



NAVFAC
Naval Facilities Engineering Command

ENGINEERING SERVICE CENTER
Port Hueneme, California 93043-4370

TECHNICAL REPORT
TR-2284-ENV

**BIOMONITORING: GUIDE FOR THE USE OF
BIOLOGICAL ENDPOINTS IN MONITORING
SPECIES, HABITATS, AND PROJECTS**

by

NAVFAC Risk Assessment Workgroup and Argonne National Laboratory
POC: Amy Hawkins

November 2007

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

| | | | | | |
|---|------------------------------------|-------------------------------------|----------------------------|--|---------------------------------|
| 1. REPORT DATE NOV 2007 | | 2. REPORT TYPE | | 3. DATES COVERED | |
| 4. TITLE AND SUBTITLE BIOMONITORING: GUIDE FOR THE USE OF BIOLOGICAL ENDPOINTS IN MONITORING SPECIES, HABITATS, AND PROJECTS | | | | 5a. CONTRACT NUMBER | |
| | | | | 5b. GRANT NUMBER | |
| | | | | 5c. PROGRAM ELEMENT NUMBER | |
| 6. AUTHOR(S) Amy Hawkins | | | | 5d. PROJECT NUMBER | |
| | | | | 5e. TASK NUMBER | |
| | | | | 5f. WORK UNIT NUMBER | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Commanding Officer, Naval Facilities Engineering Command, 1100 23rd Avenue, Port Hueneme, CA, 93043-4370 | | | | 8. PERFORMING ORGANIZATION REPORT NUMBER TR-2284-ENV | |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | | | | 10. SPONSOR/MONITOR'S ACRONYM(S) | |
| | | | | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) | |
| 12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited. | | | | | |
| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT This guidance document presents a framework for using biological endpoints in monitoring species, habitats and projects associated with Department of Navy (Navy) Environmental Restoration Program (NERP) sites undergoing remediation in compliance with the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). | | | | | |
| 15. SUBJECT TERMS | | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES 147 | 19a. NAME OF RESPONSIBLE PERSON |
| a. REPORT unclassified | b. ABSTRACT unclassified | c. THIS PAGE unclassified | | | |

**BIOMONITORING: GUIDE FOR THE USE OF BIOLOGICAL ENDPOINTS IN
MONITORING SPECIES, HABITATS, AND PROJECTS**

Prepared by

NAVFAC Risk Assessment Workgroup and Argonne National Laboratory
POC: Amy Hawkins

November 2007

This page intentionally blank.

EXECUTIVE SUMMARY

This guidance document presents a framework for using biological endpoints in monitoring species, habitats and projects associated with Department of Navy (Navy) Environmental Restoration Program (NERP) sites undergoing remediation in compliance with the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA).

The purpose of this guidance is to:

1. Provide a framework for the development and implementation of scientifically defensible monitoring plans that use biological endpoints,
2. Facilitate consistency in the use of biological endpoints and biota in monitoring programs across NERP, and
3. Provide direction for determining when the use of biological endpoints may be appropriate and for selecting biological endpoints and biota for use in biomonitoring programs.

This guidance presents a six-step framework for designing biomonitoring programs. This framework, which is fully consistent with U.S. Environmental Protection Agency guidance on monitoring plan development, includes identification of biomonitoring objectives and the development of biomonitoring hypotheses to focus the monitoring plan, the development of decision criteria that include action levels and alternative actions for terminating or continuing the bio monitoring program, and the design of data collection and analysis methods, including identification of appropriate biological endpoints and biota for use in the biomonitoring program.

This guidance is not intended to specify the scale, complexity, protocols, data needs, or investigation methods for meeting the needs of site-specific biomonitoring. Rather, it presents a framework that can be used to develop and implement scientifically defensible and appropriate biomonitoring plans at NERP sites. This guidance addresses the development of the logic and rationale needed to support a decision to design and implement at NERP sites a monitoring program using biological endpoints. Specifically, this guidance addresses the development of defensible monitoring objectives and hypotheses that focus the biomonitoring program, and the development of decision criteria that will support site management decisions related to the biomonitoring program. This guidance identifies general categories of biomonitoring endpoints and the use of captive and naturally-occurring biota, and identifies a number of factors such as ease of collection and exposure potential to be considered when selecting biological endpoints and biota for use in a biomonitoring program.

This page intentionally blank.

CONTENTS

| | |
|--|------|
| EXECUTIVE SUMMARY | iii |
| LIST OF FIGURES | vii |
| LIST OF TABLES | vii |
| 1 INTRODUCTION | 1-1 |
| 1.1 PURPOSE OF THE GUIDE..... | 1-1 |
| 1.2 WHAT IS BIOMONITORING | 1-1 |
| 1.3 ADVANTAGES AND LIMITATIONS OF BIOMONITORING | 1-2 |
| 1.4 BIOMONITORING OBJECTIVES AND APPROACHES..... | 1-4 |
| 1.5 DETERMINING WHETHER BIOMONITORING IS APPROPRIATE | 1-5 |
| 1.5.1 Understanding Overall Project Goals and Management Objectives..... | 1-6 |
| 1.5.2 Biomonitoring vs. Other Types of Monitoring | 1-7 |
| 1.6 FRAMEWORK FOR BIOMONITORING | 1-8 |
| 1.7 GUIDE ORGANIZATION..... | 1-10 |
| 2 BIOMONITORING OBJECTIVES AND DECISION CRITERIA..... | 2-1 |
| 2.1 THE BIOMONITORING OBJECTIVES AND ASSOCIATED MONITORING QUESTIONS..... | 2-1 |
| 2.2 DEVELOPING BIOMONITORING DECISION CRITERIA | 2-3 |
| 2.2.1 Elements of Biomonitoring Decision Criteria | 2-3 |
| 2.2.2 Biomonitoring Action Levels | 2-4 |
| 2.2.3 Temporal Considerations | 2-5 |
| 2.2.4 Alternative Actions | 2-7 |
| 2.3 ADAPTIVE MANAGEMENT..... | 2-8 |
| 2.4 REGULATOR AND STAKEHOLDER CONSIDERATIONS | 2-8 |
| 3 BIOMONITORING BIOTA AND ENDPOINTS..... | 3-1 |
| 3.1 BIOMONITORING BIOTA..... | 3-1 |
| 3.2 CATEGORIES OF BIOMONITORING SPECIES AND ENDPOINTS | 3-2 |
| 3.2.1 Levels of Biological Organization | 3-2 |
| 3.2.2 Ecological Habitats | 3-3 |
| 3.2.3 Cellular and Subcellular Endpoints | 3-4 |
| 3.2.4 Organismal Endpoints | 3-4 |
| 3.2.5 Population and Community Endpoints | 3-5 |
| 3.2.6 Bioaccumulative Endpoints | 3-5 |
| 3.3 BIOMARKERS | 3-6 |
| 4 BIOMONITORING STUDY DESIGN..... | 4-1 |

| | | |
|---------|--|------|
| 4.1 | BIOMONITORING BIOTA | 4-2 |
| 4.1.1 | General Categories of Biomonitoring Biota | 4-2 |
| 4.1.2 | Biota Attributes | 4-3 |
| 4.2 | CAPTIVE VS. NATURALLY-OCCURRING BIOTA | 4-5 |
| 4.2.1 | Captive Biota | 4-5 |
| 4.2.2 | Naturally-Occurring Biota | 4-6 |
| 4.3 | SELECTING BIOTA | 4-7 |
| 4.3.1 | Identifying Candidate Biota for Biomonitoring | 4-8 |
| 4.3.1.1 | Exposure Potential | 4-8 |
| 4.3.1.2 | Spatial and Temporal Occurrence | 4-10 |
| 4.3.1.3 | Ease of Collection | 4-10 |
| 4.3.1.4 | Existing Exposure and Effects Data | 4-12 |
| 4.3.1.5 | Sensitivity | 4-12 |
| 4.3.1.6 | Natural Recovery | 4-12 |
| 4.3.2 | Selecting among Candidate Biota | 4-13 |
| 4.4 | BIOMONITORING ENDPOINTS | 4-16 |
| 4.4.1 | Endpoint Attributes | 4-16 |
| 4.4.2 | Temporal Considerations | 4-17 |
| 4.5 | REFERENCE SITES AND BASELINE CONDITIONS | 4-18 |
| 4.5.1 | Reference Sites | 4-18 |
| 4.5.2 | Baseline Conditions | 4-19 |
| 4.6 | SAMPLE LOCATION AND DATA ANALYSIS | 4-20 |
| 4.7 | QUALITY ASSURANCE AND QUALITY CONTROL | 4-20 |
| 5 | REFERENCES | 5-1 |
| | | |
| | APPENDIX A. Example of Biomonitoring for Protection of a Sensitive Ecological Resource - Rare Turtle Oversight Monitoring Program, Naval Air Station Weymouth..... | A-1 |
| | | |
| | APPENDIX B. Example of Biomonitoring Habitat Restoration Success – Habitat Mitigation Work Plan for McAllister Point Dredging at Naval Station Newport..... | B-1 |
| | | |
| | APPENDIX C. Example of Biomonitoring Wetland Restoration Success - Long-Term Monitoring Report for Restored Wetlands at Naval Submarine Base-New London Newport..... | C-1 |
| | | |
| | APPENDIX D. Example of a Fish Tissue Biomonitoring Program – Washington State Toxics Monitoring Program..... | D-1 |
| | | |
| | APPENDIX E. Example of a Fish Tissue Biomonitoring Program – New Jersey Final Work Plan for Routine Monitoring Program for Toxics in Fish | E-1 |

LIST OF FIGURES

| | |
|--|------|
| Figure 1.1 Framework for Designing a Biomonitoring Program | 1-9 |
| Figure 4.1 Process for Selecting Naturally Occurring Biomonitoring Species | 4-15 |

LIST OF TABLES

| | |
|--|------|
| Table 1.1 Advantages and Limitations of Biomonitoring | 1-3 |
| Table 1.2 Biomonitoring Objectives, Biological scales, and Endpoints | 1-5 |
| Table 1.3 Understanding Overall Project Goals/Management Objectives - Questions to Consider | 1-7 |
| Table 1.4 Biomonitoring vs. Other Types of Monitoring – Questions to Consider .. | 1-8 |
| Table 2.1 Developing Biomonitoring Objectives – Questions to Consider | 2-2 |
| Table 3.1 Levels of Biological Organization..... | 3-3 |
| Table 4.1 Examples of Data Needs for Different Project and Biomonitoring Goals | 4-1 |
| Table 4.2 Characteristics for Evaluating Candidate Biomonitoring Biota | 4-4 |
| Table 4.3 Advantages and Disadvantages of using Naturally-Occurring Biota for Biomonitoring | 4-7 |
| Table 4.4 Evaluating Exposure Potential of Candidate Biota | 4-9 |
| Table 4.5 Evaluating Ease of Collection of Candidate Biota | 4-11 |
| Table 4.6 Potential Sensitivity Factors for Different Types of Contaminants | 4-13 |
| Table 4.7 Example Calculation of Mean Numerical Ranking for the Suitability Factor “Ease of Collection” | 4-15 |
| Table 4.8 Example Calculation of an Overall Suitability Score | 4-16 |

This page intentionally blank.

1 INTRODUCTION

1.1 PURPOSE OF THE GUIDE

This document presents guidelines when considering implementing a biomonitoring program at the Department of the Navy (Navy) Environmental Restoration Program (NERP) sites undergoing investigation or remediation in compliance with the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). This guide is also applicable to sites where habitat restoration activities are being considered to address natural resource injuries that may have occurred as a result of past chemical releases or ongoing Navy remediation activities.

This guide is not intended to specify the scale, complexity, protocols, or data needs that should be implemented, nor be a comprehensive methods manual, for a biomonitoring program. Rather, this guide focuses on the applicability, planning considerations and issues, and methods related to the use of biomonitoring approaches to:

1. Evaluate exposure to, and effects of, chemical releases from Navy NERP sites on human health and the environment,
2. Evaluate the effectiveness of mitigation measures implemented during remedial activities to protect sensitive ecological resources, and
3. Evaluate the progress and effectiveness of habitat restoration activities that may be part of a remedial action.

This guide identifies and discusses important issues that need to be considered when deciding to initiate a biomonitoring program, or that may be encountered during biomonitoring. The guide is intended to assist RPMs in discussions with Navy staff, regulators, and stakeholders regarding the need and applicability of a biomonitoring program for a Navy NERP site or activity, and in the design of appropriate biomonitoring programs.

1.2 WHAT IS BIOMONITORING?

Monitoring may be defined as the collection and analysis of environmental data (biological, chemical, and physical) over a sufficient period of time and frequency to determine the status and/or trend in one or more environmental parameters or characteristic toward meeting a management objective (Elzinga et al., 1998). Monitoring is conducted for two primary reasons: (1) to establish a baseline that represents the current status of the environment; and (2) to detect changes over time that are outside the natural variation of the baseline (Hicks and Bridges 1994). Environmental data collected during monitoring may be chemical (e.g., cadmium concentration in soil), physical (e.g., temperature, soil moisture), or biological (e.g., biomass, community structure) in nature.

