,000 g for 50 min. The resulting pellet will be resuspended in phosphate buffer. The EROD assay will be performed on the same day as the microsomal preparation.

Enzymatic activity of CYP1A in triplicate samples per fish will be measured as the conversion of 7-ethoxyresorufin to resorufin (EROD) (Pohl and Fouts, 1980). Microsomal samples (5 nL) added to 96-well microtitre plates will be mixed with 50 nL of 10 µM ethoxyresorufin, 50 nL of 4.3 mM NADPH and 50 nL of phosphate buffer (all reagents at 25°C). Plates are incubated for 10 min at 25°C and then scanned on a Cytofluor 2300 plate reading fluorometer (Perseptive Biosystems, Framingham, MA) with the following settings: emission filter (590 nm), excitation filter (530 nm), sensitivity 3, and 10 scans at a scan cycle of 60. All resorufin concentrations will be calculated based on a resorufin standard curve. Immediately following the EROD scan, protein content of the mixture will be determined fluorometrically using the method of Kennedy et al. (1994).

3.7.5 Steroid Hormone Analysis

Planar halogenated hydrocarbons can affect natural steroid hormone levels, potentially affecting reproduction in fish (Munkittrick et al., 1992). To determine if PCBs in the Housatonic are interfering with steroid hormone regulation, we will measure levels of 17ß-estradiol and testosterone in plasma from fish collected at the four studies sites. The radioimmunoassay (RIA) technique described in Van der Kraak et al. (1984) will be used. Briefly, plasma samples (50 nL) will be combined with 1-3 mL of phosphate buffer (0.05 M, pH 7.6, containing 0.1% gelatin) and heated at 70°C for 1 hour. 17ß-Estradiol and testosterone will be measured using rabbit anti-17ß-estradiol and anti-testosterone serum. Diluted, heated plasma or appropriate standards (200 nL each) will be combined with 200 nL of tritiated estradiol or testosterone and 200 nL of diluted antiserum. Samples are then incubated for 16 to 20 h at room temperature, cooled on ice for 15 min prior to the addition of 200 nL of phosphate buffer containing 0.5% Norit A charcoal and 0.05% Dextran T-70. Samples will then be incubated 10 min on ice followed by a 10-min centrifugation at 4,000 rpm and 4°C. The resulting supernatant will be poured directly into scintillation vials and combined with 6 mL of scintillation fluid for counting. The antibody concentrations in the RIA procedure will be adjusted

so that 45 to 55% of the radiolabeled steroids will be bound in the absence of competitor. All plasma samples will be analyzed in duplicate.

3.7.6 Vitellogenin Determination

To ascertain the presence of estrogenic contaminants in the Housatonic river, induction of vitellogenin synthesis in male fish will be compared to that in females. Since it is the major source of protein-bound phosphate in fish plasma, the amount of vitellogenin can be quantified accurately using a phosphoprotein-phosphate assay (Ng and Idler, 1983). Plasma vitellogenin will be estimated in duplicate plasma samples as described in Mount et al. (1988). Five mL of cold 10% trichloroacetic acid (TCA) will be added to 20-40 nL of undiluted plasma in a 12-mm by 75-mm glass test tube and incubated overnight at 4°C to precipitate plasma proteins. After 24 h, tubes will be centrifuged at 2,000 g for 10 min and the supernatant discarded. To remove lipids, the pellet will then be washed twice with 2 mL of acetone followed by centrifugation. After the second wash, the pellet will be blown to dryness under air. The dried pellet will be dissolved in 1 mL of 2 N NaOH and incubated in water at 90°C for 15 min, liberating protein-bound phosphate. Tubes will then be cooled in an ice bath and neutralized with 167 nL of concentrated HCl. Four mL of cold 10% TCA will be added and incubated overnight at 4°C to precipitate any remaining proteins. Tubes will then be centrifuged and the supernatant decanted into 13-mL by 150-mL tubes. Duplicate standards of 0, 1, 2, 3, 4, 5, and 6 mg PO4/L in 5 mL TCA will be made and 5 mL of 3% (v/v) HCl will be added to both standards and samples. Phosphate content is then determined colorimetrically by reaction with ammonium molybdate.

3.8 DATA ANALYSIS

Mortality and gross pathologies exhibited during the egg and sac-fry stages of development will be recorded and evaluated for contaminant-related increases by chi-square analysis (Snedecor and Cochran, 1980). Data that exhibit treatment-related increases will be used to generate continuous dose-response curves and 95% fiducial limits using a probit procedure (SAS, 1988). The probit procedure corrects for control mortality analogous to using Abbott's formula. In addition, this procedure uses chi-square goodness of fit, estimates the slope and intercept, and is based on the assumption that mortality/effect is independent for fish within a dose group and among dose groups.

The slopes for the apoptosis-response curves will be compared with the mortality-response curves as described in Cantrell et al. (1998). Determination of statistical differences in slopes will be accomplished using analysis of covariance with interaction of the data curves (SAS 1988). Confidence level will be set to 95% (type I error set at 5% or $p < 0.05$).

Individual dose groups for egg injections will be tested against controls using ANOVA and a multiple post-hoc comparison, the least-significant difference (LSD) test. This method will also be used to ascertain differences among sites for EROD induction, vitellogenin synthesis, and steroid hormone levels in adult fish from the Housatonic. Relationships between measured parameters will be determined by linear regression and Pearson's pairwise correlations. Significance levels are set at $p \leq 0.05$.

The statistical analysis of the analytical chemistry data will consist of mean concentration comparisons among sites. Concentrations of total PCBs and TEQs generated from the analytical chemistry and an additive model of toxicity will be compared among sites by ANOVA (SAS, 1988) if assumptions of normality and homogeneity of variance are met, or Kruskul-Wallace one-way analysis of variance (Snedecor and Cochran, 1980) if assumptions are not met.

4. QUALITY ASSURANCE /QUALITY CONTROL

4.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

4.1.1 Data Quality Objectives

The data quality objectives for the proposed study are: 1) to ensure that the analytical measurements, biological/toxicological assays, and biochemical analyses are accurate and precise measurements of the samples collected in the field or laboratory portions of the study, and 2) to ensure that the potency of the mixtures measured in these toxicological tests of embryo development are reflective of the toxicity expected to be observed in feral fish.

To meet these objectives, a quality assurance plan has been designed whose general approach includes:

- Replication of various stages of the study,
- Comparison and calibration of analytical results against known standards,
- Proper maintenance and calibration of equipment,
- Accurate sample tracking and custody,
- Proper documentation at all steps of sample processing,
- Other considerations of Good Laboratory Practice (GLP).

The specific aspects of the QA Plan for this study are given below.

4.1.1.1 Fish Sampling Procedures

Brood fish will be collected from three study locations on the Housatonic River that are expected to be representative of the PCB exposure of fish throughout the study area. A reference location also will be selected that is expected to be representative of the background PCB levels in the Housatonic River watershed. The collection of brood fish will be performed by the US Fish and Wildlife Service according to the fish collection standard operating procedure (SOP) provided in Appendix A.20. The quality assurance procedures for the collection, holding and shipping of brood stock fish are further supplemented in CERC's *Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants* (CERC SOP P.326 [Schmitt et al., 1999]).

4.1.1.2 Fish Processing and Preservation Procedures

Fish collected but not used as brood stock will be processed in accordance with the protocols set forth in CERC SOP P.326. All fish (brood stock and fish collected but not used as brood stock) carcasses will be logged in a centralized sample tracking system and stored at –20 ºC until extracted and analyzed. Sample tracking forms will be completed and retained with the samples to provide exact information on the samples. These forms are the Sample Inventory Listing form and the Sample Batch History Information form (both forms can be found in CERC SOP P. 326 - Appendix 1).

The Sample Inventory Listing form is a list of all the samples to be transmitted and has the sample label (identifier with year, project #, study #, and personal sample ID #) and a brief description of each sample. The Sample Batch History Information form contains information on sample collection dates and location, how samples were collected, where they were collected, how they were preserved, sample transmission dates and modes, and other pertinent information about the samples and how they have been handled.

All samples will be given an independent identification number for internal tracking and all of the information is computerized on a central sample tracking system. A complete description of the sample tracking system is provided in CERC SOP P. 326 - Appendix 1.

4.1.1.3 Extraction and Analytical Procedures

The matrices for analysis include whole fish tissue and extracts of fish tissue. Detailed methods for the extraction and subsequent chemical analysis to be conducted by CERC laboratory on fish tissue and fish tissue extracts and their respective SOPs include:

Lipid analysis	*Extraction of Animal Tissues for Residue Analysis* (CERC SOP P. 461)
Organochlorine pesticide analysis	*Organochlorine Pesticide Analysis: Fractionation of Complex Mixtures on Silica Gel/ODS* (CERC SOP P. 460)
Total & congener-specific PCB analysis	*Capillary Gas Chromotography with Electron Capture Detection Procedure for Congener Specific Polychlorinated Biphenyl Analysis* (CERC SOP P.195)
Non-ortho PCB analysis	*Analysis of Selected Non-O-Chloro-substituted Polychlorinated Biphenyls by Gas Chromotography – High Resolution Mass Spectrometry* (CERC SOP P. 481)
Chlorinated dioxin and furan analysis	*Analysis of Tetra- through Octa- Substituted Polychlorinated Dibenzo-p-dioxins and Dibenzofurans by Gas Chromotography – High Resolution Mass Spectrometry* (CERC SOP P. 482).

Ethoxyresurofin-O-deethylase (EROD) Analysis	*Microsomal Preparation of Liver Tissue* (CERC SOP P. 123).
	Procedure for the determination of 7-Ethoxyresurofin-O Deethylase (EROD) Activity on Microsomes from Liver Tissue Using 96-Well Microtiter Plates (CERC SOP P. 124)

Analyses will be performed by GC/ECD and/or GC/MS. Method limits of detection will be 1-5.0 ppt (pg/g) for dioxins and furans and <1 ppb (ng/g) for the PCB congeners. These detection limits were selected to ensure the detection of chemicals at concentrations that may cause adverse effects.

QA/QC procedures for the analyses presented above will include at a minimum, analyses of spiked samples with appropriate standards, analysis of replicates, analysis of procedural and matrix blanks, and the demonstration of correct chemical identifications. The general QA procedures for chemical analyses that will be followed by CERC for this project are provided in CERC's *Minimum Quality Assurance Standards for Trace Organic Residue Analysis.*

4.2 DATA QUALITY INDICATORS

Data developed in the Fish Reproductive Health Assessment must meet standards of precision, accuracy, completeness, representativeness, comparability and sensitivity that are appropriate to the data quality objectives. Each of these data quality indicators is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Precise measurements of the various chemical analyses, as well as measures of effect are crucial to the interpretation of study results. To ensure adequate precision, replication and subsequent performance checks are performed throughout a number of stages of this study. Quality control considerations to ensure precision of chemical analyses will follow those performance criteria outlined in each of the aforementioned CERC SOPs. For measuring effects in the offspring development phase of the study, the study design includes an increase in the number of replicates to increase the statistical power. For offspring development phase of the study, each spawning event will be split into six replicates and each replicate will contain 50 to 100 eggs. For the egg injection phase, the performance criteria for precision will include the replication of each delivered dose (3 replicates/dose level) and a targeted number of individuals in each replicate (20 eggs/replicate). Each chemical or chemical mixture (extract) will be tested at five separate doses.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study, accuracy is defined as meaning that the chemical and biochemical analyses represent the true measure of that chemical and that the effects are correctly characterized and measured. To ensure accuracy of analytical results, calibration of equipment and calibration of chemical results against known standards will be performed throughout the performance of the study. Quality control considerations to ensure accuracy of chemical and biochemical analyses will follow those performance criteria outlined in each of the aforementioned CERC SOPs. To ensure accuracy of effects measurements, QA procedures include replication, the use of "sham" doses, as well as

vehicle controls. Moreover the egg injection phase will incorporate surrogate species for which a historical database will provide a measure of comparison. In addition, all effects monitoring will be directed in the laboratory by senior scientists. Technical support will include only those staff that are properly trained to ensure the collection of quality data. All equipment used in these studies is routinely inspected and preventive maintenance is performed. A logbook is kept for each instrument to document its use, performance, and maintenance

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the Fish Reproductive Health Assessment. An accurate record of information on sample transmittal, receipt and inventory will be ensured by following CERCs SOP C5. 162. Completeness also refers to the percentage of the planned number of bioassays and bioassay design elements (e.g. proposed doses) that have been proposed in the study design. Completeness of this information will be ensured by the recording of all procedures and results in the study notebooks. All completeness goals will be as defined in the project QAPP (WESTON, 2000).

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of the sampling. Representativeness for this study is ensured through the establishment of an approved sampling design and through careful implementation of the sample processing, handling, and maintenance measures as well as the consistent use of standardized chemical and biochemical methods. The use of chemical extracts provides a direct measure of embryo toxicity to exact chemical mixtures found in the fish in the Housatonic River. Additional aspects of representativeness will be evaluated by comparing the study results with known and/or expected results from previous studies of the chemicals and chemical mixtures being evaluated.

Comparability is a measure of the confidence with which comparisons of data within and among stations can be made. Comparability will be evaluated by examining the in-station variability as well as the dose-response variability in key measurements as determined from the large numbers of replicates that will be monitored relative to fish species type, the location from which those fish were harvested and the number of laboratory treatments. Comparability will also be evaluated for this data through comparison with known and/or expected results from previous studies of the chemicals and chemical mixtures.

4.3 DATA VALIDATION, VERIFICATION, AND USABILITY

Validation and verification of chemical and biochemical analyses data will follow the general procedures of validation and verification outlined in CERC's *Minimum Quality Assurance Standards for Trace Organic Residue Analysis*. Specific procedures for validation, verification, and usability of chemical and biochemical analyses are presented in each of the CERC SOPs (referred to previously) and will be used wherever applicable in this study.

Data on the measurement effects (assay endpoints) will be collected and reported as specified by the appropriate SOP (e.g., CERC P.326). These data will be review and verified by the senior investigator who will judge the data against data quality indicators appropriate to that data. In many

cases, the senior investigator will use his professional judgment in the determination of data usability.

All experimental information will be recorded on datasheets specified in the various CERC SOPs, signed, and copies maintained in a separate secured area. Instrument printouts and computerized data tables are uniquely labeled and cross-referenced to the project notebook as appropriate. The accuracy of all such measurements will be independently checked. Copies of the computerized data files are maintained in a project notebook and file, on floppy disk in the project file and by archived tape back up.

4.4 QA AUDITS

Internal audits are continuously performed by the Principal Investigators with routine checks by independent CERC personnel.

4.5 CORRECTIVE ACTION

Problems will be identified as they occur or through weekly staff meetings. Remedial actions will be taken as deemed appropriate and in accordance with the QA performance criteria. All such problems and corrective actions will be recorded in the project notebook(s) and reported to management or the Project Officer, if necessary.

4.6 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated by this study is described in the previous section "Data Analysis." Routine analyses will be performed and an allowance for Type I errors will be set at 5% ($\propto = 0.05$). Outliers will be determined as described by Gill (1978). Reporting of the data will initially be in draft form to the appropriate collaborators. Following review and approval, an internal review of the draft report will be made and a final report sent to the Project Officer. The study findings will be incorporated in the ecological risk assessment including all data, analyses, and interpretation and will be prepared with specific reference to both the data quality objectives specific to the fish reproductive health assessment and CERC SOP P.326 *Biomonitoring of Environmental Status and Trends (BEST) Programs: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants.*

4.7 EXPECTED BENEFITS

Results from the proposed research will significantly contribute to the risk assessment of the Housatonic River through: (1) characterization of the PCB exposure to fish in the four selected areas; (2) development of dose-response models for fish embryotoxicity of the mixture of chemicals present in fish from the study areas; (3) confirmation and validation of the dose-response models with fish eggs from the Housatonic River; and (4) elucidation and evaluation of any causal relationship for PCB-related effects on fish health in the Housatonic River.

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APPENDIX A.22

FIELD SAMPLING AND ANALYSIS PLAN FOR SOIL INVERTEBRATES

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APPENDIX A.22

FIELD SAMPLING AND ANALYSIS PLAN FOR SOIL INVERTEBRATES

1. INTRODUCTION

1.1 BACKGROUND

Soil invertebrates, particularly earthworms, have proven to be useful indicators of the environmental effects of contaminants, including PCBs (Edwards and Thompson, 1973; Goats and Edwards, 1988; Fitzpatrick et al., 1992; and Beyer and Stafford, 1993). Being in nearly constant contact with the soil, soil invertebrates are continually exposed to soil contamination. In addition, they account for the majority of animal biomass in soil, and are preyed upon by a variety of secondary consumers. Thus, soil invertebrates form a pathway by which soil contamination may be passed on to receptors such as short-tailed shrews (*Blarina brevicauda*), American robin (*Turdus migratorius*), and American woodcock (*Scolopax minor*) that rely on earthworms for a major portion of their diet.

For the purposes of this Work Plan, soil invertebrates are divided into two separate groups of interest based on their availability to receptors and their degree of exposure to contaminated soils. These two groups consist of: (1) those invertebrates living in the soil itself, as represented by earthworms, and (2) those living primarily in the litter or detritus layer, as represented by adult beetles and other arthropods, hereinafter referred to as litter invertebrates.

Invertebrates will not be sampled from the surface of terrestrial vegetation, since these invertebrates form a relatively small proportion of the diet of American robins and short-tailed shrews. For example, Whittaker and Ferraro (1963) reported that summer short-tailed shrew diets consisted of only 4.3% lepidopteran larvae (found primarily on vegetation), while earthworms, slugs, and snails (found in litter or soil) accounted for over 58.5% of the diet.

Robins may forage on invertebrates in terrestrial vegetation to a larger degree than shrews or woodcock, but these invertebrates are not a dominant item in their diet. For example, Howell (1942) found that lepidopteran larvae accounted for less than 25% of the robin's summer diet based upon stomach content data. In addition, the large proportion of earthworms and similar soft-bodied ground-dwelling invertebrates that robins ingest are likely to be greatly underrepresented in stomach content data because these invertebrates are easily digested (Wheelwright, 1986).

1.2 OBJECTIVES

The principal objective of this study is to collect representative soil invertebrate samples for the Lower Housatonic River Supplemental Investigation (SI) for analysis of PCBs, dioxins/furans, and organochlorine (OC) pesticide concentrations in tissue. Results will be used in the ecological risk assessment to model exposure through the food chain of higher trophic level consumers such

as robins, woodcock, and shrews. In addition, the results of tissue analyses and co-occurring soil analyses will be used to determine the relationship between earthworm tissue concentrations and corresponding soil concentrations.

2. STUDY DESIGN

2.1 FIELD SAMPLING DESIGN

2.1.1 Sampling Locations

Soil invertebrates will be collected at three different sampling areas corresponding to where small mammals were collected, including a reference site with soil and habitat characteristics similar to the contaminated sites.

The total PCB concentration ranges of the three sites are:

- < 1 mg/kg (reference location)
- 1 mg/kg to 30 mg/kg
- > 30 mg/kg

All three sampling areas represent suitable habitat for potential ecological receptors (e.g., robins, shrews, woodcock) for which risk will be assessed. Soil chemistry data are also available from these areas, and will be used to select specific collecting locations. In selecting sampling locations for soil invertebrates at each site, particular weight will be given to prior analytical results from surficial floodplain soils (0 to 6 inches below ground surface [bgs]), since soil invertebrates feeding within this zone are most likely to be preyed upon by the ecological receptors that may be modeled.

2.1.2 Number of Samples

A target goal of 30 earthworm samples and 9 litter invertebrate samples is proposed for tissue analysis. Ten earthworm samples (individual worms or, if necessary, composites) and three composite samples of litter invertebrates will be collected in each of the three sampling areas.

The number of proposed samples for earthworms is based primarily upon a power analysis (Attachment 1) conducted after a literature review. The power analysis focused on variability in tissue uptake in depurated worms, since that was the most applicable study found in the literature. In this investigation, however, the worms will not be depurated since their consumers (e.g., robins and shrews) ingest the entire worm. The number of samples for litter invertebrates is based on the anticipated difficulty in collecting sufficient biomass for tissue analysis.

2.1.3 Collection Methods

2.1.3.1 Field Reconnaissance/Pilot Study

A field reconnaissance and brief pilot study will be conducted in the spring of 2000 in order to refine the proposed study design. The primary objectives of the field reconnaissance are to:

- Determine the sampling plot size that will provide sufficient biomass for both earthworm and litter invertebrate tissue analysis.
- Determine the dominant species of earthworms present in different parts of the study area. (If possible, only the single dominant species of earthworm in the study area will be sampled and analyzed, in order to minimize potential interspecific variation in PCB uptake or alimentary tract content).
- Evaluate and confirm the usefulness of the different soil invertebrate sampling methods proposed.
- Evaluate plot locations based on existing surface soil PCB concentrations.

2.1.3.2 Plot Selection and Soil PCB Screening

Sample plots will be established within each of the three sampling areas to be compared. A pilot study using an initial plot size of 1 m^2 will be conducted to determine the size of plots required for the collection of sufficient tissue mass for chemical analysis of earthworms. The plot size needed to obtain a minimum of 10 g (wet weight) of earthworm tissue per plot will be determined. Individual plots will be selected on the basis of considerations identified in Subsection 2.1.1.

In addition, one set of drift fences with pit traps will be constructed in each of the three sample areas. These traps will be used to determine the sampling effort required to obtain at least 10 g of litter invertebrates. Pit traps will be constructed from No. 10, or similar, metal cans paired inside and outside the drift fence. Pit traps will be checked daily and captured individuals placed in resealable plastic bags labeled with the litter invertebrate sample plot number and transported to a central processing area.

Approximately three composite soil samples (0 to 6 inches bgs) will be collected from each potential sampling area to ensure that plot locations are representative of the desired range of soil PCB contamination. Surface soils will be screened for total PCBs. Once identified, plots and pit traps will be marked with pin flags, labeled with a plot number, and the location surveyed using global positioning system (GPS) equipment.

Pilot plots will be sampled for earthworms by excavating and sorting through soil collected to a depth of approximately 0.5 ft until at least 10 g (wet weight) of tissue mass is obtained. This information will be used to determine the appropriate plot size for earthworm sampling. The earthworm samples will be placed in resealable plastic bags labeled with the sample plot number and transported to a central processing area (see Subsection 2.1.4.1) for taxonomic identification.

2.1.3.3 Sample Plot and Pit Trap Locations

Using the results of the pilot study to determine plot size, 10 sample plots and three sets of drift fences with pit traps will be established in each sampling area. Plots will be marked with pin flags, labeled with a plot number, and the location surveyed using GPS equipment. Pit traps will be placed at select locations in each sampling area and located using GPS.

2.1.3.4 Soil Invertebrate and Soil Sampling

All sampling will be conducted in spring 2000. It is best to conduct this sampling during spring, since that is when invertebrates form the majority of the diet of the American robin. Other potential receptors such as woodcock and shrews forage on invertebrates from spring through fall.

The two methods discussed in Subsection 2.1.3.2 will be used to sample for soil invertebrates. One involves the collection of earthworms by removing and sorting through soil collected to a depth of approximately 0.5 ft. The second involves the establishment of pit traps and drift nets to collect litter invertebrates. Litter invertebrates collected daily from pit traps will be combined as necessary to meet the minimum sample size requirements for tissue analysis. Litter invertebrates will be collected from sample plots only if less than 10 g of litter invertebrates are collected from a pitfall trap.

Individual sampling plots will be sampled for earthworms. Surface litter and detritus from the sample plots will only be collected and sorted if less than 10 g of litter invertebrates are collected from a pit trap.

Each plot that is established will be sampled for earthworms according to the following approach. After the plot is delineated with pin flags, soil samples will be collected at three locations in the plot, minimizing any disturbance to the litter layer. The soil collected will be homogenized in a dedicated stainless-steel pan, and apportioned into appropriate laboratory glassware labeled by plot number. The soil will be submitted for chemistry analysis to confirm the PCB concentration range in the plot.

When it is time to sample the plot for earthworms, all surface litter and detritus will be removed and placed into one or more decontaminated 5-gallon buckets labeled with the plot location if less than 10 g of litter invertebrates have been collected from an adjacent pit trap. After litter removal, the 5-gallon buckets will be covered with cheesecloth held in place by a large rubber band. The buckets will be transported to a refrigerator in a central processing area. Earthworms will be collected by removing soil from the plot to a depth of approximately 6 inches bgs using a decontaminated shovel, and removing earthworms by hand. If necessary, worms will also be screened from the soil through standard 1/8- to 1/4-inch mesh sieves or through a larger decontaminated screen constructed from 2-inch by 4-inch lumber and 1/4-inch hardware cloth. The earthworm samples will be placed in resealable plastic bags labeled with the sample plot number and transported to a central processing area for processing and taxonomic identification (see Subsection 2.1.4.1).

If sufficient numbers of worms are not located by digging and soil conditions appear suitable, a mustard-based extraction technique may be used (Stair et al., 1995). A mustard/water suspension will be applied to each plot and surfacing earthworms will be collected by hand. The earthworm samples will be placed in resealable plastic bags labeled with the sample plot number and transported to a central processing area for processing and taxonomic identification (see Subsection 2.1.4.1).

Earthworm collection at each plot will continue until at least 10 g (wet weight) of tissue mass is collected, preferably of the same species of earthworm. If sufficient earthworm populations are still not found at a location, the professional judgment of field personnel will be used to determine if sampling at a location should be suspended or if a reduced sample volume will be collected (the analytical laboratory is able to analyze as little as 0.1 g of tissue, but with somewhat higher detection limits). Additional earthworm samples will be collected from plots for duplicate and matrix spike/matrix spike duplicate (MS/MSD) samples.

The primary sampling method for litter invertebrates will be pit traps, supplemented by hand sampling as necessary. Pit traps and drift nets will be installed adjacent to soil invertebrate sampling plots and checked daily over a 1-month period until at least 10 g of tissue mass (wet weight) is collected, plus any additional sample required for duplicate and MS/MSD samples. Invertebrates collected from the traps will be placed in resealable plastic bags labeled with the litter invertebrate sampling plot number and transported to a central processing area. Processing procedures are described in Subsection 2.1.4.

2.1.4 Invertebrate Field Sample Processing

2.1.4.1 Earthworm Sample Processing

Earthworms will not be depurated prior to processing. Specific processing steps are described in Subsection 3.2.1.

Earthworm processing will consist of:

1. Rinsing the worms with distilled water.
2. Segregating the worms into adult and juvenile age classes.
3. Identifying and segregating the species of the worms.
4. Weighing the worms and recording their individual and combined composite sample weights.
5. Freezing the worms pending shipment to the analytical laboratory.

Each sample will be placed in aluminum foil that has been rinsed with hexane and air dried (dull side toward the sample) and then labeled by plot number. Samples will be frozen in resealable plastic bags at -10°C until they are shipped to the analytical laboratory.

If possible, a single species and size class composite will be submitted for analysis. Efforts will be made to match the composition of any reference samples collected with those collected in contaminated areas. The age and size distribution and species of earthworms collected will be recorded for each composite sample collected.

A representative sample of individuals (i.e., voucher specimens) will also be retained in isopropyl alcohol for subsequent species identification.

2.1.4.2 Litter Invertebrate Sample Processing

Litter invertebrate samples retained for analysis will be processed as follows. Invertebrates collected from pit traps will be taxonomically identified to order, then weighed, returned to the plastic bag, and held in a freezer at -20° to -30°C until shipment. Samples collected from ensuing weeks will be added to the same bag after taxonomic identification and weighing. Cumulative sample weights will be recorded on sample data sheets; before shipment the final sample weight will be recorded to ensure sufficient tissue mass is available for analysis. If necessary, the sample will be supplemented with invertebrates collected from the leaf litter of the closest sampling plot to form a composite sample.

Soil invertebrates collected from litter and detritus will be processed as follows. The litter and detritus will be hand-picked until at least 10 g of invertebrate tissue is collected. No effort will be made to remove all invertebrates; rather, sampling will be biased toward larger food items since these are most likely to be taken by shrews (Churchfield, 1990) and robins (Howell, 1942). Invertebrates within these samples will be taxonomically identified before the samples are snap frozen in petri dishes, placed in aluminum foil that has been rinsed with hexane and air dried (dull side toward the sample), and bagged in a labeled resealable plastic bag.

2.2 ANALYSES

Each composite earthworm and composite litter invertebrate sample will be submitted to a contract laboratory for analysis of PCBs (total, Aroclors, congeners, and homologs), percent lipids, and percent moisture. A subset of the samples (at least one per location) will also be analyzed for dioxin/furans and select OC pesticides.

Composite soil samples will be analyzed for PCBs (total and Aroclors), total organic carbon (TOC), and grain size. A subset of all composite samples (at least one per group per location) will also be analyzed for dioxin/furans and Appendix IX OC pesticides.

The analytical results will be used in the ecological risk assessment to model exposure to higher consumers such as robins, woodcock, and shrews. The results of tissues analyses and co-occurring soil analyses will be used to determine earthworm concentration factors.

2.3 QUALITY ASSURANCE/QUALITY CONTROL SAMPLES

Duplicate analyses will be conducted for each parameter on 5% of the earthworm and litter invertebrate samples. Duplicate samples will be collected from the sample plot location as the

original sample; an additional 10 g of tissue will be required for each set of analyses beyond the 10 g required for the original analyses.

In addition, a matrix spike/matrix spike duplicate (MS/MSD) sample is required for every 20 samples. An additional 20 g of tissue will be required for each set of MS/MSD analyses, beyond the 10 g required for the original analyses.

3. QUALITY ASSURANCE/QUALITY CONTROL

3.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

3.1.1 DATA QUALITY OBJECTIVES

The two primary data quality objectives of the soil invertebrate evaluation are outlined in Subsection 1.2. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Taxonomic identification of earthworms to LPIL (lowest practical identification level): earthworms must be identified to the species level whenever possible. When identification to the species level is not possible, the LPIL will be consistent with standard practice for invertebrate taxonomy. Of equal importance is that the level of taxonomy is consistent for all samples. Other soil invertebrates will be identified to the level of Order.

- Biomass (wet weight) for each taxon or larger taxonomic group: Biomass must be determined accurately and recorded to 1 mg (.001 g) using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude. Accurate determination of biomass is also partly determined by following the field sampling methodologies discussed above.

- Soil chemistry for PCBs and selected other contaminants: Analysis of soil for chemical constituents must result in data that are consistent in all respects with other sediment/soil contaminant data collected as part of the project. Satisfactory results will be ensured by submitting samples to the same laboratories that are analyzing samples for other components of the program. Quality control specifications for these data are delineated in the project QAPP (WESTON, 2000).

- Soil grain size distribution: Quality control considerations to ensure achievement of DQOs for this parameter will follow the QAPP.

- Tissue residue concentrations for PCBs and other contaminants of soil invertebrate samples: Quality control considerations to ensure achievement of DQOs for this parameter will follow the QAPP.

3.1.2 Data Quality Indicators

Data developed in the soil invertebrate study must meet standards of precision, accuracy, completeness, representativeness, comparability and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. Each of these data quality indicators, some of which are not readily quantifiable for soil invertebrate data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Because of the small-scale spatial heterogeneity inherent in soil invertebrate communities, it is not possible to take repeated independent measurements of the biological parameters. Rather than control and measure precision, the study design includes an increase in the number of replicates to increase the statistical resolution; for this study the large number of replicates (10) is used for earthworms in this manner. Precision may also be evaluated by assessment of the degree to which sample collection procedures are able to ensure collection of consistent sample volumes. For the measurements that are not unique to the soil invertebrate study, such as soil chemistry and grain size, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (soil invertebrate taxonomy and biomass), accuracy is defined as meaning that the taxa are correctly represented and identified in each sample, and correctly weighed. The data generated by this study will also be evaluated for accuracy via comparison with known and/or expected results from similar studies conducted in the Housatonic River area or in similar New England systems. For parameters such as soil contaminants and area grain size, accuracy is as defined in the QAPP.

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for all components of the soil invertebrate program. To ensure achieving the planned statistical resolution, it is important that completeness of 100% be achieved for all components of this study with the exception of the tissue residue analyses. For this latter study component, the number of analyses will be determined by the material available for collection; therefore, establishment of an *a priori* completeness goal is not possible.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of representativeness will also be evaluated via comparison with known and/or expected results based on previous investigations of the Lower Housatonic River area and other similar systems.

Comparability is a measure of the confidence with which the soil invertebrate data may be compared to another similar data set. Comparability will be evaluated by examination of the variability in key parameters as determined from the large numbers of samples to be collected at each sample site.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is related for soil invertebrate investigations to the ability of the taxonomic analysis to resolve the various soil invertebrates into individual species and/or

orders. Sensitivity is applicable and important for the chemistry parameters that will be analyzed as part of the soil invertebrate study. For these parameters, the detection limits for chemistry and grain-size parameters specified in the QAPP will provide appropriate sensitivity for the purpose of providing insight into factors controlling abundance and distribution of the soil invertebrate taxa and populations.

3.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the chemical and physical data are discussed in various sections of the project QAPP and will be used whenever applicable in this study. For the biological data, usability will be largely be determined by three factors: (1) the experience of the senior investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field; (2) an evaluation of the taxonomic data both within the study and compared with previous studies in the Housatonic River and in the New England area; and (3) a direct comparison between the chemistry and grain-size data and similar data developed from co-located samples that have been collected as part of other project components.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

3.2 SAMPLING DESIGN

The rationale for selection of the three locations to be sampled in the soil invertebrate study is presented in Subsection 2.1.1. The locations are not intended to be representative of the entire area but rather are intended to encompass a range of sediment PCB concentrations typical of the area; one of the locations with near-background PCB levels will be used as a reference.

Soil invertebrate community data are typically highly variable in nature. To achieve acceptable statistical resolution for earthworms it is necessary to collect large numbers of samples from each sampling site. Data will be collected from 10 samples at each of 3 sampling sites. This number of replicates was selected based on power analyses for this sampling plan (Attachment 1).