Biomonitoring is the collection and analysis of biological data to assess environmental changes (often associated with anthropogenic causes) or to identify hazards to human health and the environment. The evaluation and interpretation of these data are then used to support a management objective related to the causative factors of the observed environmental changes or hazards. Because biological measures are based on living organisms that directly respond to the conditions around them, biomonitoring may be helpful in diagnosing chemical, physical, or biological changes in the environment and cumulative environmental impacts.

| |
|--|
| <p>Biomonitoring</p> <p>The collection and analysis of biological data to assess changes in the environment or to identify hazards to human health and the environment.</p> |
|--|

This guide focuses on biomonitoring most commonly conducted in the NERP, namely biomonitoring contaminant tissue concentrations for estimating human and ecological exposures, biomonitoring to ensure that NERP activities are not resulting in adverse impacts to important ecological resources (such as endangered species or sensitive habitats), and biomonitoring to evaluate habitat restoration success. Additional information for developing and implementing habitat restoration monitoring can be found in NAVFAC 2004a, User's Guide UG-2061-ENV, Guidance for Habitat Restoration Monitoring.

1.3 ADVANTAGES AND LIMITATIONS OF BIOMONITORING

The use of living organisms to monitor environmental conditions has both advantages and limitations that may influence the decision to conduct biomonitoring (Table 1.1). Major limitations of biomonitoring include data variability and the characterization of exposure or effects. Because biomonitoring involves the collection and evaluation of data from living organisms under at least partially natural conditions, the monitoring results will be affected by the environmental conditions and associated natural variability present prior to and during data collection. Native biota in particular will be influenced by many natural factors that can be difficult if not impossible to identify, characterize, and control. These factors will also be unrelated to NERP-related activities or releases. For example, a decrease in the abundance of a monitored species could be due to such factors as unfavorable climate conditions, a reduced food supply, or a disease outbreak, all of which may be completely unrelated to NERP activities or conditions. Unless this variability is considered within the monitoring design, the biomonitoring results may be incorrectly interpreted.

Biomonitoring is often employed to identify the level of exposure that human or ecological receptors may be experiencing to an environmental contaminant. While biomonitoring data by itself may indicate that exposure has been or is currently occurring, it may not necessarily tell about the source of the exposure nor its timing, magnitude, duration, and frequency. In addition, the presence of a chemical is not necessarily an indication that there is a harmful effect. The magnitude of these limitations (not knowing the exposure source or its effects) will be directly related to the

mobility of the organism being used for biomonitoring, and also the sensitivity of the organism to an exposure. For example, a contaminant is detected in an animal collected from a NERP site, indicating exposure of the animal to that contaminant. However, if the animal is highly mobile and normally ranges over considerable distances in its day-to-day activities, linking the exposure to the NERP site may not be possible, although that is how the results might be viewed. In some cases, the measured concentrations may be the result of exposure to multiple sources at multiple locations. Even if there is exposure occurring at the NERP site, it would not be possible to determine the contribution of the NERP-related exposure to the total concentration measured in the animal.

The principal advantage of biomonitoring is that it can provide site-specific, real world information on the environment (Table 1.1). Living organisms act as integrators of abiotic and biotic conditions where they live and thus reflect the status of the environment at that location. By evaluating biota from a specific site or location, biomonitoring may provide a much more accurate depiction of actual environmental conditions or hazards than would modeling or extrapolation from only physical or chemical data. Thus, a change in a biological variable may provide a direct link to changing conditions in the environment (such as an increase in contaminant concentrations or an increase in habitat quality). Depending on the biological variables being measured, monitoring using biological measures may also be less costly than monitoring abiotic variables (such as media concentrations). For some activities, such as habitat restoration, the measurement of biological variables is the primary means by which to determine activity success.

Table 1.1 Advantages and Limitations of Biomonitoring

| ADVANTAGES | LIMITATIONS |
|---|---|
| Allows for evaluation of real-world, site-specific exposure | Natural variability may make interpretation difficult |
| Can provide assessment of long-term exposure and effects | Results may reflect exposure to all environmental stressors, not just site-related stressors |
| Can provide indications of cumulative exposure and impacts from contaminant mixtures | Results may indicate exposure, but not the source, timing, magnitude, or duration of the exposure |
| Because of relatively short life cycles of many organisms, adverse responses of all life stages to contaminant exposure may be quickly observed | Data from highly mobile biota will likely reflect exposure from other areas |
| Biota can serve as real world, site-specific surrogates for human exposure | Newly developed methods may have difficulty in obtaining regulatory and stakeholder acceptance |
| Because some biological endpoints are rapidly affected by changing environmental conditions, biomonitoring can provide an assessment of rapidly changing conditions | A large number of samples collected over a long time period may be needed to identify population-level responses in some species. |
| Many biomonitoring methods are relatively inexpensive and quicker than chemical monitoring methods | Extrapolating biomonitoring data to project goals, such as sediment remediation, may be difficult. |

While monitoring programs associated with contaminant issues are typically based on chemical analyses of environmental media (water, air, soil, sediment), the collection, analysis, and interpretation of chemical data are often constrained by available methodology and detection limits, and require time series of data to provide more than a snap shot in time picture of environmental conditions or events. In contrast, biomonitoring programs may use time series data or one-time sampling events. Organisms integrate effects over time, and sampling of naturally occurring biota will reflect past exposures and effects. As such, the results observed from a one-time sampling event in a biomonitoring program likely reflect exposure over a length of time. Therefore, biomonitoring programs are capable of detecting the effects of episodic events (such as spills) as well as cumulative effects of multiple contaminants.

Biomonitoring may provide exposure and effects information in a more rapid and less expensive manner than traditional chemical analyses or toxicological studies. Because many biomonitoring tools may be rapidly and relatively inexpensively implemented, a well designed biomonitoring program may include more samples and cover a greater area than a monitoring program using other approaches, and thus provide a much more robust database on which to base management decisions.

1.4 BIOMONITORING OBJECTIVES AND APPROACHES

Biomonitoring has been used to evaluate the exposure and effects of ecological resources to chemical releases, to evaluate the effectiveness of remediation activities in reducing chemical exposure and effects, to evaluate the success of species management activities and habitat restoration projects, and to provide early warning to potential environmental and human health impacts (for example, see NRC 1991).

Biomonitoring has also been used to evaluate the effects of a particular activity before, during, and after an activity is completed. In these cases, biomonitoring would be conducted to document an improvement in environmental conditions such as a decrease in contaminant exposure, or to show a success in habitat restoration. Biomonitoring is also often used to evaluate the effectiveness of mitigation measures that target ecological resources such as protected species or habitats and are meant to reduce or eliminate impacts to those resources from the activity. This type of biomonitoring may be appropriate during NERP cleanup activities and restoration projects. Biomonitoring has also been used to ensure compliance with various environmental regulations, such as the Clean Water Act and Endangered Species Act.

As previously mentioned, biomonitoring involves the measurement of biological parameters. The evaluation of these parameters may be conducted at any of a variety of biological scales, ranging from the subcellular level to the level of an entire habitat (ecosystem) (Table 1.2). Biomonitoring methods may evaluate biological responses at the physiological, biochemical, or histological level (i.e., chromosome damage, reduced enzyme activity, increase in incidence of tumors), or at the individual, species,

population, or community level (i.e., tissue concentration, presence or absence of a species, reproductive success, species diversity, community structure).

Terrestrial and aquatic biota that have been used in biomonitoring programs include bacteria, algae, vegetation, benthic and soil invertebrates, zooplankton, fish, amphibians, reptiles, birds, and mammals. Which biota and biological parameter is evaluated, and the methods used to evaluate that parameter, will depend on the objectives of the specific nature of the NERP activity and its monitoring needs and objectives.

Table 1.2 Biomonitoring Objectives, Biological Scales, and Endpoints

| Common Biomonitoring Objectives | Scale at Which Biomonitoring May Occur | Types of Endpoints Evaluated by Biomonitoring |
|---|--|---|
| Track Condition of a Natural Resource for Management Purposes | | Development Physiological Responses |
| Evaluate Effectiveness of a Remedy | | Genetic Changes Histological Changes |
| Evaluate Success of Mitigation Measures | Subcellular | Tissue Concentrations |
| Track Exposure of Biota to Site Contaminants | Cellular | Behavior |
| Track Exposure of Biota to Site Contaminants | Species | Mortality |
| Provide Early Warning of a Contaminant Release | Population | Presence/Absence of a Species or Group |
| Provide Early Warning of a Contaminant Release | Community | Abundance/Biomass of a Species or Group |
| Track Potential Human Exposure to Environmental Contaminants from the Ingestion of Contaminated Foods | Habitat | Species Diversity |
| Track Progress/Success of a Habitat Restoration Project | | Population Structure |
| | | Community Structure and Function |

1.5 DETERMINING WHETHER BIOMONITORING IS APPROPRIATE

The decision to conduct biomonitoring should consider three factors:

1. The overall project goals and management objectives (and especially the management decisions to be made) for the site or activity;

2. How the biomonitoring objectives will support the overall project goals and management objectives; and
3. Whether a different form of monitoring (such as chemical) may be as or more effective in supporting the overall project goals and management objectives.

Within the NERP, biomonitoring is conducted primarily to monitor (1) human health exposure to Navy-related environmental contaminants, (2) the condition of sensitive ecological resources during NERP activities, and (3) the success of habitat restoration following site remediation.

1.5.1 Understanding Overall Project Goals and Management Objectives

A clear understanding of the overall project goals and management objectives is critical for deciding whether biomonitoring is appropriate. This understanding is also important in ensuring that the biomonitoring program is designed so that it will support future management decisions.

The overall project goals are directly related to the specific objectives that the project manager has for the site or activity (such as restoration of an impacted habitat or the cleanup of environmental contamination). When these goals and objectives are clearly understood, a more effective determination may be made regarding whether:

- 1) a biomonitoring program will provide information that supports the project goals and management objectives; or
- 2) the project goals and management objectives could be more effectively supported by some other form of monitoring (such as chemical analyses of site soils).

Examples of Project, Management, and Biomonitoring Goals and Objectives

Habitat Restoration Project:

- Project and Management Goals/Objectives
 - Conduct restoration activities to establish a desired habitat type and size.
- Biomonitoring Goals/Objectives
 - Determine whether the desired habitat type and size is being/has been reached.

NERP Remediation Project

- Project and Management Goals/Objectives
 - Ensure unacceptable human exposure to site-related contaminants is not occurring during or after remediation.
- Biomonitoring Goals/Objectives
 - Monitor contaminant concentrations in fish and shellfish to identify if/when concentrations could result in unacceptable human exposure from ingestion. Monitoring will indicate whether a particular unacceptable level is exceeded over a given length of time.

For example, suppose a NERP site consists of a capped landfill where monitored natural attenuation is being implemented to address contaminants within the landfill. The landfill is located adjacent to a coastal area that receives heavy recreational use (boating and fishing). Because of public concerns that during remediation, contaminants may be moving from the landfill to aquatic habitats where they may be taken up by fish and shellfish which in turn may be harvested and consumed by people fishing in the area. For this site, the overall management objectives are to remediate the contaminants in the landfill while being protective of human health. By providing data that could be used to predict human exposure, a biomonitoring program that measures fish and mussel tissue concentrations would assist in meeting the management objective of protecting human

health during remediation. Tissue measurements would identify real-world exposure levels for people catching and eating fish and shellfish from the area and provide early warning of potentially unacceptable exposures.

Understanding the management objectives will also aid in deciding whether another type of monitoring may be more effective (either in terms of cost, timeliness, or uncertainty) in supporting management decisions for the site. In the above example, fish tissue and shellfish concentrations or human exposure levels estimated from the measured tissue concentrations could be used to estimate the level of human exposure to site-related contaminants. Alternately, a monitoring program could be implemented that estimates human exposure from measured contaminant concentrations not in biological tissues but in surface water and sediment from area where fishing occurs. While both approaches would provide exposure estimates, the direct measurement of fish and shellfish would provide a more realistic estimate of potential human exposure than would the measurement of surface water and sediment; the latter requiring an additional modeling estimate (with an associated increase in uncertainty) to predict human exposure. The project manager must decide on how important any differences in exposure uncertainty might be in the overall protection of human health. If the uncertainty differences are acceptable, differences in the sample collection and analysis costs as well as in the timeliness of obtaining the results may be important in choosing one form of monitoring over another.

Table 1.3 Understanding Overall Project Goals/Management Objectives – Questions to Consider

| |
|---|
| What are the overall management objectives for the site or activity? |
| What are the decisions to be made regarding these objectives? |
| How would a monitoring program help make those decisions? |
| Why is a biomonitoring program being considered or suggested? |
| Could a different type of monitoring support the management decision in a similar or more effective manner? |

1.5.2 Biomonitoring vs. Other Types of Monitoring

When biomonitoring is being considered, it is important to consider whether some other type of monitoring, such as chemical analysis of abiotic media, could also provide data that would meet management objectives and support a management decision. When evaluating other types of monitoring, factors such as cost, ease of data collection, analytical turn-around time, and regulator and stakeholder acceptance should be examined and compared to the same factors for biomonitoring. The lack of suitable biota or quantifiable endpoints to sample, lack of representative reference sites, or unacceptable levels of natural variability may preclude a biomonitoring program to evaluate remedial actions. In such cases, other types of monitoring may be preferred.

The applicability of other types of monitoring will certainly be influenced by the overall project goals and management objectives. For some projects, such as a habitat restoration project, the overall objective will be the attainment of a specific type of habitat condition, such as the establishment of a particular plant community. For a cleanup project, one of the goals may be that a specific ecological resource is not adversely affected during site remediation. In both examples, a biomonitoring program, with the collection and measurement of biological data, would likely be the most appropriate type of monitoring.

| Table 1.4 Biomonitoring vs. Other Types of Monitoring – Questions to Consider |
|---|
| <p>Could other types of data support the monitoring objectives and decisions? If so, what types of data could also be applicable?</p> <p>What are the cost and implementation differences among the monitoring options?</p> <p>How quickly are monitoring results needed following sampling, and are there differences in analytical times of the different monitoring approaches?</p> |

Depending on the importance given to the ecological resources in the system, a combination of biological and chemical monitoring approaches may be appropriate. Alternately, if the project goal and management objective is to limit ecological or human exposure at offsite locations during site remediation, then the monitoring of environmental contaminant concentrations (i.e., chemical analyses of media) may be more appropriate than a biomonitoring program.

1.6 FRAMEWORK FOR BIOMONITORING

Biomonitoring is widely used by a variety of Federal, state, and local agencies and programs, as well as by private industry. While each may have or follow a specific process or framework for conducting biomonitoring, all have two major, shared, components or features: (1) the development and understanding of the biomonitoring objectives for a specific project or activity; and (2) the design of a scientifically-defensible biomonitoring program that meets the biomonitoring objectives and supports management decisions for the project or activity. Figure 1.1 illustrates a framework that incorporates these two components into a process for designing biomonitoring programs that support Navy projects.

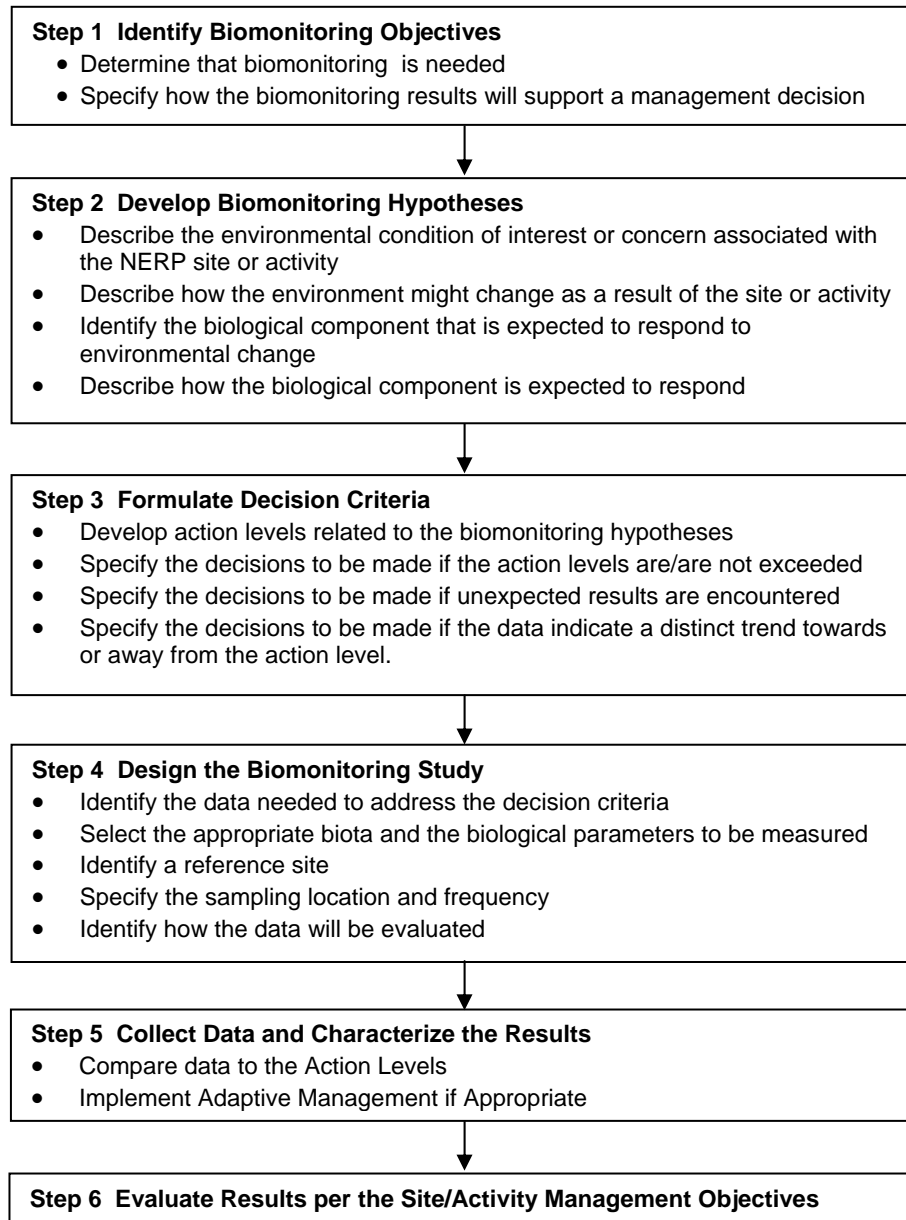


Figure 1.1 Framework for Designing a Biomonitoring Program (modified from NAVFAC, 2004a)

Critical to this framework is the identification of appropriate action levels and decision criteria against which the monitoring results are applied in order to support a defensible management decision. This framework is fully consistent with recent USEPA monitoring guidance (USEPA 2004), and relies on the use of the data quality objectives (DQO) process to design scientifically defensible biomonitoring study designs (UFP-QAPP; IDQTF, 2005). This framework is also fully consistent with the NAVFAC guidance for habitat restoration monitoring (NAVFAC 2004a), which must be used together with this guide when designing biomonitoring programs for habitat restoration activities.

1.7 GUIDE ORGANIZATION

This guide is organized along 6 sections. Section 1 presents an overview and general discussion of biomonitoring. Section 2 discusses the development of the biomonitoring objectives, hypotheses, and decision criteria (Figure 1-1, Steps 1-3). Section 3 discusses the selection of biota and endpoints for biomonitoring programs (Step 4), while Section 4 discusses study design and data analysis (Steps 4 and 5). Section 5 presents the references cited in the earlier section. Appendices A-E provide examples of biomonitoring plans.

2 BIOMONITORING OBJECTIVES AND DECISION CRITERIA

A biomonitoring program, regardless of its details, involves the repeated measurement of one or more biological variables in order to show whether changes are occurring in a biological or environmental condition. Regardless of the intent of the biomonitoring program, a number of issues need to be considered when deciding on whether biomonitoring is appropriate and for developing such a program. These issues include:

- An understanding of the overall project goals and management objectives and why biomonitoring is needed to support those goals and objectives;
- The development of well-defined and defensible biomonitoring objectives that are directly linked to the project goals and management objectives;
- The development of well-defined and scientifically defensible biomonitoring decision criteria that support management decisions and overall project goals;
- The selection of appropriate biomonitoring methods;
- The availability of reference sites and controls;
- The need for quality assurance/quality control;
- The ability to link biomonitoring results, project goals, and management objectives to the Navy site of concern; and
- Stakeholder involvement and support.

Success of a biomonitoring program depends on the attention given to understanding and addressing these issues, especially during program design. Too often biomonitoring is implemented without clear-cut objectives and decision criteria, with the results ultimately providing little or no support to overall project goals or management decisions.

2.1 THE BIOMONITORING OBJECTIVES AND ASSOCIATED MONITORING QUESTIONS

Once the project goals and management objectives are understood, biomonitoring objectives related to the project goals and management objectives should be developed. The biomonitoring objectives should identify the purpose and be linked to the overall project goals and management objectives. The development of the biomonitoring objectives should also include development of questions that relate biological exposure to the project goals and management objectives.

For example, suppose a remedy has been selected for a NERP site that includes the “hot-spot” removal of

Biomonitoring Objectives May Include

- Providing data for evaluating habitat restoration success at NERP sites.
- Providing data for the rapid estimation of human exposure to site contaminants originating from NERP sites.
- Providing data for evaluating the condition of sensitive ecological resources and for protecting those resources from being impacted by NERP activities.

contaminated soil followed by *in-situ* bioremediation of the residual contamination. During the bioremediation period, which may take some time, there is concern that there may be future human or ecological exposure as a result of bioconcentration or food chain uptake by fish and other biota at the site and in surrounding areas.

In this example, the overall project goal and management objective may be the reduction of contaminant concentrations to levels protective of human health and the environment. The objective of a biomonitoring program may be to provide biologically-based data that would provide for the determination of contaminant uptake by exposed biota and subsequent estimation of human exposure.

Once the biomonitoring objectives are determined, one or more questions can be developed that link the objectives with specific human health or ecological concerns. In the previous example, the overall project goal and management objective is the reduction of contaminant concentrations to protective levels while the biomonitoring objective is to provide data for predicting human and ecological exposure. Biomonitoring questions may be:

1. Are local biota being exposed (via direct uptake from media or through food chain uptake) to the residual contaminants at the site?
2. Could the exposure levels pose a threat to ecological receptors? and
3. Could consumption of local biota pose a threat to human health?

Note how questions link the overall project goals and management objectives to the biomonitoring objectives by asking whether exposure is occurring and if so, could the exposure pose a threat to human health and the environment. These questions may be used in biomonitoring study design to help with the identification of the biota and biological parameters to be evaluated and with the selection of data collection and analysis methods and sampling design. In addition, the biomonitoring questions will help in making management decisions regarding how well the overall project goals and management objectives are being met, and will aid in decisions regarding the need for additional action (such as additional remediation, data collection and analysis, or revision of the biomonitoring program).

| Table 2.1 Developing Biomonitoring Objectives – Questions to Consider |
|--|
| What are you trying to accomplish with biomonitoring (what are the biomonitoring objectives)? |
| How are the biomonitoring objectives related to the overall project goals and management objectives? |
| What are the associated biomonitoring questions? |
| Will the answers to the biomonitoring questions support a management decision? |

2.2 DEVELOPING BIOMONITORING DECISION CRITERIA

Decision criteria are quantitative statements that take the form of “if...then...” statements. The criteria specify the threshold levels (the action levels) for making a choice between management options and selecting a path forward from among specific alternative actions. As the biomonitoring data are collected and analyzed, they are compared to the action levels. A determination can then be made of how well project and management objectives (e.g., protection of a sensitive ecological resource, the restoration of a specific habitat type) are being met, and whether or not additional or further project and biomonitoring activities are necessary.

Biomonitoring Decision Criteria

Statements that establish the criteria for deciding whether or not a specific condition has been or is being met by the NERP activity. The decision criteria also identify when an action should be taken relative to the monitoring results, and what that action should be.

Decision criteria are important because they:

- Provide a basis for concluding that a desired condition has been or is being met.
- Support adaptive management with regard to the biomonitoring program, overall project activities, or both, and;
- Minimize potential for ambiguous or wrong decision-making.

The development of defensible decision criteria is critical for effective project management and decision making. Well thought-out decision criteria provide a quantitative basis for initiating a particular response (e.g., to take or not to take a specific action) to the biomonitoring results, and support adaptive management strategies related to overall project activities and goals as new biomonitoring data become available.

Decision criteria also provide a defensible basis for continuing, ceasing, or modifying not only the biomonitoring program but also the NERP activities that biomonitoring is supporting. By specifying the conditions under which the biomonitoring program may be terminated, decision criteria act to minimize the potential for “indefinite” monitoring thereby avoiding perpetual or inappropriate monitoring. Without clearly stated and rationale decision criteria, unnecessary data collection and analyses may occur, providing little support to the project goals and management objectives. For additional discussion on the importance of decision criteria, see guidance for habitat restoration monitoring (NAVFAC 2004a) and also USEPA guidance for monitoring at hazardous waste sites (USEPA 2004). Examples of decision criteria are provided in Appendixes B and C.

2.2.1 Elements of Biomonitoring Decision Criteria

In general, there are five main elements to a biomonitoring decision criterion:

- The biomonitoring parameter being measured (e.g., a tissue concentration, plant species diversity of an area);
- The metric used to measure the biological parameter (e.g., ug of a contaminant/g of tissue, a Shannon-Weiner diversity index);

- An action level (e.g., a specific tissue concentration, a minimum diversity index value) against which the monitoring results are compared and which results in an action when met or exceeded;
- The temporal considerations (e.g., endpoint measurements over time) in the development of the decision criteria; and
- The alternative actions to be considered for implementation when an action level has/has not been met or exceeded (e.g., issue a fish consumption ban when target tissue levels are exceeded, conclude restoration monitoring when the desired plant community is established).