3.3 SAMPLING METHODOLOGY

3.3.1 Sampling Procedures

Sampling methods, as discussed in Subsection 2.1.4, were chosen to ensure unbiased (i.e., accurate) samples that will facilitate comparisons with other soil invertebrate data, both from the Housatonic River and from other areas. Steps taken to ensure that sampling does not unnecessarily induce bias include: visual inspection of each sample to confirm satisfactory collection, and confirmation of visual similarity of soil type within a location. All samples will be collected by trained and experienced personnel; senior oversight of all aspects of the sampling and sample processing will further promote comparability and reduce potential bias. Subsamples for physical and chemical analyses will be collected following procedures documented in the

project QAPP (WESTON, 2000) and will therefore be comparable with procedures followed for all other similar efforts throughout the Supplemental Investigation.

3.3.2 Quality Control Samples

The nature of soil invertebrate sampling does not allow the incorporation of typical duplicate and blank samples as part of the study design.

Duplicate and MS/MSD samples for chemistry will collected in this study. Quality control of chemistry analyses will be provided and processed in accordance with the QAPP.

3.3.3 Sample Processing and Preservation

Detailed procedures for collection and initial processing of all samples to be collected as part of the soil invertebrate study are provided in Section 4. Subsampling, homogenization, and decontamination between samples will follow procedures established in the QAPP. All samples will be held on wet ice and returned to the field laboratory daily and will be either refrigerated, frozen (physical, chemical samples), or preserved (taxonomic samples) at that time. Holding time for physical and chemical samples will follow procedures established in the QAPP; there is no holding time for taxonomic samples.

3.3.4 Training

All sampling will be directed in the field by senior scientists with experience in the collection of soil invertebrate samples. Supporting staff will receive training from the senior scientist(s) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

3.4 SAMPLE ANALYSIS

3.4.1 Taxonomy Samples

Processing of taxonomy samples will follow standard procedures established for both earthworms and other soil invertebrates. All samples will be processed by experienced staff who have received specific training in the SOP and whose work is checked periodically by their supervisors and peers. Depending on sample volume and other factors, samples will be processed by eye or under low-power microscopes.

Quality of taxonomic identification will be ensured by maintaining voucher collections and requiring a consensus among all taxonomists at the processing laboratory prior to an identification becoming accepted as a type for the voucher collection. In the event that the taxonomists are unable to agree on an identification, specimens will be sent to a third party for determination.

3.4.2 Physical/Chemical Samples

Samples for soil grain size, soil chemistry, and tissue chemistry will be processed following procedures and SOPs provided in the QAPP. These samples will be submitted in catalogs (sample delivery groups) and batches with other samples from the larger project and data validation will be performed on a catalog basis in accordance with procedures established and described in the QAPP.

3.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Subsection 2.4. The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations and will be prepared with specific reference to both the data quality objectives specific to the soil invertebrate study (Subsection 2.3.1, above) and Subsection 4.1 of the QAPP.

4. PROCEDURES

4.1 FIELD SAMPLING PROCEDURES

Working in two-person teams, identify and mark plot locations using four pin flags (one for each plot corner). The plot area is assumed for planning purposes to be 1 m^2, but may be adjusted based on the results of the pilot study. Label the flags with a sequential location number, and note the location on a field map and in a bound logbook. Survey the plot location using GPS equipment.

Scrape sufficient leaf litter from the plot area to collect three random soil grab samples using a decontaminated stainless-steel trowel. Be careful to minimize removal of surface litter and detritus that may be used by invertebrates. Homogenize the sample in a dedicated stainless steel tray or on a clean plastic sheet, and apportion it into a 4-ounce glass jar. Label the sample jar with an indelible marker and fill out a sample data sheet. The soil sample will be submitted for confirmatory analysis following successful collection of earthworms and other invertebrates from the plot.

Invertebrates should be removed daily from the pit traps during a 1-month period, or until sufficient tissue mass is obtained for analysis. If, after 1 week, it is apparent that insufficient tissue mass will be available from the pitfall traps over a 1-month period, the sample will be supplemented by invertebrate sampling of leaf litter and detritus within sampling plots.

If possible, plots should be sampled for earthworms after a heavy precipitation event, when earthworms are closest to the surface. All leaf litter and detritus will be removed from the plot area by hand, while wearing protective gloves. New gloves will be donned before sampling at a new plot to avoid cross contamination of samples. Leaf litter will be placed into decontaminated 5-gallon plastic buckets (or equivalent). The buckets will be labeled by plot number, and cheesecloth will be placed over the top and secured with a large rubber band. The buckets will be

returned to the central processing area and refrigerated until they are processed (only if necessary; see Subsection 2.1.3.4).

Plots will then be sampled for earthworms. Worms will be removed from the plot by digging to a maximum depth of approximately 0.5 ft with a decontaminated shovel. If sufficient sample mass is not achievable by this method, the soils may be screened using a standard 1/4-inch sieve or equivalent.

An alternative means of sampling earthworms is to apply a mustard/water solution to the ground surface. The solution is prepared by mixing approximately 1 tablespoon of dried mustard to 5 gallons of distilled water. Apply 5 gallons per 1 m^2 of plot area, or until the ground is fully saturated with the solution. Wait until the earthworms surface and collect them from the surface.

Individually rinse each worm with distilled water, using a spray or squeeze bottle. Then place all the worms from the plot into an appropriate precleaned sample container. Label the container with the plot location, the date, time, and collector's initials.

Place the containers in a cooler with ice and transport them to the central processing area.

All sampling equipment will be decontaminated following the project-specific SOP for equipment decontamination, including detergent/water wash, potable water rinse, hexane rinse, isopropyl alcohol rinse, and deionized water rinse.

4.2 SAMPLE PROCESSING PROCEDURES

4.2.1 Earthworm Sample Processing

1. At the central processing area, place earthworms in the refrigerator in their labeled locations until ready to process.

2. Segregate and taxonomically identify earthworm species to determine the dominant species collected within the study area.

3. Once the dominant species is determined, process each container individually. Segregate the species by placing them on decontaminated aluminum foil or paper toweling. Group the largest individuals into a composite sample and weigh the group to ensure that 10 g of tissue are available for analysis. Then weigh each earthworm separately. Record all data on a sample data sheet.

4. Note any external lesions or other abnormalities, such as a "pinched" appearance caused by constriction of the coelom.

5. Place the sample in aluminum foil that has been rinsed with hexane and air dried (dull side toward the sample), add a label with the sample number, and double bag it using resealable plastic bags. Label the outer bag with the sample number, and place in freezer at -10 °C.

6. Complete a sample attribute form for each sample.

4.2.2 Invertebrate Sample Processing

1. At the central processing area, store invertebrates in sampling containers until ready to process.

2. For litter samples from sample plots, pick the invertebrates from the soil litter from each location/container, and segregate live individuals by taxonomic order into decontaminated petri dishes with lids. The lid of the petri dish should be labeled with the sample location number.

3. Taxonomically identify individuals for both pit trap and sample plots to the level of order, and record the number of individuals per order on a data form for each sample collected. Obtain a wet weight for each order and sample.

4. If sufficient sample mass is present, group the largest individuals into a composite sample for that location, and weigh it. Record the weights, which should be at least 10 g per sample. If a composited pit trap sample weighs less than 10 g then add the litter invertebrates from the closest sample plot. If the total biomass is still less than 10 g then add the litter invertebrates from the next closest sample plot.

5. Wrap samples in decontaminated aluminum foil that has been rinsed with hexane and air dried (dull side toward the sample), and place in resealable plastic bags. Attach a label to each sample indicating the sample number and place in the freezer at -10 °C.

6. Complete a sample attribute form for each sample.

4.2.3 Sample Handling and Shipping

1. Keep samples in a -10 ºC freezer until shipment to the laboratory.

2. When ready to ship, place the samples (wrapped in labeled foil and enclosed in labeled resealable plastic bags) in a large plastic bag into a cooler lined with vermiculite.

3. Complete a chain-of-custody form listing the contents of each cooler, and place it in a resealable plastic bag. Tape the resealable plastic bag to the inside of the top lid of the cooler, or place it on top of the samples.

4. Seal the cooler with two custody seals, and label the cooler with appropriate WESTON shipping labels, including the WESTON return address, and U.S. Fish and Wildlife Service (USFWS) laboratory address.

5. Samples will be delivered by courier or overnight delivery to the analytical laboratory. Earthworm tissue samples should be sent by overnight delivery service

(next morning delivery) or hand delivered. Samples sent to USFWS should be shipped to:

Ken Carr/Ken Munney/Drew Major
USFWS
22 Bridge St., Unit 1 Phone: 603-225-1411
Concord, NH 03301 Federal Express Acct: 1510-1036-9

Shippers should notify the receiving laboratory or USFWS that samples are being sent for next-day delivery. Samples should not be sent to USFWS if Ken Munney, Ken Carr, or Drew Major are not available for receipt of the shipment. Samples need to be sent for arrival on a weekday only. Therefore, Thursday is the last day of the week to ship samples. Shippers should also call the receiving laboratory of USFWS the day of delivery to verify the receipt of samples. Composite soil samples collected will be shipped to the appropriate contract laboratory following procedures outlined in the FSP (WESTON, 1999).

4.2.4 Sample Documentation

All sample documentation will follow project-specific SOPs for field sample ID, data sheet, chain-of-custody form, and custody seal procedures.

Use a field logbook to record the location, date and time, amount of time spent in collecting activities at each area, method of collection, name(s) of collector(s), the number of earthworms collected, and any other pertinent information such as problems encountered.

Complete an earthworm specimen data sheet for each location sampled. Specimen data sheets should include location; date and time of collection; method of collection; collector's initials; earthworm species; total weight of earthworm composite sample; and total weight of individual earthworms retained for analysis. Numbers of individuals of other earthworm species collected should also be noted.

An invertebrate community data sheet should also be completed for each location sampled. Data recorded should include the location; date and time of collection; method of collection; collector's initials; species collected and number of each per sample; and weight of the litter invertebrate sample.

Complete a sample attribute form for each tissue sample (earthworm and litter invertebrates). Put the sample number for each sample and the date and processor's initials on the form.

Complete a chain-of-custody form for each cooler of samples shipped to the USFWS laboratory. Provide copies to the task manager, who will retain them in the WESTON files.

5. EQUIPMENT LIST

5.1 FIELD

- First aid kit
- 5-gallon (or equivalent) buckets for litter/detritus collection with lids and holes for ventilation or cheesecloth and rubber bands to secure the cheesecloth to the top of the container
- Plastic buckets (1-gallon or less) for collection of earthworms
- 6 ¼-inch standard soil sieves and/or a 2-ft by 2-ft sieve constructed from hardware, cloth, and 2-inch by 4-inch lumber
- Indelible markers, duct/labeling tape
- Pin flags
- Wooden stakes
- Heavy duty stapler
- Hammer
- Plastic sheeting (or other appropriate material) to use for drift fences
- No. 10 (or similar) cans with covers
- Field logbook
- Rubber gloves
- Resealable plastic bags
- Dry mustard
- Distilled water (5 gallons per 1 m^2 plot) to mix with mustard and pour on sampling plots, if necessary
- GPS receiver
- Wet and dry ice
- Coolers for sample storage and transport
- Soil sampling equipment: stainless-steel trowels, bowls, glassware for soil sample

5.2 PROCESSING AREA

- 2 folding tables
- Polyethylene plastic sheets
- 4 boxes of Nitrile gloves
- 10 boxes of gallon-size resealable plastic bags
- Data sheets
- Four sets of forceps
- 200 plastic petri dishes
- Invertebrate taxonomic keys
- 2 dissecting scopes, each 2X minimum and illuminated
- Weighing scale for up to 100 grams
- 4 boxes of aluminum foil
- 2 large coolers for freezing samples
- 1 to 2 shipping coolers
- Ice to fill cooler, in plastic resealable bags

- Dry ice for shipping
- Gloves for handling dry ice and/or liquid nitrogen
- Indelible markers (fine and wide)
- Ballpoint pens
- Hexane in rinse bottle
- Isopropyl alcohol in rinse bottle
- Distilled, deionized water in rinse bottle
- Large bucket for decontamination solutions
- Packaging tape
- Laboratory sample labels with unique sample numbers
- WESTON or USFWS QA/QC labels

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ATTACHMENT 1

POWER ANALYSES FOR EARTHWORMS

ATTACHMENT 1

POWER ANALYSES FOR SOIL INVERTEBRATES

Earthworms consume large quantities of soil and may thus accumulate polychlorinated biphenyls (PCBs) that occur in soils of contaminated areas. A comparison of PCB concentrations in earthworm tissues among three areas with different degrees of exposure to these chemicals will be tested with analysis of variance (ANOVA). The anticipated power of such a test was studied both analytically and by means of simulation.

Analytical Approach

For the smallest difference δ between means that we wish to detect and desired power, the adequate sample size n is a function of the non-centrality parameter, which is related to

$$\phi = \sqrt{\frac{n\delta^2}{2k\,s^2}}$$

where k is the number of groups being compared and s^2 is an estimate of the error mean square (variation within groups). Using published charts (Zar, 1984) that relate the power of ANOVA to ϕ, for different degrees of freedom (v) and levels of significance (α), it is possible to solve the above equation for n by guessing its value, calculating ϕ, and from the chart obtain the corresponding power. With a few iterations of this process, it is easy to find n for the desired power. For this earthworm study, the number of groups k=3. The within-group variance was estimated from Diercxsens et al. (1985) to be s^2=8110. Figure 1 displays the required sample sizes to detect a wide range of differences between means. Ten samples per site would be sufficient to detect a difference of 150 mg/kg wet mass. Detection of a difference of 100 mg/kg wet mass would require almost twice the sample size.

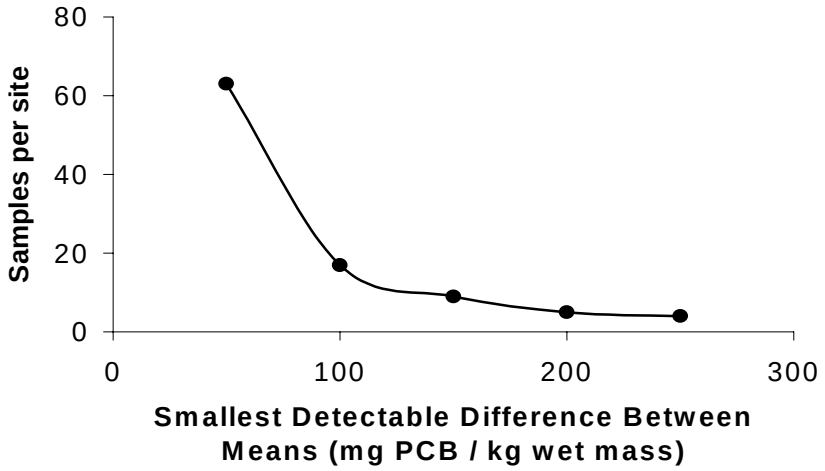

Figure 1. Number of samples required to detect the smallest difference between mean concentrations of PCB in earthworm tissues from *k*=3 sites with distinct concentrations of these chemicals in the soil. Power ≥ 0.8, α = 0.05, s^2 = 8110 (estimate based on data from Diercxsens et al. 1985).

Simulation Approach

Implicit in the analytical approach is the assumption that Housatonic earthworms have the same response to variation in PCB concentrations in the soil as earthworms from a nature reserve and a vineyard in Switzerland, the sites from which the estimate of s^2 was obtained (Diercxsens et al., 1985). We relax this assumption somewhat by modeling variable responses of PCB concentrations in earthworm tissues we might expect for the Housatonic. Log-transformed concentrations of PCB in earthworm tissues can be modeled as a linear function of log-transformed PCB concentrations in soils. Figure 2 shows an apparently good fit to this regression on the available empirical data pooled from Diercxsens et al. (1985) and Kreis et al. (1987).

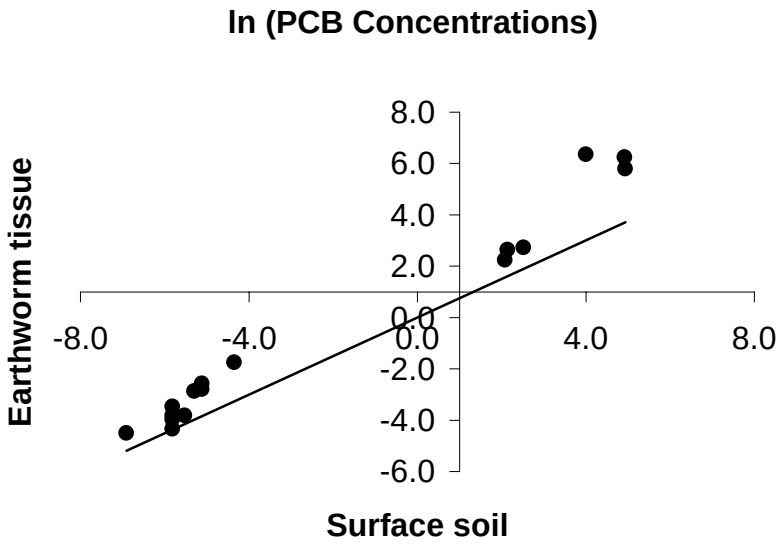

Figure 2. Linear relationship between log-transformed PCB concentrations in soils and earthworm tissues collected at agricultural fields (Kreis et al. 1987, points in the lower left quadrant), a vineyard, and a nature reserve (Diercxsens et al. 1985, points in the upper right quadrant) in Switzerland.

Monte Carlo simulations were used to estimate statistical power for an analysis of variance for each of several sample sizes. In these simulations, the anticipated means and dispersions of earthworm PCB concentrations among the three Housatonic sites were modeled with the regression function above. Because the sites are expected to have low, moderate, or high contamination, a soil concentration for each site was first simulated by randomly sampling from a loguniform distribution, having the range [0.01, 1], [1, 30], or [30, 100] mg/kg dry mass, respectively. These loguniform deviates correspond to variation due to differences in soil

concentrations. Each deviate is an X value from which a concentration Y in worm tissue is simulated using the expression

$$Y = \exp(\delta \ln(X) + \varepsilon(0,\sigma))$$

where $\varepsilon(0,\sigma)$ represents a normally distributed error term with zero mean and standard deviation estimated as the root residual mean square

$$\sigma = \sqrt{\sum (Y - \overline{Y})^2 / (n-1)} = 1.47$$

observed in the regression on the pooled data in Figure 2, and δ (the slope in log-log relationship) represents the magnitude of differences due to any bioaccumulation in earthworm tissues. The simulated Y values were log-transformed and subjected to an ANOVA test. A tally was kept of the number of times the ANOVA was statistically significant at the α=0.05 level of significance. The number of significant results divided by the total number of tests constitutes an estimate of the power for a particular sample size (under the prevailing hypothesis about the true difference among means). A Pascal program was used to conduct these simulations. The results of this analysis are shown in Figure 3, which displays the sample sizes required for a power of no less than 80% as a function of δ (the slope of the underlying log-log regression).

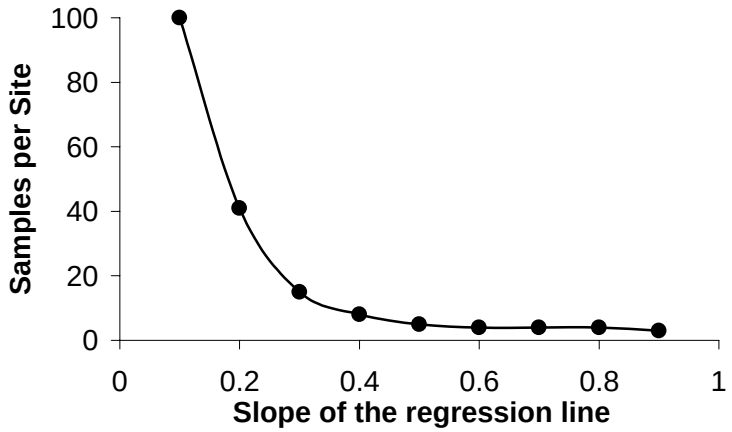

Figure 3. Estimated number of samples, for each of the three sites, required to detect significant differences in mean concentration of PCB in earthworm tissues as a function of the slope of the regression line relating log transformed concentrations of PCB in earthworm tissues (wet mass) and soils (dry mass). Power ≥ 0.8, α = 0.05.

Once the sample sizes that achieve the desired power (≥ 0.8) are identified, the selection of a specific sample size should balance costs against the ability to detect differences. Both the analytical and simulation approaches indicate that above ~10 samples per site sharp increases in sample sizes are required to detect smaller differences.

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APPENDIX A.23

WORK PLAN FOR WATERFOWL COLLECTION AND TISSUE SAMPLING (TECHLAW, INC.)

APPENDIX A.23

WORK PLAN FOR WATERFOWL COLLECTION AND TISSUE SAMPLING

Submitted to:

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1.0 Sample Site and Size Selection

Elevated levels of PCBs have been detected in sediment and floodplain soils in the Housatonic River downstream from the GE facility in Pittsfield, MA. Waterfowl, including mallards and wood ducks, have been observed using Woods Pond and upstream floodplain wetlands for breeding, brood rearing, and feeding, and waterfowl hunting is a common activity along this reach of the river. Therefore, these areas were chosen as collection sites to evaluate the potential for risk to the waterfowl directly and to humans consuming the waterfowl due to PCB accumulation in the tissue.

To determine a potentially suitable sample size for waterfowl tissue analysis, *Power and Precision: A computer program for statistical power analysis and confidence intervals* was used (Borenstein et al., 1997). PCB tissue levels from waterfowl collected at the Fox River in Wisconsin (U.S. Fish and Wildlife Service, T. Custer, pers. comm., 1998) were used for the study area population mean and standard deviation. An assumed off-site control mean of 0.5 and a similar standard deviation of 5.5 was used in a power analysis for determining sample size. A sample size of 30 was expected to yield a statistically significant result; however, a permit was issued for a sample size of 20 birds by the MADEP, restricting the sample size below that desired after the power analysis. Because the objective of determining if a risk to humans was posed through waterfowl consumption was critical to address, the decision was made to proceed with a smaller sample size to assess if a concern exists, in which case follow-up studies can be conducted if necessary.

To identify an appropriate reference site outside of the study area for the two target species, the Massachusetts Department of Fisheries and Wildlife (MDFW) was consulted for information on waterfowl habitat near the study area. MDFW suggested the Three-Mile Pond Wildlife Management Area (WMA) in Sheffield as a potential reference area. Three-Mile Pond WMA, approximately 15.6 miles south of the study area, consists of a shallow pond dominated by submerged aquatic vegetation with pockets of emergent and scrub-shrub wetlands on the periphery of the pond. This habitat contains similar water levels, similar amounts of submerged aquatic vegetation, emergent marsh, and surrounding undeveloped habitat as that of the lower portions of the study area near and upstream of Woods Pond. These factors, in combination with the fact that MDFW had conducted waterfowl banding at the site in the past because the area was known to contain populations of both local and migratory waterfowl, made it a suitable potential candidate as a waterfowl collection reference area. In addition, wood ducks and mallards were observed using the WMA during site surveys, and were known to breed at the WMA (T. Keefe, pers. comm., 1998).

A large cove with shallow (less than 1 ft deep) water and some exposed sediment at the north end of the pond was chosen as the potential trapping location due to the similarity in the habitat to that chosen for collection areas in the river study area. Samples will be collected and analyzed the sediment in the Three-Mile Pond WMA trapping location to verify that it is an appropriate reference location uncontaminated by PCBs.

Captured waterfowl were identified to species, sex, and age, therefore allowing comparisons between samples. Because waterfowl are migratory it was important to age the birds to determine if they were raised in or near the capture location.

2.0 Capture Methods

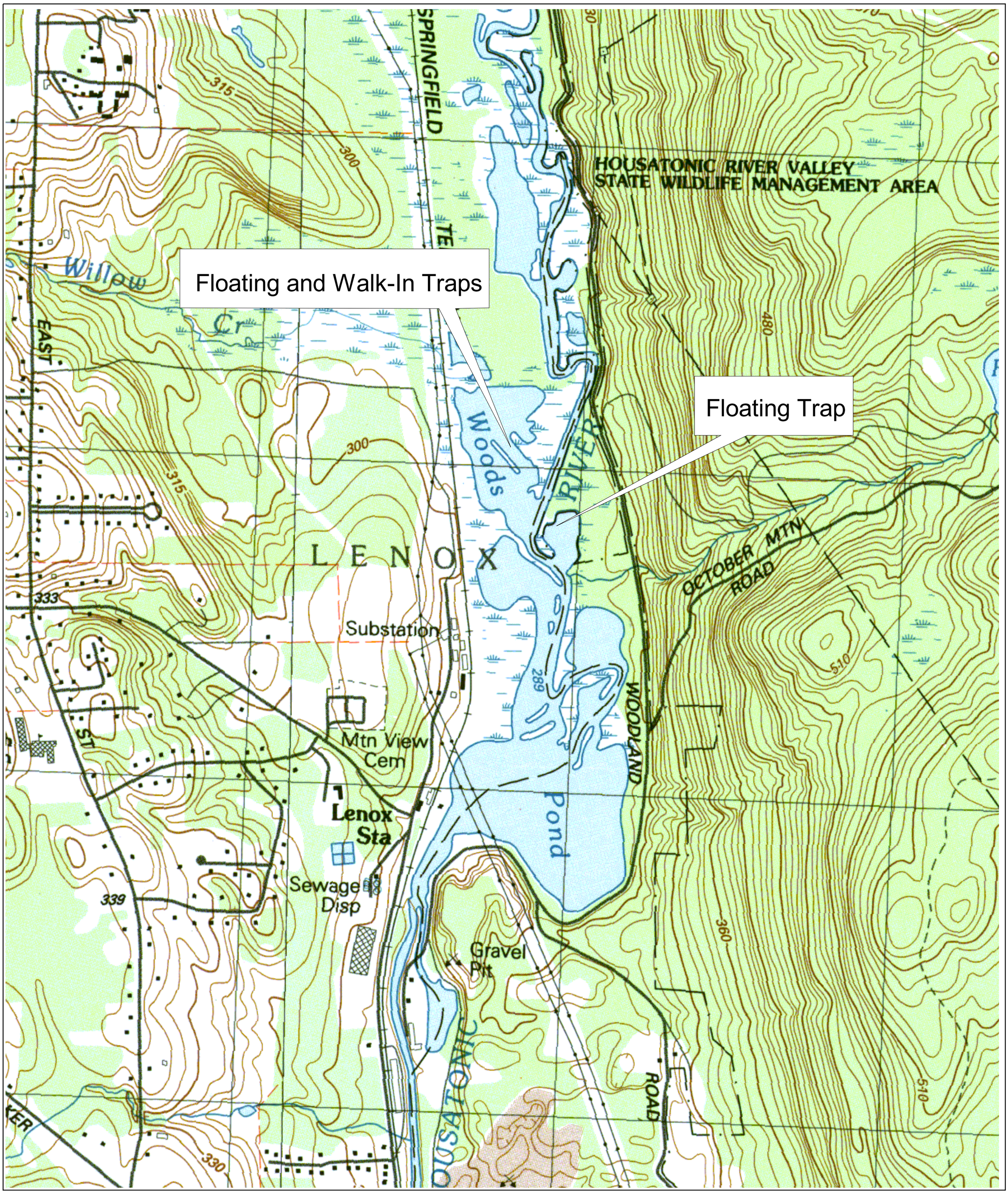
Air boating and bait trapping were used to capture wood ducks and mallards. Air boating efforts were conducted by MDFW as part of annual banding operations, which typically take place in late summer or early fall in the Woods Pond and upstream floodplain wetland areas. During this effort an air boat equipped with high-beam halogen headlights was used on the night of August 28, 1998 to locate waterfowl. Two people captured birds with hand nets while the boat was driven through non-persistent emergent vegetation and in open water areas. Birds were placed in crates and transported to the Woods Pond boat ramp, where they were aged, sexed, and banded. Eleven wood ducks and four mallards were placed in crates for tissue analysis while the banded birds were transported back to their capture location and released.

Baited traps were then used to capture the remainder of the birds needed for analysis. Two floating box traps designed by H. Heusemann, of MDFW, and one walk-in clover trap were each placed in the greater Woods Pond area and in the Three-Mile Pond reference area. Traps were deployed from August 30 to September 16 in the greater Woods Pond area and from August 27 to September 15 at Three-Mile Pond. Approximate trap locations are shown in Figures 1 and 2.

Trap sites were baited with whole and cracked corn for approximately one week before traps were set to capture birds. While in operation, traps were checked one to two times each day and all captured ducks were removed from the traps and placed in crates for transportation to the dissection area. Ducks that were kept overnight before being dissected were placed in a pet travel kennel with hay (for bedding), corn, and water. Composite samples of the corn and water fed to the ducks were collected for PCB analysis.

3.0 Dissection Methods

Dissection methods, performed using chemically clean instruments, were based on necropsy procedures outlined in Friend (1987) and Wobeser and Spraker (1980). Sample birds were aged, sexed, and weighed prior to dissection. Birds were euthanized by severing the head from the body with sharp shears and breast feathers were removed. The brain, liver and breast were removed for analysis. The brain was removed by cutting the top of the skull between the anterior end of each eye, intersecting the front end of the brain case. Brain tissue will be retained for future potential use.Two more incisions were made, one on each side of the brain, back to the foramen magnum. The top of the skull was then peeled back and the brain was removed. The breast was removed with the skin on. Finally, the ribs were cut and the breast bone was removed to expose the liver and other organs. The liver was removed whole.



SOURCE: USGS East Lee quadrangle map.

N

Scale In Feet

700 0 700 1400

SI Work Plan
Lower Housatonic River
Massachusetts

FIGURE 1
WATERFOWL TRAP LOCATIONS
AT WOODS POND

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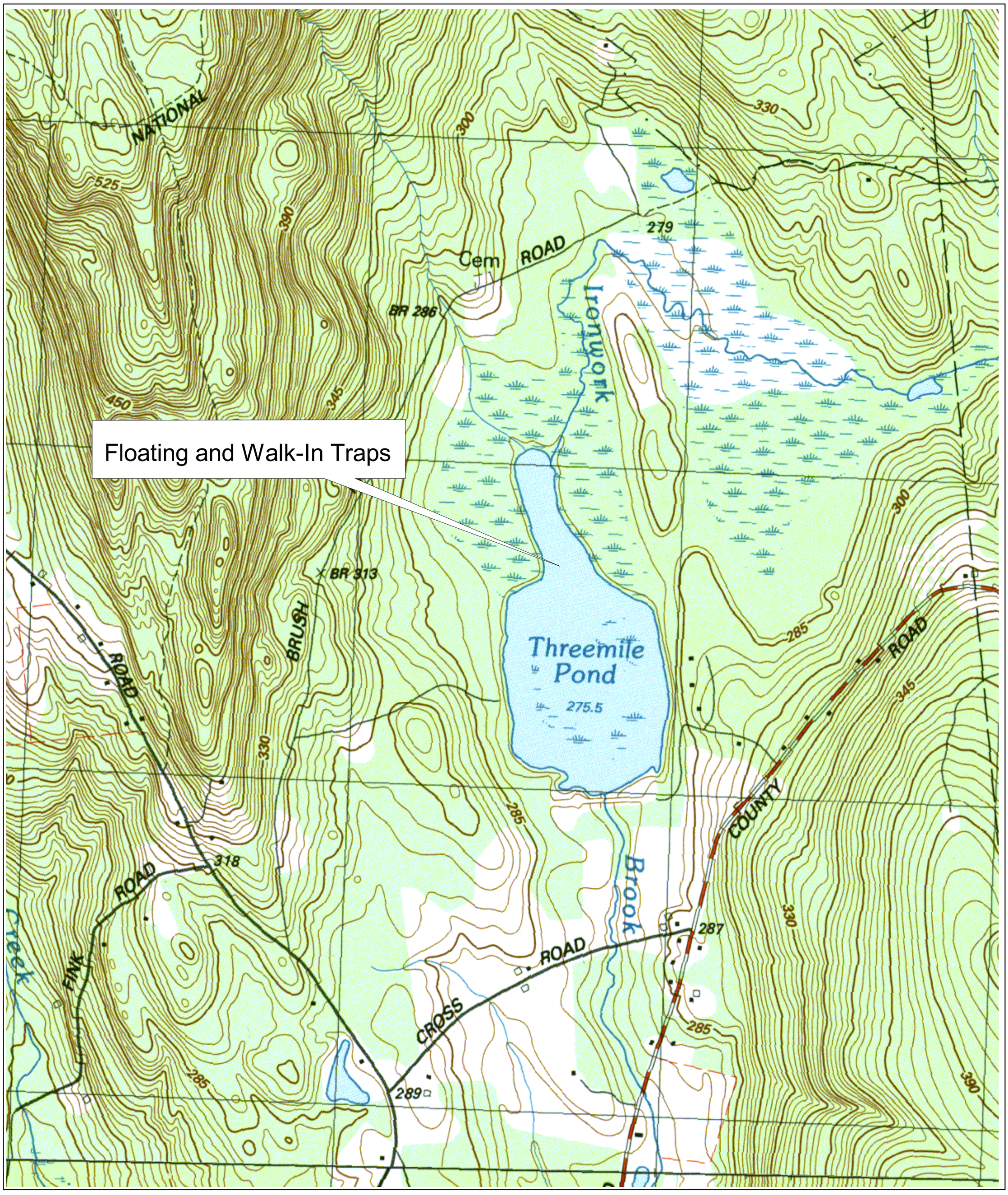

SOURCE: USGS Great Barrington quadrangle map.

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Scale In Feet

700 0 700 1400

SI Work Plan
Lower Housatonic River
Massachusetts

FIGURE 2
WATERFOWL TRAP LOCATIONS
AT THREE-MILE POND

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All sample tissues were wrapped in aluminum foil (dull side in contact with the sample), tagged, and double bagged in resealable bags with a second sample ID tag. All dissection tools, stainless steel dissection trays, and aluminum foil were decontaminated according to EPA standards and consisted of a four part process: 1) a wash with a mild detergent, 2) a rinse with potable water, 3) a spraying wash with hexane, and 4) a spraying rinse with deionized water. Samples were placed on dry ice and shipped to the USFWS for cataloging and analysis of PCBs (total and Aroclors), PCB congeners and homologs, dioxins/furans, select OC pesticides, percent lipids, and percent moisture.