2.2.2 Biomonitoring Action Levels

An action level represents a success criterion or performance standard that specifies the value of the measured parameter that when met or exceeded will require a management decision and action. Action levels must be carefully selected so that if met, there is certainty that not only the monitoring objectives but also the overall project objectives have been or are being achieved. In some cases, the action level(s) may be based on regulatory values, while in other cases, they may be based on discussions with appropriate stakeholders as well as considerations of time and money.

| Action Levels |
|---|
| An action level is the threshold value or condition that provides the basis for choosing between alternative actions. |

Depending on the biomonitoring objectives and the environmental conditions being targeted by the biomonitoring program, action levels may indicate either a positive or a negative environmental condition. For example, an action level for a habitat restoration project may be the establishment of a specific type of plant community over a specific area of restored habitat (i.e., at least 60% of the restoration site consists of plant community A). In this example, if plant community A occurs on at least 60% of the site, then the restoration is considered a success. On this basis, the management decision may be to end restoration activities and the biomonitoring program.

Alternately, an action level for a biomonitoring program targeting human exposure to site-derived contaminants may be a contaminant-specific tissues concentration in fish collected from the site. In this case, the target tissue concentration represents the maximum tissue level considered safe for human consumption. Exceeding this tissue concentration would be considered indicative of an unacceptable risk to human health from the consumption of fish from the site. In this example, when the action level is exceeded the management decision and action may be to issue a fish consumption ban for the site and initiate an investigation to determine the cause of the tissue concentrations.

In some cases, the decision criterion may actually be specified by several different action levels. For example, the management objective of a wetland restoration project may be the development of a wetland that replaces the previously impacted wetland plant community, provides habitat for certain wildlife, and enhances water quality. In this

case, the success of the project will depend on a number of parameters: the composition of the restored wetland plant community, the level of use of the restored wetland by the target wildlife, and an increase in water quality. Each of these conditions will require development of a specific action level (success criterion) which, when taken together, will provide the basis for determining whether the restoration has been successful.

Example 2.1 Multiple Action Levels for a Wetland Restoration Biomonitoring Program

About 2.9 acres of palustrine wetlands were disturbed during the removal of contaminated soil and sediments at Naval Submarine Base-New London at Groton, Connecticut (Appendix C). A Wetlands Restoration Plan was developed with the long-term goal of re-establishing the disturbed wetlands. There were four specific project objectives identified in the plan, including the restoration and enhancement of pre-remediation wetland function. For this objective, four action levels were identified:

- All streams and ponds associated with the affected wetland system show a trend toward greater biological diversity in the benthic invertebrate community.
- Post-remedial functions and values equal to or greater than pre-remedial functions and values.
- Predicted potential habitat for 27% of all wetland-dependent amphibians, reptiles and mammals evaluated by the WEThings Method (Whitlock et al., 1994).
- Restoration of 1.26 acres of emergent wetland, 1.17 acres of scrub/shrub/forested wetland and 0.47 acres of open water.

2.2.3 Temporal Considerations

In its simplest form, a decision criterion will include a specific action level that triggers a management decision following a single attainment or exceedance of that action level. For example, a single exceedance of a target fish tissue concentration may be sufficient to trigger an immediate consumption ban. Alternately, a decision criterion may combine a specific action level with time component in order to provide the basis for making a management decision and response. For example, while an action level may identify a specific tissue concentration (e.g., 5 ug/g), the decision criterion may also require multiple and consecutive exceedances of the specific tissue concentration (e.g., the action level must be exceeded for three consecutive sampling periods) before a management decision and response is triggered.

Trends in the biomonitoring data may also play an important role in interpreting the data relative to the action levels and overall project goals and management objectives. For example, the evaluation of fish tissue data over a period of time may show a distinct downward trend in contaminant concentrations over time. Observance of such a trend would provide confidence in project success. Alternately, an observed upward trend in

Time Series and Data Trends

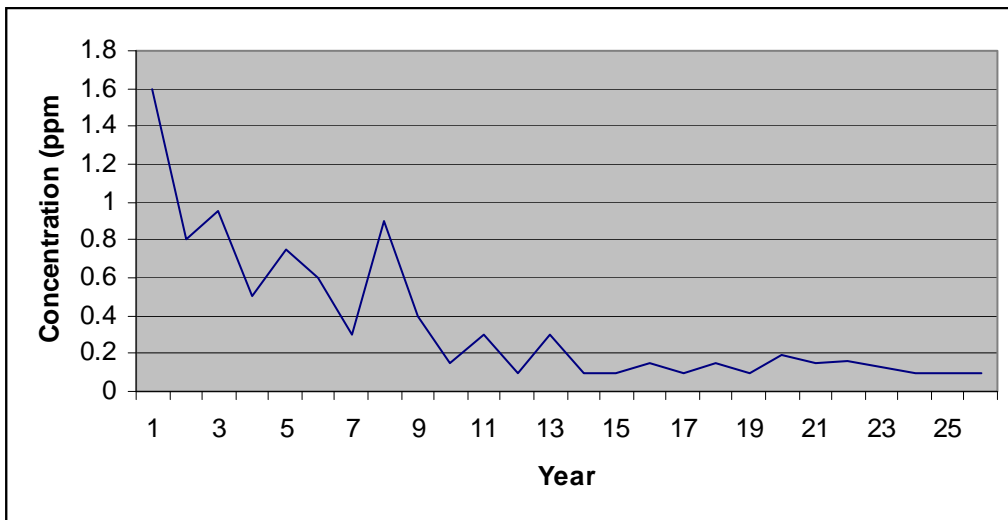
Data collected at regular intervals over time often show regular patterns, whether decreasing, increasing, or oscillating in nature. Examination of these data trends may provide insight into how environmental conditions are responding to project activities and how well project goals are being met. Interpretation of such trends may lead to changes in project activities, the biomonitoring program, or both, and may result in early termination of project and/or biomonitoring activities.

contaminant concentration would suggest that not only would the action level be exceeded, but that the project is not effective in controlling potential human exposure.

The temporal aspects of decision criteria will be a function of a variety of factors, especially the biomonitoring objectives. If biomonitoring is being conducted to provide warning of an unacceptable contaminant exposure, a single exceedance of the action level may be enough to trigger a management decision and response. In contrast, biomonitoring to evaluate habitat restoration success often includes trend analysis of the biomonitoring data. Habitat restoration involves the development of complex relationships among biotic and abiotic components of the local ecosystem. In addition, certain groups of organisms and habitats will recover following remediation more quickly than other groups or habitats. For example, earthworm populations have been reported to take from 3 to 15 years to recover following open-pit mining and reclamation (Rushton 1986). Specific relationships may become established at different rates, and all may be affected by the natural annual variability in climate. As a result, the restoration of a desired habitat may take many years (e.g., >10 years). In this case, the decision criterion may be based not on a single attainment of an action level, but rather on observed, long-term trend in the biomonitoring data towards a desired action level (indicating that restoration is progressing towards the desired condition). For example, four years of data may indicate that a desired action level (e.g., 60% of the site supports plant community X) has not yet been met. However, analysis of the data indicates that as a result of restoration activities the desired plant has increased its distribution across the site in each of the past four years and will likely reach the desired action level in the next few years.

Example 2.2 Trend Analysis

This example shows a long-term downward trend in PCB concentrations (aroclor 1248 + 1254 + 1260) measured in fish tissues collected from Iowa waters over a 25-year period (1980-2005) (modified from IDNR 2006).



2.2.4 Alternative Actions

Associated with each action level will be two or more alternative actions that represent the management options from which the decision maker will choose. The nature of these choices must be directly related to the overall project management goals and objectives. For example, if a site is undergoing habitat restoration, the overall management objective may be to restore the habitat to a particular condition. In this case, the alternative actions may involve continuing, modifying, or ending the restoration activities and/or the biomonitoring program. If biomonitoring is being conducted to provide early warning of unacceptable contaminant exposure of humans from the consumption of local fish and wildlife, then the alternative actions may be to continue monitoring, institute a consumption ban, and/or initiate characterization, risk assessment, and/or remediation activities.

Example 2.3
Decision Criteria, Action Levels, and Alternative Actions for a Navy Habitat Restoration Biomonitoring Program

1. Eelgrass Restoration for McAllister Point Dredging at Naval Station Newport
 - Project objective/management goal was to restore eelgrass habitat that was impacted by dredging.
 - Decision Criterion No. 1
 - If eelgrass seedling success is low, then additional planting will be implemented.
 - Action Level: Seedling success considered good if there a minimum of 50 shoots/m² are established over more than 50% of the seeded area.
 - Alternative Actions: 1) Conduct remedial planting if action level is not met or 2) no need for remedial planting if action level met or exceeded.
 - Decision Criterion No. 2
 - If at least 75% of the bottom area is covered with eelgrass, then the habitat restoration is considered successful
 - Action Level: Eelgrass covers at least 0.45 acres of the 0.6-acre restoration area after three years.
 - Alternative Actions: 1) Conclude restoration is successful if action level attained, and end restoration activities, 2) with regulatory concurrence, identify alternate methods of restoration, or 3) conclude that despite best efforts, successful mitigation is unlikely.

When selecting among alternative actions, there are three possible outcomes that could have serious negative consequences: 1) taking an action that is not needed; 2) not taking an action when one is needed; or 3) taking the wrong action. Each of these outcomes may result in unnecessary impacts to human health and/or the environment and affect overall project costs. Because of the consequences of selecting an inappropriate alternative, it is critical that each action alternative be clearly linked to a specific biomonitoring action level and management response, and that the rationale for selecting a particular alternative is clear-cut and well described and can be scientifically defended.

2.3 ADAPTIVE MANAGEMENT

Critical to any biomonitoring program is the incorporation of adaptive management principles and strategies into the interpretation of the biomonitoring data. As a biomonitoring program is developed and monitoring approaches and methods selected, it is important that not only the program, approaches, and methods but also project management be sufficiently flexible to respond to unexpected changes in abiotic and biotic conditions that affect the behavior of the biological parameters being evaluated. The inclusion of adaptive management strategies into the biomonitoring program will ensure that appropriate data may be collected to understand the cause and effects of unexpected environmental changes that may be affecting the biomonitoring results. The final decision rules should incorporate ongoing evaluations of the biomonitoring data as it is collected and analyzed, and support changes to the biomonitoring design as appropriate. This adaptive management approach should be integrated with adaptive management of the overall project, as the biomonitoring data may show the need to adjust some aspects of overall project activities.

Adaptive Management

Adaptive management allows the project manager to make “real-time” adjustments in project components and activities in response to data generated during biomonitoring. As biomonitoring data are collected and analyzed, the project and biomonitoring goals and objectives are reviewed and a determination is made as to whether adjustments can or should be made in project activities and/or biomonitoring activities that could help in reaching the overall project goals and management objectives for the site.

Adaptive management provides the flexibility for optimizing project activities in order to provide for the greatest likelihood of project success.

2.4 REGULATOR AND STAKEHOLDER CONSIDERATIONS

When developing a biomonitoring program, it is important to take into account how the regulatory community and other stakeholders might respond to the program. Depending on site-specific issues, some stakeholders may not be readily receptive to a biomonitoring program. Issues such as repeatability of results and technical soundness of biomonitoring methods may affect the acceptability of biomonitoring at the site. Many of these issues will be the same ones that were considered when deciding whether a biomonitoring program would be appropriate for the site.

Because of the need to gain stakeholder acceptance of not only biomonitoring but also of the use of the results in making management decisions, it is important to involve regulators and stakeholders early in development of the biomonitoring program.

3 BIOMONITORING BIOTA AND ENDPOINTS

An effective biomonitoring program requires the use of appropriate biota as well as chemical or biological parameters to be measured in that biota. In a biomonitoring program, organisms are exposed to existing site conditions (such as ambient contaminant concentrations, remediation activities, or a habitat undergoing restoration) while an endpoint is regularly and systematically measured during the exposure period. The measured results are then used to determine the direction and magnitude of change (if any) in the measured parameter away from or towards a baseline or predetermined condition. When these results are compared to monitoring action levels, decisions may be made regarding the condition of the environment, whether a project activity or site is adversely affecting the environment, and whether the biomonitoring and overall project objectives have been (or are being) met (Lower and Kendall 1990, NRC 1991, Beeby 2001, Golden and Rattner 2003).

3.1 BIOMONITORING BIOTA

The underlying assumption with biomonitoring is that the presence and/or condition of the biota being monitored directly reflect environmental conditions at a location (Johnson et al., 1993). Any change in presence, abundance, morphology, physiology, or behavior of the biomonitoring species is considered to indicate that one or more of its physical or chemical requirements are outside of preferred limits as a consequence of some environmental condition. Therefore, biomonitoring requires the use of species with one or more very specific and particular requirements for a known set of physical and/or chemical variables or conditions that can be directly linked to the ER site being monitored.

A wide variety of biota has been used for biomonitoring. Birds, for example, are one of the earliest animal groups used to monitor environmental conditions. Because of their increased sensitivity to carbon monoxide and quick response to decreasing air quality, canaries were used in mines to provide early warning of the presence of gas. The U.S. Forest Service currently includes selected bird species in many of its Land and Resource Management Plans (e.g., USFS 1995) as indicator species for monitoring forest health. In these plans, changes in the populations of these species are considered to reflect the effects of management activities on overall forest condition.

In aquatic systems, fish and invertebrates are commonly used as indicators of overall ecosystem health and condition. For example, Hillsenhoff (1982) and others have characterized various freshwater invertebrates on their relative intolerance of poor water quality conditions. The presence or absence of these species provides an indication of overall water quality of the area being monitored. Within the Great Lakes, the lake trout has been used as a regional indicator of water quality. Monitoring of annual harvests provides measures of overall lake productivity and the condition of the fishery, while contaminant measurements of lake trout tissue provide information regarding ecological and human exposure to hazardous chemicals as well as tracking contaminant levels in the

lake ecosystems (e.g., DeVault et al., 1985, Mac and Edsall 1991). Monitoring lake trout also may show the effects of multiple stressors on parts of the lakes, including nutrient enrichment, fishing intensity, releases of hazardous chemicals, invasive species, and other stressors. Elsewhere, the incidence of tumors in fish has been used to evaluate chemical exposure in industrial harbors and rivers (Balch et al., 1995).

In addition to the particular species used for biomonitoring, there must also be one or more specific parameters that are measured in the species. These parameters, the biomonitoring endpoints, are specific characteristic (such as abundance, a morphological character, or survival) of the biomonitoring species that is measured during the biomonitoring program. Measured changes in the endpoint are used to infer environmental conditions, and when evaluated against the biomonitoring action levels (see Section 2.2.2) serve as the basis for making decisions regarding the monitoring program and overall project objectives.

Biomonitoring Species: The species on which data are collected during the biomonitoring program.

Biomonitoring Endpoint: A specific parameter of the biomonitoring species that is measured during biomonitoring and compared against the biomonitoring action levels to support a management decision.

3.2 CATEGORIES OF BIOMONITORING SPECIES AND ENDPOINTS

3.2.1 Levels of Biological Organization

Biomonitoring may occur at, and utilize biota and endpoints, from a variety of levels of biological organization. These levels fall into three broad categories that represent a hierarchy of structural, taxonomic, and ecological organization and complexity ranging from molecules to ecosystems (Table 3.1).

Biomonitoring at the cellular level evaluates endpoints associated with changes in biochemical processes (e.g., enzyme activity) or subcellular structures (e.g., chromosome deformities) of individual cells or tissues, and often involve laboratory analyses with specialized analytical techniques and instrumentation.

Biomonitoring at the organismal level employs single- or multi-cellular biota and endpoints associated with whole organism responses, such as changes in abundance, biomass, and behavior. These variables are typically evaluated in the field using naturally occurring or caged biota and observational data (e.g., number counts, weight measurements). The most complex organizational category is the ecosystem. Ecosystem evaluations may address populations, communities, and habitats, and can include endpoints from multiple levels of organization which are integrated to draw conclusions regarding larger ecosystem conditions and effects. Biomonitoring that is typically

conducted in support of NERP activities is primarily carried out at the organismal or ecosystem level of organization.

Each organizational level has unique and distinct series of attributes which cannot be evaluated in other levels. For example, species diversity and a community structure are measurable characteristics of communities, but are meaningless attributes at a subcellular or organismal level. Similarly, measures of enzyme activity or evidence of chromosome damage may have little or no application to evaluating populations, communities, or habitats.

| Table 3.1. Levels of Biological Organization (modified from Krebs 1978). | | |
|---|--|---|
| Organizational Category | Level of Organization | Examples of Potential Biomonitoring Variables |
| Cellular | Molecules | Enzyme levels or activity; protein level; bioluminescence |
| | Subcellular Organelles | Chromatid structure |
| | Cells | Abundance, growth; shape; survival |
| Organismal | Species (individuals, including single-cell organisms) | Abundance, growth; behavior; survival; reproduction; tissue concentration |
| Ecosystem | Populations | Abundance (number); productivity or biomass; sex and age structure |
| | Communities | Species diversity; species richness; presence of sensitive species; community structure |
| | Habitats | Habitat structure; physical-chemical properties; habitat function; area |

The level of organization will affect not only the overall complexity of the biomonitoring program, but also its design, cost, technical requirements, regulator and stakeholder acceptance, and its interpretation. For example, methods that evaluate subcellular responses typically require specialized sampling and laboratory analyses, which may be costly and require extensive training, very specialized sample preparation, or unique analytical or laboratory procedures and instrumentation.

Alternately, studies evaluating ecosystems must deal with the many interrelationships between biotic and abiotic components of the ecosystem, which in many cases are not well understood and thus may add considerable uncertainty into interpretation of the biomonitoring results. To address these interrelationships and uncertainty, ecosystem studies often employ multiple biota and endpoints, thus increasing the complexity and cost of the biomonitoring program. It is important to keep in mind that because of their complexity, ecosystem-level studies may be the most difficult to conduct and interpret.

3.2.2 Ecological Habitats

Ecological habitats may vary in climate, geography, and other physical properties. As such, each habitat type supports biota adapted to conditions for that habitat and not found

in other habitat types. Due to differences in physical properties and species composition among habitat types, therefore, the processes leading to natural recovery may vary by habitat type. A biomonitoring program within a particular habitat type should take into consideration the biological, chemical, and physical properties that would affect the fate of the contaminant in the biota.

3.2.3 Cellular and Subcellular Endpoints

Biomonitoring at the cellular and subcellular level employs biochemical, physiological, or histopathological (structural) endpoints to indicate adverse effects from exposure to contaminants and/or provide early evidence of unacceptable levels of contaminants in the environment (Mayer et al., 1992; Johnson et al., 1993; White and MacNaughton 1997). In some cases, these endpoints are measured in naturally occurring biota, while in other cases a laboratory strain is used. The measurement of cellular or subcellular endpoints should be directly related to the site goals and objectives in accordance with the program's decision criteria. Examples of biochemical endpoints include enzyme activity (Custer et al., 2001; Whyte et al., 2004), immunosuppression (Weeks et al., 1992), bioluminescence (Sakai and Takigami 2003), presence of metabolites (Angerer et al., 1997; Bundy et al., 2004), and DNA alterations (Shugart 1988; Malins et al., 2004). Examples of physiological endpoints include changes in hemoglobin content (Oikari et al., 1985; Everaarts et al., 1993), genetic effects (Bickham et al., 2000), and a variety of organo-somatic indices (Everaarts et al., 1993; Hoque et al., 1998). Examples of histopathological (structural) endpoints include changes in kidney structure (Hinton et al., 1992; Hinton 1993), changes in red blood cell volume and/or number (Handy and Depledge 1999), changes in skin structure (Hinton et al., 1992), and chromosomal aberrations (Gómez-Arroyo and Villaobos-Pietrini 1995).

A major advantage of using cellular and subcellular endpoints is that many of the responses at this level occur within a short time of exposure to a stressor and thus allow for relatively rapid determinations of environmental conditions. This is especially desirable if the biomonitoring program is intended to provide early warning to unacceptable environmental conditions (such as contaminant concentrations in groundwater). In addition, the measurement of cellular and subcellular endpoints may be faster and less expensive than the collection and analysis of chemical data.

3.2.4 Organismal Endpoints

Organismal endpoints reflect stress-related cellular and subcellular changes but are evaluated at the whole organism level. For example, while growth can be measured using an entire organism, it is a function of metabolic and physiological processes. Organismal endpoints used to monitor environmental conditions have included measures of growth (Gagnon et al., 1995; Salazar and Salazar 1997a,b), presence/absence (Hilsenhoff 1987; Moore et al., 1991; Welsh and Ollivier 1998), behavior (Daly et al., 1995; Maltby et al., 2002; van der Schalie et al., 2004), survival (Buikema and Voshell

1993), reproduction and development (Donaldson 1990, Wilke 1991), respiration (Rockwood et al., 1990); gross physical abnormalities (Goede and Barton 1990, Blazer 2000, Khan 2000); and contaminant tissue concentrations (Seiders and Yake 2002, VDEQ 2003, NJDEP 2004). Many of these measures have been used in monitoring programs to provide early warning of environmental contamination and toxic conditions (van der Schalie et al., 2001).

3.2.5 Population and Community Endpoints

Population and community endpoints are used to evaluate environmental conditions by measuring changes at the population or community level using native (naturally occurring) biota. Population endpoints include changes in abundance (Welsh and Ollivier 1998) and growth (Salazar and Salazar 1997a, b), while community endpoints include changes in species diversity and community structure (Karr 1981; Bramblett and Fausch 1991; Barbour et al., 1999; Zweig and Rabeni 2001; Martin et al., 2005). Population and community endpoints are often used to monitor overall ecosystem conditions (e.g., Welsh and Ollivier 1998; Barbour et al., 1999). Because population and community endpoints may not be stressor-specific (i.e., permit identification of specific factors responsible for the observed biomonitoring results), they are often used together with cellular (and subcellular) and organismal endpoints to support a more definitive and defensible interpretation of results for biomonitoring programs targeting contaminants. Population and community endpoints are typically used in monitoring habitat restoration success and in monitoring the status of individual species.

3.2.6 Bioaccumulative Endpoints

In some biomonitoring programs, conclusions on environmental conditions and potential ecological or human health risks are based on a contaminant concentration measured in the tissues of exposed biota (Hellawell 1986; Beeby 2001; Custer et al., 2001; van der Oost et al., 2003). In this type of biomonitoring, individual organisms are exposed (either naturally or experimentally) to the environment, collected after some predetermined time, and analyzed for the contaminant of interest (Bargagli et al., 1995; Kim et al., 1996). The tissue concentration is a result of uptake of a contaminant directly from the surrounding environmental media (bioconcentration), from the ingestion of contaminated food, or both (bioaccumulation). Depending on the contaminant of concern, concentrations may be measured in specific tissues or in the entire body (i.e., whole body analysis). The results of these analyses provide information regarding 1) the presence and environmental transport of the contaminant of interest, 2) the movement of the contaminant through a food web, or 3) the bioavailability of the contaminant in a specific environmental medium.

3.3 BIOMARKERS

A relatively common biomonitoring approach is to measure a biochemical, physiological, and histological endpoint and use this value to estimate chemical exposure or effects. These endpoints are commonly referred to as biomarkers. While some environmental scientists broadly use the term biomarker to include measures of exposure and/or effects at any level of biological organization, the most common usage (and that adopted in this guide) is for biochemical, physiological, or histological indicators of exposure or effects at the suborganismal or organismal level (Huggett et al, 1992). An example of a widely used biomarker is the measurement of acetylcholinesterase (AChE) activity to provide evidence of exposure to organophosphate and carbamate insecticides (Xavier et al., 1998; Jebali et al., 2006). The toxicity of such insecticides is based on their inhibition of AChE, which results in interference with proper neurotransmission (Taylor and Brown 1994). The measurement of AChE activity is well accepted as a method to diagnose exposure to such insecticides in vertebrates (Stansley 1993).

Some biomarkers respond to a variety of chemicals and thus may be useful as general indicators of exposure to chemical mixtures, while others are specific to individual chemicals or classes of chemicals. Biological specificity also varies, with certain biomarkers having greater applicability to certain groups of organisms (see Huggett et al., 1992). In general, biomarkers may be more applicable for monitoring exposure (e.g., early warning) to chemical stressors associated with a chemical release, and may be less appropriate for evaluating the recovery of populations, communities, and habitats following remediation (i.e., during habitat restoration).

4 BIOMONITORING STUDY DESIGN

Once the biomonitoring objectives and associated questions and decision criteria have been developed and agreed upon (Figure 1.1), the next step in developing the biomonitoring program is the design of the biomonitoring study. The study design should be consistent with the UFP-QAPP (IDQTF, 2005). Study design will include the identification of the data needed to address the decision criteria, the identification of the biota and endpoints best suited for providing the necessary data, identification of an appropriate reference site, selection of sampling locations and sampling regime, and the identification of methods for analyzing the biomonitoring data.

In order to fully support the biomonitoring objectives and management decisions, a well designed biomonitoring study should have the following characteristics:

- It should be effective at readily detecting environmental changes;
- It should be readily implementable; and
- Its objectives and approach should be clear, understandable, and acceptable to stakeholders.

Thus, the selection of biomonitoring biota, endpoints, and methods should be conducted with the intent to optimize (to the extent possible) these three characteristics. Some biota, endpoints, and methods will be better at meeting some of these characteristics and less effective at meeting others. By considering these three characteristics, the project team can select a combination of biota, endpoints, and methods that best meets all three characteristics.