4.0 Quality Assurance/Quality Control

4.1 Data Quality Objectives, Indicators, and Assessment

4.1.1 Data Quality Objectives

The objectives of the waterfowl collection and tissue sampling effort were described in Section 1.0. To achieve these objectives, the following types of data will be required:

- Collection and taxonomic classification of organisms--Mallards and wood ducks must be captured from the study area and a reference area and accurately identified to species, sex, and age.

- Concentrations of PCBs in tissues of waterfowl--Quality control considerations for PCB concentration analysis for biological samples will follow those identified in the QAPP.

4.1.2 Data Quality Indicators

Data developed in the waterfowl collection and tissue sampling study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000). For all waterfowl tissue residue analyses, data quality indicator values and standards will be in accordance with those defined in the QAPP.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, laboratory equipment will be used that has the capability to provide measurements that fall within the allowable precision limits identified in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. Standard sample processing, preservation, and laboratory analysis will be conducted using standards and equipment identified in the project QAPP. Those standards have been established in order to eliminate sample contamination and reduce researcher bias, which results in accurate measures of PCB concentrations in tissue.

Completeness is defined as the percentage of the planned samples actually collected and processed. A power analysis was conducted to determine the sample sizes needed for this study. Completeness, therefore, will be measure of number of samples collected compared to the number of planned samples.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study will be ensured through establishment of an approved, thorough sampling design and through careful implementation of the sample processing and analytical methods.

Comparability is defined as the measure of confidence with which the results of this study may be compared to another similar data set. Comparability will be attained through use of laboratory procedures that provide data in units that can be compared to other similar studies.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability to identify waterfowl to species and age from the study area. Sensitivity of non-biological parameters is defined in the QAPP.

4.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the analysis of waterfowl tissue PCB concentrations are discussed in various sections of the project QAPP and will be used whenever applicable in this study.

4.2 Sampling Design

Two study sites were chosen for the assessment of PCB concentrations in waterfowl tissue and included the Newell Street to Woods Pond study area and one site located outside the formal study area but within the Housatonic River drainage that represents a control. Within the Newell Street to Woods Pond study area, waterfowl collection took place in floodplain and backwater wetlands from the downstream half of the study area: from New Lenox Road, south to Woods Pond.

Sample sizes were determined by using PCB concentration data from a waterfowl tissue PCB analysis study conducted in Wisconsin and *Power and Precision: A computer program for statistical power analysis and confidence intervals* (Borenstein et al., 1997); however, they were later restricted by the MADEP in issuing the collection permit.

4.3 Sampling Methodology

4.3.1 Sampling Procedures

Sampling methods were chosen to ensure unbiased samples that will facilitate comparisons with other studies on waterfowl tissue PCB concentrations and will be suitable for the evaluation of the potential ecological risk to waterfowl directly and the risk to humans consuming waterfowl from the study area.

Steps taken to ensure that sampling does not unnecessarily induce bias include: adopting relatively short holding times for live waterfowl specimens planned for sampling, establishment of a relatively undisturbed (controlled climate, reduced noise, shaded from direct sunlight) holding location for sample specimens prior to tissue collection, and nourishment for specimens prior to tissue collection.

4.3.2 Quality Control Samples

Duplicate samples for the chemical analysis of waterfowl tissues will be in accordance with the QAPP and through comparisons with results from split samples provided to GE.

4.3.3 Sample Processing and Preservation

Detailed procedures for the collection of waterfowl used for tissue analysis are provided in Section 2.0. Biological sample dissection methodology are described in Section 3.0. These procedures are in accordance with measures identified in the QAPP. Holding time for biological samples (processed and preserved tissue samples after animal dissection) will follow procedures established in the QAPP.

4.3.4 Training

Animal collection will be directed by two senior scientists with experience in waterfowl collection methodologies and identification, measurements, and dissection will be directly conducted by the two senior scientists, again with experience in animal identification and dissection.

4.4 Sample Analysis

Samples for chemical analysis were processed following procedures and SOPs provided in the QAPP. These samples will be submitted to the appropriate analytical laboratory and data validation will be performed in accordance with procedures established and described in the QAPP.

4.5 Data Analysis and Reporting

The study findings will be included in the ecological risk assessment including all data, analyses, and interpretations.

5.0 Literature Cited

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APPENDIX A.24

TREE SWALLOW STUDY PROTOCOL AND PROCEDURES

APPENDIX A.24

RESEARCH PROTOCOL

Bioaccumulation and effects of PCBs on tree swallows nesting along the Housatonic River, Massachusetts

Testing Facility

Upper Mississippi Science Center
Biological Resources Division, U.S. Geological Survey,
P.O. Box 818
La Crosse, Wisconsin 54602-0818

Study Number	WE-98-Cont-08
Study Director	Christine M. Custer, Ph.D. Wildlife Biologist (Research)
Proposed Starting Date	April 1998
Proposed Ending Date	September 2001

Reviewed and Approved by:

Christine M. Custer
Christine M. Custer, Ph.D.
Study Director

April 29, 1998
Date

Carl E Korschgen
Carl E. Korschgen, Ph.D.
Section Leader, Wildlife Ecology

11/3/98
Date

Guy R. Stehly
Guy R. Stehly, Ph.D.
Animal Care and Use

June 30, 1998
Date

Steve Gutreuter, Ph.D.
Statistician

11/10/98
Date

Leslie Holland-Bartels, Ph.D.
Center Director

11/12/98
Date

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APPENDIX A.24

TREE SWALLOWS RESEARCH PROTOCOL

1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) Region 1, Boston, Massachusetts, and the U.S. Fish and Wildlife Service (USFWS), Region 5, Concord, New Hampshire Ecological Services Office, have requested assistance from the Upper Midwest Environmental Sciences Center (UMESC) in an evaluation of the effects of PCBs on wildlife downstream from the General Electric (GE) facility located on the Housatonic River near Pittsfield, Massachusetts.

The GE facility has contaminated the main stem and many of the backwaters of the Housatonic River with polychlorinated biphenyls (PCBs) (ChemRisk, 1997). Contamination originated from numerous sources including direct discharge to the river, a spill of liquid PCBs in the late 1960s at Building 68 that contaminated the riverbank and river sediments, and from contaminated fill that was placed in former oxbows. The extent of PCB contamination in the floodplain coincides roughly with the 10-year floodplain of the Housatonic River. The Housatonic River has been closed to all but catch and release fishing from Pittsfield, Massachusetts extending 80 miles downstream because of high concentrations of PCBs in fish tissues. The USFWS will be deciding whether additional management actions are warranted. To make this determination, data on PCB concentrations, accumulation, and injury to wildlife are required.

Little published information is available on concentrations of PCBs in avian tissues of birds nesting along the Housatonic River and no information is available on possible effect levels. In 1993 the nest success of 8 passerine species, within and outside the 10-year floodplain of the Housatonic River, was monitored (Henning et al., 1997). No data, however, on PCB concentrations in their eggs or food were collected during that study. Sample sizes were generally small in that study; however, for barn swallows (*Hirundo rustica*), clutch size was reduced at nests within the 10-year floodplain compared to reference sites at $P = 0.06$. Probably because of the reduced clutch size, mean number of young hatched in barn swallow nests from nests within the 10-year floodplain was also reduced compared to the mean number of young hatched per nest at reference sites. Again this difference may be considered significant ($P = 0.06$). Of the 4 species studied that Henning et al. (1997) had sufficient sample size to conduct statistical analyses on ($N > 5$ nests), the barn swallow would be the most likely to consume aquatic insects and hence be affected by PCBs. The food of American robins (*Turdus migratorius*), red-winged blackbirds (*Agelaius phoeniceus*), and wood thrushes (*Hylocichla mustelina*), the other 3 species studied, are terrestrial insects, and hence are not as likely to be exposed to the high concentrations of PCBs that are found in aquatic sediments (Ankley et al., 1993).

Swallows, especially tree swallows (*Tachycineta bicolor*) are now being more widely used as indicators of local sediment contamination (Shaw, 1983; DeWeese, 1985; Kraus, 1989; Ankley et al., 1993; King et al., 1994; Bishop et al., 1995; Nichols et al., 1995; Custer et al., 1998). Tree

swallows will readily use nest boxes, so study sites can be established at specific locations of interest. They feed near their nest box (± 400 m, Quinney and Ankney, 1985) on emergent aquatic insects (Blancher and McNicol, 1991) so residues in their tissues reflect sediment contamination for those chemicals that transfer into the biota (Fairchild et al., 1992). They also will nest relatively densely so that adequate sample sizes can be obtained. Data are now available on contaminant levels in tree swallows at a number of locations across the U.S. for PCBs (Bishop et al., 1995; Custer et al., 1998), other organochlorines (Shaw, 1983; DeWeese et al., 1985; Elliott et al., 1994), and metals (Kraus, 1989; King et al., 1994).

2. OBJECTIVES

This study has three primary data quality objectives. In addition, the data also will support the DQOs outlined in Subsection 4.1 of the Final Quality Assurance Project Plan (QAPP) (WESTON, 2000). The three primary objectives of the study are to:

- Determine PCB concentrations in tree swallow eggs and/or just-hatched young in the Lower Housatonic River.
- Determine PCB accumulation rates in nestlings.
- Determine whether PCBs are impairing reproduction in tree swallows.

3. STUDY DESIGN

3.1 TEST ORGANISM AND LOCATION

The test organisms are tree swallows. Identification of bird species will be made by the study director according to National Geographic Society *Birds of North America* or similar book. The study area is along the Housatonic River between Pittsfield, Massachusetts and Woods Pond (see map). The reference site will be a nearby tributary or river, such as the West Branch of the Housatonic, or the Housatonic River itself above the suspected contamination source. Other reference sites will be added if appropriate, such as Three-Mile Pond.

3.2 SAMPLE COLLECTION

Approximately 120 swallow boxes, 30 at each of 4 sites, will be attached to posts, trees, or other suitable structures in suitable habitat. Predator guards will be used as needed. Boxes will be placed approximately 20 - 30 m apart, but this can vary depending on the structure of the habitat. Additional boxes may be added in subsequent years of the study. Three sites (upper, middle, and lower reaches) will be below the GE facility on the Housatonic River and reference sites will be on a nearby waterbody such as the West Branch of the Housatonic River (Fig. 1). Location of boxes and specific study sites will be determined during the first visit to the area, but may be

modified in subsequent years of the study based on the professional judgment of the Study Director.

Each nest box will be visited approximately once per week until egg laying begins. After that time, nests will be visited up to 2 - 3 times per week, or more often as needed to collect egg or just-hatched young samples. After the eggs have hatched, boxes will be visited at least once per week until the young reach 12 days of age. Once the majority of eggs at a site have hatched, spot checks of selected nests are permissible and can be made at an appropriate interval to assess hatching and fledging success. Whether eggs or young are present in the nest box and the number of eggs and young present will be recorded on data sheets (SOP WE 408, Attachment 3).

A sample of 2 - 3 eggs and/or just-hatched eggs (hereafter termed pippers) and a sibling 12-day-old tree swallow nestling will be collected from a minimum of 5 - 10 boxes at each site. All sample collections will be covered by appropriate Federal and State collecting permits. The exact number of samples collected at each site will be dependent on the number of tree swallows that nest at each site and the analytical dollars available. If sufficient analytical dollars are available pipper samples will be collected and analyzed from all active boxes at each site. The exact number of pippers to be collected from each nest box will depend on the clutch size. Clutches $\leq$ 5 eggs will have only 2 pippers collected; clutches $\geq$ 6 eggs may have 3 pippers collected. Pippers and the 12-day-old nestlings will be collected from the first 5 - 10 clutches that are initiated at each site, however, unforeseen circumstances, such as nest predation or flooding, may preclude this.

Food samples from the stomachs of tree swallow nestlings will be removed at the time they are collected and dissected. A pooled food sample, from each site, will be analyzed for organochlorine chemicals, including total PCBs and perhaps PCB congeners. Nestling tree swallows may be ligatured to obtain additional food samples for insect species identification and for chemical analysis of their food. Adult and nestling tree swallows may be banded with standard USFWS aluminum bands.

In addition, WESTON will collect sediment samples within the tree swallows' expected foraging radius around the nest boxes to support this study.

3.3 SAMPLE PREPARATION

Pippers or 12-day-old nestlings will be removed from the nest box and weighed. Pippers and nestlings will be visually examined for gross abnormalities and this information noted. Nestlings will be decapitated with a sharp pair of scissors (Anonymous, 1993), contents in the upper gastrointestinal tract removed with forceps after an incision is made along the length of the stomach, and the carcass remainder placed in a chemically clean jar, which has been purchased in that condition. For in-depth methodology see SOP WE 409. The above will be done within 2 hours after removal from the nest box. The carcass remainders and food samples will be maintained frozen until transported to the Upper Midwest Environmental Sciences Center for storage in a freezer. Samples will be shipped to the analytical laboratory following chain of custody procedure in SOP WE 410.0.

3.4 SAMPLE ANALYSIS

Carcass remainders and pooled food samples will be analyzed for total PCBs, Aroclors, homologs, and congeners, with a subsample of pipper and nestling samples pooled by site being analyzed for polychlorinated dibenzodioxins/polychlorinated dibenzofurans (PCDDs/PCDFs), organochlorine pesticides, and trace elements. Analyses will be performed according to standard operating procedures of a contract lab approved by the U.S. Fish and Wildlife Service Patuxent Analytical Control Facility (PACF), Laurel, Maryland. Quality assurance and control for chemical analysis will be the responsibility of the PACF. The samples will be analyzed for the following specific contaminants:

PCBs

- Total PCBs
- Aroclors
- PCB homologs
- PCB congeners (including but not limited to the 12 congeners with dioxin-like activity)

PCDDs/PCDFs

- 2,3,7,8-TCDD
- 1,2,3,7,8-PentaCDD
- 1,2,3,4,7,8,-HexaCDD
- 1,2,3,6,7,8-HexaCDD
- 1,2,3,7,8,9-HexaCDD
- 1,2,3,4,6,7,8-HeptaCDD
- OCDD
- 2,3,7,8-TCDF
- 1,2,3,7,8-PentaCDF
- 2,3,4,7,8-PentaCDF
- 1,2,3,4,7,8-HexaCDF
- 2,3,4,6,7,8-HexaCDF
- 1,2,3,6,7,8-HexaCDF
- 1,2,3,7,8,9-HexaCDF
- 1,2,3,4,6,7,8-HeptaCDF
- 1,2,3,4,7,8,9-HeptaCDF
- OCDF

Organochlorines

- aldrin
- α-, β-, γ- and δ-benzene hexachloride (BHC)
- α- and γ –chlordane
- o,p'-DDD; o,p'-DDE; o,p'-DDT; p,p'-DDD; p,p'-DDE; and p,p'-DDT

- dieldrin
- endrin
- hexachlorobenzene (HCB)
- heptachlor epoxide
- mirex
- *cis*- and *trans*-nonachlor
- oxychlordane
- toxaphene

Trace Elements

- Aluminum
- Arsenic
- Cadmium
- Chromium
- Mercury

The nominal limit of detection will be 0.1 ng/g wet weight for PCB congeners, 0.05 µg/g wet weight for total PCBs, and 0.01 µg/g wet weight for the remainder of the organochlorines. The number of spikes, duplicates, and blanks will be at least 5% of the total number of samples analyzed for PCB congeners and organochlorines. Total PCBs and p,p'-DDE will be confirmed by GC/mass spectrometry in at least 10% of the samples. From congener data, toxic equivalents (TEQs) will be calculated with a variety of methods including those of Safe (1990) and Kennedy et al. (1996).

PCDDs/PCDFs will be analyzed with matrix-specific extraction, analyte-specific cleanup, and HRGC/HRMS analysis techniques by a method of internal standard quantification with [$^{13}C_{12}$]PCDD surrogates. The nominal limit of detection for PCDDs/PCDFs will be 0.05 ng/g wet weight. Concentrations will not be adjusted for recovery.

Tissue samples will also be analyzed for lipid and moisture content. Sediment samples will be analyzed for PCBs (total and Aroclors), TOC, and grain size, with a subset being analyzed for modified Appendix IX parameters including PCB congeners.

4. SAFETY CONCERNS

Wasp and bees will often use nest boxes, so field personnel should look and listen for bee or wasp activity before opening a box. Personnel should be aware of whether they are allergic to bee or wasp stings and prepare accordingly. Bee or wasp nests in the nest boxes may be dispatched as necessary either by smashing them, burning them with a butane lighter, or other method.

5. STATISTICAL ANALYSIS

Prior to any statistical analysis, the data will be verified for accuracy. Any unduly influential observations that may be outliers will be identified and further checked. Contaminant levels in biota will be compared among the 4 study areas with 1-way analysis of variance. Conformation of the data to model assumptions, specifically homogeneity of variances, will be checked with Bartlett's test, and remedial measures will be implemented where necessary (Neter et al., 1990). Comparisons of chemical concentrations, TEQs, and accumulation rates, between contaminated sites and the reference site, will be made with Bonferroni's multiple comparison or other appropriate procedure. Principal component analyses of the PCB congeners, PCDF, and PCDD concentrations may be done. Reproductive success will be quantified with the Mayfield method (Mayfield, 1961, 1975) and compared among sites according to Hensler and Nichols (1981). Regression techniques, including logistic regression, will be used to correlate levels of PCB contamination and TEQs with nest success measures. Bioaccumulation factors (average concentrations in nestlings divided by concentrations in their food) will be calculated at each site. Type-I error rate of 0.05 will be used for all statistical analyses.

6. STUDY RECORDS

All data generated in the study will be recorded in bound laboratory data books or kept in file folders (SOP No. GEN 008). All data sheets and laboratory data books will be encoded with the study number when the data are generated and stored in secure files. Raw data, laboratory data books, computer disks, and the completion product will be filed in the archives (SOP No. GEN 023) of the Upper Mississippi Science Center, La Crosse, Wisconsin.

7. GOOD SCIENTIFIC PRACTICES

Data collection, storage, and retrieval procedures for the study will be conducted in compliance with good scientific practices. The Study Director has the responsibility of ensuring that all procedures used in conjunction with the study conform to good scientific practices.

All changes from the research protocol will be documented in the laboratory data book or on appropriate data sheets and reviewed by the Study Director, who will make a judgment on the impact of the deviations.

8. QUALITY ASSURANCE/QUALITY CONTROL

8.1 DATA QUALITY OBJECTIVES, INDICATORS, AND ASSESSMENT

8.1.1 Data Quality Objectives

The three primary data quality objectives of the tree swallow study were delineated in Section 2. To achieve these objectives, the following types of data and specific quality criteria will be required:

- Tissue concentrations for PCBs and selected other contaminants in food samples of emergent insects and tree swallow pippers and 12-day-old nestlings: Quality control considerations to ensure achievement of the Data Quality Objectives (DQOs) for these parameters will follow the QAPP (WESTON, 2000).

- Sediment chemistry for PCBs and selected other contaminants: Analysis of sediment for chemical constituents must result in data that are consistent in all respects with other sediment contaminant data collected as part of the larger project. Satisfactory results will be ensured by submitting samples to the same laboratories that are analyzing samples for other components of the program. Quality control specifications for these data are delineated in the project QAPP (WESTON, 2000).

- Sediment grain size distribution: Quality control considerations to ensure achievement of DQOs for this parameter will follow the QAPP (WESTON, 2000).

- Condition of nests: Assessments of nest conditions must be made and recorded accurately. Accurate assessment of nest conditions is readily achievable with proper field techniques.

- Number of eggs and young produced: Counts must be made and recorded accurately. Accurate counts are readily achievable with proper field techniques.

- Weight of pippers and 12-day-old nestlings: Tissue weights must be determined accurately and recorded to 0.1 g using a calibrated balance designed and intended by the manufacturer to be capable of accurately measuring masses of this magnitude.

8.1.2 Data Quality Indicators

Data developed in the tree swallow study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives. Each of these data quality indicators, some of which are not readily quantifiable for the tree swallow study data, is discussed below.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Because of the small number of eggs in a tree swallow clutch, it is not possible to take repeated independent measurements of the biological parameters. Rather than control and measure precision, the study design includes a large number of what may be called replicates (based on samples from 30-50 nest boxes within an area), to increase the statistical resolution. For the measurements that are not unique to the tree swallow study, such as sediment chemistry and grain size, precision is evaluated as defined in the QAPP (WESTON, 2000).

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (e.g., tree swallow nest completeness, enumeration and weight of eggs and young), accuracy is defined as meaning that the nest completeness scores are correctly assigned; numbers of eggs and nestlings within each box are correctly enumerated and weighed (as in SOPs WE 409 and 410 and recorded on data sheets following SOP WE 408); correct assessment of live versus dead embryos and nestlings; and developmental status of embryos correctly assessed. Accuracy is a function of consistent field techniques and proper training. The data generated by this study will also be evaluated for accuracy via comparison with known and/or expected results from similar studies. For parameters such as tissue residue and sediment concentrations and grain size, accuracy is as defined in the QAPP (WESTON, 2000).

Completeness is defined as the percentage of the planned samples actually collected and processed. Completeness can be evaluated for only the sediment collection portion of this program. To ensure achieving the planned statistical resolution, it is important that completeness of 100% be achieved for sediment collection. For the tissue residue study component, the number of analyses will be determined by the weight of the material available for collection; therefore, establishment of an *a priori* completeness goal is not possible.

Representativeness refers to the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study is ensured through establishment of an approved thorough sampling design and through careful implementation of the sample processing and analytical methods. Specific aspects of representativeness will also be evaluated via comparison with known and/or expected results based on previous investigations of the Lower Housatonic River and other similar systems.

Comparability is a measure of the confidence with which the tree swallow data may be compared to another similar data set. Comparability will be evaluated by examination of the in-station variability in key parameters as determined from the large numbers of replicates to be collected at each location. Comparability will also be evaluated for this data set through comparison with data among the three years of this study in the Lower Housatonic River and with tree swallow data in similar systems.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is related for this tree swallow study to the ability of the field personnel to examine nests without causing abandonment. This data quality indicator may be evaluated by comparing the initial number of active well-defined nests to the number of well-defined nests that remain active throughout a study season. As nest abandonment of well-defined nests increases, the availability of young decreases, and the ability to tell differences among reproductive effects from different areas decreases. In addition, sensitivity is applicable and

important for the chemistry parameters that will be analyzed as part of the tree swallow study. For these parameters, the detection limits for chemistry specified in the QAPP (WESTON, 2000) will provide appropriate sensitivity for the purpose of providing insight into factors contributing to the exposure of and reproductive effects in tree swallows.

8.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the tissue and sediment chemistry and physical (e.g., sediment grain size, lipid content in tissue) data are discussed in the project QAPP (WESTON, 2000) For the biological data, usability will be largely be determined by two factors: (1) the experience of the study investigator in establishing that the field sampling was conducted following the SOP and that accuracy and precision were not compromised by an inability to control the sampling procedures in the field and (2) a comparison of the data both within the study and with previous tree swallow studies.

Sediment subsamples for physical and chemical analyses will be collected following procedures documented in the project QAPP (WESTON, 2000) and will therefore be comparable with procedures followed for other similar efforts throughout the Supplemental Investigation.

The purpose of the remainder of this section of the study plan is to document the measures included in the study to ensure that the standards discussed above are met.

8.2 SAMPLING DESIGN

The selection of the four general swallow box locations is based on (1) the location containing sufficient contiguous area of tree swallow habitat on the banks of the Housatonic River to accommodate 30 to 50 nest boxes; (2) the desire that each site be owned by the state or other governmental entity to facilitate access for the duration of the study; and (3) the sites covering a range of sediment PCB contamination in the Lower River between the confluence and Woods Pond. Appropriate reference locations in a nearby tributary or other waterbodies, such as the West Branch of the Housatonic River, Three-Mile Pond, or the river itself upstream of suspected contamination will also be sampled. The three “target” locations are sufficient to achieve the study data quality objectives consistent with the resources available for the study.

To achieve acceptable statistical resolution, data will be collected from a minimum of 5-10 boxes at each of the 4 stations, with the number of pippers and 12-day-old nestling samples varying depending upon the clutch size as described in the “Sample Collection” subsection of the “Study Design” (planned minimum of 2 individuals collected/box). Power analyses for logistic regressions indicated that a sample size of 75 is adequate to have a >80% probability of detecting a relationship between PCBs and hatching success if one exists. In addition, power analysis to detect differences among sites in PCB concentrations as small as 2 µg/g was 99% with as few as 5 samples per site.

8.3 SAMPLING METHODOLOGY

8.3.1 Sampling Procedures

Nest box checking procedures and sampling procedures are discussed extensively in Attachment 2, SOP WE 409 and 410, respectively. Procedures have been selected to minimize disturbance and nest abandonment. In addition, sample processing procedures have been set to avoid cross contamination between the site and reference areas, and among the site areas.

8.3.2 Quality Control Samples

The nature of tree swallow biological data does not allow the incorporation of typical duplicate and blank samples as part of the study design. For field observations, there is no acceptable method of obtaining such samples in a manner analogous to that for duplicates and blanks collected for chemistry analysis.

Duplicate samples for chemistry will be determined in the laboratory based upon the availability of tissue mass, with a target goal of 5% of the samples. Quality control of chemistry analyses for sediments will be provided by taking larger volumes of sediments at a number of locations and processing in accordance with the QAPP (WESTON, 2000).

8.3.3 Sample Processing and Preservation

Detailed procedures for collection and processing of all tissue samples to be collected as part of the tree swallow study are provided in SOP WE 410. All samples will be processed at the field laboratory within 2 hours of removal from the nest box. Food samples and carcasses will be frozen after initial processing. Holding time for samples undergoing physical and chemical analysis will follow procedures established in the QAPP (WESTON, 2000).

8.3.4 Training

All tree swallow data collection and sampling will be under the direction of the Study Director. Senior scientists experienced in sediment sample collection will direct those sampling efforts. Supporting staff will receive training from the Study Director and senior scientists (for the collection of tree swallow data and sediment samples, respectively) in the overall goals of the study and in techniques to be followed to ensure collection of quality data.

8.4 SAMPLE ANALYSIS

Samples for tissue and sediment chemistry and physical parameters (e.g., grain size and tissue lipid content) will be processed following procedures and SOPs provided in the QAPP (WESTON, 2000). These samples will be submitted in catalogs (sample delivery groups) and batches with other samples from the larger project and data validation will be performed on a

catalog basis in accordance with procedures established and described in the QAPP (WESTON, 2000).

8.5 DATA ANALYSIS AND REPORTING

The overall analytical approach for data generated under this study is described in Section 5. The study findings will be included in a stand-alone report including all field observations, tissue chemical data, analyses, and interpretations. This report will be prepared with specific reference to both the data quality objectives specific to the tree swallow study as presented in Section 2. This data will, in turn be incorporated in the ecological risk assessment. All associated sediment chemistry data will be presented in the risk assessment report with specific reference to both the data quality objectives specific to the tree swallow study and Subsection 4.1 of the QAPP (WESTON, 2000).

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ATTACHMENT 1.

KEY STAFF:

Christine M. Custer
Thomas W. Custer

ATTACHMENT 2.

Standard operating procedures (SOPs) to be used in study WE-98-Cont-05

SOP	Title
GEN 008	Laboratory data books and recording data
GEN 023	Archives management for nonregulated studies
WE 409	Nest box checking procedure
WE 410	Tree swallow sample collection and processing procedures

ORIGINAL

Upper Mississippi Science Center
2630 Fanta Reed Road, P.O. Box 818
La Crosse, Wisconsin 54602

GENERAL OPERATING PROCEDURE

PROCEDURE TITLE: Laboratory Data Books and Recording of Data

AREA OF APPLICABILITY: All research staff of Upper Mississippi Science Center-La Crosse (UMSC).

PURPOSE: To describe the organization of laboratory data books and the procedures for properly recording daily activities for studies. The laboratory data book is the key document that will facilitate replication of a study.

PROCEDURES:

A. Laboratory Data Books

1. A separate data book(s) shall be maintained for each study.

2. Data books shall be bound so that pages cannot be removed or replaced without leaving clear evidence that this has occurred. Data books that use carbon paper to make duplicate copies and have perforated pages for easy removal of the carbon copy are encouraged.

3. The front cover of the laboratory data book shall be labelled with the study number, the title of the study, the date on which the study was begun, and the name of the study director.

4. The printed name, written signature, and written initials of anyone assigned to the study shall be placed inside the front cover of the book.

5. The page opposite the front cover shall list recording error codes (see B.4). Commonly used abbreviations for the study can also be written on this page. Example BNZ = benzocaine. Do not use abbreviations in the data book unless they are defined on this page or found on the equipment list in the study guide (SOP GEN 002).

6. The first three pages of the data book may be left blank so that a table of contents can be entered.

B. Recording of information

1. Entries to laboratory data books can be made only by personnel assigned to the study.

2. Entries should be made chronologically, beginning on or before the day on which the study begins and ending on the day on which the study ends. All interim events should be entries in the laboratory

data book.

3. Entries into the data book must be made in black or blue ink.

4. Errors in recording are corrected by making a single line through the incorrect entry; the original entry must be legible. The strike-out must be initialed and dated by the individual making the correction and an explanation for the correction must be written in the margin. The explanation must be sufficient for an auditor to clearly determine what has occurred. When applicable, it is permissible to use the following error codes:

SP= spelling error	example bo~~o~~nk SP GmK 3/May 96
GE= grammatical error	example ~~was~~ were GE GmK 3 May 96
TE= transposition error	example ~~97~~ 79 TE GmK 3 May 96
NL= not legible	example ~~[illegible]~~ was NL GmK 3/May 96

5. Each entry into the data book shall begin with the date the entry was made and, if appropriate, the time.

   Next, a brief outline of the procedure or methods -- referencing the approved protocol or SOP's (whenever possible) and what is to be accomplished -- shall be given. Any materials used to generate the data must be listed. If these items are listed in an approved study guide, it can be referenced; otherwise, the items must be listed. This information must be given in sufficient detail to allow the experiment to be repeated. This description shall include the following information:

   a. The identity, name, model number, serial number, FWS property number (if available), and present location of specific pieces of equipment used to produce numerical data. Such equipment includes balances, pH and dissolved oxygen meters, spectrophotometers, gas chromatographs (GC), high performance liquid chromatographs (HPLC), osmometers, physiographs, sample oxidizers, liquid scintillation counters, and computers.

   b. The identity, name, model, serial number, FWS property number (if available), and present location of any appliance or equipment used to store or prepare samples, including refrigerators, freezers, special storage cabinets, temperature-controlled ovens or rooms, water baths, and special chromatography columns.

   c. Types and sizes of glassware, plasticware, vials, filters, and syringes.

6. Results of the study shall be recorded neatly and sequentially. All entries should be well thought out before being made.

7. If the form or volume of data being obtained makes it impossible to

enter all data into the data book, a file folder can be made to store this information. The file folder and each page of data shall contain the study number (a rubber stamp can be used), which irrefutably links the data sheets and associated information to the study. Reference should be given in the laboratory data book to the location of the study file. The file then becomes part of the data book and must be safeguarded accordingly.

a. Examples of data that might be in this category could include printouts from analytical instrumentation or absorbance traces from GCs or HPLCs, autoradiographs, original copies of thin layer chromatography traces, preprinted forms for recording mortality, and water quality data from acute and chronic toxicological testing, and spreadsheets (e.g. Lotus).

8. Anyone making an entry in a data book must initial and date the end of the entry.

   If the form or volume of data is such that the provisions of B.7. of this SOP are being used, the first and last pages of the data set must be signed and dated by the individual producing the data. In addition, the total number of pages involved shall be indicated.

9. When all entries for a given date have been made and signed in the bound data book, a heavy blue or black line shall be drawn horizontally across the page directly below the last entry for each date. One blank line may be left between the black line and the next day's entries. No blank areas of more than one line are to be left in the bound data book, except after the final entry.

10. The Study Director must review and certify all entries and must countersign the bottom of each page of the bound data book. Multiple page data sets (see B.7. and B.8.) only need to be signed and dated on the first and last pages by the study director. These reviews shall be conducted at least every two weeks; however, more frequent reviews are recommended.

11. If more than one laboratory data book is required for a study, the last entry in a full book should indicate that the study data is continued in another laboratory data book. At the end of the study, the front cover of each laboratory data book must indicate the number of laboratory data books used during the study (e.g. laboratory data book 2 of 3).

12. The last entry made in the laboratory data book(s) shall denote the ending date of the study.

ORIGINAL

C. Security: Laboratory data books and raw data files must be secured at the end of each working day in a locked file cabinet, desk drawer, storage container or vehicle. Only personnel assigned to the study shall have access to these files. Certified copies of the bound data book shall be made at least every two weeks after reviews by the study director (see B.10. above) (carbon copies are acceptable). The certified copies shall be placed in a secured location separate from the location of the laboratory data books.

Electronic data files that have been printed do not have to be copied to produce another paper copy if the electronic file and disk name are placed on the front page of the printed data set. The paper file and the electronic file shall be stored in separate locations.

D. Archives: All laboratory data books and associated raw data files are to be archived according to the UMSC Standard Operating Procedure on archiving at the end of each study.

APPROVED BY: David M. Kennedy DATE 8 March 1996
Quality Assurance Officer

APPROVED BY: [signature] DATE 3/8/96
Center Director

Upper Mississippi Science Center
2630 Fanta Reed Road, P.O. Box 818
La Crosse, Wisconsin 54602-0818

GENERAL OPERATING PROCEDURES

PROCEDURE TITLE: Archives Management for Nonregulated Studies

APPLICABILITY: All Center personnel

PURPOSE: To securely maintain all raw data, supporting documentation and ancillary information generated at the Upper Mississippi Science Center (UMSC), La Crosse, WI during nonregulated studies. Nonregulated studies are defined as studies not intended to support applications for research or marketing permits for products regulated by the U.S. Food and Drug Administration (FDA) and pesticide products regulated by the U.S. Environmental Protection Agency (EPA).