In addition, the selection of biomonitoring biota, endpoints, and methods will be related to, and affected by, the data needed for the biomonitoring decision criteria and associated action levels. Table 4.1 illustrates the types of data that may be needed for general types of project and biomonitoring objectives.

Table 4.1 Examples of Data Needs for Different Project and Biomonitoring Goals

| Example Project Goal | Biomonitoring Objective | Decision Criteria | Potential Data Need |
|---|--|--|--|
| Control offsite contaminant transport in order to avoid exposure of biota in offsite habitats | Provide early warning of contaminant exposure of biota at offsite habitats | Tissue concentration of contaminant of concern exceeds specified value for three consecutive sampling events | Tissue concentration of contaminant of concern in biota resident at offsite habitats |
| Manage offsite effluent discharge to avoid environmental | Provide early warning of effluent discharges that could result in | Effluent exhibits prescribed level of toxicity | Effluent toxicity tests and effluent chemical analyses |

Table 4.1 Examples of Data Needs for Different Project and Biomonitoring Goals

| Example Project Goal | Biomonitoring Objective | Decision Criteria | Potential Data Need |
|---|--|---|---|
| concentrations that are toxic to fish | toxic conditions to the resident fish community | | |
| Restoration of aquatic habitat to pre-impacted conditions | Evaluate effectiveness of habitat restoration activities and determine when successful restoration has been attained | Biotic communities exhibit specified minimal desired characteristics for specific number of sampling events | Community structure metrics for communities of interest |
| Restoration of native terrestrial vegetation communities | Evaluate effectiveness of restoration activities | The vegetation community meets the minimum desired characteristics | Metrics of vegetation community structure |

4.1 BIOMONITORING BIOTA

4.1.1 General Categories of Biomonitoring Biota

The biota used for biomonitoring may be placed into three broad categories based on the overall biomonitoring objectives (NRC 1991; Johnson et al, 1993; Beeby 2001). *Monitoring species* are used to evaluate an activity or environmental condition on the basis of a measurable change in a biological structure or performance (e.g., chromosome damage, reduced enzyme activity).

Indicator species are used to evaluate environmental conditions on the basis of the species absence or abundance (rather than on changes in a chemical, physical, or biological parameter) within the environment of interest. Lastly, *sentinel species* are used to evaluate and provide early warning of adversely changing environmental conditions on the basis of observed levels of pollutants in their tissues (Rabinowitz et al., 1999; van der Schalie et al., 1999; Beeby 2001). These general categories are not exclusive with the types of biota that may be used, and all the categories may be used to draw conclusions regarding threats to human health and the environment, or on the success of remediation or habitat restoration activities of projects.

Categories of Biomonitoring Biota

- Monitoring Species – examined for changes in physical, chemical, or biological attributes.
- Indicator Species – examined for absence or abundance.
- Sentinel Species – examined for levels of contaminants in their tissues.

An underlying assumption for all biomonitoring biota is that their presence, abundance, or condition is directly related to their ecological requirements (food, shelter, climate), their tolerance of environmental stressors, and the nature and magnitude of stressors in their environment. If a species is present in numbers or in a biological condition expected for individuals living in a “healthy” environment, then it may be assumed that its ecological requirements are being met and that there are no adverse environmental conditions (stressors). However, a change in the expected abundance, behavior, or other parameter is considered to reflect a change in one or more environmental conditions that are affecting exposed individuals. These changes may be reflective of positive or negative environmental conditions and affects. For example, an increase in the abundance of a desired species at a habitat restoration site could be considered to reflect an increase in habitat quantity or quality for that species.

4.1.2 Biota Attributes

In general, the best choice of a species for biomonitoring has 1) well understood environmental and ecological requirements, 2) well understood but narrow tolerance of the environmental stressor of concern, and 3) responds quickly and consistently to changes in environmental conditions. In contrast, a species with a wide range of environmental requirements and a wide tolerance of environmental stressors may be expected to be much less affected by a change in environmental conditions, and thus would probably be a poor choice for use in biomonitoring (Johnson et al., 1993). In addition, a species with a large home range may confound attempts to attribute effects to the site and would likely be a poor choice for use in biomonitoring.

In biomonitoring, the presence, abundance, or condition of the organism is assumed to be directly related to 1) the ecological requirements of the organism, 2) its tolerance of environmental stressors, and 3) the nature and magnitude of environmental stressors to which the organism has been exposed.

When evaluating species for use in a biomonitoring program, there are a number of common characteristics that should be considered (Table 4.2). These characteristics relate to the susceptibility of the species for exposure to the environmental conditions of interest (whether a site-related contaminant, a project-related activity, or a habitat undergoing restoration) and also take into account aspects of data collection and natural variability.

To minimize the potential for environmental conditions that are not associated with the site affecting the biomonitoring results, an ideal biomonitoring species should have a narrow tolerance of, or a distinct preference for, specific environmental conditions or requirements (such as an intolerance to a specific contaminant or a preference for particular type of habitat). It is also helpful if the relationship of the

The better known the ecological and environmental requirements of a species and its responses to the specific stressor of interest, the less uncertainty there will be in interpreting the biomonitoring results.

Table 4.2 Characteristics for Evaluating Candidate Biomonitoring Species

| Evaluation Characteristic | Importance |
|--|---|
| For free-ranging species, the species should be numerous and easily sampled (counted or captured). | Reduces sampling effort and cost and increases likelihood of attaining statistically valid sample sizes. |
| If a free-range species is considered, it should be relatively common and widely distributed. | Increases likelihood of identifying a suitable reference site and determining the contribution of background conditions and natural variability to the observed results. |
| The species should be readily identifiable by non-specialists. | Reduces sampling effort and costs. |
| Sampling of the species should not adversely affect its population. | Minimizes or avoids potential for the biomonitoring program to cause adverse population-level impacts to the species of interest. |
| The species should have limited mobility and have a territory or home range that overlaps the site or area of interest. | Maximizes site-specific exposure and reduces the potential for exposure to environmental conditions outside of the area of interest. |
| The species should be long-lived. | Allows for long-term exposure and evaluation of all life stages. |
| The relationships between the environmental requirements and biological characteristics (ecology, behavior, physiology, etc.) of the species should be well known. | Increases understanding of the relationship between any observed changes in biological parameters and environmental conditions, which in turn aids in the evaluation of the biomonitoring data. |
| The species should have a measurable response to the activity or environmental condition of interest. | Allows stronger linkage of results to the site activity or environmental conditions. |
| The species should exhibit relatively low and narrow ecological and environmental requirements. | Reduces contribution of natural variability in the observed biological responses and strengthens linkage between observed biological and environmental changes. |
| For monitoring bioaccumulative contaminants, the species should exhibit ready and rapid uptake of, but be largely unaffected by exposure to, the contaminants of interest. | Allows for rapid and relatively real-time evaluation of potential exposure of human and ecological receptors to contaminants in the environment. |

Source: Rosenberg and Weins 1976; Hellawell 1986; Johnson et al., 1993; Sheffield et al., 1998

species to the environmental stressor of concern (e.g., a contaminant) is well documented and understood, and that other factors that may similarly affect the species are known. The better these relationships are understood, the less justification will be needed to support use of that species for biomonitoring, and the less uncertainty there will be in interpreting the biomonitoring results and making subsequent management decisions. This holds true for all species being considered for biomonitoring, regardless of the level

at which the biomonitoring will occur (e.g., organism, community, or ecosystem) or the category of monitoring species (i.e., monitoring, sentinel, or indicator).

4.2 CAPTIVE VS. NATURALLY-OCCURRING BIOTA

Some biomonitoring programs use methods that employ “standardized” biota that are evaluated under very controlled exposure conditions. Examples include Microtox™, which evaluates changes in bioluminescence in a laboratory strain of bacteria following exposure to contaminated media, and any number of toxicity bioassays that utilize specific biota (e.g., certain types of alga, invertebrates, fish, and amphibians) to measure stressor effects. In these cases, method-specific chambers are used to produce the exposure, and the exposed biota are evaluated under laboratory or “laboratory-like” conditions.

In contrast, other biomonitoring programs are conducted *in situ* (i.e., ‘in the field’). These programs evaluate real-world exposures and effects by using either captive or naturally-occurring biota (or a combination of both). Captive and naturally-occurring biota each has advantages and limitations for use in a biomonitoring program.

4.2.1 Captive Biota

Captive biota are organisms that are restricted to a site or area of interest (i.e., a contaminated stream bank) through the use of a cage or other type of test chamber (Burton et al., 2005), and are not permitted to freely move about. By directly placing the organisms in specific locations and restricting their movement, the biomonitoring program can evaluate exposure and effects at specific locations and under very site-specific environmental conditions. Captive organisms are most often used in biomonitoring programs in aquatic habitats; these programs typically employ benthic invertebrates such as molluscs (Maltby et al., 2002; Applied Biomonitoring 2004). These species may or may not be naturally occurring in the monitoring area.

The use of captive biota provides a number of advantages. Because the organisms are exposed in the field, realistic exposure levels can be evaluated, and the affect of natural variability and some confounding factors may be reduced or evaluated. The use of caged organisms also allows for the experimental testing of exposure and effects. For example, captive biota may be placed along a contamination gradient, in hot spots, or in specific areas of interest. The use of captive biota also ensures adequate sample sizes. To limit the amount of stress that would be

Advantages of Using Caged Biota

- Ensures exposure to site-specific conditions.
- Effects of confounding factors can be controlled or evaluated.
- Allows for experimental testing of exposure and effects.
- Attainment of adequate sample sizes.
- Baseline data on biological characteristics of interest may be available, increasing discernment of site-specific effects.

incurred by a captive organism, biomonitoring programs using captive biota typically use relatively small organisms that exhibit little or no mobility. Disadvantages of using captive organisms include difficulty of deployment in some environments (such as high current areas and shorelines with heavy wave action), locating appropriate reference sites, potential effects of the confining chamber, depredation of the captive biota while deployed, and vandalism of chambers during the exposure period.

4.2.2 Naturally-Occurring Biota

Naturally-occurring biota as considered in this guide are those that normally inhabit or use the area of interest (such as the NERP site) and exhibit natural activities and behaviors. Thus, naturally-occurring biota provide the best measure of actual site-specific exposure, uptake and effects. Naturally-occurring biota includes mobile organisms as well as plants. Biomonitoring programs at habitat restoration sites typically evaluate both naturally-occurring biota, which colonize the restoration site from surrounding areas, as well as planted native vegetation. Biomonitoring of sensitive resources during remediation activities focuses exclusively on naturally-occurring biota, while biomonitoring programs based on tissue analyses may utilize caged biota, naturally-occurring biota, or both.

A variety of factors should be examined when considering the use of naturally-occurring biota for biomonitoring. Factors such as the distribution, abundance and reproductive capability of the candidate biota will help indicate the level of exposure that the organisms may be exposed to at the site or area of interest, as well as which life stages may be present and exposed. From a practical standpoint, it is also important to consider the species susceptibility to capture; naturally-occurring biota should be present in sufficient numbers and be distributed so that it will be readily encountered for counting or capture, and provide for a sufficient sample size. It is also important to ensure that any collection of organisms will not adversely affect populations of the biota being monitored.

When considering the use of mobile species, such as fish and birds, the territory size or home range of the candidate species should be examined. Unless the home range is completely encompassed by the site boundaries, wide-ranging species will spend time outside of the area of interest, while highly mobile and active species may spend relatively little time in the area of interest during their normal daily activities. Thus, it will be difficult to determine the actual amount of time (and thus level of exposure to site conditions) spent by mobile, wide-ranging species at a site. In addition, wide-ranging biota will be exposed at off-site locations to environmental stressors (including contaminants that may be of interest at the site) from non-Navy sites and activities. Thus, it will also be difficult to equivocally link any measured exposure or effects to Navy sites or activities, or Navy contributions to any measured exposures or effects.

Mobile species that are seasonal residents, however, may be appropriate for a biomonitoring program if the variable to be evaluated is associated with that residence

period. For example, migratory birds may not be year-round residents at a site, but adults that have returned to an area in spring typically exhibit a high degree of site fidelity (i.e., they stay at the site and surrounding vicinity) during the reproductive season, while young will be completely restricted to the site. Thus both adults and young will experience high levels of exposure to environmental conditions around the nest site. The advantages and disadvantages of using naturally occurring biota are summarized in Table 4.3.

Table 4.3 Advantages and Disadvantages of using Naturally Occurring Biota for Biomonitoring.

| Advantages | Disadvantages |
|---|---|
| Exposure and effects reflect real-world exposure due to natural activities and behaviors at the site. | For highly mobile and wide-ranging biota, measured exposure and effects may be due to other, non-site associated conditions or factors. |
| Exposure and effects reflect natural variability in biota and the environment. | Baseline data for natural variability likely limited or unavailable, making it difficult to differentiate between natural variability and site-related effects. |
| | Ability to control confounding factors limited. |

4.3 SELECTING BIOTA

The selection of biota for biomonitoring will be strongly affected by the project for which the biomonitoring program is being developed and the overall goals and objectives of the biomonitoring program (i.e., early warning of human exposure, measuring restoration success, protecting sensitive resources). For example, biomonitoring of habitat restoration projects or of sensitive species or ecological resources (such as habitats) during remediation activities will most likely involve native naturally-occurring species that are the target of the overall project. Naturally-occurring species are native species found within the biomonitoring program’s area of interest. For these types of projects, the overall project goals and objectives will be directed towards specific native species, communities, and habitats, and thus the biota for monitoring these projects will be drawn from the target species of interest.

In contrast, biota used for biomonitoring of contaminant-related projects (such as site cleanup or early warning) may be sedentary or captive, and may or may not include naturally-occurring species. Contaminant-related biomonitoring programs provide information on whether, and if so at what level, human health and/or ecological exposure are occurring from a NERP site or activity. When considering which species to use for contaminant-related biomonitoring, the selected species must meet certain requirements: 1) it must be native to the area surrounding the biomonitoring program’s area of interest, 2) it must be exposed to the contaminant of interest, and 3) it must exhibit a measurable response.

Navy staff responsible for managing natural resources on the Navy facility can be very helpful in identifying naturally-occurring species at the site from which candidate biomonitoring species may be selected.

4.3.1 Identifying Candidate Biota for Biomonitoring

The identification of candidate biota should consider a number of factors related to the exposure potential of the species under consideration, how susceptible the candidate species may be to incurring adverse effects (i.e., if exposed how likely will an individual be affected?), and the nature and magnitude of those effects (i.e., if individuals are affected, what will be the effect on the population and how easily could the population recover?) (Lower and Kendall 1990; Golden and Rattner 2003). Factors to be considered when evaluating candidate biota include:

- the exposure potential of the species,
- the spatial and temporal distribution and occurrence of the species,
- how easily the species can be collected,
- the availability of exposure and effects data for the species,
- the sensitivity of the species for being adversely affected by an exposure, and
- how readily an adversely affected population can naturally recover (its resilience),

The lower the likelihood of a species for exposure to a site-related activity or contaminant, the less suitable that species is for biomonitoring.

Different species will have differing characteristics associated with these factors, and the most suitable species for biomonitoring will be those species that best optimize these factors. Some of these factors will apply only to naturally-occurring species, and may not be applicable for selecting species for biomonitoring programs employing captive biota.

4.3.1.1 Exposure Potential

Exposure potential considers the likelihood that individuals of the candidate species would be exposed to site related activities and/or contaminants. This exposure could occur by one or more of the following exposure routes: direct contact, ingestion, inhalation, and dermal uptake. Factors that could affect the potential exposure of a species include its habitat preferences, diet preferences or requirements, life span, foraging technique (how it feeds), and other behaviors (such as burrowing) (Table 4.4). The lower the exposure potential of a species, the less suitable that species is for biomonitoring. Consideration of exposure potential would not be applicable for selecting captive biota for biomonitoring.

Table 4.4. Evaluating Exposure Potential of Candidate Biota.

| Evaluation Factor | Consideration Questions | Role in Affecting Potential Exposure |
|--------------------------|---|--|
| Diet | What does the species prefer to eat? What is its primary diet component? What is the trophic level of this species? | Relates to the likelihood of contaminant exposure via ingestion. Also incorporates knowledge of where in food chains the contaminant of concern is likely to occur and at greatest concentrations. |
| Foraging Style | How does the species obtain its food? | The potential for exposure via incidental ingestion of contaminated soil, sediment or surface water will differ if food is obtained from within, from the surface, or from above, the contaminated medium. |
| Life Span | How long does the animal live? | Directly relates to the duration of exposure that an individual may experience during its lifetime. |
| Preferred Habitats | Where does the organism live? What habitats does the organism use for feeding, resting, and reproduction? | If the habitat that a species prefers or requires is not present, or only present in minimal quantities, exposure potential will be limited. If the stressor of interest does not occur in the preferred habitat, then exposure will be unlikely. |
| Other Behaviors | Could any of the normal behaviors of the species result in exposure to the stressor of interest? Does the species utilize or avoid certain types of land use? | Other behaviors can directly affect the nature and duration of direct exposure to an environmental stressor. For example, burrowing could increase the likelihood of exposure to soil contaminants due to the incidental ingestion, dermal uptake, and inhalation of soil while burrowing. Similarly, some species may avoid certain areas (such as industrial settings) where the stressor of interest occurs, thereby reducing the likelihood of exposure. |

4.3.1.2 Spatial and Temporal Occurrence

Spatial and temporal occurrence considers the geographic distribution of a candidate species (where is it found?) as well as its daily and seasonal occurrence at the site or area of interest (when is it present?). The consideration of spatial and temporal occurrence is not applicable for evaluating candidate biota for a captive biomonitoring program. Some species (e.g., vegetation, small mammals) will be year-round residents and are present at the site or area of interest year-round. Other species may be considered as year-round residents but exhibit daily movements onto and from the site or area (e.g., fish that move with daily tidal patterns into shallow habitats to feed). Such species may therefore only be exposed for a portion of any 24-hr period.

Some species of fish and wildlife exhibit extensive seasonal movements which result in extended periods of absence from certain habitats. For example, in the eastern U.S. there are many bird species that are common summer residents and breed in suitable habitats, but migrate south to the Gulf Coast or beyond and are absent from the East in winter. In

contrast, other birds breed in northern latitudes (Canada, the Arctic) and thus are absent from the East in summer, but migrate southward in fall and may overwinter in portions of the East. Finally, there are many bird and some bat species that can be considered as transitory visitors. These species are only present for a brief period of time when they are passing through the area as part of long distance migration.

Species that occur in the area of interest for all or most of their lives have a greater potential for exposure to site-related conditions or activities than do species that exhibit daily or seasonal movements to and from a site or area. Among seasonally migratory species, those that breed in the area of interest would be preferable. Although these species are present for only a specific time period, they occur at the site throughout the breeding season, and their offspring represent individuals that are completely restricted to the site during their growth and development and may be most sensitive to site-related activities or conditions.

The less time an individual organism spends in the area of interest, the less likely that individual is to be exposed and thus the less suitable it is for biomonitoring.

4.3.1.3 Ease of Collection

To be useful for biomonitoring, a species must be easily collected. Species that are easy to capture should be favored over those that are highly mobile and/or require specialized equipment and complicated or complex collection schemes. Factors that affect the collectivity of a species include its population size (small or large), social structure (is it solitary, social, or colonial?), accessibility (is it easy to locate and get to?), ease of capture, and its management status (is it protected regulations) (Table 4.5) (Golden and Rattner 2003). It will be easier to find and collect individuals when the population size is large than when small. Similarly, it will be easier to find and collect individuals when they aggregated in a colony or flock. Finally, some species may be protected by federal, state, or local environmental regulations, which may limit or prevent their collection and thus their use for biomonitoring. For biomonitoring programs designed to protect threatened or endangered species, surrogate species should be considered for monitoring. These surrogate species should have similar life-history traits and habitat requirements as the protected species but be locally common and easier to sample.

The easier to find, identify, and capture individuals of a species, the better that species for biomonitoring.

The ease of collection should also be evaluated when a captive biomonitoring program using naturally-occurring species is being considered. For example, naturally occurring biota (such as mussels) may be collected from an unaffected area, caged, and placed into the area of interest. Depending on the desired exposure and monitoring frequency, the ease of collection of the biota may directly affect how often caged biota may be deployed at the site

Table 4.5. Evaluating Ease of Collection of Candidate Biota for Biomonitoring.

| Evaluation Factor | Consideration Questions | Role in Affecting Potential Exposure |
|--------------------------|---|---|
| Population Size | Is the species common and individuals abundant or uncommon in the area of interest? | The more abundant the species, the easier to not only collect individuals but also to collect sufficient numbers for analysis. |
| Social Structure | Is the species solitary, or does it occur in colonies, flocks, or schools? | It may be less costly and labor intensive, and more likely to obtain a desired sample size, when collecting individuals of a species that occur in colonies, flocks, or schools that when collect individuals of species that are solitary. |
| Accessibility | How easy is it to get to where individuals of the species can be encountered and collected? | Some areas will be much more difficult to collect in than other areas. For example, sampling in a high current or high wave action area is more difficult than sampling in a shallow water habitat in a protected shoreline. |
| Ease of Capture | Is the species mobile or sedentary? What would be the best way to capture individuals? Are there readily available methods and what do those methods involve? | Cost, effort, and complexity of collection methods will vary among species and the habitats they inhabit. Highly mobile organisms will be more difficult to capture than more sedentary ones. |
| Management Status | Is the species protected or managed under federal, state, or local regulations? | Regulatory protection will likely include restrictions or prohibitions on sampling and collection. |

4.3.1.4 Existing Exposure and Effects Data

Biomonitoring assumes that the presence or condition of the biomonitoring organisms will be a direct reflection of site-specific exposure of that organism to the environmental conditions of the site or area of interest and the nature of the stressor of interest (e.g., a contaminant). The availability of existing data regarding species-specific exposure and effects is an important factor to consider when evaluating candidate biota. Available exposure and effects data can provide baseline information on the nature and magnitude of exposure and effects that the biota may incur from the stressor. Such information may be found in the EPA Wildlife Exposure Factors Handbook (USEPA, 1993a, b) or primary literature sources. If little is known about how a species may be exposed or affected by a particular stressor, it will not be possible to identify appropriate endpoints to evaluate in that species, nor to draw conclusions regarding the relationship

The availability of species- and stressor-specific exposure and effects data allows for better evaluation of the biomonitoring results and decreases uncertainty in the interpretation of the biomonitoring data.

between observed or measured effects and site conditions. Species with greater amounts of existing data should be given preference for potential use in a biomonitoring program.

4.3.1.5 Sensitivity

Sensitivity refers to the likelihood that an individual will be affected following exposure to an environmental condition or stressor (Golden and Rattner 2003). The sensitivity of a species will be a function of the environmental condition or stressor (such as a restored habitat or contaminant) and one or more species-specific parameters (such as ability to metabolize and eliminate a contaminant, growth rate, or population size). Sensitivity to chemical effects may be especially important to consider in biomonitoring programs that provide early warning of contaminant exposure. Depending on the objectives of the biomonitoring program, a very low sensitivity to a condition or stressor may be desirable. For example, biomonitoring that employs tissue concentration as an indicator of environmental contaminant levels would be best served by a species that readily uptakes the chemical of interest but is otherwise unaffected by the chemical. If the species is highly sensitive to the toxic effects of the chemical, individuals might not survive long enough to attain measurable tissue concentrations, nor be present in sufficient numbers for sampling. Table 4.6 presents sensitivity factors that have been proposed for several types of contaminants.

The greater the sensitivity of a species to a stressor, the more likely that species is to incur a measurable adverse effect from an exposure.

4.3.1.6 Natural Recovery

Natural recovery refers to the ability of an affected population to recover following an adverse impact or harmful exposure. Factors that affect the ability and speed of an affected population to naturally recover include mobility, its abundance both within and outside the area of interest, its reproductive potential, and the age at which breeding begins. Each of these factors may affect how quickly population numbers could be restored via immigration and the production and recruitment at the site of young into the adult population. Some types of habitats, communities, and populations may be capable of recovering more quickly than others. The more quickly a population can recover, the less likely it is that a monitoring program will be able to detect an adverse effect in that population and its members (Golden and Rattner 2003). Consideration of natural recovery may be especially important if sampling is expected to occur infrequently (i.e., because of funding constraints or difficulties in site accessibility). Natural recovery would not be a factor to consider when selecting species for a captive biomonitoring program. For identifying

The lower the ability of an affected population to recover to pre-disturbance levels, the more susceptible that species is to exposure to a stressor and the more likely more likely it will be to detect an adverse effect through biomonitoring.

biomonitoring species for use in the types of biomonitoring typically conducted at NERP sites, the consideration of natural recovery may be less important than consideration of the other factors.