PROCEDURES:

A. Scope - All raw data and supporting documentation generated during a nonregulated study, referred to as the "study package" (Attachment #1, Section A), will be stored in UMSC archives managed by the Center's Quality Assurance Unit (QAU). Ancillary information in support of the study package (Attachment #1, Section B) will be stored as described in Section E.2., "Filing System".

B. Location and Characteristics of Archives - USMC archives for nonregulated study packages and for ancillary information such as training files of former employees, closed-out instrument log books and fish culture records are locked file cabinets adjacent to the southeast stairway (C-2) and in Room 22 in the Administration Building (Segment C, lower level). These cabinets are kept locked at all times except when accessed by a member of the QAU.

C. Access and Return Procedure - Only members of the QAU are authorized to access to retrieve items from the archives, but UMSC personnel can request access to archived materials via the QAU. The QAU will arrange a time to escort the requesting party to the archives to retrieve the requested materials and will record the transaction in the Archives Access Log Book (Form GEN 007a). The requesting party must sign for receipt of archived materials in the Archive Access Log Book. After use, item(s) must be returned to a member of the QAU, who will sign and date the Archive Access Log Book, verifying the item(s) have been returned.

D. Responsibilities

1. The QAU is responsible for managing the Center archives.

2. The Study Director is responsible for indexing and submitting a study package to the QAU for placement into the archives. Study packages will not be accepted by the QAU unless accompanied by completed index forms (Form GEN 023.1a), signed and dated by the Study Director. In addition, a Master Index for each study must be prepared on Form GEN 023.1a. This index will be placed in the archives as the first file folder for each study.

3. The Study Director is responsible for timely submission of ancillary information, such as closed-out instrument logbooks and completed temperature recording charts, to the QAU for placement in UMSC archives.

4. The Center fish culturist is responsible for submitting receipt and rearing records from the Center fish culture facility to the QAU annually, in January, for the previous year.

5. The person signing the Archive Access Log Book for receipt of archive items is responsible for those items until they are returned to the QAU and the Archive Access Log Book is signed and dated, verifying their return.

E. Filing System

1. Study packages will be filed in the archives by study number.

2. Ancillary information will be filed as follows:

   a. Instrument Use, Calibration and Maintenance Log Books and records that are no longer in use will be maintained in archive file cabinets assigned by the QAU.

   b. Temperature-recording charts from temperature-recording devices will be archived annually, in January, for the previous year.

   c. The index of nonregulated studies, copies of protocols, quality assurance inspection reports and personnel training records will be secured in locked QAU file cabinets. Training records of personnel leaving the Center's employment will be transferred to the archives.

   d. Personnel position descriptions will be stored with other facility employment records in a secure area by the Administrative Officer.

   e. Receipt and rearing records of all aquatic invertebrates and vertebrates entering the UMSC fish culture facility will be archived annually, in January, for the previous year.

F. Storage Time

1. Study packages and ancillary information will be securely maintained indefinitely at UMSC. In the event this Center closes, study packages will be transferred to the study sponsors, or if the study sponsor and the Center are the same, will become the property of the parent organization, the U.S. Department of the Interior.

2. Biological specimens will be retained by the Study Director only as long as he/she feels the quality of the preparation affords evaluation. Disposition of biological specimens will be performed at the discretion of the Study Director[1] (**Note:** regulated studies must follow SOP No. GEN 007 for disposal of biological specimens).

G. Transfer of Raw Data - Under no circumstances except closure of this Center will original study packages, or any parts thereof, be permanently transferred outside of the UMSC, La Crosse. If a sponsor requests a study package or specific study data, copies of the originals will be sent.

REFERENCES:

1. UMSC SOP No. GEN 001, *Responsibilities and Procedures of the Quality Assurance Unit.*
2. UMSC SOP No. GEN 007, *Archives Management for Regulated Studies.*
3. UMSC SOP No. GEN 132, *Care, Maintenance and Disposal of Aquatic Vertebrates*
4. UMSC SOP No. GEN 133, *Care and Maintenance Record Keeping for Aquatic Vertebrates.*
5. UMSC SOP No. GEN 134, *Approval of Housing and Care of Vertebrate Animals During Experiments.*

APPROVED BY: David M. Kennedy DATE 4 Sept 1999
Quality Assurance Officer

APPROVED BY: James C. Kitchel DATE Sept 5,
Center Director

[1] See Step F.1. for required procedures to transfer study packages if the Center ever closes permanently.

A. Study Package includes all applicable items in A.1.-A.11.: the study package must be archived at the conclusion of the study, after the study completion report is signed by the Study Director.

1. A Master Index to all files being archived (on Form GEN 023.1a)
2. Protocol, protocol amendments and protocol deviations.
3. Study guide:
   a. Verification statement signed by all study personnel.
   b. List of instruments used during the study.
   c. List of TOPs and IOPs used during the study.
4. Test chemical & test substance origin, purity, stability & storage information.
5. Test animal origin, Daily Care and Maintenance Records, Vertebrate Use and Approval (Form GEN 132a), Approval for Housing and Care (Form GEN 134a), Record of Fish Transfers (Form GEN 133d), necropsies and other raw data items associated with test animals.
6. Identity and source of test animal feed.
7. Laboratory notebooks.
8. Raw data generated from direct observation during the study.
9. Reduced data generated from analysis of raw data.
10. Significant correspondence related to the study, excluding QA inspection reports (SOP No. GEN 001).
11. Directory of computer-generated file information associated with raw and reduced data generated during the study.
12. Final report and any amendments to final report and/or other completion products (see GEN No. 014) (**Note:** Publications resulting from a study can be added to the study package at a later date by submitting them to the QAU).

B. Ancillary Information includes all applicable items in B.1.-B.4.:

1. Instrument Calibration, Use and Maintenance Log Books will be retained with the instrument until the log book is full, has been in use for five years, or the instrument is no longer in use. When any of the preceding conditions are met, the log book will be closed-out. A final entry should be written regarding the closure of the log book and the entry must be signed and dated. A line should be drawn diagonally on the remainder of the page, invalidating additional entries. The log book should then be submitted to the QAU for archiving.
2. The index of nonregulated studies, copies of protocol, QAU inspection records, final report reviews and personnel training records will be secured in locked cabinets by the QAU. Training files of personnel leaving the Center's employment will be transferred to the archives.
3. Personnel position descriptions will be retained in a secure location by the Administrative Officer with other facility employment records.
4. Receipt and rearing records of aquatic invertebrates and vertebrates entering the UMSC fish culture facility will be archived annually, in January, for the previous year.

ARCHIVES RECORDS INDEX
Upper Mississippi Science Center

Form GEN 023.1a
Page 1 of ___

Study Number: _____ Study Director: _____
Study Title: _____
_____ Test Substance: _____

GIVE TITLE OF FILE & THEN LIST ALL ITEMS THAT ARE TO BE ARCHIVED (BE SPECIFIC)

_____ _____
Study Director Date

ARCHIVES RECORDS INDEX
Upper Mississippi Science Center-La Crosse

Form GEN 023.1a
Page ___ of ___

Study Number ____________________ (Continued)

GIVE TITLE OF FILE & THEN LIST ALL ITEMS THAT ARE TO BE ARCHIVED (BE SPECIFIC)

ORIGINAL

Upper Mississippi Science Center
2630 Fanta Reed Road, P.O. Box 818
La Crosse, Wisconsin 54602-0818

TECHNICAL OPERATING PROCEDURE

PROCEDURE TITLE: Nest Box Checking Procedure

APPLICABILITY: All personnel in the Section of Wildlife Ecology who are checking tree swallow nest boxes

PRINCIPAL: To describe nest box checking procedures.

PRECAUTIONS: None

PROCEDURE:

1. Prior to beginning the nest checks, fill in the top two lines of the data collection form (Form WE 409.0a) with your name, the names of anyone assisting you during the nest check, the date, the time of day, and a brief description of the weather. For example, temp. in the mid-70s, partly cloudy with a slight breeze would be an adequate description of the weather.

2. Proceed to visit each nest box and note the contents on the data sheet (Form WE 409.0a) after the appropriate nest box number.

3. To check a box, open the door by lifting up on the nail located on the top of the door. Look inside. It may help to stand on an overturned bucket or small stool, or use an automotive inspection mirror on the end of a short pole to clearly see inside the nest.

4. If feathers obscure the nest contents move them gently aside to get a clear count of the nest contents.

5. **Do not touch** or remove the eggs from the nest. The eggs are extremely fragile and can be easily damaged or broken.

6. If a bird is on the nest gently remove her being careful not to roll eggs or young out of the nest. Her feet (it is usually always a female on the nest) can often be clutching nest material that can cause eggs or young to be displaced. Release her by gently tossing her into the air towards an area without obstructions, such as tree branches.

7. Record the quality of a nest or beginning of a nest in the "Nest" column. The choices are 0, small, medium, or good. Also note if feathers are present. 0 means no nest present, small means just a few grass blades are present, medium means there is 1/2 inch (plus or minus) of grass blades forming the nest, and good means there is a well defined nest. Even though we are categorizing the "quality" of the nest in categories, the amount of nest material is a continuum so use your best judgement as to which category the nest fits into. This column just assists us in anticipating when eggs might be laid.

8. The next 2 columns on the data sheet, "Prev. Data (#eggs and #yg)", will be transcribed from the previous nest check data form before going into the field. Unless otherwise instructed, these 2 columns will be filled out by the Study Director. This information is used to cross check the new data. If the contents are different than what was recorded on the previous check, the number of eggs or young should be recounted to verify the egg or young count or to look more carefully for missing or broken eggs or dead young. **It is very easy to miscount**. For example, if you count 6 eggs on this visit and there were 7 eggs on the last visit then you would want to recount to verify the correct number. If an egg really is missing, determine whether it might have been rolled to the back of the box, is crushed in the nest, or if it really is missing.

9. In the "New Data" columns, write in, for each box number, the number of eggs or young which are present. Draw a short horizontal line in the "#egg" column if no eggs or young are present. Be sure that either a number or horizontal line is written in the "#egg" column. Initial the data sheet for each nest you check. This ensures that we know that a nest box was check and not inadvertently missed.

10. If there are damaged eggs or dead young then include those in the total count in the "#eggs" or "#yg" columns and then note in the Comments section that dead young or damaged eggs were present. For example, if there were 3 live young and 1 dead young in the box, record it as 4 young in the "#yg" column and then put in the comments column that 1 young was dead. See attached sample for examples.

11. Note in the comments section any other item of interest for that nest.

12. **Be sure and check that the box door is securely closed** before moving on to the next box. Once the nail has been replaced, give a light tug on the door to be sure it is secure. If a door flops open, the nest will usually be abandoned.

13. After all boxes have been checked and the data recorded, photocopy the original data sheets. Keep the photocopy and fax, mail or give the original to the Study Director.

14. Electronic versions of the data form will be used containing the updated information from the previous visit. Prior to each visit update the Prev. Data columns with the current number of eggs and young, save the file, and print-off a clean copy to use in the field. Use the following naming convention for files. Begin with the two letter state abbreviation and the date of the data in the Prev. Data columns. Use .dat as the file extension. For example, data from a May 5 check from Agassiz would be called mnmay4.dat. Data from a May 11 nest check in Massachusetts would be mamay11.dat.

APPROVED BY: Carl E Korschgen DATE: 8/6/98
Leader, Section of Wildlife Ecology

APPROVED BY: David A. Kennedy DATE: 7 Aug 1998
Quality Assurance Officer

Form WE 409.0a
Page 1 of 1

Colony: Investigator(s): Study Protocol #

Date: Time: Weather:

		Previous Data		New Data		
Box #	Nest[1]	# Eggs	# yg	# Eggs	# yg	Comments

[1] Choices: 0 = no nest
Small = a few blades of grass blades
Medium = ½ inch of grass
Large = well defined nest

ORIGINAL

Upper Mississippi Science Center
2630 Fanta Reed Road, P.O. Box 818
La Crosse, Wisconsin 54602-0818

TECHNICAL OPERATING PROCEDURE

PROCEDURE TITLE: Tree Swallow Sample Collection and Processing Procedures

APPLICABILITY: Any UMSC study personnel responsible for collecting and processing tree swallow samples from nest boxes.

PRINCIPLE: To describe tree swallow sample selection, collection, processing and chain-of-custody procedures

PRECAUTIONS: Safety

A. Wear latex or similar gloves while dissecting tree swallow samples.

B. Adhere to Material Safety Data Sheets (MSDS) for any chemicals used during sample processing

PROCEDURE:

A. Equipment, Reagents and other Supplies Needed

1. Plastic compartment boxes and containers
2. Styrofoam or other type of cooler or ice chest
3. Frozen cooler pack(s) ("blue ice")
4. Aluminum foil
5. Latex or similar gloves
6. Jars for cleaning reagents
7. Tap water
8. Hexane
9. Acetone
10. Labels
11. Indelible marker
12. Chemically clean jars, various sizes
13. Portable field balance
14. Plastic weigh boats or weigh paper
15. Dissection scissors
16. Forceps
17. Paper towels

B. General Sample Collection Information

1. Two types of tree swallow samples are targeted for collection from each nest box: just-hatched young or unhatched eggs (both referred to as pippers) and 12 ± 1 day old young (nestlings).

2. Pippers should be collected on the day they are hatching. A nestling will be collected 12 ± 1 days later from the same nest box, whenever sample numbers permit. Other collection schedules may be implemented if a nest box has insufficient numbers of either pippers or nestlings, or as hatching rate dictates (Section C). When the collection schedule is altered, the change and reason will be documented in the field notebook and on Form WE 410.0a or Form WE 410.0b.

C. Selection of Pipper Samples

1. Two or three pippers will be collected from each nest box; two pippers from clutches of ≤ 5, 3 pippers from clutches ≥ 6. Because the eggs hatch over a 12 - 24 hour period, a variable number of eggs may be hatched when samples are collected from the nest. If all eggs are hatched, select the smaller young for the sample.

2. If there are more just-hatched young than eggs present in the nest, collect one just-hatched young and two eggs for the sample. **For example, if there are four just-hatched young and two eggs, collect one just-hatched young and both eggs.**

3. If there are fewer just-hatched young than eggs, collect three eggs only, for the sample. **For example, if there are two young and three eggs, collect the three eggs.**

4. **Important!** Once eggs have started to hatch, never collect all of the young and leave only eggs in the nest because this will cause the parents to abandon the nest.

D. Selection of Nestling Samples - Count the number of nestlings in the nest. Number them clockwise or linearly, depending on how they are arranged in the nest. Randomly select one nestling using a list of random numbers between 1 and 4 generated from a random number table.

E. Sample Collection

1. Before collecting samples, place a small amount of grass or other vegetation in the bottom of the pipper compartment box or plastic

nestling containers to cushion the sample(s). For nestlings, be sure the container is aerated either with air holes punched into the container or by leaving the lid cracked slightly. Use an indelible ink marker to label each sample container with the nest box number.

2. Pipper collection - Gently transfer the pippers, by hand, from the nest box to the corresponding, labeled compartments of a compartmentalized plastic box.

3. Nestlings - Gently transfer the nestling, by hand, from the nest box into the corresponding, labeled plastic container.

4. Sample containers must be handled carefully to prevent injury or damage to the pippers or nestlings. If samples are stored in a vehicle before being dissected, they should be placed out of the sun and kept as cool as possible. If the temperature inside the vehicle is uncomfortable to the occupants without venting or air conditioning then the samples can be stored in an ice chest with ice packs ("blue ice") to provide a cooler environment. Samples should be processed within 0.5 - 2 hours of collection to preserve the stomach contents.

F. Sample Processing - **NOTE**: If the samples being processed are from both reference and contaminated sites, process the reference site samples first to avoid cross contamination.

1. Field Laboratory Set Up

   a. Place a sheet of aluminum foil on top of an ice chest or other flat surface.

   b. Fill three glass jars with one each of the following: clean tap water, hexane, and acetone.

   c. Place the balance on a flat surface and verify its accuracy according to the balance Instrument Operating Procedure (IOP).

2. Data Collection Form - Prepare Form WE 410.0a or 410.0b for data collection by listing the study number, location and date of collection, and species collected.

3. Label Sample Vials - Each label must include the:

a. Nest box number followed by a dash and the letter "P" for pippers or "N" for nestling

b. Current date

c. Initials of person performing the sample processing or the Study Director's initials; and

d. Collection site including state if state is not part of nest box numbering system. Other information may be included.

Example: This label indicates that the sample was a nestling collected at Lenox Road for study number WE-98-CONT-08 from nest box ML 845 by Christine Custer

ML845-N 6/10/98 CMC, Lenox Road

e. The study protocol number will be on the sample label or on the container box, ice chest, etc., that contains the samples.

G. Processing Pipper Samples

1. Label plastic weigh dishes alphabetically (A,B,C,..), one each for the number of pippers collected from a specific nest box.

2. Write the nest box number, the alphabet designation and the pipper type, "egg" or "young (yg)" on Form WE 410.0a.

3. Tare the first plastic weigh boat. Place the pipper in the weigh boat and record the pipper's weight (0.0 g) on Form WE 410.0a. Repeat steps E.2 and E.3 for each pipper from the specified nest box.

4. Label one chemically-clean vial to contain all pippers from a single nest box. Process the pippers beginning with just-hatched young followed by unhatched eggs.

5. Unhatched egg processing - Tare the sample vial. Hold the egg over the tared vial and cut along the air cell of the egg with a small pair of scissors. Allow the contents of the egg to drop into the tared vial.

If a 1/2 or greater developed embryo is present in the egg, it may have to be removed from the shell with a pair of forceps and placed in the tared vial. If the embryo is alive and has a visible head, snip the head off with a sharp scissors into the tared collection vial then place the remaining embryo into the vial. Discard the egg shell. Record the weight of the embryo and the stage of embryonic development on Form WE 410.0a. **Note**: Development options are: no visible development (none), 1/4, 1/2, 3/4, or full term embryo. Also record if the embryo is alive or dead.

6. Repeat the process for each unhatched egg from a single nest box. The weight on Form WE 410.0a refers to the sample weight. Either cumulative or individual sample weight may be recorded.

7. Just-hatched young processing - While still in the plastic weigh dish, euthanize the bird by cutting off its head with a sharp pair of scissors. Next make an incision in the bird's abdominal wall and remove the stomach using a forceps and small scissors. Make a slit along the stomach, then use a forceps to remove any stomach contents (food) onto a clean weigh dish. Set the food sample aside. Food samples from all just-hatched young from a specific nest box will be pooled, but can be weighed individually. See section I for additional information and labeling requirements for food samples.

8. Place the head, carcass and stomach (without contents) into the tared, chemically clean vial for that nest box and record the weight on the data sheet.

9. Repeat steps G.6 and G.7 for each pipper until all pippers from a nest box have been processed.

10. Screw the vial lid tightly onto the sample vial and double check that the nest box number is the same as that on the data sheet (Form WE 410.0a).

11. If individual food weights are not taken, then tare a clean plastic weigh boat. Transfer the pooled food sample into the clean, tared weigh boat. Record the sample weight on Form WE 410.0a. Place the food into the sample jar for the corresponding site and type (pipper or nesting).

12. Complete the form by making any other notes or comments as necessary and be sure that all data have been recorded.

13. After processing all pippers from a single nest box, clean and rinse all dissecting tools in the tap water jar to remove residual blood or tissue. Next rinse tools in the acetone then hexane before laying the tools on a clean surface to dry. Discard the foil covering the dissection surface and replace with clean foil. These cleaning procedures will prevent cross contamination between samples collected from different nest boxes and should be repeated after the sampling from each nest box is completed.

H. Processing Nestling Samples

1. Prepare Form WE 410.0b by entering the nest box number, current date and other information required on the form.

2. Tare a medium-sized plastic weigh boat or other container and weigh the young. Record the weight (0.0 g) on Form WE 410.0b.

3. Label a sample jar, then tare it.

4. Euthanize the bird by cutting off its head with a sharp pair of scissors. (Note: it works well to have one person hold the body with the head stretched out slightly and have the second person wield the scissors). Next, make an incision in the bird's abdominal wall and remove the stomach using a forceps and small scissors. Slit the stomach and use a forceps to remove any stomach contents (food) onto a clean, weigh dish. Weigh and record. See Section I. for additional information and labeling requirements for food samples.

5. Place the head, stomach (without contents), and carcass of the nestling in the tared sample jar and record the weight on the data sheet.

6. Screw the lid tightly onto the sample jar and double check that the nest box number and other information on the label corresponds to the data collection Form WE 410.0b.

7. Transfer the nestling food sample into the labeled, vial for that site and record the weight on Form 410.0b if not done previously. Cap the vial tightly and verify the label information against the data sheet.

8. Complete Form WE 410.0b by making other notes or comments, as needed, and be sure the data and other required information have been completed on the form.

9. After processing each nestling, clean all tools thoroughly and place clean aluminum foil on the dissecting surface (see G.13).

I. Food Samples.

1. Food samples from a single site will be composited to provide enough mass for chemical analysis. Food samples from the pippers will be composited separately from the food samples from the nestlings, but may be combined at a later time, as needed and decided by the Study Director.

2. The label on the food sample vials should contain the following information: site designation (Example: West Branch, Lenox, Canoe Meadows, Roaring Brood, or other study site names), state, whether it is a composite for pippers or nestlings, the year, initials of person collecting and processing the sample or the Study Director's initials, and the words tree swallow food. The study protocol number will be included on the sample label or on the container, box, ice chest, etc., that contains the sample. For example, a food sample from nestlings collected at Lenox Road, in 1998 would have the following information on the label:

tree swallow food, Lenox Road, MA, Pipper, 1998, CMC

J. Transportation and Storage of Processed Samples - Samples will be kept as cool as possible during transportation from the field to a freezer. As soon as feasible, samples will be placed in a standard freezer using the following chain of custody procedures.

K. Chain of Custody

1. Samples to be place in the freezer will first be placed in a plastic bag or box. The bag or box will be labeled, at a minimum, with the study number, type of samples, and current date and initials of person packaging them or the Study Director for freezer storage. Other information may be included as desired.

2. The bag or box will be locked or sealed with security tape to allow detection of unauthorized tampering. The security seal will be signed and dated by authorized study personnel.

3. The Study Director or other designated study personnel are personally responsible for the care and custody of the samples until they are transferred to the chemical laboratory. A sample or set of samples is considered "in the custody" of an individual if any of the following apply:

   - the sample is in the individual's possession.
   - the sample is in the individuals residential freezer.
   - the sample is within view after being in possession.
   - the sample is in a locked or sealed area or container that prevents tampering after being in possession.
   - the sample is in a designated secured area.
   - the sample is in a security-sealed bag or box.

4. A chain of custody form (Form WE 410.0c) or similar form should accompany samples when shipped to analytical laboratories. The chain of custody form should include the following information: a title indicating that the document is a Chain of Custody Record; the Study Protocol title and number; where the samples are being shipped to; the method(s) of shipment (FEDEX, UPS, hand, etc.) And the sample number(s). The form should also contain a minimum of two signature lines containing (1) signature of person releasing samples and date samples were relinquished, and (2) signature of person accepting samples and date that samples were received. This form may be computer generated.

REFERENCE: EPA/FDA GLPs At a Glance. Society of Quality Assurance.

APPROVED BY: Carl E. Korschgen _____ **DATE** 8/6/98
Leader, Section of Wildlife Ecology

APPROVED BY: David M. Kennedy _____ **DATE** 7 Aug 1998
Quality Assurance Officer

ORIGINAL

Form WE 410.0a
Page 1 of 1

Egg/Pipper Dissection Data

Location _____ Date _____

Species _____ Investigator(s) _____ Study Number _____

Nest Number	Pipper Desig[1]	Pipper Type[2]	Whole Pipper Weight(g)	Embryo Develop[3]	Sample Weight(g)	Food Weight(g)	Comments

Comments:

[1] Pipper Desig = a, b, c, etc.
[2] Pipper Type = egg or young (yg)
[3] Embryo Develop = none, 1/4, 1/2, 3/4, full

ORIGINAL

Form WE 410.0b
Page 1 of 1

Tree Swallow Nestling Dissection Data

Location _____ Date _____

Species _____ Investigator _____ Study Plan _____

Nest Box Number	Weight in Grams (0.0) Live Bird	Carcass Remainder	Food	Comments

Comments:

ORIGINAL

Form WE 410.0c
Page 1 of 1

Chain of Custody Record

Study Protocol Title & Number: _____

_____

_____

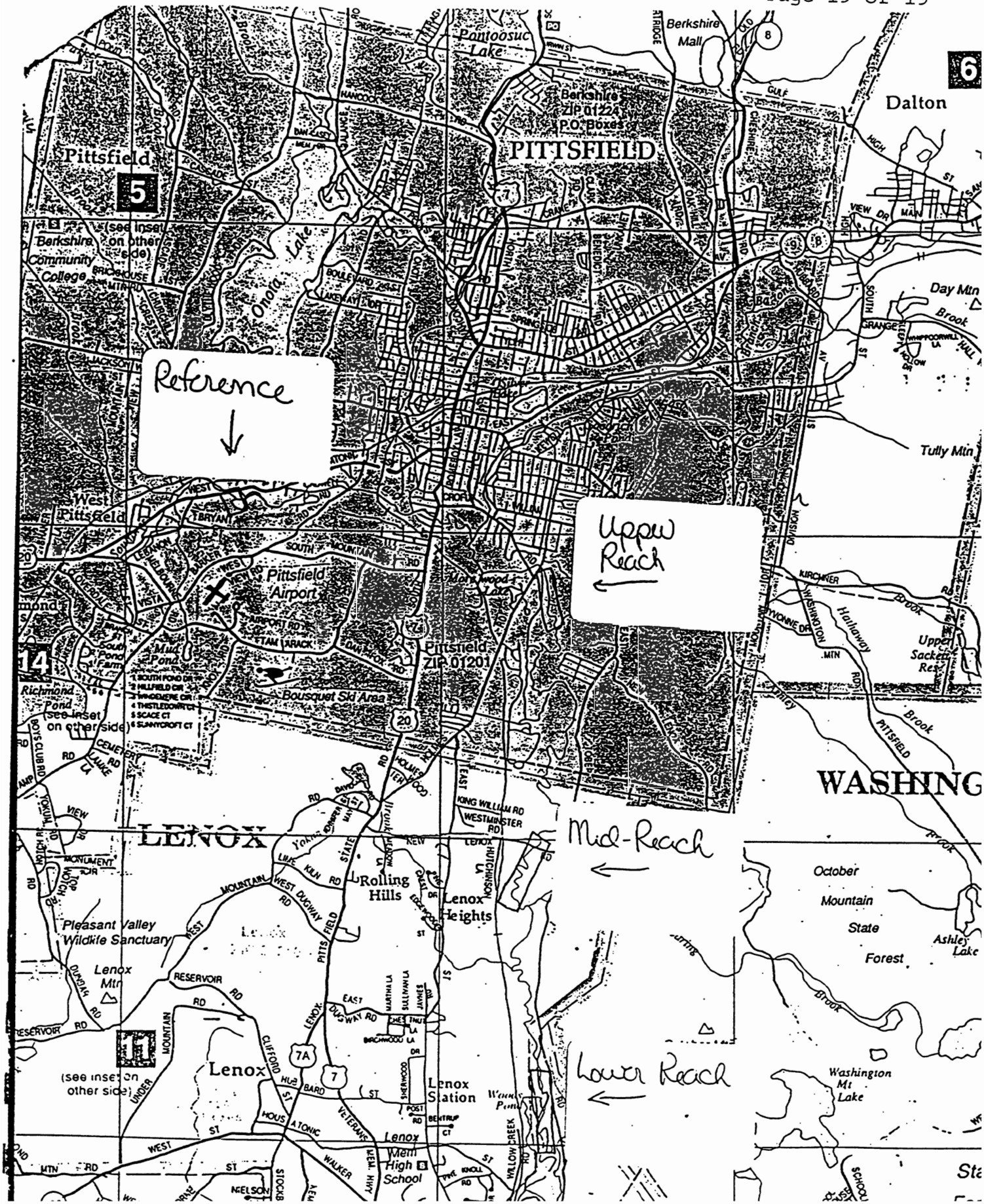
Principal Investigator: _____

Samples Shipped to (Name of Laboratory or Individual & Address): _____

_____

Item No.(s)

From: (print name) To: (print name)	Release Signature Receipt Signature	Release Date Receipt Date	Delivered via: O Federal Express O U.S. Mail O By hand O Other
From: (print name) To: (print name)	Release Signature Receipt Signature	Release Date Receipt Date	Delivered via: O Federal Express O U.S. Mail O By hand O Other


Reference
Upper Reach
Mid-Reach
Lower Reach
Pittsfield
PITTSFIELD
Dalton
Lenox
Lenox Station
Lenox Heights
Rolling Hills
Pittsfield Airport
Bousquet Ski Area
Pontoosuc Lake
Onota Lake
Berkshire Mall
Berkshire Community College
Pleasant Valley Wildlife Sanctuary
October Mountain State Forest
Washington Mt Lake
Tully Mtn
Day Mtn
Upper Sackett Res
Mud Pond
Richmond Pond
Lenox Mtn
Mem High School
Pittsfield ZIP 01201
Berkshire ZIP 01224 P.O. Boxes
(see inset on other side)

ATTACHMENT 3.

Colony: Investigator: WE-98-Cont-08
Date: Weather:

Box	Nest?	Prev. data		New data		Comments
		#eggs	#yg	#eggs	#yg	

APPENDIX A.25

WORK PLAN FOR THE STUDY OF SMALL MAMMALS OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND (WOODLOT ALTERNATIVES, INC.)

FINAL

APPENDIX A.25

WORK PLAN FOR THE STUDY OF SMALL MAMMALS OF THE HOUSATONIC RIVER FROM NEWELL STREET TO WOODS POND

Submitted to:

Roy F. Weston, Inc.
1400 Weston Way
West Chester, PA 19380-1499

Submitted by:

Woodlot Alternatives, Inc.
122 Main Street
Topsham, Maine 04086

FINAL

TABLE OF CONTENTS

1.0 Introduction

The United States Environmental Protection Agency (EPA) is characterizing the natural resources found in and adjacent to the Housatonic River in portions of Pittsfield, Lenox, and Lee, Massachusetts (hereinafter referred to as the study area) (see Figure 1 in Appendix A.6). This reach of river is approximately 12 miles long and extends from Newell Street in Pittsfield downstream to the Woods Pond Dam in Lee. Elevated levels of polychlorinated biphenyls (PCBs) that originated from the General Electric (GE) facility in Pittsfield have been found in this reach of the Housatonic River and the adjacent floodplains (Blasland, Bouck, & Lee, Inc., 1996).

The study area is inhabited by a variety of mammals that travel through, breed, and feed in the river, floodplain wetlands, and adjacent uplands. Large mammals that have recently been sighted in the study area include black bear (*Ursus americanus*), white-tailed deer (*Odocoileus virginianus*), coyote (*Canis latrans*), beaver (*Castor canadensis*), muskrat (*Ondatra zibethicus*), red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), and striped skunk (*Mephitis mephitis*) (TechLaw, Inc., 1999). Coyote and red fox scat observed in 1998 contained small mammal fur and bones, an indication that a portion of their diet consists of mice and voles (TechLaw, Inc., 1999). Small mammals known to occur in the study area include the white-footed mouse *(Peromyscus leucopus)*, southern red-backed vole (*Clethrionomys gapperi*), meadow vole (*Microtus pennsylvanicus*), smoky shrew (*Sorex fumeus*), short-tailed shrew (*Blarina brevicauda*), masked shrew (*Sorex cinereus*), meadow jumping mouse (*Zapus hudsonius*), and star-nosed mole (*Condylura cristata*) (TechLaw, Inc., 1999).

Several studies have investigated the effects of exposure to PCBs on small mammals including laboratory mice (Marks et al., 1989; Murk et al., 1991; Kholkute and Dukelow, 1997), Mongolian gerbils (*Meriones unguiculatus*) (Phillips and Batty, 1995), little brown bats (*Myotis lucifugus*) (Clark and Stafford, 1981), and white-footed mice (Linzey, 1987). Linzey (1987) reported white-footed mice exposed to food treated with PCBs at a rate of 10 parts per million (ppm) weaned significantly smaller numbers of young, suggesting potential population impacts from exposure. Another study indicated that PCBs can bioaccumulate in small mammals (Watson et al., 1985), and therefore become available to higher trophic level predators.

1.1 Objectives

The primary objective of this study is to collect small mammals for tissue residue analysis for PCBs, and possibly dioxins/furans and organochlorine pesticides, in order to provide data on the bioaccumulation of these materials in the food web. Analytical results will be used in food chain models to estimate potential risks to higher consumers, such as large mammals or raptors that feed upon small mammals. Another study objective is to determine if there are differences between numbers of placental scars and embryos in females of reproductive age from areas with varying concentrations of PCBs. These data will be considered in the ecological risk assessment.

1.2 Project Approach

To accomplish the objectives of the study, small mammals will be captured in several areas of floodplain forest habitat within the study area with varying PCB concentrations (i.e., less than one part per million [ppm], from 1 to 30 ppm, and greater than 30 ppm). Small mammal whole body tissue will be analyzed for PCBs (totals, Aroclors, congeners, and homologs), percent moisture, percent lipids, and possibly organochlorine pesticides and dioxin/furans, and the placental scars of adult females will be examined.

2.0 Study Design

2.1 Literature Review

The scientific and technical literature, including museum records, will be reviewed to determine the historic distribution of mammals in the Housatonic River drainage system. As part of this effort, local and regional experts will be consulted to obtain unpublished records regarding the historic and current distribution of mammals in the Housatonic River drainage. The Massachusetts Natural Heritage Program and the U.S. Fish and Wildlife Service (USFWS) will also be consulted to determine if any records of mammals from the Housatonic River drainage are available from surveys sponsored or conducted by these agencies.

2.2 Field Sampling Design

2.2.1 Small Mammal Trapping Locations

Preliminary surveys in 1998 indicated that several species of small mammals live in forested floodplains in the study area. White-footed mice were the most abundant small mammal captured (64% of all captures), followed by meadow voles, short-tailed shrews, southern redback voles, and masked shrews (TechLaw, Inc., 1999). Two of these species are suitable for whole body tissue analysis because they are primarily insectivorous during the summer months, and certain insects are known to take up PCBs from soil in a relatively short time period (Paine et al., 1993). Short-tailed shrews eat earthworms, slugs, snails, lepidopterous larvae, spiders, and centipedes, while white-footed mice, although omnivorous, primarily eat insects in the summer months (Whitaker and Hamilton, 1998). Meadow voles and southern redback voles seldom eat insects (Whitaker and Hamilton, 1998), and because of this, they are unlikely to bioaccumulate PCBs at the same rate as more insectivorous species.

In 1998 small mammals were captured in floodplains dominated by overstories of boxelder (*Acer negundo*) and silver maple (*Acer saccharinum*), and understories of wood nettle (*Laportea canadensis*) and ostrich fern (*Matteuccia struthiopteris*) (TechLaw, Inc., 1999). This type of habitat is common in the forested floodplains of the study area. Based on these findings, similar habitat in the study area will be selected for potential trapping locations. These areas will be identified using aerial photos and cover type maps of the study area to locate forested floodplain habitat similar to that used by small mammals in 1998. After areas of similar habitat are identified, maps showing PCB concentrations in floodplain soils will be reviewed to identify

places in the floodplain with varying levels of PCBs. Potentially suitable trap sites will then be surveyed on the ground to verify habitat suitability based on the similarity with habitat where small mammals were captured in 1998.

Only sites where signs of small mammal use including scat, tracks, and browsing are observed will be considered for selection. Sampling sites that are selected will be described both quantitatively and qualitatively using data collected on Natural Community Survey Forms (Maine Natural Areas Program, 1997). Detailed quantitative information on plant community characteristics, soils, microtopography, and hydrology will be collected for describing habitat within small mammal home ranges, which can vary from 20 to 50 m for meadow voles (Getz, 1982).

2.2.2 Data Evaluation and Statistics

Small mammal trapping data are typically normalized to captures per 100 trap nights. For less common species, or species that are more difficult to trap, such as masked shrews, captures can range from less than one animal to a few animals per 100 trap nights. Captures of abundant species, such as meadow voles or white-footed mice, can range up to 10 captures per 100 trap nights. Assuming a mean capture rate of 10 individuals (the sum of all species) per 100 trap nights, a mean of approximately 50 individuals may be captured per site under the proposed sampling regime of 500 trap nights per site.

Data available for statistical analysis are expected to include the total number of captures and the captures per 100 trap nights by species for each site; descriptive statistics (e.g., mean, variance, and range) for all measurements of continuous variables, such as weight and length; and counts of categorical variables (e.g., sex). Placental scars and embryos are technically discrete data (i.e., a female may have 1 or 2 embryos but never 1.5 embryos), but they are often reported as continuous variables. For example, many reports in the literature refer to the mean number of placental scars. Placental scars and the number of embryos, therefore, will be reported using both frequency tables (i.e., the number of females with 0 placental scars, the number with 1 scar, etc.) and descriptive statistics, including the mean, variance, and range.

Reproductive data (placental scars and number of embryos) will be primarily analyzed as discrete variables. Treating the data as discrete variables, when using a chi-square analysis, will require correction for continuity. A contingency table and chi-square analysis will be used to compare the number of placental scars per female between sites for each species (Fienberg, 1983; Zar, 1984). This is a Poisson sampling model (Fienberg, 1983:15) with a set of Poisson processes, one for each cell in the cross-classification, over a fixed period of time, and with no *a priori* knowledge regarding the total number of observations to be taken. Each process yields a count for the corresponding cell (e.g., number of females with 2 placental scars at Site 1). All analyses will be run using the *Statistica*^{TM} software package (Steiger, 1999).

For most species in the study area, the mean number of young per litter typically ranges from 4 to 6 (Whitaker and Hamilton, 1998). It is likely, however, that the number of young per litter is not normally distributed around the mean. It is more likely that most adult females have 4 to 6 young,

very few have greater than 6 young, and somewhat more have less than 4 young (i.e., the distribution function is skewed to the right). Based on this assumption, and assuming captures of 25 individuals per species at each site and 3 sites (total N = 75), a power analysis (Borenstein, et al., 1997) for the chi-square analysis was developed using hypothetical data (Table 1). This analysis is based on $\alpha = 0.1$ and a desired power ($1-\beta$) of at least 0.8. For this analysis, it was assumed that observing a mean number of placental scars roughly half the expected mean was biologically significant[a]. The results of this analysis ($\beta = 1.00$) indicate that this test would be a suitably powerful indication of statistically significant results. When the data are analyzed, the observed power will be reported. In the event that cells within the contingency table contain no observations (e.g., no females had a count of 1 placental scar), Fienberg's (1983) guidelines for fixed and random zeroes will be followed.

Once the PCB tissue data are available, regression analysis will be used to determine whether there is a relationship between PCB concentration in female small mammal tissues and the number of placental scars or embryos. Data from all sites will be pooled for this analysis and covariates will include age of the female (immature versus adult) and weight. Assuming that the covariates explain 20 percent of the increment to R^2 and the tissue PCB level explains 40 percent, which would be considered biologically significant, a sample size of 10 would be needed to achieve a power greater than 0.8 with an α of 0.1 (Borenstein, et al., 1997). The actual power of the test and the increment to R^2, however, will be reported for the data that are collected.

Table 1

Power Analysis for Hypothetical Small Mammal Placental Scars

	Proportion falling in column								Proportion in Row
	0	1	2	3	4	5	6	>7	
<1 ppm PCBs	5	5	5	5	15	45	15	5	0.33
1-30 ppm PCBs	5	5	25	40	10	5	5	5	0.33
>30 ppm PCBs	10	15	40	15	5	5	5	5	0.34

Number of cases = 75

Alpha (2-tailed) = 0.10, Power=1.00

Power computation: Non-central chi-square

[a] The actual significance of reducing the mean litter size for each species may be modeled as part of this study. For some small mammal populations, which may turn over completely on an annual basis, a modest decrease in mean litter size may be biologically significant and result in unstable or declining populations.

2.3 Analytical Requirements

2.3.1 Analyses

Based on the average size of short-tailed shrews and white-footed mice collected during preliminary surveys in 1998, sufficient tissue mass (about 10 g) for the required tissue residue analyses will be obtainable from each individual collected. In addition, preliminary surveys (TechLaw, Inc., 1999) indicated that approximately 10 individuals per target species should be captured during the approximately 500 trap night surveys. Each small mammal submitted for analysis will be analyzed for PCBs (total, Aroclors, congeners, and homologs), percent moisture, and percent lipids. A subset of samples may be analyzed for organochlorine pesticides and dioxin/furans if there is sufficient sample weight. The analytical methods and detection limits to be used are specified in Appendix C of the *Quality Assurance Project Plan* (QAPP) (WESTON, 2000).

2.3.2 Quality Assurance/Quality Control Samples

Duplicate analyses will be conducted for each parameter on approximately 1 of every 20 samples. An additional 10 g of tissue will be required for each set of analyses beyond the 10 g required for each set of original analyses, with 20 g as the total amount of tissue required. In addition, a matrix spike/matrix spike duplicate (MS/MSD) sample is required for approximately every 20 samples. An additional 20 g of tissue will be required for each set of MS/MSD analyses, beyond the 10 g required for the original analyses. Thus, the amount of tissue required to conduct the original analyses and MS/MSD analyses on a single sample is about 30 g.

3.0 Procedures

3.1 Field Sampling

At each trap site, following established protocol (Clough, 1987; Lortie and Pelletier, 1987), 100 snap-traps baited with peanut butter will be placed in an "X" pattern (when possible, some sites may require that the direction of an axis be changed due to the configuration of the habitat area), with each axis being approximately 500 feet in length. One trap will be placed at each trapping station on the line and every tenth trapping station will include a pit-trap, which is more effective for capturing shrews. Where small mammal runways are apparent, traps will be placed on the runways to increase capture efficiency. Trapping will be conducted for 5 days, for a total of approximately 500 trap nights per site. Sampling intensity of 500 trap nights per site is based on the anticipated size of the small mammal population in the habitats being sampled. For example, Getz (1982) recommends using 450 trap nights when estimating relative abundance per habitat for meadow voles and additional sampling is unlikely to result in capturing significantly more small mammals.

Because pit traps tend to be more efficient at capturing shrews (Kirkland, 1982), pit-trap arrays will also be used at each trapping site. These arrays consist of four drift fences 25 feet long and 2.5 feet high, arranged in an "+" formation with a 50-foot gap in the center of the "+." Individual

pit traps were installed on both sides and at either end of each of the four drift fences, for a total of 16 pits per array. The pit-fall trap arrays will be situated near one of the axes of the larger snap trap arrays.

3.2 Processing

3.2.1 Initial Processing

Captured small mammals will be removed from traps and placed in clean, unused, individually labeled resealable plastic bags for transport to the processing area. Bags will be labeled with date, trap site, and individual trap location. Prior to transport to the processing area, bagged small mammals will be placed in a cooler containing bagged wet ice.

Two-person teams will prepare a processing table with clean plastic sheeting. All processing equipment and supplies including aluminum foil will be decontaminated with nitric acid/hexane/ isopropyl alcohol/deionized water as specified in the QAPP (WESTON, 2000). On the data sheet for each small mammal, the sample location, date, initials of collector(s), individual small mammal identification number, WESTON sample identification number, species, sex, weight (g), total length (mm), tail length (mm), hind foot length (mm), and ear length (mm) will be recorded. Each individual small mammal will also be sexed, aged (adult versus immature), and inspected for abnormalities or deformities, which will be described on data forms. Age will be determined by comparing body size measurements, pelage color, and reproductive status (e.g., size of testes) with those in the literature for known age individuals.

Uterine tissues will be removed from female small mammals using dissecting equipment decontaminated using the process described above. The number of placental scars and embryos will be counted for captured females with the aid of a dissecting microscope. Placental scars will be grouped and counted based on the size, shape, and opacity (Harder and Kirkpatrick, 1996). A sketch will be prepared and a photograph taken of placental scars. All small mammals will then be packaged for preservation for tissue analysis.

3.2.2 Tissue Processing

After morphometric and placental scar (females) information has been collected for each small mammal, the specimen will be individually wrapped in nitric acid/hexane/isopropyl alcohol/ deionized rinsed aluminum foil. The foil will be labeled with sample identification number, location, date, collector's initials, weight, and tissue type. The foil-wrapped sample will then be placed into a resealable plastic bag, similarly labeled, and then placed immediately into a freezer. Sample attribute forms will be completed for each sample (whole body tissue, duplicate, or MS/MSD samples). The above procedures summarize those outlined in the project QAPP.

3.2.3 Sample Handling and Shipping

The procedures summarized here follow those in the project QAPP. Samples will be kept in a freezer at -22° C until shipment to the laboratory. When ready to ship, the samples (wrapped in labeled foil and enclosed in labeled resealable plastic bags) will be placed in a large plastic bag

and then into a cooler lined with vermiculite. Chain-of-custody forms listing the contents of each cooler will be completed and placed into a resealable plastic bag. The resealable plastic bag will be taped to the inside of the top lid of the cooler, or placed on top of the samples. The coolers will be sealed with two custody seals, and labeled with appropriate WESTON shipping labels, including the WESTON return address, and USFWS laboratory address. Samples will be delivered by courier or overnight delivery to the analytical laboratory.

3.2.4 Sample Documentation

Specimen data sheets for each small mammal will be completed to include: location; date of collection; method of collection; collector's initials; total weight (g), sex (if known), total length (mm), tail length (mm), hind foot length (mm), ear length (mm); and analyses. Sample attribute forms will also be completed for each tissue sample, which will include the sample number for each sample and the species Latin name, date, and individual small mammal identification number. Complete chain-of-custody forms for each cooler of samples shipped to the USFWS laboratory will be provided to the task manager, who will retain them in the WESTON files.

4.0 Quality Assurance/Quality Control

4.1 Data Quality Objectives, Indicators, and Assessment

4.1.1 Data Quality Objectives

The objectives of the study are outlined in Subsection 1.1. To achieve these objectives, the following types of data will be required:

- **Natural community characterization**: accurate descriptions of habitat characteristics in the vicinity of small mammal trapping locations must be collected.

- **Taxonomic** data: accurate species identifications of captured small mammals must be obtained.

- **Enumeration:** counts of placental scars and embryos in female small mammals of reproductive age must be accurately recorded using standard procedures.

- **Morphometric data**: accurate measurements of small mammal morphometrics (weight, length, etc.) must be acquired.

- **Concentrations of PCBs in small mammal tissues**: Quality control considerations for PCB concentration analysis for biological samples will follow those identified in Subsection 3.2, and in the project QAPP.

4.1.2 Data Quality Indicators

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000), that are appropriate to the data quality objectives.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. For this study, repeated independent measurements of species identification will be made as mammals are collected and processed using taxonomy keys. Agreement between surveyors regarding species identification must be obtained for verification. Precision may also be evaluated by assessing the degree to which surveys are consistent among sites. For measurements that are not unique to small mammals, precision is evaluated as defined in the QAPP.

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (small mammal taxonomy), accuracy means that: 1) the specimens are correctly identified and counted; 2) morphometric data are measured using methods and instruments capable of providing accurate, reliable measures; and 3) tissue analysis is conducted using laboratory procedures established in the QAPP (WESTON, 2000).

Completeness is defined as the percentage of the planned samples actually collected and processed. A power analysis was conducted to determine the sample sizes needed for statistically significant sample results. Completeness for this study, therefore, can be interpreted as the percentage of the desired sample size that was acquired.

Representativeness is defined as the degree to which the data accurately reflect the characteristics present at the sampling location at the time of sampling. Representativeness for this study will be ensured through establishment of an approved, thorough sampling design and through careful implementation of the sample processing and analytical methods. Specific methods will be used to collect target organisms in an unbiased fashion and should not select for certain components (only males, females, adults, juveniles, etc.) of the source population.

Comparability is defined as the measure of confidence with which the small mammal collection data may be compared to another similar data set. Comparability will be attained through use of standard sampling procedures for small mammals. These methods are well established and are repeatable at the sampling location or other areas, which results in comparable data. In addition, trapping results are measured on a per unit effort basis that can be compared to other studies.

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. For data specific to this study, sensitivity will pertain to the ability of measurement instruments to measure animal morphometrics, optics to magnify tissues well enough to identify and count placental scars, and laboratory instruments to detect and measure PCBs in animal tissues.

4.1.3 Data Validation, Verification, and Usability

Procedures for data validation for the analysis of tissue PCB concentrations are discussed in Subsection 2.3, above, and in the project QAPP and will be used whenever applicable in this study.

4.2 Sampling Design

Trapping sites will be chosen based on 1998 background trapping data and floodplain soil PCB data acquired in 1999. Sites will contain varying levels of PCBs in the soil and will represent areas with no to high levels, as identified in Subsection 1.2.

4.3 Sampling Methodology

4.3.1 Sampling Procedures

Field investigations for this study consists of standard methods for the capture of small mammals. Lab procedures will ensure that accurate tissue concentrations and scar counts are achieved and will be based on methods identified in the compilation of observational data to create species-habitat associations. No physical samples of site soil or water will be collected for this study; therefore, QA/QC procedures for physical samples are not required.

4.3.2 Quality Control Samples

Quality control samples (routine duplicates and MS/MSD duplicates) will be collected in accordance with standards identified in Subsection 2.3.2 and in various sections of the QAPP.

4.3.3 Sample Processing and Preservation

Processing and preservation of samples will be in accordance with Subsection 3.2 and in various sections of the QAPP.

4.3.4 Training

All field work will be directed by senior field scientists with considerable experience in small mammal trapping techniques. Supporting staff will have some experience in these types of surveys but will be trained, as needed, with respect to methodology by the senior scientists.

4.4 Sample Analysis

4.4.1 Tissue Samples

Samples will be analyzed according to the analytical methods and detection limits specified in Appendix C of the project QAPP.

4.4.2 Physical/Chemistry Samples

Physical and chemical samples were collected for use in this study in order to select study sites. These samples were collected, preserved, and analyzed in accordance with the project QAPP.

4.5 Data Analysis and Reporting

The study findings will be included in the ecological risk assessment including all pertinent data, analyses, and interpretations, particularly within the PCB fate and effects component of the modeling effort. Rare species, if found, will be reported on MNHESP Rare Animal Reporting Forms. Statistical analysis will be preformed using the *Statistica*^{TM} software package (Steiger, 1999).

5.0 Equipment List

5.1 Field

Equipment that will be needed as part of the field component of the study includes:

- Camera
- Pit traps
- Field notebooks
- Snap traps
- Binoculars
- Hand lens
- Rubber knee and hip boots
- Nitrile gloves
- Resealable plastic bags
- Peanut butter
- Flagging and pin flags
- Permanent markers

5.2 Processing Area

- Microscope slides
- Dissecting scope with light
- Dissecting kit (scissors, forceps, pins)
- Petri dishes
- Razor blades or scalpel
- Rinse bottle
- Dissecting trays
- Labels, permanent markers, resealable plastic bags, paper towels
- Coolers, ice
- Aluminum foil
- Nitric acid, deionized water, hexane, isopropyl alcohol
- Taxonomy reference books

- Garbage bags
- Disposable gloves
- Work table

6.0 Results

Numbers of small mammals captured per site per species will be compared between sites and to numbers reported in the literature. Site-specific tissue concentrations from small mammals from each trapping site will be compared between sites and with residue effect levels from the literature. Numbers of placental scars observed will be compared between trapping sites and with those present in the literature. The interpretation and use of placental scar data will take into account stochasticity, differences between habitat in sampling areas, the difficulties of identifying individual placental scars and scars per litter (i.e., some small mammals can have several litters in rapid succession and separating sets of scars can be difficult [Martin et al., 1976]), and the difficulties of aging small mammals. These factors may limit the extent of use of placental scar data.

A table of mammal species known or suspected to occur in the study area per habitat type will be prepared. This table will include the species of mammals observed in the study area during this study. Maps showing the location of survey points in relation to natural community types will be produced. Morphometric data for each trapped animal will include location, species, date of capture, sex, weight, total length, hind foot length, ear length, and miscellaneous comments. Data forms (Natural Community Survey Data Forms, Small Mammal Data Forms) will be appended to draft and final narrative reports. The reports will describe the methods used to survey mammals and the results of surveys. This information will be in the ecological characterization report, and will be used in the Ecological Risk Assessment.

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APPENDIX A.26

DIETARY EXPOSURE OF MINK TO FISH FROM THE HOUSATONIC RIVER: EFFECTS ON REPRODUCTION AND SURVIVAL (AULERICH, BURSIAN, YAMINI, AND TILLITT)

APPENDIX A.26

DIETARY EXPOSURE OF MINK TO FISH FROM THE HOUSATONIC RIVER: EFFECTS ON REPRODUCTION AND SURVIVAL

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1. INTRODUCTION

During the last two decades, there has been considerable concern regarding the presence of environmental contaminants, especially polychlorinated biphenyls (PCBs) and, to a lesser extent, other contaminants, such as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), in the biota and sediments of the Housatonic River that flows through western Massachusetts and Connecticut into Long Island Sound. PCB concentrations as high as 200 ppm have been reported in sediments and fish taken downstream from a point source of PCBs located on the East Branch of the Housatonic River at Pittsfield, Massachusetts (Smith and Coles, 1997).

Because consumption of fish containing elevated levels of PCBs might pose a serious health risk to humans, parts of the Housatonic River were closed to all but catch and release fishing in 1982 (EPA, 1999). A similar concern has been raised for piscivorous wildlife that inhabit the margins of that river. The Housatonic River flows through habitat that has historically sustained viable populations of piscivorous species, such as mink. Recent field studies have failed to observe these species at expected frequencies, either directly or by sign, in suitable habitat (Woodlot Alternatives, 1999) along the more highly contaminated sections of the river, while viable populations inhabit nearby reference areas, suggesting that PCBs potentially have an adverse effect on these species. Thus, this study will evaluate whether farm-raised mink fed diets containing PCB-contaminated fish from the Housatonic River will exhibit impaired reproductive performance and/or offspring (kit) growth and survival.

Mink (*Mustela vison*) is the species of choice for testing this hypothesis because: (1) they are a semiaquatic piscivorous species native to the area; (2) they are among the most sensitive mammalian species to PCBs (Aulerich and Ringer, 1977) and PCDDs (Hochstein et al., 1988, 1998); (3) their nutritional requirements are well documented (National Research Council, 1982); (4) stock of known genetic origin is readily available; (5) all stages of their life cycle can be successfully perpetuated in the laboratory; and (6) mink have a large biological database (Shump et al., 1976; Scientifur, 1987, 1992; Sundqvist, 1989; Aulerich et al., 1999).

The Michigan State University (MSU) Experimental Fur Farm has the facilities and experienced personnel to conduct mink toxicity studies. MSU researchers have developed guidelines for mink toxicity tests (Ringer et al., 1991) and have conducted numerous mink studies involving environmental contaminants similar to the proposed study (Heaton et al., 1995a, 1995b; Restum et al., 1998; Halbrook et al., 1999).

2. STUDY DESIGN AND METHODS

2.1 COLLECTION OF FISH

Fish will be collected from the Housatonic River from New Lenox Road to Woods Pond, which have good habitat for mink. Collection and transport of fish will be handled by the U.S. Fish and Wildlife Service (USFWS), Laconia, NH, and Roy F. Weston, Inc. (WESTON®) and in general

will follow fish shipping and handling procedures presented in Appendix A.20 of this Work Plan. When fish arrive at the MSU Experimental Fur Farm, East Lansing, MI, they will be identified, sorted, and weighed by species. All fish from each site or source will be ground and blended into a homogeneous mixture. Six "grab" samples (300 to 500 g each) of the homogenized fish tissue mixture will be collected randomly, labeled, and frozen for subsequent analysis for organochlorines (OCs), PCBs, PCDDs, PCDFs, and potentially toxic or bioaccumulative inorganics. Ocean fish will be processed and analyzed in a similar manner. Results of these analyses will determine the proportions of Housatonic River and ocean fish to be incorporated into the experimental mink diets to achieve the desired doses of contaminants in each treatment.

2.2 DIETARY TREATMENTS

The diets will be conventional mink diets formulated to meet the nutrient requirements of mink (National Research Council, 1982) as described by Ringer et al. (1991; Attachment 1). There will be six dietary treatments, each containing the same percentage of fish (for example, 30%). The control diet will contain 30% "clean" ocean fish. The remaining five diets will contain a mixture of ocean fish and the homogenized fish from the test site(s) such that targeted concentrations of total PCBs in the five treatment diets composed of fish from the test site(s) will be 0.25, 0.50, 1.0, 2.0, or 4.0 ppm (Table 1). Reproductive impairment has been reported in mink fed diets containing PCB concentrations lower than 4.0 ppm (Heaton et al., 1995a; Restum et al., 1998); however, it should be noted that the congener makeup and non-PCB chemical composition of fish used in those studies differs from fish collected from the Housatonic River.

2.3 PREPARATION OF DIETS

It is anticipated that dietary treatments will be prepared two or three times during the trial. Procedures for sampling and analysis will be identical for each batch of feed mixed. After thorough mixing of the dietary ingredients for 30 minutes, three random "grab" samples from each dietary treatment will be collected and frozen for subsequent chemical contaminant analysis and an additional sample from each dietary treatment will be collected for nutrient (proximate) analysis. Feed will be placed in sealed containers and stored frozen as described by Ringer et al. (1991). Food will be thawed slowly at room temperature within coolers, or if conditions require, under mild heat suspended above the material to be thawed both for feeding and for analysis. Each feed sample submitted for nutrient analysis will be analyzed for the following:

- Moisture
- Dry matter
- Fat
- Crude protein
- Crude fiber
- Ash
- Total digestible nutrients
- Ca, K, Mn, Mg, Fe, Na, Cu, Zn, P

Table 1

Approximate Quantity of Fish Required for Test Assuming Test Site (New Lenox Road to Woods Pond) Fish Contain 150 ppm PCBs

Type of fish	Control	0.25 ppm PCBs	0.50 ppm PCBs	1.0 ppm PCBs	2.0 ppm PCBs	4.0 ppm PCBs	Total fish (30% of diet)
Ocean fish							
kg	270	269	267	264	258	244	1572
lb	594	591	587	581	568	537	3458
Test site fish							
kg	0	1.49	2.97	5.94	11.9	23.8	46
lb	0	3.27	6.53	13.1	26.1	52.3	101

All nutrient analysis will follow standard proximate analyses SOPs presented in the Final Quality Assurance Project Plan (QAPP) (WESTON, 2000). Six random "grab" samples of feed (one from each dietary treatment) will also be analyzed for organochlorines (OCs), PCBs, PCDDs, PCDFs, and potentially toxic and bioaccumulative metals each time a batch of feed is mixed. Three samples of drinking water (one taken during the acclimation period, the study midpoint, and the end of the study) will be analyzed for OCs, PCBs, PCDDs, PCDFs, and potentially toxic and bioaccumulative metals.

Because the fish species used in the diets are known to contain thiaminase, supplemental thiamine will be provided to prevent Chastek's Paralysis (National Research Council, 1982).

2.4 ANIMALS

There will be 12 uniquely identified, first-year (virgin), natural dark, female mink (*Mustela vison*) from the MSU Experimental Fur Farm herd randomly assigned to each dietary treatment, except that litter mates will not be placed in the same treatment group to minimize genetic predisposition to PCB toxicity. If randomization results in any one treatment group being significantly larger (on a mass basis), then additional randomization within groups prior to treatment will be conducted until group masses are comparable. This procedure will ensure that any effects potentially observed are not attributable to treatment group mass differences. Male mink (untreated) will be used for breeding purposes only. All mink will have been immunized against canine distemper, viral enteritis, hemorrhagic pneumonia, and botulism.

2.5 MINK FACILITIES

Mink will be caged individually in an open-sided shed in a manner described by Ringer et al. (1991) that exceeds guidelines specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 1995). As such, mink will be exposed to ambient conditions, which, based on experience, yield superior reproductive performance compared to raising mink in a more controlled indoor environment.

2.6 ACCLIMATION PERIOD

The mink will be acclimated for 7 days prior to the initiation of the definitive trial as described in Ringer et al. (1991). They will be weighed at the beginning of the acclimation period and an attempt will be made to determine food consumption at the end of the acclimation period as described by Ringer et al. (1991), if weather permits.

2.7 DEFINITIVE TRIAL

Three females from the breeding stock will be sacrificed and livers will be analyzed for OCs, PCBs, PCDDs, PCDFs, and potentially toxic and bioaccumulative metals.

After the 7-day acclimation period, the definitive test will begin on or around 1 January 2000, which is 8 weeks prior to the initiation of breeding. Test diets will be fed daily for approximately

150 days throughout the pre-breeding, breeding (March 1 to March 21), gestation, parturition (April 21 to May 15), lactation, and weaning (June 15 to July 1) periods, at which time 12 adult females and 6 kits randomly selected from each treatment will be euthanized by asphyxiation (CO_2) and necropsied for analysis. Twelve kits from each treatment group will be maintained on their respective diets through November to assess possible effects of PCBs on developmental parameters. Although Aleutian disease has not been observed in the MSU mink breeding stock over the last several years, during the necropsy stage of the study, all individuals will be examined for histopathological abnormalities typically associated with this disease. Should any individual mink be diagnosed with Aleutian disease, it and all its associated data will be removed from the study analysis.

Husbandry and experimental procedures during the pre-breeding through lactation periods are as described in Ringer et al. (1991). These will include daily observation of mink and determination of body weights every 2 weeks and feed consumption weekly. Feed consumption will be assessed on a weekly basis by measuring food consumption for 2 days during this period. Any adult mink that loses greater than 30% of its initial (acclimation) body weight will be euthanized by asphyxiation (CO_2) and necropsied, as recommended by Michigan State University's All University Committee on Animal Use and Care. Individuals removed from the study for weight loss reasons will not be included in the subsequent analyses. Breeding of treated females with untreated males will begin on or around 1 March 2000 and will follow procedures outlined in Ringer et al. (1991). A ratio of 1 male for every 4 females will be used. To ensure that successful mating has occurred, each adult female mink will have a vaginal aspiration performed after copulation to confirm the presence of sperm and sperm motility. If any male is unsuccessful in inseminating the females, the male will be replaced with another from the herd. Attempts will be made to ensure that females will have two or more confirmed matings during the breeding period. Determination of body weights and feed consumption will be discontinued at the initiation of breeding. All other procedures related to breeding, gestation, parturition, and lactation are as described in Ringer et al. (1991). Kits will be weighed within 24 hours post-partum and at 3 and 6 weeks of age.

When the last litter whelped is 6 weeks old, the adult females and six kits (non-litter mates) from each treatment will be euthanized by asphyxiation (CO_2) and necropsied. Organs (brain, liver, kidneys, spleen, heart, thyroid gland, and adrenal glands) will be removed and weighed. Samples of organs will be stored in a 10% formalin-saline solution for subsequent histological examination. Additionally, liver samples from six adults and six kits per treatment will be frozen for subsequent contaminant and cytochrome P450 analysis by the Columbia Environmental Research Center (CERC). The P450 analysis will provide an additional line of evidence in the conformation of exposure to the dioxin-like compounds. All collected materials will be appropriately labeled (type of tissue, identification of the individual animal the tissue came from, date of collection, and project identification). As previously discussed, water provided *ad libitum* throughout the study will be sampled at the beginning, midpoint, and end of the study, each water sample will be analyzed for organochlorines, PCBs, PCDDs, PCDFs, and potentially toxic and bioaccumulative inorganics.

Twelve kits from each treatment group will be maintained on their respective diets through November. These kits will be immunized against canine distemper, viral enteritis, hemorrhagic pneumonia, and botulism at 10 weeks of age. Body weights will be determined every 4 weeks.

At the end of the growth period in November, six kits from each of the six treatment groups will be euthanized by asphyxiation (CO_2) and necropsied with tissues being handled as described above. Any mink (except unweaned kits) that die during the trial period will be submitted to MSU's Animal Health Diagnostic Laboratory for necropsy by a veterinary pathologist.

3. CHEMICAL ANALYSIS

Congener-specific PCB analysis for determination of PCBs will proceed as follows. Sample preparation and analysis will generally follow the methods described by Schwartz and Stalling (1991). Samples will be homogenized in a blender. A 5-g portion from each sample will be dried with 20 g of anhydrous sodium sulfate (Merck, USA, 99%) and ground. The samples will be homogenized with sodium sulfate and column-extracted with CH_2Cl_2. A portion of each sample will be used to gravimetrically determine the lipid content and the remainder of each extract will then be treated by two stages of reactive column cleanup, followed by high-performance gel permeation chromatography. PCB congeners will be analyzed with a Hewlett-Packard 5890A Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector (ECD) and a Hewlett-Packard 7673 autosampler. The detector temperature will be 330 °C and the injector set to follow the oven temperature. Injections will be 1 µL cool on-column onto a 30-m by 0.25-mm by 0.25-µm DB-5 capillary column (J & W, Folsom, CA, USA) with a 1-m by 0.53-mm deactivated retention gap connected to the column, with H_2 carrier gas maintained at 12 psig, linear velocity 60 cm/s. The oven temperature program will be as follows: 60°C, 10°C/min to 120°C, 2°C/min to 240°C, and then 10°C/min to 320°C with a 5-minute hold. Data will be collected with PC-based PE Nelson chromatography software (Perkin Elmer, Norwalk, CT, USA). Quantitation of approximately 100 PCB congeners will be based on an internal standard calibration.

Characterization of the fish, diets, adult livers, and kit livers will include chemical measurements of the OC pesticides, PCBs, PCDFs, PCDDs, and potentially toxic and bioaccumulative inorganics found in the extracts (Feltz et al., 1995; Peterman et al., 1996). The determination of the exact amounts of dioxin-like chemicals in the samples will proceed as follows. All samples analyzed for organics will be homogenized, dried with sodium sulfate, spiked with the appropriate standards and column-extraction with methylene chloride (Feltz et al., 1995). All of the concentrated extracts will then be treated by a two-stage reactive cleanup, using first a sulfuric acid silica gel/potassium silicate column, and second, a column of sulfuric acid silica gel/potassium silicate/silica gel. High-pressure gel permeation chromatography (HP-GPC) cleanup will follow to remove residual lipids (Feltz et al., 1995). All samples analyzed for inorganics will follow procedures presented in the QAPP (WESTON, 2000).

Aliquots of the final extracts will be fractionated using high-performance porous graphitic carbon chromatography (HP-PGC) into fractions containing: 1) bulk through mono-*ortho* chlorine substituted PCB congeners, 2) non-*ortho* chlorine substituted congeners, and 3) 2,3,7,8-PCDDs and PCDFs according to the procedures in Echols et al. (1997). The instrumental analysis for the determination of the congener-specific PCBs (fraction 1) will be as described above by GC/ECD. Non-ortho PCBs (fraction 2) will be analyzed by gas chromatography/high-resolution mass spectrometry (GC/HRMS) (Peterman et al., 1996). Finally, PCDD/PCDFs (fraction 3) will be eluted through basic alumina (according to ECRC SOP C5.152) for removal

of potential co-contaminants such as polychlorinated diphenyl ethers (PCDEs) and residual polychlorinated naphthalenes (PCNs) and PCBs. The instrumental internal standard, ^{13}C-labeled 1,2,3,4-PCDD, will be added to each semiconical autosampler vial prior to transferring the PCDDs/PCDFs (fraction 3). PCDFs and PCDDs will be determined by GC/HRMS by monitoring five sequential mass windows of selected ions during the chromatographic separation (according to ECRC SOP C5.183 and Peterman et al., 1996). GC/HRMS analysis will be performed using a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high-resolution mass spectrometer. An HP 7673 autosampler will be used to introduce 2 of 25 µL of the enriched extract from a conical vial through a spiral uniliner onto a 5-m by 320-µm deactivated fused silica retention gap via a heated (285°C) direct inlet. The analytes of interest will be separated on a 50-m by 200-µm by 0.11-µm Ultra-2 (Hewlett Packard) capillary column with an initial hold of 1 min at 120 °C followed by a ramp to 200°C at 20°C/min, another ramp to 300°C at 2.3°C/min, and a final hold of 5 min. The He carrier gas is maintained at 44 psig with an initial linear velocity of 25 cm/s. All column-to-column connections are made using fused silica press-tight connectors.

The VG GC/HRMS system is tuned to 10,000 R.P. and calibrated using perfluoro-tetradecahydro-phenanthrene, and mass windows are established for five ion groups to measure Cl_{4-8} PCDFs and PCDDs. These windows are monitored sequentially during the temperature program. Within each mass window, the two most abundant ions are measured for positive identification and quantitation of each analyte. The ion responses are quantitated and averaged, unless interferences occur. Within each mass window, additional ions monitor any responses from Cl_{5-9}-PCDEs, Cl_{5-7}-terphenyls, Cl_{6-7}-PCNs, Cl_{3-8} dibenzothiophenes, and Cl_{3-8} phenanthrene/anthracenes.

Determination of non-*ortho* PCBs (planar PCBs) in fraction 2 above is conducted by GC/HRMS analysis and performed with a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high-resolution mass spectrometer. An HP 7673 autosampler is used to introduce 2 µL of the enriched extract from a conical vial onto a 2.5-m by 530-µm deactivated fused silica retention gap via a cool on-column injection technique. A 50-m by 200-µm by 0.11-µm Ultra-1 capillary column (Hewlett-Packard's equivalent to DB-1) is used to resolve most non-*ortho*-PCBs from interferences. The GC oven is held at 120°C for 1 min, programmed to 240°C at 2.2°C/min, then ramped to 310°C at 5°C/min, for a final hold of 5 min. Helium carrier gas is maintained at 48 psig with an initial linear velocity of 25 cm/s. The analytical column is put into the MS interface and heated at 310°C. All column-to-column connections are made using fused silica press-tight connectors.

The VG GC/HRMS system is tuned to 10,000 R.P. and calibrated using perfluorodecalin, and mass windows are established for two groups of non-*ortho*-PCBs. Group 1 from 23-48:00 min included ions for Cl_4-biphenyls 77 and 81 and Cl_5-biphenyl 126; Group 2 from 48:05-65 min included ions for Cl_6-biphenyl 169. Within each mass window, the two most abundant ions are measured for positive identification and quantitation of each analyte. The ion responses are quantitated and averaged, unless interferences occur. Within each mass window, additional ions monitor the responses of higher chlorinated, potentially interfering PCB congeners, Cl_{4-8} naphthalenes (PCNs), Cl_{3-5} terphenyls (PCTs), Br_5- and Cl_6-diphenyl ethers, and Cl_4-PCDF (to ensure no breakthrough of PCDFs).

The amount of each analyte detected is inherently self-corrected for losses through the whole analysis (extraction, isolation of analytes, and instrumental analysis). A calibration curve describing the response of each native congener to that of an isotope-labeled congener is used directly in the calculations and its range of values is determined in the calibration procedure. Concentrations of the native PCB congeners in standards ranged from 0.25 to 2,500 pg/µL. Each calibration curve is specifically matched to the range of analyte responses in the sample set. All water sample analyses will follow procedures presented in the QAPP (WESTON, 2000). Dietary treatment samples will also be shipped to Litchfield Analytical Services, Litchfield, MI, for subsequent nutritional analysis (see Subsection 2.3). All nutritional analyses will follow standard analytical procedures documented in SOPs provided by Brookside Laboratory (under contract to Litchfield Analytical Services) and presented in the QAPP (WESTON, 2000).

4. BIOCHEMICAL ANALYSIS

Samples of mink livers will be flash frozen and stored in liquid nitrogen or in an ultracold freezer at -80°C until they are to be sent to the CERC for analysis. Upon arrival at the CERC, the mink liver samples will be stored in an ultracold freezer at -80°C until they are analyzed for CYP1A or CYP2B activity. The biochemical assays of CYP1A and CYP2B activity will follow previous methods (Burke and Mayer, 1975; Burke et al. , 1985; Lubet et al., 1985; Ullrich and Weber, 1972). Briefly, portions of the frozen livers will be broken off, thawed, and homogenized and microsomes will be prepared from the homogenates by differential centrifugation. Optimal assay conditions will be determined for the assays of ethoxyresorufin *O*-deethylase (EROD), ethoxycoumarin *O*-deethylase (ECOD), pentoxyresorufin *O*-deethylase (PROD), and benzyloxyresorufin *O*-deethylase (BROD). Microsomes will be prepared on the same day the catalytic activity is assayed. Protein will be measured using the fluorescamine-based assay (Undenfriend et al., 1972; Bohlen et al., 1973) calibrated against bovine serum albumin (BSA). Triplicate assays will be performed, if sample volumes allow.

All biochemical analysis conducted by CERC will follow procedures and SOPs identified in Section 8 of this study plan.

5. SUMMARY OF ENDPOINTS

Adult body weights:	At beginning of the acclimation period; at beginning of the definitive trial; every other week thereafter until initiation of breeding; at whelping; at necropsy (Ringer et al., 1991)
Adult feed consumption:	During the acclimation period; weekly during the definitive trial (if temperature above 0°C) until initiation of breeding (Ringer et al., 1991)
Number of females mated:	(Ringer et al., 1991)
Length of gestation:	(Ringer et al., 1991)
Number of females whelping/	(Ringer et al., 1991)

not whelping:		
Total newborn/female whelped:	(Ringer et al., 1991)	
Live newborn/female whelped:	(Ringer et al., 1991)	
Average kit birth weight:	(Ringer et al., 1991)	
Average litter weight:	(Ringer et al., 1991)	
Percent kit survival to 3 weeks:	(Ringer et al., 1991)	
Average 3-week body weight:	(Ringer et al., 1991)	
Percent kit survival to 6 weeks:	(Ringer et al., 1991)	
Average 6-week body weight:	(Ringer et al., 1991)	
Average adult and 6-week kit organ weights:	(Heaton et al., 1995a)	
Histopathology of adult and 6-week-old kit organs:	(Heaton et al., 1995b)	
Total PCB and congener and planar PCB, PCDD, and PCDF analyses of adult and 6-week-old kit livers:	(Tillitt et al., 1996)	
Cytochrome P450 enzyme analysis of adult and 6-week-old kit livers:	(Burke and Mayer, 1975; Burke et al. , 1985, Lubet et al., 1985)	
Average body weight (monthly) of 7-month-old kits:	(Heaton et al., 1995a)	
Average organ weights of 7-month-old kits:	(Heaton et al., 1995a)	
Histopathology of 7-month-old kit organs:	(Heaton et al., 1995b)	
Total PCB and congener and planar PCB, TCDD, and PCDF concentrations in livers of 6-week-old and 7-month-old kits:	(Tillitt et al., 1996)	
Cytochrome P450 enzyme concentration in livers of 7-month-old kits:	(Burke and Mayer, 1975; Burke et al., 1985; Lubet et al., 1985; Ullrich and Weber, 1972)	

6. STATISTICAL ANALYSIS

The data generated in this study will be evaluated by analysis of variance or by contingency tables. Significant differences will be tested by Dunnett's method for comparison with a control or by Bonferroni's Chi square test (as described by Ringer et al., 1991).

7. QUALITY ASSURANCE/QUALITY CONTROL

All work will be conducted in compliance with the project QAPP (WESTON, 2000) and in compliance with the study-specific QA plan as discussed below.

The objectives of the quality assurance (QA) plan for the proposed study are: 1) to ensure that the analytical measurements, biological/toxicological assays, and biochemical analyses are accurate and precise; and 2) to ensure that the mink reproductive toxicity tests are conducted according to protocols and SOPs of the MSU Experimental Fur Farm, and in accordance with animal use and care requirements of MSU's Department of Animal Science and All University Committee on Animal Use and Care. The general protocol includes replication of various stages, comparison and calibration against known standards, proper maintenance and calibration of equipment, accurate sample tracking and custody, proper documentation at all steps of sample processing, and other considerations of Good Laboratory Practice (GLP).

7.1 DATA QUALITY OBJECTIVES

The data quality objectives for the mink dietary exposure study are directly linked to endpoints presented in Section 7 and study objectives discussed in Section 1. In summary, the measurement endpoints in the study will be evaluated to determine if the assessment endpoints of survival, reproduction, or development of mink are being impacted by dietary exposure to PCBs. In addition, as part of the overall Housatonic River Project, the mink dietary exposure study must support and complement applicable data quality objectives established in Subsection 4.1 of the QAPP (WESTON, 2000). To achieve these objectives, the following types of data will be required:

- Reproduction, growth, and survival data for controls and treatment groups.
- Dietary exposure chemistry.
- Mink liver chemistry.
- Biomarker and pathological evaluations.

The data developed as part of the mink dietary exposure study must achieve acceptable standards of accuracy, completeness, representativeness, and comparability. The purpose of this section of the study plan is to further document the measures being taken to ensure that these standards are met.

7.2 DATA QUALITY INDICATORS

Data developed in the mink dietary study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, as defined in Section 15 of the QAPP (WESTON, 2000). Each of these data quality indicators, some of which are not readily quantifiable, are discussed below with specific reference to the mink dietary study.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristic. Rather than control and measure precision, the investigator increases the number of replicates to obtain sufficient statistical resolution; for this study the several replicates (12) per treatment group is used in this manner. For the measurements that are not unique to the mink dietary study, such as tissue chemistry, biomarkers, and water chemistry, precision is evaluated as defined in appropriate SOPs presented the QAPP (WESTON, 2000).

Accuracy is defined as the agreement of a measurement with its true value. For the parameters unique to this study (tissue weights, reproductive effects, and pathology), accuracy is defined as meaning that tissue are correctly weighed, and reproductive effects and tissue pathology were correctly assessed. The data generated by this study may be evaluated for accuracy via comparison with reference organisms, and results observed in similar dietary studies. For parameters such as tissue and water chemistry, biomarker levels, and dietary nutrient content, accuracy is as defined in the QAPP (WESTON, 2000).

Completeness is defined as the percentage of the planned samples actually evaluated and processed. Completeness can be evaluated for all components of the mink dietary study. To ensure that the desired statistical resolution is achieved, it is important that a high level of completeness be achieved for all components of this study. Mink toxicity studies have been conducted by the Department of Animal Sciences at MSU for over 20 years. During this time no studies have been discontinued or significantly impacted by non-treatment-related mortalities or sample exclusions (e.g., >30% weight loss) to such a degree that the remaining data were deemed incomplete or unacceptable for use in accessing treatment-related effects. The current statistical design of this study (i.e., 12 replicates per treatment) is adequate to account for typical non-treatment-related losses while still maintaining sufficient sample size required for a high level of data completeness.

Representativeness refers to the degree to which the data accurately reflect the effects that would be observed if a wild mink would ingest a similar diet. This data quality indicator is addressed through implementation of proper experimental design and sample processing methods and may be evaluated via comparison with expected results.

Comparability is a measure of the confidence with which the study data may be compared to another similar data set. Comparability may be evaluated for this data set through comparison with previous mink dietary studies with similar contamination levels.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is largely not applicable to the biological parameters. The detection limits for chemistry, biomarkers, and nutritional parameters specified in the QAPP or associated SOP, in conjunction with reproductive and pathological effects, will provide more

than sufficient sensitivity for the purpose of providing insight into factors potentially impacting resident mink populations.

7.3 SAMPLING AND ANALYTICAL PROCEDURES

Samples of mink, diets, livers, and original fish will be taken at MSU and sent to the CERC and/or WESTON's contract lab for subsequent analysis. Samples for biochemical analysis will be stored at -80°C prior to shipment, while those for chemical analysis will be stored at -20°C prior to shipment. Samples will be shipped from MSU to CERC frozen on dry ice for biochemical or chemical analysis. Prior to receipt of any samples, forms are sent to provide exact information on the samples. These forms are the Sample Batch History Information form and the Sample Inventory Listing form (see Attachment 2). The Sample Inventory Listing is a simple list of all the samples to be transmitted and has the sample label (identifier with year, project #, study #, and personal sample ID #) and a brief description of each sample. The Sample Batch History Information form contains information on sample collection dates, how samples were collected, how they were preserved, transmission dates and modes, and other pertinent information about the samples and how they have been handled. Upon receipt of the samples, they are assigned an independent identification number for internal tracking and all of the information is computerized on a central sample tracking system. A complete description of the system is attached (see Attachment 2). This approach is analogous to the chain-of-custody requirements specified in the project QAPP (WESTON, 2000).

The methods for extraction and subsequent chemical analysis are presented in Section 3. SOPs of these methods are included in Attachment 2. The matrices for analysis include homogenates of whole fish that will be used to form the diets, time-weighted sub-samples of the actual diets, adult mink liver samples, and kit livers/tissue. The measurements to be made by the CERC include lipid analysis (CERC SOP P.461), organochlorine pesticide analysis (CERC SOP P.460), total and congener-specific PCB analysis (CERC SOP P.195), non-*ortho* PCB analysis (CERC SOP P.481), and chlorinated dioxin and furan analysis (CERC SOP P.482). Analyses will be performed by GC/ECD and/or GC/MS. Method limits of detection will be 1 to 5.0 ppt (pg/g) for dioxins and furans and <1 ppb (ng/g) for the PCB congeners. QA/QC procedures will include analyses of spiked samples with appropriate standards, analysis of replicates, analysis of procedural and matrix blanks, and the demonstration of correct chemical identifications. The general QA procedures for chemical analysis by the CERC for this project are presented in the QAPP (WESTON, 2000).

Tissue samples submitted to WESTON's contract lab for inorganic residue analysis will follow QA/QC procedures presented in the QAPP (WESTON, 2000). Nutritional analyses of dietary treatment samples will follow QA/QC procedures provided in the SOPs submitted by Brookside Laboratory, which are presented in the QAPP (WESTON, 2000).

7.4 DATA REDUCTION, VALIDATION, AND REPORTING

All experimental information is recorded in bound notebooks and signed. Copies are maintained in a separate, secured area. Instrument printouts and computerized data tables are uniquely labeled and cross-referenced to the project notebook. The accuracy of all such measurements will

be independently checked. Copies of the computerized data files are maintained in a project notebook and file, on floppy disk in the project file, and by archived tape backup.

Reporting of the data will initially be in draft form to the appropriate collaborators. After their review and approval, an internal review of the draft report will be made and a final report sent to the Project Officer. The results of this work will be coordinated with that of the other collaborators into one or more articles to be submitted to peer-reviewed journals.

7.5 SAMPLING METHODOLOGY

7.5.1 Sampling Procedures

Fish sampling in the Housatonic River will be conducted in Woods Pond and associated upstream backwater areas below New Lenox Road. Fish sampling will follow techniques and procedures presented in Appendix A.20. Carp *(Cyprinus carpio)* and goldfish *(Carassius auratus)* are the primary target fish for this sampling activity because previous fish sampling activities identified populations of sufficient size and number so that collecting these species at these locations would have minimal impact on the resident populations and could be accomplished in a time-efficient manner.

Samples will be collected by a team led by trained USFWS personnel supported by WESTON, EPA, and Woodlot Alternatives team members.

7.6 QUALITY CONTROL SAMPLES

The nature of the pathological and reproductive effects portion of the mink dietary study does not allow the incorporation of typical duplicate and blank samples as part of the study design. For effects endpoints there is no acceptable method of obtaining such samples in a manner analogous to that developed for duplicates and blanks collected for chemistry analysis; however, reference samples and evaluations will be collected from study control treatment mink.

The number of quality control samples such as duplicates and blanks for chemistry, nutrient, and biomarker analysis are presented in the QAPP (WESTON, 2000).

7.7 EQUIPMENT

All equipment used in these studies is routinely inspected and preventive maintenance performed. A logbook is kept for each instrument to document its use, performance, and maintenance.

7.8 STATISTICAL ANALYSIS OF DATA AND SAMPLING DESIGN

The statistical treatment of the data is described in Section 6 of the study plan. Routine analyses will be performed and an allowance for Type I errors will be set at 5% ($p = 0.05$). Outliers will

be determined as described by Gill (1978). Performance criteria for MLOD, MLOQ, precision, and accuracy are given above. Sampling design in general follows procedures described by Ringer et al., 1991 (Attachment 1).

7.9 QA AUDITS

Internal audits are continuously performed by the Principal Investigator and are performed quarterly by the CERC QA Officer. Quality assurance procedures outlined in the QAPP (WESTON, 2000) will be followed for any analyses conducted by WESTON's contract laboratories.

7.10 CORRECTIVE ACTION

Problems will be identified as they occur or through weekly staff meetings. Remedial actions will be taken as deemed appropriate and in accordance with the QA performance criteria. All such problems and corrective actions will be recorded in the project notebook(s) and reported to management or the Project Officer, if necessary.

7.11 TRAINING

All sampling and analyses will be directed by senior scientists with experience in the collection and shipping of samples, the analyses of tissue and diet chemistry, biomarker analysis, and the evaluation of mink reproductive endpoints or mink pathology. Supporting staff will receive training from the senior scientist(s) in overall goals of the study and in techniques to be followed to ensure collection of quality data.

8. STANDARD OPERATING PROCEDURES

Standard Operating Procedures (SOPs) and Documents of Procedure for the portion of the study conducted at the Columbia Environmental Research Center, Department of Interior, Columbia, MO are provided in the QAPP (WESTON, 2000).

Contents

SOP Number	Title of Standard Operating Procedure
SOP P.461	Extraction of Animal Tissues for Residue Analysis and Percent Lipid Determination
SOP C5.162	Sample Transmittal, Receipt, and Inventory
SOP P.123	Microsomal Preparation of Liver Tissue

SOP Number	Title of Standard Operating Procedure
SOP P.475	Calibration Check of 96 Well Microplate Absorbance and Fluorescence Readers
SOP P.124	Procedure for the determination of 7-Ethoxyresorufin-O-Deethylase (EROD) Activity in Microsomes from Liver Tissue Using 96-Well Microtiter Plates
SOP P.270	Preparation of Sulfuric Acid/Silica Gel (SA/SG)
SOP P.271	Preparation of Potassium Hydroxide-Treated Silica Gel
SOP P.193	Alumina Cleanup of PCDD/PCDF Fractions from HPLC-Carbon
SOP P.186	Tissue Analysis for PCBs and Low-Level Planar Halogenated Hydrocarbons
SOP P.460	Organochlorine Pesticide Analysis: Fractionation of Complex Mixtures of Silica Gel/ODS
SOP P.482	Analysis of Tetra- through Octa-Substituted Polychlorinated Dibenzo-p-dioxins and Dibenzofurans by Gas Chromatography-High Resolution Mass Spectrometry
SOP P.481	Analysis of Selected Non-O-Chloro-Substituted Polychlorinated Biphenyls by Gas Chromatography-High Resolution Mass Spectrometry
SOP P.195	Capillary Gas Chromatography with Electron Capture Detection Procedure for Congener Specific Polychlorinated Biphenyl Analysis
General Analytical	Minimum Quality Assurance Standards for Trace Organic Residue Chemistry QA Analysis

9. LITERATURE CITED

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ATTACHMENT 1

MAMMALIAN WILDLIFE (MINK AND FERRET) TOXICITY TEST PROTOCOLS

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MAMMALIAN WILDLIFE (MINK AND FERRET) TOXICITY TEST PROTOCOLS (LC50, Reproduction, and Secondary Toxicity)

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16. ABSTRACT

Protocols describing guidelines for conducting dietary LC_{50} and reproduction toxicity tests and for assessing the primary versus secondary toxicity of a test substance using carnivorous mammalian wildlife, specifically mink (Mustela vison) or European ferrets (Mustela putorius furo) are presented. These protocols go beyond the procedural steps and include the rationale behind each recommendation. Appropriate statistical procedures for assessing the dat are presented for each protocol.

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ABSTRACT

Protocols describing guidelines for conducting dietary LC_{50} and reproduction toxicity tests and for assessing the primary versus secondary toxicity of a test substance using carnivorous mammalian wildlife, specifically mink (Mustela vison) or European ferrets (Mustela putorius furo) are presented. These protocols go beyond the procedural steps and include the rationale behind each recommendation. In the LC_{50} test, test species are fed diets that contain several concentrations of a test substance for 28 days in which signs of toxicity and mortality are recorded and toxicity is expressed as the mean lethal concentration of the test substance. The reproduction protocol contains guidelines for determining the reproductive toxicity of a test substance administered to males and females at several concentrations in their daily diet prior to and during the breeding period and through gestation and lactation. Adverse effects on adult survival, oogenesis and/or spermatogenesis, reproductive indices, embryo or fetal development, and offspring growth and survival are measured. In the third protocol (primary vs secondary toxicity), the toxicity and lethality (LC_{50} value) of a test substance, in the form of the parent compound, administered via the diet in several concentrations to males and females (primary toxicity test) is compared with the toxicity and lethality of the same test substance fed at identical concentrations but contained in animal tissue (prey) contaminated by previous exposure to the same parent test substance (secondary toxicity test). Appropriate statistical procedures for assessing the data are presented for each protocol.

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TABLES

FIGURE

MAMMALIAN WILDLIFE (MINK AND FERRET) DIETARY LC_{50} TEST[1]

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INTRODUCTION

The U.S. Environmental protection Agency (EPA) has been charged, under several laws, with the regulation of many toxic or hazardous substances. The key to regulation of these substances is toxicological testing of the compounds with appropriate test species. The species of choice for testing the toxicity of a compound to man and domestic animals, as specified in federal guidelines (U.S. EPA, 1975, 1978, 1982a,b), have been common laboratory animals such as rats, mice, rabbits, guinea pigs, and hamsters. No representative mammalian wildlife species have been designated in the federal guidelines. Adequacy of risk assessment for wildlife mammals has normally been based on toxicity data for evaluating hazards to humans and domestic animals (U.S. EPA, 1978; Ringer, 1988).

Under special circumstances of hazard assessment, such as secondary toxicity poisoning or evaluation of certain chemicals that bioconcentrate in the food chain, special tests involving wildlife mammals may be required. Carnivorous animals are generally the species of choice in toxicological testing for secondary poisoning (Schitoskey, 1975; Townsend et al., 1984; Aulerich et al., 1986, 1987) and represent the top of the food chain for chemicals that bioconcentrate.

Prompted by these concerns, the mink (Mustela vison) (U.S. EPA, 1975; Aulerich and Bleavins, 1981) and European ferret (Mustela purotius furo) (Thornton et al., 1979; Hoar, 1984) were suggested as representative mammalian carnivores for toxicological testing. The mink may be preferred because it is indigenous to the U.S. and Canada and occurs in the wild throughout much of North America. Its nutritional requirements are well known (National Research Council, 1982), stock of known genetic origin is available, all stages of its life cycle can be successfully perpetuated in the laboratory, and it has a large biological

data base (Shump et al., 1976; Scientifur, 1987; Sundqvist, 1989). The European ferret, although not indigenous to North America, has been examined as a laboratory model for toxicology (Willis and Barrow, 1971; Thornton et al., 1979; Anon., 1981; Hoar, 1984). The mink has been shown to be among the most sensitive, if not the single most sensitive, mammalian species to polychlorinated biphenyl (PCB) toxicity (Aulerich and Ringer, 1977). Research performed with mink was instrumental in setting US water quality standards for PCBs (Aulerich and Bleavins, 1981). Subsequent studies have shown this species to be similarly sensitive to polybrominated biphenyls (Aulerich and Ringer, 1979), hexachlorobenzene (Bleavins et al., 1984), aflatoxins (Chou et al., 1976; Bonna et al., 1991), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Hochstein et al., 1988). Additional toxicological studies with mink and ferrets have been reported in the literature (Frederick and Babish, 1985; Scientifur, 1987; Sundqvist, 1989).

The following protocol for conducting dietary LC_{50} tests with mink and ferrets was developed based on procedures used in previous toxicology studies from our laboratory and refined by additional studies on four chemicals (Hornshaw et al., 1986a,b,c; 1987) providing a range of solubilities, volatilities, toxicities, and modes of action. If a measure of secondary toxicity is also needed, see protocol entitled "Mammalian Wildlife (Mink and Ferret) Dietary LC_{50} Tests to Assess Primary and Secondary Toxicity".

1. Scope

1.1. This protocol describes a method for determining the subacute dietary toxicity of a test substance (that can be mixed uniformly into the diet) administered to animals in their daily diet. Toxicity is expressed as the median lethal concentration of the test substance (LC_{50}) and the slope of the dose-response curve.

1.2. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and the European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

2. Summary

2.1. Both males and females of the same species and age are fed diets containing a test substance to determine a subacute dietary toxicity (LC_{50}). Concentrations of the test substance are administered in a geometric series of doses for 28 days to measure lethality. This exposure period may be followed by a withdrawal period during which lethality is also measured.

2.2. Daily observations for signs of toxicity and mortality are reported.

2.3. Data derived from treatment and control groups are compared statistically to detect changes in body weight, feed consumption, and an index of toxicity.

3. Significance

3.1. This protocol provides a means of measuring the toxicity of a test substance in the daily diet of a carnivore under controlled conditions. The use of a 28-day dietary exposure period allows metabolic transformations of the test substance to occur. It is recommended that, when possible, tests be conducted indoors. Indoor tests allow greater control of environmental test conditions, and therefore, greater reproducibility. Indoor facilities, if heated, allow conducting tests at any time of the year. Indoor facilities also make the possibility of escape of test animals less likely.

3.2. This protocol provides data for assessing the potential adverse effects of chemicals to mammalian carnivores exposed through dietary intake, the normal exposure route in the environment. The mammalian carnivore occupies a position high on the food chain, thus it may be subject to the

effects of bioaccumulation of chemicals.

3.3. This protocol permits collection of data on signs of toxicity in addition to mortality.

3.4. The dose-response curve provides additional information about the susceptibility of carnivores to a test substance.

3.5. This test provides a basis for deciding whether additional toxicity testing should be conducted. Results from the 28-day test may indicate the need for subsequent reproduction or chronic tests with the test species. These results might also indicate the need for other types of tests, such as aquatic, inhalation, secondary toxicity, etc., or tests designed for a target organ or organ system.

4. Definitions

4.1. LC_{50}: The calculated concentration of a test substance which causes 50 percent lethality of a test animal population under the conditions of the test.

4.2. Test Substance: The element, chemical compound, formulation, known mixture, or material mixed in diets and fed for the purpose of determining an LC_{50}.

4.3. Concentration: The weight of the test substance per unit weight of the diet (expressed as mg/kg of diet).

4.3.1. Theoretical (nominal) concentration: The targeted concentration of test substance mixed into the diet.

4.3.2. Measured concentration: The concentration of test substance in the diet determined by analysis.

4.4. Acclimination period: A period of at least 7 days immediately preceding the exposure period when the test animals are housed in the test

facilities under test conditions and fed the untreated diet and drinking water to be used during the exposure period.

4.5. Range-finding test: Test conducted on a few animals to determine the concentrations of the test substance to be used in the definitive test.

4.6. Palatability test: Test where the highest proposed dietary concentration of a test substance for use in the definitive test is fed to a few animals to determine if they will consume the diet containing this concentration of the test substance.

4.7. Definitive test: Test where a substance is fed to a prescribed number of animals at several geometrically-spaced concentrations for a specified period of time (exposure period may be followed by a withdrawal period) when parameters of toxicity, including lethality, are measured.

4.8. Exposure period: The 28-day period when the test animals are fed diets containing the test substance.

4.9. Withdrawal period: The period following an exposure period when all animals are fed an untreated diet to allow for observation of delayed mortality.

4.10. Conventional diet: Feed consisting of both fresh and dried ingredients with water added to provide a semi-solid (hamburger-like) consistency.

4.11. Dry diet: Feed consisting of only dried ingredients fed in pelleted form.

5. Precautions

5.1. Contact with all test substances, solutions, and mixed diets should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Information on other mammalian toxicity

and special handling procedures should be known before this protocol is used.

5.2. Disposal of excess test substances, solutions, mixed diets, etc., and treated animals should be done with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

5.3. Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in well-ventilated areas.

5.4. Because this protocol addresses the use of carnivores, appropriate precautions should be used in the handling of test animals. Mink are by nature extremely aggressive and may attack a handler if given the opportunity. Appropriate precautions, such as leather gloves and arm-coverings, should be used. Ferrets are normally much less aggressive than mink but precautions should also be taken when they are handled. Mink and ferrets can transmit certain diseases, most importantly tetanus, and also tuberculosis (TB) and rabies if contacted from outside sources, so researchers should take precautions for these diseases. The possibility of TB infection can be reduced by avoiding the use of pork products in the diet. Proper caging and isolation of test facilities from wild animals can similarly reduce the possibility of rabies infections.

6. Test Animals

6.1. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications, such as housing and diet.

6.2. All animals for a given test must come from one source and strain and

be of approximately the same age to minimize variability. Test animals may be obtained from commercial sources or may be reared in laboratory colonies, but they must not have been used in a previous test. It is recommended that test animals be immunized against diseases commonly afflicting the test species. Immunization for mink should include canine distemper, virus enteritis, infectious pneumonia, and botulism (Joergensen, 1985). It is recommended that ferrets be vaccinated against canine distemper and botulism (Fox, 1988). Mink and ferrets are also susceptible to Aleutian Disease (AD). Since no vaccine exists for this viral disease, testing and certification of the animals as AD-free is recommended. Animals that are deformed, injured, emaciated, or phenotypically different from normal animals must not be used as test subjects. The population of animals from which the test subjects (treated and control) are selected should be considered unsuitable for testing if mortality exceeds 5% during the acclimation period.

6.3. It is preferable to use animals that have approached their mature body size (for mink and ferrets that is about 18-20 weeks of age). Older animals can also be used to determine the LC_{50}. The use of younger animals may yield a distorted LC_{50} value because the change in body weight far exceeds the change in feed consumption resulting in a decreased amount of test substance consumed per unit of body weight over the 28-day period. Because of the sex difference in size of mink and ferrets, the two sexes should be treated as separate sub-groups.

7. Facilities

7.1. Space requirements for most carnivores have not been standardized. However, adherence to the guidelines of the Fur Farm Animal Welfare

Coalition (1988) should provide a basis for adequate space and husbandry requirements. This space requirement is currently 32,774 cu cm or 2,000 cu in. Cages measuring 61 (L) x 76 (W) x 46 (H) cm (24 x 30 x 18 in) have proven adequate for housing individual mink or ferrets for tests performed in conjunction with the development of this protocol. Cages must be constructed to prevent both cross-contamination of treatment groups and contact between individual animals. To prevent aggressive mink from attacking neighboring animals, use solid dividers between adjoining cages or provide adequate space between adjoining cages if wire mesh cage material is used throughout the cage. Species not conducive to colony rearing, such as mink, must be caged individually.

7.2. Construction materials in contact with animals should not be toxic, nor be capable of excessively adsorbing or absorbing test substances. Stainless steel, galvanized steel, or materials coated with perfluorocarbon plastics are acceptable materials.

7.3. Adequate ventilation should be provided at all times. The Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 1985) recommends 10 to 15 room air changes/hour for animal facilities.

7.4. If the animals have been reared outdoors and the test is conducted indoors, the photoperiod should simulate ambient daylight conditions because altered photoperiods may subject mink and ferrets to slight adjustments in normal body rhythms with respect to feeding, sleeping, fat mobilization or deposition, coat development, and hormonal activity. It should be noted that the effects of the length of the light/dark cycle on toxicity have not been investigated in these species. If the animals are reared indoors, the

photoperiod should not be altered.

8. Diets

8.1. Diets must be formulated in accordance with the nutrient requirements of the test species (National Research Council, 1982). Suggested ranges of composition of conventional diets for mink are shown in Table 1. Any unmedicated commercial diet that meets the minimum nutritional requirements of the test species is acceptable.

8.2. Fresh diets and water must be provided daily and fed ad libitum.

9. Diet Preparation

9.1. Knowledge of the physical, chemical, and biological properties of the test substance is important in test diet preparation.

9.2. Test diets can be prepared by mixing the test substance directly into the feed or by dissolving or suspending the test substance in an appropriate solvent or carrier prior to mixing with the feed. The use of solvents or carriers may be necessary to achieve uniformity. If solvents or carriers are used, they must also be added to the control diet in equivalent volumes. It is very important to assure uniform distribution of a test substance in the diet. In many instances, this will be more easily accomplished using a conventional diet, since many substances can be mixed into a diet more uniformly if the diet is semi-solid and capable of being machine-mixed. For some test substances, especially water soluble ones, this may be the only method of assuring uniform distribution, since pelleted diets are not conducive to being coated with aqueous solutions, but rather tend to become a mash. No matter which type of diet is used, it is recommended that innocuous solvents or carriers, such as distilled water or corn oil, be used whenever possible. (It is recommended that, unless the amount of test

substance to be added is large, solvents or carriers be used to introduce the substance to the diet to ensure uniform distribution). If an innocuous solvent or carrier cannot be found, it is recommended that a volatile solvent, such as acetone or hexane, be used to dissolve the test substance, if possible, and the resultant solution added to a small amount of either a dry diet or a dry ingredient (e.g., cereal) of a conventional diet. After the solvent is evaporated, the pre-mix can be uniformly mixed with the rest of the diet. (If this procedure is used, it must likewise be used on the control diet).

9.3. When conventional diets are used, sufficient diet should be mixed to provide adequate feed for the 28-day exposure period. The amount of feed to be mixed may be based upon the feed consumption data obtained during the acclimation period. When mixed, the diet should be stored frozen in containers large enough to hold 1 to 2 day's feed. It is important not to freeze the diets in containers too large, because the diets will not remain fresh under refrigeration for more than 2 or 3 days. In testing volatile substances, sealable containers must be used and stored upside down. The feed should fill the container allowing no head space. The amount stored in a container should be equal to one day's volume of feed. When dry diets are used, they should be stored to maintain the stability of the test substance in the diet. The frequency with which the feed is mixed is dependent upon the physical/chemical properties of the test substance.

9.4. All diets must be analyzed to determine the measured concentration of the test substance in the diet. Analysis of several samples should be conducted to determine homogeneity of the test substance in the diet.

10. Procedure

10.1. Range-finding test:

10.1.1. In most cases, LD_{50} estimates for mink or ferrets will not be available to aid in setting dietary concentrations for the LC_{50} test. Therefore, range-finding procedures can be used to save both time and animals by reducing errors or miscalculations in setting these concentrations. LD_{50} estimates for other species may be helpful in setting dietary concentrations, although, in general, mink and ferrets are more sensitive to toxic compounds that other mammals. For this reason, if LD_{50} estimates are available for other species, these values can be used as the upper limit of doses in the range-finding procedure. If range-finding is to be conducted, a geometrically-spaced series of doses (e.g., in multiples of 2 or ½) administered by gavage to 2 animals per dose can be used, in which case the approximate LD_{50} is the dose at which 1 or 2 animals die after an appropriate period of observation (often one week). It is suggested that, when administering an oral dose to mink or ferrets by gavage, a piece of plastic large enough to force the animal's mouth open, with a small hole in the center, be used. The tube can then be inserted through the opening without the animal biting it (see Figure 1). A three inch, 14 gauge, curved, stainless steel animal feeding needle can also be used to administer the test substance. If LD_{50} estimates are not available for other species, widely-spaced doses (e.g., 1, 10, 100, and 1000 mg/kg) can be administered to one animal per dose to find a lethal dose. The range-finding procedure described above can then be employed, centering on the lethal dose. If range-finding procedures yield an approximate LD_{50} value, the highest dietary

concentration should then be set to ensure that an animal will consume the equivalent of an LD_{50} dose in one day's feed. If a lethal dose is not found, the highest dietary concentration should be set at 5000 mg/kg because concentrations above this value are assumed to be nontoxic. Palatability tests should also be employed to save time and animals. It is recommended that the highest proposed dietary concentration be fed for several days to 2-4 animals to determine if they will eat the diet at this concentration of the test substance. If not, it may be necessary to reduce the highest concentration in the diet to a level that will be eaten.

10.2 Acclimation period:

10.2.1. All animals should be conditioned to the test facilities, including: photoperiod, temperature, and caging for a minimum of 7 days. A minimum of 7 days is required, but a longer period may be necessary, especially if the animals to be used in a test are changed from outdoor to indoor housing (or vice-versa) or if the diet or water to be used in a test is different from that which the animals are accustomed to consuming. During this period all animals should be given the untreated (control) diet and drinking water, as used during the definitive test. It is important to measure feed consumption during this period as these measurements can provide an indication of how much feed the animals should consume once the test starts, how much diet to mix for the test, and can also serve as a control value for each group. Test animals should be weighed at the start of the acclimation period.

10.3. Definitive test:

10.3.1. Each test animal should be randomly assigned according to weight

class to a specific test diet concentration and be uniquely identified.

10.3.2. The test diets must be fed for 28 days. For some test substances, it may be necessary to include a withdrawal period, during which the test diets are replaced with untreated feed, in order to observe prolonged or delayed toxicity. A withdrawal period is recommended when animals are still exhibiting signs of toxicity at the end of the exposure period. This period provides a more accurate estimation of the true toxicity of a test substance, especially if the substance causes delayed or cumulative injury. By observing the animals and measuring feed consumption during this period, the permanence of the injury can also be estimated. It is recommended that a withdrawal period not exceed 14 days.

10.3.3. Individual body weights must be recorded at the initiation of the definitive test and at weekly intervals thereafter, and on the day of death. Feed consumption must be measured weekly for the exposure and withdrawal periods, and should be based on a minimum of two consecutive day's feed consumption. In estimating feed consumption by mink or ferrets, several precautions are necessary. Since feed consumption can be adversely affected on a short-term basis by temperature, weather, and other factors, estimates should be based on at least two consecutive day's consumption. These days should also be days when the animals are not handled (e.g., during weighing, moving, etc.), since handling can produce a temporary reduction in feed consumption.

10.3.4. Mortality, behavioral abnormalities (lethargy, nervousness, etc.), and other signs of toxicity (unthrifty appearance, convulsions, incoordination, unusual vocalizations, etc.) should be recorded daily

during the test.

10.3.5. For tests conducted indoors, the photoperiod should be maintained at the same schedule in effect at the conclusion of the acclimation period because a changing photoperiod may subject mink and ferrets to slight adjustments in normal body rhythms with respect to feeding, sleeping, fat mobilization or deposition, coat development, and hormonal activity. It should be noted that the effects of the length of the light/dark cycle on toxicity have not been investigated in these species.

10.3.6. A minimum of eight animals for each test concentration should be used. The test concentrations should be geometrically spaced so as to result in at least 2 dietary concentrations yielding 10-90% mortality. These results usually can be obtained with 4-6 dietary concentrations, including a control. It is possible to conduct an LC_{50} test with as few as 3 dietary concentrations and 8 animals per concentration if a good estimate of the LD_{50} for the test species is available. In many instances, however, accurate results can be achieved with 5 dietary concentrations and 10 animals per concentration if a good estimate of the LD_{50} is available from range-finding procedures.

10.3.7. The prescribed length of the mammalian dietary LC_{50} test is 28 days for several reasons. A 28-day test allows time for absorption, distribution, metabolism, enzyme induction, redistribution, bioconcentration, and elimination to occur, similar to that which might occur in animals subacutely exposed to a substance via diet in the environment. A 28-day test also allows testing of slow-acting or bioaccumulating substances. Such tests could prove negative or

misleading in a test of shorter duration. For example, prolonged mortality patterns were observed in 28-day tests with mink (Table 2) and ferrets (Table 3) fed Compound 1080, in which mortalities were observed up to the end of the test (Hornshaw et al., 1986b). Delayed mortalities were observed in a 28-day test with mink fed Aroclor 1254 (Table 4), in which mortalities were observed during a 7-day withdrawal period as well as during the exposure period (Hornshaw et al., 1986c). In some instances, it may be possible to achieve satisfactory results with a test of shorter duration using higher concentrations of the test substance, but the possibility of feed rejection or avoidance becomes greater with increasing concentrations. For example, in the 1080 tests already noted, signs of feed avoidance appeared in the first week of both tests in a dose-related manner. Increasing the concentration in these tests may have resulted in nearly complete avoidance of the feed and subsequent removal of the highest dietary concentrations from the test for humane reasons (Hornshaw et al., 1986b). Also, certain substances cause delayed mortality, whether administered as a single dose or multiple dosages. Increasing the concentration of the substance does not necessarily shorten the time to death. An example of this phenomenon is seen in the Aroclor 1254 test (Table 4; Hornshaw et al., 1986c). A test should be considered invalid if more than 12.5% of the control animals die during the definitive test.

10.3.8. It is strongly recommended that a dietary concentration group be removed from testing when food consumption measurements indicate that 10% or less feed, compared to controls and/or acclimation period values, is consumed daily for the first two week's feed consumption measurements

or the animals lose 30% of their original body weight.

10.3.9. Necropsies should be performed on all mortalities. At the termination of the test, all surviving test animals should be killed by accepted humane methods (AVMA Panel on Euthanasia, 1986) and necropsies performed. It is suggested that necropsies be performed on all test animals, either on the day of death or at the termination of the test. Valuable information on the mode of action of the test substance and its effect on target organs or organ systems can sometimes be gained from gross observation of the test animals at necropsy. Histopatho-logical examination of organs and tissues can often provide more information. Weights of internal organs of control and treated animals can be compared statistically to determine effects of the substance, although the effects of starvation can sometimes be confounded with effects of the substance.

10.4. Statistical analysis:

10.4.1. Body weight changes and feed consumption may be analyzed by analysis of variance (Sokal and Rohlf, 1969) and significant differences may be located by Dunnett's method for comparison with the control (Dunnett, 1964).

10.4.2. An LC_{50} value, including confidence limits and slope of the dose-response curve may be calculated by the method of Litchfield and Wilcoxon (1949).

10.4.3. Other valid statistical procedures may also be used to analyze the data.

11. Quality Assurance

11.1. In order to assure the quality and reliability of data developed

using this protocol, testing facilities should have a quality assurance unit that is responsible for monitoring the test along with the investigator, to assure that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with good laboratory practice standards (U.S. EPA, 1989) and are legally defensible and appropriate for the intended use.

12. Reporting Requirements

12.1. All data and information required by this protocol should be recorded in black ink on appropriate forms and/or bound laboratory notebooks. A duplicate set of records should be kept at a location other than the test site. All data should be maintained in a secure location to prevent tampering or destruction of the records. The following information should be reported:

12.1.1. Name of the investigator(s), laboratory, laboratory address, location of raw data, and date of initiation and termination of test.

12.1.2. Name of species tested, including scientific name, source, and age of the animals at the beginning of the test.

12.1.3. A detailed description of the test substance including its chemical name, synonyms, structure, formulations, purity, source, batch, lot number, and physical/chemical properties and name and source of solvent or carrier, if used.

12.1.4. Description of the test facilities and housing conditions, including test cages, temperature, and photoperiod.

12.1.5. Name and source of feed, including description and proximate analysis of diet.

12.1.6. The theoretical and measured dietary concentrations; number of

animals per concentration; body weights; feed consumption; signs of toxicity; behavioral changes; % mortality for each concentration; significant necropsy findings; calculated LC_{50} values and 95% confidence limits, slope of the dose-response curve and 95% confidence limits, and the name and reference of the statistical methods used; highest dietary concentration at which no signs of toxicity were observed; anything unusual about the test; any deviations from the protocol; and other relevant information.

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MAMMALIAN WILDLIFE (MINK AND FERRET) REPRODUCTION TEST[1]

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INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has been charged, under several laws, with the regulation of many toxic or hazardous substances. The key to regulation of these substances is toxicological testing of the compounds with appropriate test species. The species of choice for testing the toxicity of a compound to man and domestic animals, as specified in federal guidelines (U.S. EPA, 1975, 1978, 1982a,b), have been common laboratory animals such as rats, mice, rabbits, guinea pigs, and hamsters. No representative mammalian wildlife species have been designated in the federal guidelines. Adequacy of risk assessment for wildlife mammals has normally been based on toxicity data for evaluating hazards to humans and domestic animals (U.S. EPA, 1978; Ringer, 1988).

Under special circumstances of hazard assessment, such as secondary toxicity poisoning or evaluation of certain chemicals that bioconcentrate in the food chain, special tests involving wildlife mammals may be required. Carnivorous animals are generally the species of choice in toxicological testing for secondary poisoning (Schitoskey, 1975; Townsend et al., 1984; Aulerich et al., 1986, 1987) and represent the top of the food chain for chemicals that bioconcentrate.

Prompted by these concerns, the mink (Mustela vison) (U.S. EPA, 1975; Aulerich and Bleavins, 1981) and European ferret (Mustela putorious furo) (Thornton et al., 1979; Hoar, 1984) were suggested as representative mammalian carnivores for toxicological testing. The mink may be preferred because it is indigenous to the U.S. and Canada and occurs in the wild throughout much of North America. Its nutritional requirements are well known (National Research Council, 1982), stock of known genetic origin is available, all stages of its life cycle can be successfully perpetuated in the laboratory, and it has a large biological

data base (Shump et al., 1976; Scientifur, 1987; Sundqvist, 1989). The European ferret, although not indigenous to North America, has been examined as a laboratory model for toxicology (Willis and Barrow, 1971; Thornton et al., 1979; Anon., 1981; Hoar, 1984). The mink has been shown to be among the most sensitive, if not the single most sensitive, mammalian species to polychlorinated biphenyl (PCB) toxicity (Aulerich and Ringer, 1977). Research performed with mink was instrumental in setting U.S. water quality standards for PCBs (Aulerich and Bleavins, 1981). Subsequent studies have shown this species to be similarly sensitive to polybrominated biphenyls (Aulerich and Ringer, 1979), hexachlorobenzene (Bleavins et al., 1984), aflatoxins (Chou et al., 1976; Bonna et al., 1991), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Hochstein et al., 1988). Additional toxicological studies with mink and ferrets have been reported in the literature (Frederick and Babish, 1985; Scientifur, 1987; Sundqvist, 1989).

The following protocol for conducting reproduction tests with mink and ferrets was based on procedures used in previous toxicology studies from our laboratory and refined by additional studies on four chemicals (Hornshaw et al., 1986a,b,c, 1987) providing a range of solubilities, volatilities, toxicities, and modes of action.

1. Scope

1.1. This protocol describes a method for determining the reproductive toxicity of a test substance (that can be mixed uniformly into the diet) administered to animals in their daily diet. Reproductive toxicity may be expressed as an adverse effect on: (a) adult survival; (b) oogenesis and/or spermatogenesis; (c) embryo or fetus development; (d) reproductive indices; or (e) offspring growth and survival.

1.2. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

2. Summary

2.1. Groups of animals of the same species and age (both sexes) are fed diets containing a test substance in a series of concentrations, plus a control, for 8 weeks prior to breeding, during breeding, gestation, and parturition, and for 3 weeks of lactation (approximately 23 weeks) to measure reproductive toxicity.

2.2. Animals are observed daily and mortalities are reported.

2.3. Data derived from treatment and control groups are compared statistically to detect changes in body weight; feed consumption; length of gestation; percent of females bearing offspring; total offspring born per female (live and dead); average birth weight of offspring; average live litter weight; average weight of offspring at 3 weeks; and percent offspring survival to 3 weeks.

3. Significance

3.1. This protocol provides a means of measuring the reproductive toxicity of a test substance in the daily diet of a carnivore under controlled

conditions. It is recommended that, if possible, tests be conducted indoors. Indoor tests allow greater control of test conditions, and therefore, greater reproducibility. Indoor facilities, if heated, allow more accurate measurements of feed consumption than outdoors, especially during sub-freezing conditions. Indoor facilities also make the possibility of escape of test animals less likely.

3.2. This protocol provides data for assessing the potential adverse effects of chemicals to mammalian carnivores exposed through dietary intake, the normal exposure route in the environment. The mammalian carnivore occupies a position high on the food chain, thus it may be subject to the effects of bioaccumulation of chemicals.

3.3. This protocol permits collection of data on signs of toxicity and mortality over an extended period of dietary exposure, such as may occur in nature.

3.4. This test provides a basis for deciding whether additional toxicity testing should be conducted. Results from a reproduction test may indicate the need for subsequent chronic tests with the test species. These results might also indicate the need for other types of tests, such as aquatic or inhalation, or tests designed for a target organ or organ system. This protocol can provide limited data on the effects of a substance on male reproductive performance. However, if such effects are noted, it would be necessary to conduct further tests employing a different experimental design than the one described in the protocol to quantify male effects.

4. Definitions

4.1. Test substances: The element, chemical compound, formulation, known mixture, or material mixed in diets and fed for the purpose of determining

reproductive toxicity.

4.2. Concentration: The weight of the test substance per unit weight of the diet (expressed as mg/kg of diet).

4.2.1. Theoretical (nominal) concentration: Targeted concentration of the test substance in the diet.

4.2.2. Measured concentration: Concentration of the test substance in the diet as determined by analysis.

4.3. Acclimation period: A period of at least 7 days immediately preceding the exposure period during which the test animals are housed in the test facilities under test conditions and fed the untreated diet and drinking water to be used during the exposure period.

4.4 Definitive test: Test where a substance is fed to a prescribed number of animals at several geometrically-spaced concentrations for a specified period of time during which parameters of toxicity are measured.

4.5. Conventional diet: Feed consisting of both fresh and dried ingredients with water added to provide a semi-solid (hamburger) consistency.

4.6. Dry diet: Feed consisting of only dried ingredients usually fed in pelleted form.

5. Precautions

5.1. Contact with all test substances, solutions, and mixed diets should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Information on other mammalian toxicity and special handling procedures should be known before this protocol is used.

5.2. Disposal of excess test substances, solutions, mixed diets, etc., and

treated animals should be done with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

5.3. Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in well-ventilated areas.

5.4. Because this protocol addresses the use of carnivores, appropriate precautions should be used in the handling of test animals, especially mink. Mink are by nature extremely aggressive and may attack a handler if given the opportunity. Appropriate precautions, such as leather gloves and arm-coverings, should be used. Mink and ferrets can transmit certain diseases, most importantly tetanus, and also tuberculosis (TB) and rabies if contacted from outside sources. Researchers should take precautions for these diseases. The possibility of TB infection can be reduced by avoiding the use of pork products in the diet. Proper caging and isolation of test facilities from wild animals can similarly reduce the possibility of rabies infections.

5.5. Mink and ferrets are known to be sensitive to handling and other disturbances during the first 2 weeks post-partum; contact and outside disturbances should be minimized during this period.

6. Test Animals

6.1. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications, such as diet, cages, etc.

6.2. All animals for a given test must come from one source and strain and be of approximately the same age to minimize variability. Test animals may

be obtained from commercial sources or may be reared in laboratory colonies, but they must not have been used in a previous test. It is recommended that test animals be immunized against diseases commonly afflicting the test species. Immunization for mink should include canine distemper, virus enteritis, infectious pneumonia, and botulism (Joergensen, 1985). It is recommended that ferrets be vaccinated against canine distemper and botulism (Fox, 1988). Mink and ferrets are also susceptible to Aleutian Disease (AD). Since no vaccine exists for this viral disease, testing and certification of the animals as AD-free is recommended. Animals that are deformed, injured, emaciated, or phenotypically different from normal animals must not be used as test subjects. The population of animals from which the test subjects (treated and control) are selected should be considered unsuitable for testing if mortality exceeds 5% during the acclimation period.

6.3. It is preferable to use animals that are proven breeders. However, availability and cost may dictate that animals in their first breeding season be used.

7. Facilities

7.1. Space requirements for most carnivores have not been standardized. However, adherence to the guidelines of the Fur Farm Animal Welfare Coalition (1988) should provide a basis for adequate space and husbandry requirements. Individual cages measuring 61 x 76 x 46 cm (24 x 30 x 18 in) and nest boxes measuring 38.1 x 27.9 x 26.7 cm (15 x 11 x 10.5 in) have proven adequate for tests performed in conjunction with the development of this protocol. Mink and ferrets must be caged individually. In designing a caging system for carnivores, it is important to prevent both cross-

contamination of treatment groups and contact between individual animals. To prevent aggressive animals from attacking neighboring animals, use solid dividers between adjoining cages or provide adequate space between adjoining cages if wire mesh cage material is used.

7.2. Construction materials in contact with animals should not be toxic, nor be capable of excessively adsorbing or absorbing test substances. Stainless steel, galvanized steel, or materials coated with perfluorocarbon plastics are acceptable materials, but other construction materials may also be useful.

7.3. A nest area or nest box containing nesting material (such as straw, shredded wood, or marsh hay) must be provided for all females prior to the parturition period. It is very important to ensure that newborn are protected from toxic compounds. A particular area of concern is wood by-products which may be contaminated with compounds to which mink are sensitive.

7.4. Adequate ventilation should be provided at all times. The Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 1985) recommends 10 to 15 room air changes/hour for animal facilities.

7.5. This protocol addresses the use of mink and ferrets during their natural breeding seasons. Photoperiodic manipulations may permit the use of this protocol at other seasons. If the animals were raised outdoors and the test is conducted indoors, the photoperiod should simulate ambient conditions appropriate to maintain the normal reproductive status throughout the acclimation period and definitive test. In order to bring mink and ferrets into breeding condition indoors, it is necessary to gradually

increase the length of photoperiod during the test. If the animals are held indoors for an extended period of time prior to the test, it is also necessary to gradually decrease the photoperiod prior to the acclimation period to provide a necessary quiescent period of sexual development for the animals. Since very low intensities of light may alter the reproductive cycle, care must be taken to ensure that total darkness is maintained during the appropriate periods. If the test is conducted outdoors, care must be taken to ensure that the photoperiod is not altered by extraneous light sources.

8. Diets

8.1. Diets must be formulated in accordance with the nutrient requirements of the test species (National Research Council, 1982). Suggested ranges of composition of conventional mink diets are shown in Table 1. Any unmedicated commercial diet that meets the minimum nutritional standards of the test species is acceptable.

8.2. Fresh diets and water must be provided daily and fed ad libitum.

9. Diet Preparation

9.1. Knowledge of the physical, chemical, and biological properties of the test substance is important in test diet preparation.

9.2. Test diets can be prepared by mixing the test substance directly into the feed or by dissolving or suspending the test substance in a solvent or carrier prior to mixing with the feed. The use of solvents or carriers may be necessary to achieve uniformity. If solvents or carriers are used, they must also be added to the control diet in an equivalent volume. It is very important to assure uniform distribution of a test substance in the diet. In many instances, this will be more easily accomplished using the

conventional diet, because many substances can be mixed into a diet more uniformly if the diet is semi-solid and capable of being machine-mixed. For some test substances, especially water soluble ones, this may be the only method of assuring uniform distribution, since pelleted diets are not conducive to being coated with aqueous solutions, but rather tend to become a mash. No matter which type of diet is used, it is recommended that innocuous solvents or carriers, such as distilled water or corn oil, be used if possible. (It is recommended that, unless the amount of test substance to be added is large, solvents or carriers be used to introduce the substance to the diet to ensure uniform distribution). If an innocuous solvent or carrier cannot be found, it is recommended that a volatile solvent, such as acetone or hexane, be used to dissolve the test substance, if possible, and the resultant solution be added to a small amount of either the dry diet or a dry ingredient (e.g., cereal) of the conventional diet. After the solvent is evaporated, the pre-mix can then be mixed with the rest of the diet uniformly. (If this procedure is used, it must likewise be used on the control diet).

9.3. If the researcher chooses to use the conventional diet, it is important not to freeze the diets in containers too large, because the diets will not remain fresh under refrigeration for more than 2-3 days. When conventional diets are used, sufficient diet should be mixed to provide adequate feed for approximately 4 weeks. The amount of feed to be mixed may be based upon the feed consumption data obtained during the acclimation period. When mixed, the diet should be stored frozen in containers large enough to hold 1-2 day's feed. In testing volatile substances, sealable containers must be used and stored upside down, one day's feed per container. The feed should

fill the container, allowing no headspace. When dry diets are used, they should be stored so as to maintain the stability of the test substance in the diet. The frequency with which the feed is mixed is dependent upon the physical/chemical properties of the test substance.

9.4. It is imperative that all diets be analyzed for the concentration of the test substance in the diet and the measured concentration reported with the test results. A significant number of samples should be analyzed to accurately determine both the concentration of test substance in each diet as well as the homogeneity of the mixture.

10. Procedure

10.1 Dietary concentrations of test substance.

10.1.1. Establishing the dietary concentrations of a test substance for a reproductive study is a difficult but essential first step in determining an environmental effect of a chemical substance upon reproduction. A number of procedures exist for establishing the dietary concentrations to be used. Three are presented in this protocol.

10.1.1.1. If a dietary LC_{50} test has been conducted with the species under consideration, the highest dietary concentration at which no signs of toxicity were observed should approximate the highest of a series of geometrically-spaced dietary concentrations, plus a control.

10.1.1.2. Another method of establishing dietary concentrations utilizes known or expected environmental concentrations of the test substance. Two or more dietary concentrations, plus a control, should be used. Examples of series of concentrations that may be used include 1X, 3X, and 5X or 1X, 3X, and 10X, where X equals the

measured environmental concentration.

10.1.1.3. If LC_{50} data are lacking, it is useful to conduct a preliminary study with several widely spaced dietary concentrations of the test substance. The dietary concentrations for the definitive test may be established from these preliminary studies. It is recommended that 3 or more dietary concentrations plus a control be tested in the definitive test if this procedure is followed. If an estimate of a dietary concentration at which signs of toxicity are not observed is lacking, it is recommended that a preliminary study be conducted to aid in establishing dietary concentrations for the definitive test. This study may be patterned after the protocol for mammalian dietary LC_{50} tests, using several widely-spaced concentrations over a short period (e.g., 7-14 days) to determine an approximate no effect concentration. Because the data from a study such as this would be expected to be fragmentary, it is suggested that at least 3 dietary concentrations be tested in the definitive test in order to maximize the possibility of meeting the criteria for an acceptable test while minimizing the possibility of wasting time, money, and animals.

10.2. Experimental design.

10.2.1. This protocol is intended for use with individually caged animals only. Males and females will be paired only during breeding attempts, and one male will be assigned to a treatment group for each 3 or 4 females. Thus, this protocol is primarily designed to test female reproductive effects, and provides only limited data on male reproductive effects. If data on male reproductive effects are desired, a

different experimental design will be necessary.

10.2.2. Relatively few background data are available to aid in determining the proper number of male and female mink and ferrets to use to detect a significant difference for a given reproductive parameter. Due to considerations of cost and availability of proven breeders, it may be necessary to use animals which have not had breeding experience. If this is the case, based on reproduction tests performed in conjunction with the development of this protocol and on other reproduction tests with mink and ferrets, it is recommended that a minimum of 12 females per treatment group be used to provide a margin of safety against females which will not accept males, are barren, or do not have proper maternal instincts (each of these reproductive anomalies will be exhibited by a small percentage of first year females within a cohort). Because the male's only function in reproduction is the mating act, it is not necessary to house equal numbers of males and females, unless male reproductive effects are expected. Thus, it is only necessary to house one male for every three or four females per dietary concentration. Again, if first year animals are used, it is suggested that the male:female ratio be 1:3, to provide a margin of safety against males which will not attempt to mate or which produce no viable spermatozoa. If proven breeders are used, it may be possible to meet the criteria for an acceptable test with as few as eight females and two males per dietary concentration. It is recommended that breeding attempts be made only between males and females within the same treatment group.

10.2.3. If this experimental design is selected, one of the following

criteria must be met:

A. One dietary concentration must produce an effect.

B. The highest dietary concentration must contain at least 1000 mg/kg.

C. The highest dietary concentration must be at least 100 times the highest known or expected environmental concentration.

If the researcher selects an experimental design based on considerations of Type I and Type II error, the number of females per treatment group may be specified by the researcher's levels of power, significance, and difference between means to be detected.

10.2.4. Each test animal should be randomly assigned to a specific test diet concentration and be uniquely identified.

10.3. Acclimation period.

10.3.1. All animals should be conditioned to the test facilities including: photoperiod, temperature, and caging for a minimum of 7 days. A longer acclimation period may be desirable especially if the animals to be used in a test are changed from outdoor to indoor housing (or vice versa) or if the diet or water to be used in a test is different from what the animals are accustomed to consuming. During this period all animals should be given the untreated (control) diet and drinking water, as used during the definitive test. Test animals should be weighed at the start of the acclimation period. It is recommended that feed consumption be measured during the latter part of the acclimation period as these measurements can provide an indication of how much feed the animals should consume once the test starts, how much diet to mix for the test, and can also serve as a control value for each group.

10.4. Definitive test.

10.4.1. The test diets must be fed daily throughout the pre-breeding, breeding, gestation, parturition, and lactation periods, a duration of approximately 20-23 weeks. The suggested length of the mammalian reproduction test of approximately 20-23 weeks is designed to conform to the normal reproductive seasons of mink (March through June) and European ferrets (April through July), with an 8 week exposure period prior to the reproductive season. The total time of the mink exposure period can be expected to be somewhat longer than the ferret exposure period because mink exhibit a variable delay in implantation of fertilized ova, while ferrets do not. Thus, the gestation period for mink can range naturally from approximately 42 to 60 days, whereas for ferrets the gestation period will normally be approximately 42 days. The length of this test allows ample time for absorption, distribution, metabolism, enzyme induction, re-distribution, bioconcentration, and elimination to occur, and for tolerance to be acquired, similar to that which might occur to animals chronically exposed to a substance in the environment.

10.4.1.1. Pre-breeding period: Individual body weights must be recorded at the initiation of the definitive test and bi-weekly (once every other week) thereafter for the 8 weeks of the pre-breeding period. Feed consumption must also be measured weekly during the pre-breeding period, and should be based on a minimum of two consecutive day's consumption because feed consumption can be adversely affected on a short-term basis by temperature, weather, and other factors. Feed consumption should be measured on days when

the animals are not handled (e.g., during weighing, moving, etc.), because handling can produce a temporary reduction in feed consumption.

10.4.1.2. Breeding period: Under natural conditions, mating attempts begin at the first of March for mink and at the end of April for ferrets. In breeding mink, a female is presented to a male and, if receptive, is allowed to mate. If not receptive, the female is removed and presented to a male approximately 4 days later. Once a successful mating occurs (as verified by the presence of viable spermatozoa in a vaginal aspiration taken just after copulation), the female is given the opportunity to mate a second time (with the same male or a different male), either 8 days after the initial mating or the next day (if the first mating occurs late in the breeding season). In breeding ferrets, females are presented to males when they are judged to be in estrus (determined by the extent of vulvar swelling) and left overnight. They are not normally given the opportunity for additional matings. If the researcher has reason to suspect male reproductive effects, vaginal aspirations may be taken for examination of spermatozoa. Generally, it is advisable to discontinue recording body weights and measuring feed consumption once the breeding attempts begin. The increased handling of the animals during the breeding period causes perturbations in the animals' daily routines, resulting in decreased feed consumption by some animals. In addition, some animals respond to increased handling by becoming excitable. Repeated breeding attempts, coupled with routine weighings, may produce some females

that are so excitable that breeding them becomes extremely difficult. Once the breeding period is over, it is best that the animals are left undisturbed as much as possible, especially during the first 2 weeks post-partum.

10.4.1.3. Gestation period: This period lasts approximately 6 weeks for ferrets and 6-8 weeks for mink. During this period, the animals should not be weighed, handled, or unduly disturbed.

10.4.1.4. Parturition period: This period lasts up to 3 weeks, depending on species. During this period females are checked daily for newborn. All newborns are counted, weighed, sexed, and recorded within 24 hours post-partum. It is suggested that in checking for newborn, care is taken not to disturb the females more than necessary. If a nest box is not employed, visual inspection often is sufficient to determine whether a litter has been born. If a nest box is employed, it may be necessary to exclude the female from the nest box while checking the nest for newborn. When the female refuses to leave the nest box, it is often an indication that parturition has occurred.

10.4.1.5. Lactation period: Individual body weights of all surviving newborn are recorded at the end of this 3 or more week period. This period should not extend beyond 6 weeks, the normal weaning time for mink and ferret offspring. During this period, offspring may come in contact with or eat (after 3 weeks) the maternal diet.

10.4.1.6. Termination: At the termination of the test, all males and at least an equal number of females chosen at random from each dietary group should be killed by accepted humane procedures (AVMA

Panel on Euthanasia, 1986) and necropsies performed. Valuable information on the mode of action of the test substance and its effect on target organs or organ systems can sometimes be gained from gross observation of the test animals at necropsy. Histopathological examination of tissues and tissue residue analyses can often provide more information. Weights of internal organs and blood parameters of controls and treated animals can also be compared statistically to determine effects of the substance.

10.4.2. General considerations.

10.4.2.1. All animals must be observed daily. All overt clinical signs and any abnormal behavior must be recorded. If mortality occurs, the date and body weight must be recorded and necropsy performed.

10.4.2.2. A test must be considered invalid if more than 20% of the control animals die during the definitive test. It is highly unlikely, based on the results of tests conducted in conjunction with the development of this protocol and on general mortality patterns observed in the fur industry, that more than 20% of a population of healthy mink or ferrets would die over the course of a 23 week reproduction test (Joergensen, 1985). If a researcher suffers the loss of greater than 20% of control animals in a test, it is possible that problems may exist in the diet or husbandry practices, or that disease has affected the stock.

10.5. Reproductive indices.

10.5.1. The reproductive indices required in this protocol were selected based on features of the reproductive performance of mink and ferrets.

Weights of all offspring (live and dead) are not required to be tested and reported because mink and ferrets are known to consume dead or stillborn young, thus, testing this reproductive index may produce incorrect or misleading results. Percent survival and weights of offspring are required at 3 weeks to allow minimal disturbance of dams and offspring during the critical period after birth and to ensure that nourishment received by offspring is almost totally of maternal origin. Percent survival and weights of offspring at 6 weeks is not required because the young usually begin consuming at least some solid feed by 4 weeks of age. As mentioned previously, mink are known to exhibit a variable delay in implantation of fertilized ova, thus the length of gestation may not be useful in assessing effects of a substance on gestation in mink. It may, however, by very useful in assessing these effects in ferrets.

10.5.2. The following reproductive indices must be calculated:

A. Length of gestation: The time, in days from the last confirmed mating until parturition.

B. Number whelped, not whelped: The number of females giving birth and not giving birth in a treatment group. Number whelped includes females that die during the process of whelping from problems associated with parturition. This value is expressed as the number of females whelped or not whelped per the number of females with confirmed matings in a treatment group.

C. Live newborn/female whelped: The average number of live newborn produced by all females that give birth in a treatment group. This value does not include females that die during the process

of whelping from problems associated with parturition.

D. Average birth weight: The average weight of all live newborn born in a treatment group, weighed to the nearest tenth of a gram within 24 hours post-partum.

E. Average litter weight: The average weight of all litters (live newborn only) born in a treatment group, weighed to the nearest tenth of a gram within 24 hours post-partum.

F. Percent newborn survival to 3 weeks: The number of live newborn in a treatment group surviving to 21 days of age, expressed as a percentage of all live newborn born in a treatment group.

G. Average 3 week body weight: The average weight of all live newborn in a treatment group, weighed to the nearest gram on the 21st day after birth.

10.5.3. The following reproductive indices may also be useful:

A. Total newborn/female whelped: The average number of all newborn (alive and dead) produced by all females that give birth in a treatment group. This value includes females that die during the process of whelping from problems associated with parturition.

B. Percent newborn survival to 6 weeks: Identical to 21 day survival, but extended to 42 days.

C. Average 6 week body weight: Identical to 21 day weights, but measured at 42 days of age.

10.6. Statistical analysis.

10.6.1. The following variables may be analyzed by analysis of variance (Sokal and Rohlf, 1969) and significant differences may be tested by

Dunnett's method for comparison with control (Dunnett, 1964):

A. Body weight changes

B. Feed consumption

C. Length of gestation

D. Live offspring/female whelped

E. Total offspring/female whelped

F. Average birth weight

G. Average litter weight

H. Average 3 week body weight

I. Average 6 week body weight

10.6.2. The following variables may be analyzed by contingency tables (Zar, 1974) and significant differences may be tested by Bonferroni's Chi-square test (Gill, 1978):

A. Number whelped, not whelped

B. Percent newborn survival to 3 weeks

C. Percent newborn survival to 6 weeks

10.6.3. The statistical procedures suggested are only a few of the valid statistical methods which may be used. Use of other methods may prove more appropriate in detecting significant differences. Certain procedures may permit testing two or more combined reproductive indices to assess the true effect of a substance on reproductive performance, even though none of the indices by themselves are statistically significant (Brown, 1975).

11. Quality Assurance

11.1. In order to assure the quality and reliability of data developed using this protocol, testing facilities should have a quality assurance unit that

is responsible for monitoring the test along with the investigator to assure that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with good laboratory practice standards (U.S. EPA, 1989) and are legally defensible and appropriate for the intended use.

12. Reporting Requirements

12.1. All data and information required by this protocol should be recorded in black ink on appropriate forms and/or bound laboratory notebooks. A duplicate set of records should be kept at a location other than at the test site. The following information must be recorded.

12.1.1. Name of the investigator(s), laboratory, laboratory address, location of raw data, and date of initiation and termination of test.

12.1.2. Name of species tested, including scientific name, source, and age of the animals at the beginning of the test.

12.1.3. A detailed description of the test substance including its chemical name, synonyms, structure, formulations, purity, source, batch, lot number, and physical/chemical properties and name and source of solvent or carrier, if used.

12.1.4. Description of the test facilities and housing conditions, including test cages, temperature, and photoperiod. If conducted outdoors, adverse weather conditions may alter test results, especially during the parturition period, and should be reported.

12.1.5. Name and source of feed, including description and proximate analysis of diet.

12.1.6. The dietary concentration; number of males and females per concentration; body weights; feed consumption; signs of toxicity; abnormal behavior; mortality; reproductive indices; statistical methods

employed; significant necropsy findings (including organ weights, if recorded); anything unusual about the test; any deviations from the protocol; and other relevant information.

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MAMMALIAN WILDLIFE (MINK AND FERRET) DIETARY LC_{50} TESTS TO ASSESS PRIMARY AND SECONDARY TOXICITY[1]

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INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has been charged, under several laws, with the regulation of many toxic or hazardous substances. The key to regulation of these substances is toxicological testing of the compounds with appropriate test species. The species of choice for testing the toxicity of a compound to man and domestic animals, as specified in federal guidelines (U.S. EPA, 1975, 1978, 1982a,b), have been common laboratory animals such as rats, mice, rabbits, guinea pigs, and hamsters. No representative mammalian wildlife species have been designated in the federal guidelines. Adequacy of risk assessment for wildlife mammals has normally been based on toxicity data for evaluating hazards to humans and domestic animals (U.S. EPA, 1978; Ringer, 1988).

Prompted by these concerns, the mink (Mustela vison) (U.S. EPA, 1975; Aulerich and Bleavins, 1981) and European ferret (Mustela putorius furo) (Thornton et al., 1979; Hoar, 1984) were suggested as representative mammalian carnivores for toxicological testing. The mink may be preferred because it is indigenous to the U.S. and Canada and occurs in the wild throughout much of North America. Its nutritional requirements are well known (National Research Council, 1982), stock of known genetic origin is available, all stages of its life cycle can be successfully perpetuated in the laboratory, and it has a large biological data base (Shump et al., 1976; Scientifur, 1987; Sundqvist, 1989). The European ferret, although not indigenous to North America, has been examined as a laboratory model for toxicology (Willis and Barrow, 1971; Thornton et al., 1979; Anon., 1981; Hoar, 1984). The mink has been shown to be among the most sensitive, if not the single most sensitive, mammalian species to polychlorinated biphenyl (PCB) toxicity (Aulerich and Ringer, 1977). Research performed with mink was instrumental in setting U.S. water quality standards for PCBs (Aulerich

and Bleavins, 1981). Subsequent studies have shown this species to be similarly sensitive to polybrominated biphenyls (Aulerich and Ringer, 1979), hexachlorobenzene (Bleavins et al., 1984), aflatoxins (Chou et al., 1976; Bonna et al., 1991), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Hochstein et al., 1988). Additional toxicological studies with mink and ferrets have been reported in the literature (Frederick and Babish, 1984; Scientifur, 1987; Sundqvist, 1989).

Under special circumstances of hazard assessment, such as secondary toxicity poisoning, special tests involving wildlife mammals may be required. Carnivorous animals are generally the species of choice in toxicological testing for secondary poisoning (Schitoskey, 1975; Townsend et al., 1984; Aulerich et al., 1986, 1987) and represent the top of the food chain for chemicals that bioconcentrate. However, protocols have not been developed for mammalian wildlife species to compare the toxicity of metabolized forms of a xenobiotic (as contaminated prey substituted for similar uncontaminated animal products in the diet) with comparable concentrations of an unmetabolized form of the chemical added to the feed of test species. Standardized test protocols are needed to provide experimental techniques suitable for routine assessment of secondary toxicity.

The results of a secondary toxicity test may be difficult to interpret. For example, if the resulting primary and secondary LC_{50} test results were different, it could be because of differences in the bioavailability of the test chemical due to biological incorporation in the tissues of the prey species, or it could be due to formation of toxic metabolites. If the latter case is suspected, additional analytical chemistry would be required to identify the metabolites. If the metabolites are known, then the total toxicity of the parent compound plus the metabolites could, in some cases, be compared to the parent compound alone.

The following protocol was developed to provide a means of assessing primary versus secondary toxicity of chemicals to mammalian wildlife (mink and ferrets). The protocol is based on procedures used in previous toxicology studies from our laboratory (Aulerich et al., 1986; 1987).

1. Scope

1.1. This protocol describes a method for determining the subacute dietary toxicity of a test substance as used in field applications administered to animals in their daily diet (primary toxicity) and for comparing the primary toxicity of the compound to the toxicity of the same test substance contained within contaminated prey animal tissues (secondary toxicity). Toxicity is expressed as the median lethal concentration of the test substance (LC_{50}) and the slope of the dose-response curve.

1.2. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

2. Summary

2.1. Groups of animals of the same species and age (both sexes) are fed diets containing a substance in a geometric series of concentrations for 28 days to measure lethality. This exposure period may be followed by a withdrawal period during which lethality is also measured. At the same time, an equal number of groups of animals of the same species and age (both sexes) are fed the identical concentrations contained in animal tissues contaminated by previous exposure to the same test substance. Data derived from the two tests are compared.

2.2. Daily observations for signs of toxicity and mortality are reported.

2.3. Data derived from treatment and control groups are compared statisti-

cally within and between groups to detect changes in body weight, feed consumption, and an index of toxicity.

3. Significance

3.1. This protocol provides a means of measuring and comparing the toxicity of a test substance in the daily diet of a carnivore, as the result of primary and secondary exposure, under controlled conditions. The use of a 28-day dietary exposure period allows metabolic transformations of the test substance to occur. It is recommended that, when possible, tests be conducted indoors. Indoor tests allow greater control of environmental test conditions and, therefore, greater reproducibility. Indoor facilities, if heated, allow conducting tests at any time of the year. Indoor facilities also make the possibility of escape of test animals less likely.

3.2. This protocol permits collection of data on signs of toxicity in addition to mortality.

3.3. The dose-response curves provide a basis for comparison of primary and secondary toxicity of a test substance to an animal.

4. Definitions

4.1. LC_{50}: The calculated concentration of a test substance which causes 50% lethality of a test animal population under the conditions of the test.

4.2. Primary toxicity: Poisoning or intoxication of an animal due to the consumption of a technical grade or formulated chemical.

4.3. Secondary toxicity: Poisoning or intoxication of an animal due to consumption of contaminated prey tissues.

4.4. Test substance: The element, chemical compound, formulation, known mixture, or material mixed in diets and fed for the purpose of determining an LC_{50}.

4.5. Concentration: The weight of the test substance per unit weight of the diet (expressed as mg/kg of diet).

4.5.1. Theoretical (nominal) concentration: The targeted concentration of test substance mixed into the diet.

4.5.2. Measured concentration: The concentration of test substance in the diet determined by analysis.

4.6. Acclimation period: A period of at least 7 days immediately preceding the exposure period when the test animals are housed in the test facilities under test conditions and fed the untreated diet and drinking water to be used during the exposure period.

4.7. Range-finding test: Test conducted to determine the concentration of the test substance to be used in the definitive test.

4.8. Palatability test: Test where the highest proposed dietary concentration of a test substance for use in a definitive test is fed to a few animals to determine if they will consume the diet containing this concentration of the test substance.

4.9. Definitive test: Test where a substance is fed to a prescribed number of animals at several geometrically-spaced concentrations for a specified period of time (exposure period may be followed by a withdrawal period) when parameters of toxicity, including lethality, are measured.

4.10. Exposure period: The 28-day period when the test animals are fed diets containing the test substance.

4.11. Withdrawal period: The period following an exposure period when all animals are fed an untreated diet to allow for observation of delayed mortality.

4.12. Diet: Feed consisting of both fresh and dried ingredients with water

added to provide a semi-solid (hamburger-like) consistency.

5. Precautions

5.1. Contact with all test substances, solutions, and mixed diets should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Information on other mammalian toxicity and special handling procedures should be known before this protocol is used.

5.2. Disposal of excess test substances, solutions, mixed diets, etc., and treated animals should be done with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

5.3. Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in well-ventilated areas.

5.4. Because this protocol addresses the use of carnivores, appropriate precautions should be used in the handling of test animals. Mink are by nature extremely aggressive and may attack a handler if given the opportunity. Appropriate precautions, such as leather gloves and arm-coverings, should be used. Ferrets are normally much less aggressive than mink but precautions should also be taken when they are handled. Mink and ferrets can transmit certain diseases, most importantly tetanus, and also tuberculosis (TB) and rabies if contacted from outside sources, so researchers should take precautions for these diseases. The possibility of TB infection can be reduced by avoiding the use of pork products in the diet. Proper caging and isolation of test facilities from wild animals can similarly reduce the possibility of rabies infections.

6. Test Animals

6.1. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications, such as housing and diet.

6.2. All animals for a given test must come form one source and strain and be of approximately the same age to minimize variability. Test animals may be obtained from commercial sources or may be reared in laboratory colonies, but they must not have been used in a previous test. It is recommended that test animals be immunized against diseases commonly afflicting the test species. Immunization for mink should include canine distemper, virus enteritis, infectious pneumonia, and botulism (Joergensen, 1985). It is recommended that ferrets be vaccinated against canine distemper and botulism (Fox, 1988). Mink and ferrets are also susceptible to Aleutian Disease (AD). Since no vaccine exists for this viral disease, testing and certification of animals as AD-free is recommended. Animals that are deformed, injured, emaciated, or phenotypically different from normal animals must not be used as test subjects. The population of animals from which the test subjects (treated and control) are selected should be considered unsuitable for testing if mortality exceeds 5% during the acclimation period.

6.3. It is preferable to use animals that have approached their mature body size (for mink and ferrets this is about 18-20 weeks of age). Older animals can also be used to determine the LC_{50}. The use of young, rapidly growing animals may yield a distorted LC_{50} value because the change in body weight far exceeds the change in feed consumption resulting in a decreased amount

of test substance consumed per unit of body weight over the 28-day period. Because of the sex difference in size of mink and ferrets, the two sexes should be treated as separate sub-groups.

7. Facilities

7.1. Space requirements for mink and ferrets have not been determined. However, adherence to the guidelines of the Fur Farm Animal Welfare Coalition (1988) should provide a basis for adequate space and husbandry requirements. This space requirement is currently 32,774 cu cm or 2000 cu in. Individual cages measuring 61 (L) x 76 (W) x 46 (H) cm (24 x 30 x 18 in) have proven adequate for tests performed in conjunction with the development of this and other protocols. Cages must be constructed to prevent cross contamination of treatment groups and contact between individual animals. To prevent aggressive mink from attacking neighboring animals, use solid dividers between adjoining cages, or provide adequate space between adjoining cages if wire mesh cage material is used. Species not conducive to colony rearing, such as mink, must be caged individually.

7.2. Construction materials in contact with animals should not be toxic, nor be capable of excessively adsorbing or absorbing test substances. Stainless steel, galvanized steel, or materials coated with perfluorocarbon plastics are acceptable materials, but other construction materials may also be useful.

7.3. Adequate ventilation should be provided at all times. The Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 1985) recommends 10 to 15 room air changes/hour for animal facilities.

7.4. If the animals have been reared outdoors and the test is conducted

indoors, the photoperiod should simulate ambient daylight conditions because altered photoperiods may subject mink and ferrets to slight adjustments in normal body rhythms with respect to feeding, sleeping, fat mobilization or deposition, coat development, and hormonal activity. It should be noted that the effects of the length of the light/dark cycle on toxicity have not been investigated in these species. If the animals are reared indoors, the photoperiod should not be altered.

8. Prey (Contaminated) Animals

8.1. Prey animals for carnivores include any species that may be contaminated with the test substance and consumed by the test animals. Laboratory studies using contaminated prey animals in dietary, secondary toxicity trials have utilized fish (salmon, perch, alewife, sucker, carp, and bloater chubs), birds (chickens), and mammals (cattle, nutria, rabbits, prairie voles, pocket gophers, rats, and mice).

8.2. Contamination of prey animals may be via dietary, inhalation, or dermal routes. The prey animals should be exposed to the same test substance (same source and lot number) as fed in the definitive, primary toxicity test.

8.3. Before prey are contaminated, it may be necessary to conduct a range-finding trial using several widely-spaced concentrations to determine that concentration necessary to cause approximately 50% lethality in the test animals. Then it must be determined, through analytical procedures, whether sufficient body burdens can be achieved in the prey species. This body burden should allow for dilution of the tissues by the remainder of the dietary ingredients as per the nutrient requirements of the test animal (e.g., given that 10 mg/kg causes a 50% lethality in range-finding tests and

40% prey tissue is desired in the diet, then a prey body burden of 25 mg/kg is needed to yield a final dietary concentration of 10 mg/kg). LC_{50} estimates for other species may be helpful in setting dietary concentrations, although in general mink and ferrets are more sensitive to toxic compounds than laboratory animals. Palatability tests may also be employed to save time and animals. It is recommended that the highest proposed dietary concentration be fed for several days to 2-4 animals to determine if they will eat the diet at this concentration of the test substance. If not, it may be necessary to reduce the highest concentration to a level at which the diet will be eaten.

8.4. Prey animals that do not succumb to the test substance should be killed rapidly at the desired time by accepted humane methods (AVMA Panel on Euthanasia, 1986) that will not interfere with the test results. Prey animals that die should be frozen and stored to be used as part of the diet.

8.5. Depending on the nature of the test substance and the purpose of the test, it may be desirable to remove the contents from the alimentary tract before chemical analyses and/or incorporation into the diet. The removal of the alimentary tract contents eliminates the possibility of the primary chemical that has not been digested from being incorporated into the final diet. However, it should be noted that with certain test substances, the removal of the digestive tract contents may yield little or no body burden due to rapid metabolism and elimination by the prey species, although, considerable test substance may be present if the entire carcass including the alimentary tract content is utilized. Compounds that bioaccumulate, such as halogenated hydrocarbon compounds and certain metals, result in secondary poisoning with or without inclusion of digestive tract contents;

however, chemicals such as organophosphates, carbamates, and many rodenticides are metabolized rapidly and removal of contents from the digestive tract often renders the prey tissues non-toxic. With test substances such as organophosphates, it may be necessary to gavage the prey animals with high concentrations that may cause rapid death in order to secure tissue concentrations sufficient to be lethal to the test animals.

8.6. Carcasses of all clean prey animals should be ground and blended thoroughly to yield a homogeneous mixture. Samples should be taken for chemical analyses of test substance. The contaminated prey animals should be processed in the same manner and a sufficient number of samples to determine the concentration and homogeneity of the test substance taken for analyses. All ground carcasses should be stored in a frozen condition in tightly sealed containers until incorporated into the final diet.

9. Diets

9.1. Diets may be formulated in accordance with the nutrient requirements of the test species (Table 1 and National Research Council, 1982). Any unmedicated commercial diet that meets the minimum nutritional standards of the test species is acceptable. Diets must be formulated so that the same percentage of prey animal tissue is incorporated into each primary and secondary diet. The prey animals should be of the same species and source in all diets.

9.2. Fresh diets and water must be provided daily and fed ad libitum.

10. Diet Preparation

10.1. Knowledge of the physical, chemical, and biological properties of the test substance is important in test diet preparation.

10.2. Diets for the primary toxicity test can be prepared by mixing the

test substance directly into the feed or by dissolving or suspending the test substance in a solvent or carrier prior to mixing with the feed. The use of solvents or carriers may be necessary to achieve uniformity. If solvents or carriers are used, they must also be added to the control diet and the secondary toxicity test diets. It is very important to assure uniform distribution of a test substance in the diet. It is recommended that innocuous solvents or carriers, such as distilled water or corn oil, be used if possible. (It is recommended that, unless the amount of test substance to be added is large, solvents or carriers be used to introduce the substance to the diet to ensure uniform distribution). If an innocuous solvent or carrier cannot be found, it is recommended that a volatile solvent, such as acetone or hexane, be used to dissolve the test substance, if possible, and the resultant solution be added to a small amount of a dry ingredient (e.g., cereal). After the solvent is evaporated, the pre-mix can then be mixed with the rest of the diet uniformly.

10.3. To yield geometrically-spaced concentrations of the test substance in prey animals for the secondary toxicity test, appropriate quantities of contaminated and clean animal tissue, based on chemical analyses, should be thoroughly blended together and then mixed with the other dietary ingredients.

10.4. Sufficient diet should be prepared to provide adequate feed for the 28-day exposure period. The amount of feed to be mixed may be based upon the feed consumption data obtained during the acclimation period. When mixed, the diet should be stored frozen in containers large enough to hold 1-2 day's feed. It is important not to freeze diets in containers too large, because they will not remain fresh under refrigeration for more than

2-3 days. When testing volatile substances, sealable containers must be used. One day's volume of feed should be stored in each container and the feed should fill the container allowing no headspace. The containers should be stored upside down.

10.5. It is critical to analyze all diets for the concentration of the test substance in the diet.

11. Procedure

11.1. Both the primary and secondary portions of the test should be conducted simultaneously.

11.2. Range-finding test: In most cases, LD_{50} estimates for mink or ferrets will not be available to aid in setting dietary concentrations for the LC_{50} test. Therefore, range-finding procedures can be used to save both time and animals by reducing errors or miscalculations in setting these concentrations. LD_{50} estimates for other species may be helpful in setting dietary concentrations, although, in general, mink and ferrets are more sensitive to toxic compounds than other animals. For this reason, if LD_{50} estimates are available for other species, these values can be used as the upper limit of doses in the range-finding procedure. This procedure can be a geometrically-spaced series of doses (e.g., in multiples of 2 or ½) administered by gavage to 2 animals per dose, in which case the approximate LD_{50} is the dose at which 1 or 2 animals die after an appropriate period of observation (often one week). It is suggested that, when administering an oral dose to mink or ferrets by gavage, a piece of plastic large enough to force the animal's mouth open, with a small hole in the center, be used. The tube can then be inserted through the opening without the animal biting it (Figure 1). A three inch, 14 gauge, curved, stainless steel animal feeding needle

can also be used to administer the test substance. If LD_{50} estimates are not available for other species, widely-spaced doses (e.g., 1, 10, 100, and 1000 mg/kg) can be administered to one animal per dose to find a lethal dose. The range-finding procedure described above can then be employed, centering on the lethal dose. If range-finding procedures yield an approximate LD_{50} value, the highest dietary concentration should then be set to ensure that an animal will consume the equivalent of an LD_{50} dose in one day's feed. If a lethal dose is not found, the highest dietary concentration should be set at 5000 mg/kg because concentrations above this value are assumed to be nontoxic. Palatability tests should also be employed to save time and animals. It is recommended that the highest proposed dietary concentration be fed for several days to 2-4 animals to determine if they will eat the diet at this concentration of the test substance. If not, it may be necessary to reduce the highest concentration in the diet to a level that will be eaten.

11.3. Acclimation period: All animals should be conditioned to the test facilities, including: photoperiod, temperature, and caging for a minimum of 7 days. A minimum of 7 days is required, but a longer period may be necessary, especially if the animals to be used in a test are changed from outdoor to indoor housing (or vice-versa) or if the diet or water to be used in a test is different from that which the animals are accustomed to consuming. During this period all animals should be given the untreated (control) diet and drinking water, as used during the definitive test. It is important to measure feed consumption during this period as these measurements can provide an indication of how much feed the animals should consume once the test starts, how much diet to mix for the test, and can

also serve as a control value of each group. Test animals should be weighed at the start of the acclimation period.

11.4. Definitive test:

11.4.1. Each test animal should be randomly assigned according to weight class to a specific test diet concentration, individually caged, and be uniquely identified.

11.4.2. The test diets must be fed for 28 days. In some instances, it may be possible to achieve satisfactory results with a test of shorter duration using higher concentrations of the test substance, but the possibility of feed rejection or avoidance becomes greater with increasing concentrations. Also, certain substances cause delayed mortality, whether administered as a single dose or multiple dosages. Increasing the concentration of the substance does not necessarily shorten the time to death. A 28-day test allows time for absorption, distribution, metabolism, enzyme induction, redistribution, bioconcentration, and elimination to occur, similar to what might occur in animals subacutely exposed to a substance via diet in the environment. A 28-day test also allows testing of slow-acting or bioaccumulating substances. Tests of shorter duration could yield negative or misleading results. For some test substances, it may be necessary to include a withdrawal period, when the test diets are replaced with untreated feed, in order to observe prolonged or delayed toxicity. A withdrawal period is recommended when animals are still exhibiting signs of toxicity at the end of the exposure period. This period provides a more accurate estimation of the true toxicity of a test substance, especially if the substance causes delayed or cumulative injury. By

observing the animals and measuring feed consumption during this period, the permanence of the injury can also be estimated. It is recommended that a withdrawal period not exceed 14 days.

11.4.3. Individual body weights must be recorded at the initiation of the definitive test and at weekly intervals thereafter, and on the day of death. Feed consumption must be measured weekly for the exposure and withdrawal periods, and should be based on a minimum of two consecutive days' feed consumption. In estimating feed consumption by mink or ferrets, several precautions are necessary. Because feed consumption can be adversely affected on a short-term basis by temperature, weather, and other factors, estimates should be based on at least two consecutive days' consumption. These days should also be days when the animals are not handled (e.g., during weighing, moving, etc.), because handling can produce a temporary reduction in feed consumption.

11.4.4. Mortality, behavioral abnormalities, and other signs of toxicity should be recorded daily during the test.

11.4.5. For tests conducted indoors, the photoperiod should be maintained at the same schedule in effect at the conclusion of the acclimation period because a changing photoperiod may subject mink or ferrets to slight adjustments in normal body rhythms with respect to feeding, sleeping, fat mobilization or deposition, coat development, and hormonal activity. It should be noted that the effects of the length of the light/dark cycle on toxicity have not been investigated in these species.

11.4.6. A minimum of eight animals for each test concentration should be used. The test concentrations should be geometrically spaced so as to

result in at least 2 dietary concentrations yielding 10-90% mortality. These results usually can be obtained with 4-6 dietary concentrations including a control. It is possible to conduct an LC_{50} test with as few as 3 dietary concentrations and 8 animals per concentration if a good estimate of the LC_{50} for the test species is available. In many instances, however, accurate results can be achieved with 5 dietary concentrations and 10 animals per concentration if a good estimate of the LD_{50} is available from range-finding procedures.

11.4.7. A test should be considered invalid if more than 12.5% of the control animals die during the definitive test.

11.4.8. It is strongly recommended that a dietary concentration group should be removed from testing when food consumption measurements indicate that 10% or less feed, compared to controls and/or acclimation period values, is consumed daily for the first two weeks' feed consumption measurements or the animals lose 30% of their original body weight.

11.4.9. Necropsies should be performed on all mortalities. At the termination of the test, all surviving test animals should be killed by accepted humane methods (AVMA Panel on Euthanasia, 1986) and necropsies performed. It is suggested that necropsies be performed on all test animals, either the day of death or at the termination of the test. Valuable information on the mode of action of the test substance and its effect on target organs or organ systems can sometimes be gained from gross observation of the test animals at necropsy. Histopathological examination of organs and tissues can often provide more information. Weights of internal organs of control and treated animals can be

compared statistically to determine effects of the substance, although the effects of starvation can sometimes be confounded with effects of the substance.

11.4.10. Body weight changes and feed consumption may be analyzed by analysis of variance (Sokal and Rohlf, 1969) and significant differences may be located by Dunnett's method for comparison with the control (Dunnett, 1964). An LC_{50} value, including confidence limits and slope of the dose-response curve may be calculated for the primary and secondary test by the method of Litchfield and Wilcoxon (1949). Other valid statistical procedures may also be used to analyze the data.

12. Quality Assurance

12.1. In order to assure the quality and reliability of data developed using this protocol, testing facilities should have a quality assurance unit that is responsible for monitoring the test along with the investigator to assure that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with good laboratory practice standards (U.S. EPA, 1989) and are legally defensible and appropriate for the intended use.

13. Reporting Requirements

13.1. All data and information required by this protocol should be recorded in black ink on appropriate forms and/or bound laboratory notebooks. A duplicate set of records should be kept at a location other than the test site. All data should be maintained in a secure location to prevent tempering or destruction of the records. The following information should be reported.

13.1.1. Name of the investigator(s), laboratory, laboratory address,

location of raw data, and date of initiation and termination of test.

13.1.2. Name of species tested, including scientific name, source, history, and age of the animals at the beginning of the test.

13.1.3. A detailed description of the test substance including its chemical name, synonyms, structure, formulations, purity, source, batch, lot number, and physical/chemical properties and name and source of solvent or carrier, if used.

13.1.4. Description of the test facilities and housing conditions, including test cages, temperature, and photoperiod.

13.1.5. Analyses of contaminated prey carcasses and details of contamination methodology.

13.1.6. Name and source of feed and/or ingredients, including description and proximate analysis of diets.

13.1.7. The theoretical and measured dietary concentrations; number of animals per concentration; body weights; feed consumption; signs of toxicity; behavioral changes; % mortality for each concentration; significant necropsy findings; calculated LC_{50} values and 95% confidence limits, slope of the dose-response curves and 95% confidence limits, and the name and reference of the statistical method used; highest dietary concentration at which no signs of toxicity were observed; anything unusual about the test; any deviations from the protocol; and other relevant information.

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Table 1. Suggested ranges of composition of conventional diets for mink[1].

Ingredients	Percent
Fortified cereal[2]	15-30
Liver	0-10[3]
Quality protein feedstuffs (cooked eggs, whole poultry, whole fish, horsemeat, rabbits, nutria, etc.)	0-30[4]
Beef by-products (tripe, lungs, lips, udders, spleen, etc.)	10-30
Poultry by-products (heads, entrails, feet)	10-70
Fish scrap	10-50
Fat supplementation (rendered animal fat or vegetable oils)	0- 6[5]
Proximate analysis[6] of diet	
Protein	25-40
Fat	18-30
Carbohydrate	20-50
Ash	6-12

[1] From: National Research Council, 1982.

[2] May consist of single-cooked grains such as oat groats or wheat in combination with vitamin and trace mineral supplementation or commercially prepared fortified cereal mixtures.

[3] Reproduction-lactation diets (March-May) often contain 5-10% beef liver, although necessity for this has not been universally accepted.

[4] Level of quality-protein feedstuffs is often increased during the critical fur development and reproduction-lactation phases - a practice consistent with the higher protein requirements of the mink during these critical periods.

[5] That level of fat supplementation that provides proper protein/energy balance for each phase of the life cycle.

[6] That proximate analysis consistent with the optimum nutritional balance for each phase of the life cycle.

Table 2. Mortality pattern of mink fed sodium monofluoroacetate (Compound 1080) during a 28 day LC_{50} test.[1]

Concentration (mg/kg)	No. of animals dying/day of test																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
0																												
0.05																												
0.90																												
1.62																												
2.90																							1	2			1	
5.25					1			1			1				1				1	1	1					1	1	

[1] Hornshaw et al., 1986b

Table 3. Mortality pattern of ferrets fed sodium monofluoroacetate (Compound 1980) during a 28 day LC_{50} test.[1]

Concentration (mg/kg)	No. of animals dying/day of test																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
0																												
4.76										1																		
8.56														1														
15.40										2											1	1	1			1	1	
																												-

[1] Hornshaw et al., 1986b

Table 4. Mortality pattern of mink fed Aroclor 1254 during a 28-day LC_{50} test.[1]

Concentration (mg/kg)	No of animals dying/day of test																																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
0																																			
10.0																														1					
18.0																																			
32.4																																1			
58.3																				1	1				1		1	1				1			
105.0																				1	1				3	1		2				1			

1 Hornshaw et al., 1986c

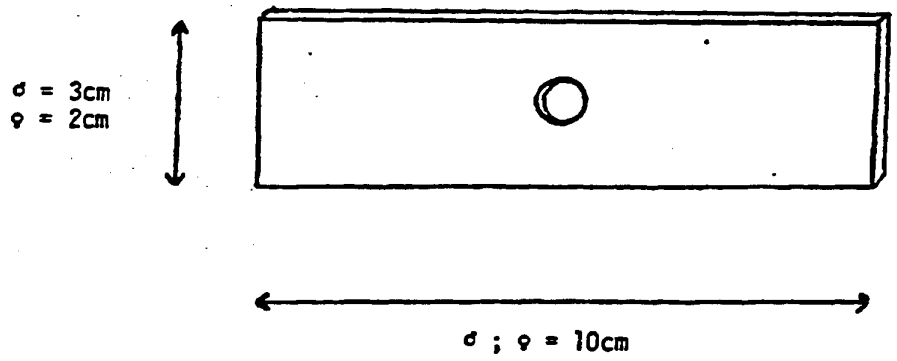

Figure 1. Plastic device for gavage.

ATTACHMENT 2

SAMPLE TRANSMITTAL, RECEIPT, AND INVENTORY (SOP P.200 CF.162)

SAMPLE TRANSMITTAL, RECEIPT, AND INVENTORY

The Environmental Chemistry Branch of the Columbia Environmental Research Center (CERC) has certain procedures which must be followed in the transmittal and receipt of samples that are to be processed by the Branch. Adherence to these instructions will facilitate incorporation of samples into the Branch's sample management system.

Sample Transmittal

1. **The individual responsible for transmission of samples (from outside CERC or internally)** must contact Jesse Arms of CERC via phone (573-876-1856) PRIOR TO any sample transmission[1]. He will send you the necessary forms to fill out and provide additional instructions. Information on methods of preservation used during transmission can be provided at this time. **NOTE: PARTICULARLY SPECIFY ANY SAMPLES REQUIRING -80°C STORAGE REQUIREMENTS; CONTACT PERSONALLY - DO NOT LEAVE VOICE MAIL.**

2. The person responsible for the samples, or designated individuals, must fill out the attached "Batch History" form in detail. Failure to complete this form in its entirety will delay and may jeopardize sample processing.

3. The form entitled "Chain of Custody Record" must be completed. Each sample is to be listed on an individual line along with other requested information. The header and appropriate footer information must be filled out for each sheet that is used.

4. For samples being transmitted to CERC **from outside:** place all completed forms in protective covering and put in packaging with samples. Seal all boxes, coolers, or other packaging so that it can be indicated if tampering has occurred. Once transmission (shipment) has taken place, immediately contact Jesse Arms (573 876-1856) and provide shipment and expected date of arrival information. **Do not leave a voice mail; a direct contact with specified CERC personnel prior to shipment must be established.** Following this phone call, specified CERC personnel must complete the "Pre-Shipment" section of the form entitled "Sample Shipment Record."

5. For samples being transmitted **from one group to another within CERC:** steps 1-4 above must be completed. Following notification of J. Arms or designate, manual transmission (hand-carrying) of samples to the Analytical Building is appropriate. However, contact J. Arms beforehand to assure completion of the transmission process. Completion of "Pre-Shipment" section of "Sample Shipment Record" is still required by specified CERC personnel (J. Arms).

[1]If Jesse Arms is unavailable, contact Tom May at 573-876-1858.

6. For samples being transmitted from CERC Chemistry to a second party outside CERC: A yellow Batch History form (samples out) must be completed along with "Chain of Custody Record" for all samples, following instructions in steps 2 and 3. This documentation is packaged and shipped with samples to the outside party. A copy of documentation is placed in a green pocket file and filed in specified Analytical Building Rm. 5 file drawer. At time of shipment, CERC personnel (J.Arms or alternate) will call the outside party and inform them of shipment details. Once the shipping details have been verified, fill out the "Pre-Shipment" section of the "Sample Shipment Record." When the outside party notifies CERC of sample receipt, J. Arms or alternate will obtain information to fill out "Receipt" portion of "Sample Shipment Record." This completed "Sample Shipment Record" is filed with other documentation in the appropriate green file pocket.

Sample Receipt (receipt at CERC)

1. J.Arms or alternate CERC personnel must immediately notify responsible party at CERC when samples are received. Also J. Arms or alternate CERC personnel must complete a sample check-in procedure following guidelines depicted in "Receipt" section of form entitled "Sample Shipment Record."

Sample Inventory (at CERC)

1. Samples are logged-in or inventoried into the Chemistry Division sample management system by J.Arms or alternate CERC personnel following guidelines depicted on a form entitled "Sample Log-in."

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