Table 4.6. Potential Sensitivity Factors for Different Types of Contaminants (Golden and Rattner 2003).

| Contaminant Type | Sensitivity Factor | Basis for Factor |
|--------------------------------------|---|--|
| Persistent Organic Pollutants (POPs) | Feeding Specialization | Species with more specialized diets exhibit lower production of enzymes that metabolize POPs. |
| | Ability to Metabolize and Clear Contaminant | Elimination of POPs directly related to ability to metabolize POPs. |
| Cholinesterase-inhibiting Pesticides | Feeding Specialization | Species with more specialized diets exhibit lower production of enzymes that metabolize the pesticides. |
| | Ability to Metabolize and Clear Contaminant | Elimination of pesticide directly related to ability to metabolize the pesticide. |
| Petroleum Crude Oil | Effect of Oil on Waterproofing and Insulation | Fouling of feathers or fur results in loss of insulation, loss of buoyancy, and increased metabolic costs to compensate for heat loss. |
| | Feeding Specialization | Species with more specialized diets exhibit lower production of enzymes that metabolize crude oil. |
| | Ability to Metabolize and Clear Contaminants | Elimination of petroleum compounds directly related to ability to metabolize those compounds. |
| Mercury | Molt | Birds and fur-bearing mammals reduce mercury body burden through molting. Increased molting provides greater opportunity to eliminate mercury. |
| Lead Shot | Proportion of Protein or Calcium in Diet | Waterfowl with diets high in protein or calcium are less susceptible to toxic effects of lead. |
| | Dietary Preferences | Erosion of ingested lead shot (making available for absorption in the gut) related to degree of development of gizzard. Seed-eating birds have muscular gizzards that grind hard foods while carnivorous birds have weaker gizzards that are used primarily for storage of indigestible foods for later expulsion as pellets |
| | Relative Body Size | The extent of adverse effects in waterfowl ingesting equal amounts of lead shot have been reported to be greater in smaller species than in larger species. |

4.3.2 Selecting among Candidate Biota

If several candidate species are being considered for biomonitoring, the selection of one or more of the species may be accomplished by following a process that evaluates each

species on its suitability for biomonitoring and ranks the candidate species on the basis of overall highest suitability for biomonitoring (Figure 4.1).

Once a list of candidate species has been developed, each species should be ranked for each of the suitability factors (e.g., natural recovery, exposure potential). To assign such a ranking, a numerical ranking value can be assigned to each suitability factor that reflects the perceived suitability of the candidate for that factor. For example, ranking values may range from 1 to 5, with a value of 1 indicating poor suitability and 5 indicates best suitability. Once ranks have been assigned to each suitability factor, a total suitability rank can be calculated by summing the ranking values assigned for each suitability factor.

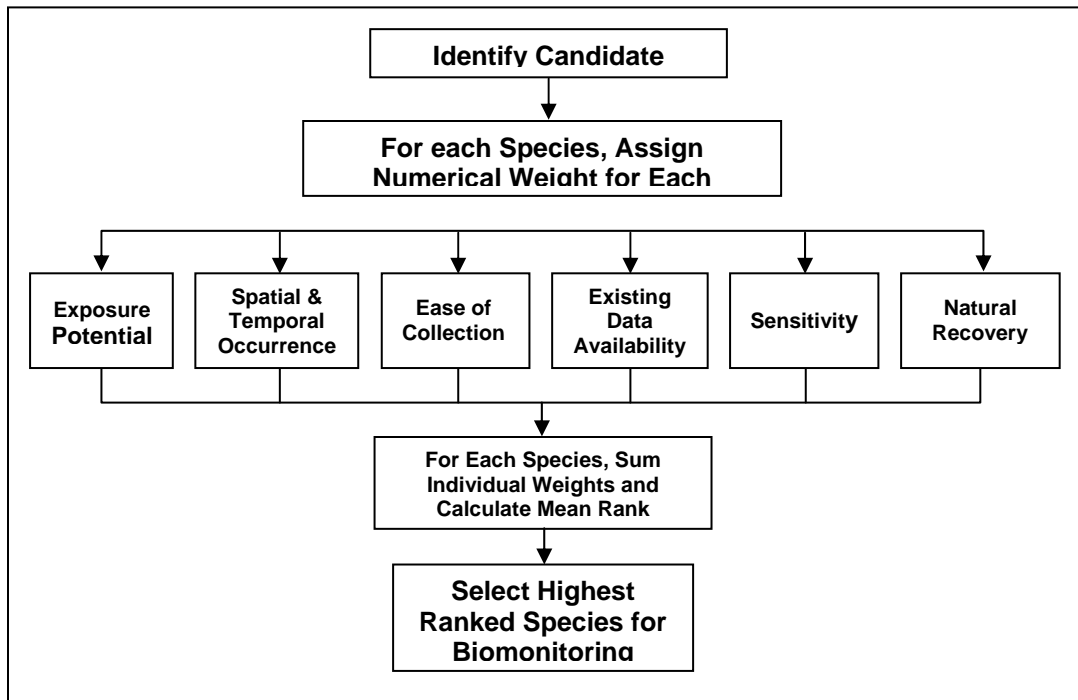


Figure 4.1 Process for Selecting Naturally Occurring Biomonitoring Species (Modified from Golden and Rattner 2003).

As previously discussed, each of the suitability factors may have one or more parameters that were evaluated to determine overall factor suitability. For example, evaluation of the ease of collection of a candidate species may have included population size, social structure, accessibility, ease of capture, and management status (Section 4.3.1.3). In this example, each of these evaluation parameters is assigned a numerical weight of 1 (worst) to 5 (best), and a mean suitability ranking value is calculated for the evaluation factor (Table 4.7).

In this example, each of the evaluation parameters were considered of equal importance, and the resulting mean ranking value represents an “unweighted” suitability rank. In some cases, the evaluation parameters may not be considered to be of equal importance.

For example, the team developing the monitoring program may feel that accessibility and ease of capture are more important than the other parameters. In this case, a weighting factor may be applied to the accessibility and ease of capture evaluation parameters to reflect their greater perceived importance (Table 4.7). When using additional weighting factors, it is important that the weighting factors be applied equally to all candidate species.

Table 4.7. Example Calculation of Mean Numerical Ranking Value for the Suitability Factor “Ease of Collection” (from Golden and Rattner, 2003).

| Ease of Collection Evaluation Parameters | Unweighted Suitability Rank (1 = poor; 5 = best) | Weighting Factor | Weighted Suitability Rank |
|---|---|-------------------------|----------------------------------|
| Population Size | 5 | 1 | 5 |
| Social Structure | 3 | 1 | 3 |
| Accessibility | 2 | 2 | 4 |
| Ease of Capture | 4 | 2 | 8 |
| Management Status | 5 | 1 | 5 |
| Mean “Ease of Collection” Ranking Value | (5+3+2+4+5)/5 = 3.8 | | (5+3+4+8+5)/5 = 5.0 |

Once a mean rank is calculated for each evaluation factor, the mean rank values are then summed to provide an overall suitability score for the candidate species (Table 4.8). A weighting scheme (as discussed for the factor-specific evaluation parameters) may also be applied to reflect any perceived differences in importance among the suitability factors. For example, exposure potential and sensitivity may be considered the most important suitability factors in selecting the biomonitoring species for a particular biomonitoring program. In this case an additional weighting scheme may be applied to each suitability factor (Table 4.8). Once calculated for all the candidate species, the overall suitability scores can be compared, with species having higher scores considered to have a higher suitability for use in the biomonitoring program than lower scoring candidates.

Such an approach supports the selection of biomonitoring biota that best optimizes multiple parameters related to exposure, effects, data collection and analysis, cost, and effort. The use of a suitability score also provides a defensible, rationale basis for selecting one or more biomonitoring species from among several candidate species. See Rattner and Golden (2003) for ranking schemes developed for selecting terrestrial vertebrate biota from Atlantic Coast habitats for monitoring different types of contaminants, such as persistent organic pollutants, petroleum crude oil, mercury, and lead shot.

Table 4.8. Example Calculation of an Overall Suitability Score (from Golden and Rattner, 2003).

| Suitability Factors | Unweighted Suitability Rank (1 = poor; 5 = best) | Weighting Factor | Weighted Suitability Rank |
|---------------------------------|---|-------------------------|--|
| Exposure Potential | 4.9 | 3 | 14.7 |
| Spatial and Temporal Occurrence | 4.2 | 2 | 8.4 |
| Ease of Collection | 3.8 | 2 | 7.6 |
| Existing Data Availability | 2.0 | 1 | 2.0 |
| Sensitivity | 5.0 | 3 | 15 |
| Natural Recovery | 3.1 | 1 | 3.1 |
| Mean Suitability Score | (4.9+4.2+3.8+2.0+5.0+3.1)/6 = 3.8 | | (14.7+8.4+7.6+2.0+15+3.1)/6 = 8.5 |

4.4 BIOMONITORING ENDPOINTS

Once the biomonitoring species are selected, endpoints are selected from each species. A biomonitoring endpoint is the characteristic of the biomonitoring biota that is measured and evaluated against the biomonitoring action levels (Section 3.2). The biomonitoring endpoints thus link the biota selected for biomonitoring with the overall goals and objectives of the biomonitoring program and ultimately the project goals and management objectives. For example, a biomonitoring program may be desired to provide early warning of potential contaminant exposure. In this case, tissue concentration may be an appropriate endpoint, and candidate biota will be selected, in part, on how effectively they take up the contaminant of interest from the environment. In the case of a habitat restoration project, the endpoint may be the presence or abundance of the species in the restored habitat.

4.4.1 Endpoint Attributes

The selection of biomonitoring endpoints should consider a variety of factors similar to those considered when selecting species for biomonitoring. To be useful, the endpoint must be sensitive to the condition or activity of interest, and must undergo a readily measurable ecological (abundance, distribution) and/or biological (behavior, physiology, survival, tissue concentration) change upon exposure to the condition or activity.

There should be a direct and well understood link between the biomonitoring endpoint and the environmental condition that is the focus of the biomonitoring program. When selecting appropriate endpoints, consideration should be given to:

- Knowledge of factors unrelated to the site or conditions of interest (such as non-Navy contaminant releases, climate conditions) that could affect the endpoint;
- The ability to control for, or discern the effects of, potentially confounding factors; and
- Knowledge of the relationship between the magnitude of the environmental condition of interest and the magnitude of the endpoint response.

Some biological endpoints may be affected by any number of factors that are unrelated to the stressor or condition of interest. If confounding factors are not well known or understood it will be difficult to discern whether the measured response in the endpoint is due to the stressor or condition of interest, or to some other factor unrelated to a Navy site or activity. The better the understanding of what and how factors unrelated to the environmental condition of interest could affect the response of the biomonitoring endpoint, the lower will be the uncertainty in evaluating the response of the endpoints and thus the better that endpoint for possible use in the biomonitoring program.

In addition to knowledge about confounding factors, it is also important to understand how the level of exposure may affect the endpoint response (i.e., the sensitivity of the endpoint response to an exposure). If a relatively high level of exposure is needed before an endpoint response is measurable, then that endpoint may not be useful, especially when the biomonitoring program is designed to provide early warning of potentially unacceptable contaminant exposure.

For many NERP biomonitoring programs, endpoints will typically include measured tissue concentrations and the presence, abundance and distribution of the biomonitoring species.

4.4.2 Temporal Considerations

The selection of biomonitoring endpoints must also take into account temporal and spatial considerations associated both with exposure and response times. The spatial and temporal considerations discussed previously with regard to selecting biomonitoring biota dealt with likelihood of the biomonitoring organism occurring at the site or area of interest and being exposed to the environmental condition of interest, and to the duration of that exposure (Section 4.3.1.2). When selecting endpoints, the response time of the endpoint relative to the duration of the environmental exposure should also be considered. Some responses, such as a change in biomass or community structure, may require much more time to detect than a behavioral or toxic response (months or years vs. hrs or days, respectively). If the environmental conditions of interest (such as residual contaminant levels) are thought to pose a potential chronic effect, then the endpoint should be appropriate for evaluating chronic exposure conditions. For example, an

environmental stressor such as a chemical contaminant may trigger an acute short-term response (e.g., mortality) under high exposure levels, while at low exposure levels the response may never be observed. In contrast, a low level of exposure, which may not trigger the acute response, may trigger a different, measurable response (e.g., reduced growth or reproductive output) in the exposed organism. The time required to sample biota that were not exposed either before or during a remedial action is important for determining remedy success. The post-remediation sampling of mature biota might indicate residual tissue contamination, which may be reflective of past exposure rather than exposure following site cleanup. On the other hand, the post-remediation sampling of juvenile biota indicates only the exposure following site cleanup. Therefore, consideration should be given to the life-history of the biota being monitored and how well juvenile biota can be distinguished from mature biota.

For monitoring habitat restoration projects, some endpoints may only be collected during very specific times of the year. For example, an endpoint such as the abundance of, or aerial coverage by, a target plant species may best be measured in late summer, near the end of growing season. Alternately, an endpoint such as seedling production of the same plant species would be best measured in spring at the start of the growing season. In both cases, the endpoint would be measured only over a relatively short but specific time period; collection during other times would provide relatively poor data.

Knowledge about the expected duration and magnitude of exposure to the environmental condition of interest can provide insight into the response time that will be needed by the endpoint. If short-term, high levels of exposure are expected and short-term effects are the issue, then the biomonitoring endpoint should have a high sensitivity to the condition of interest and be able to quickly exhibit a measurable response following exposure. If long-term exposure and effects are anticipated, then the endpoints should be sensitive enough to provide a measurable response under low levels of exposure.

4.5 REFERENCE SITES AND BASELINE CONDITIONS

4.5.1 Reference Sites

Natural variability in biota and the environmental may affect the response of the biomonitoring endpoints regardless of the environmental conditions being addressed by monitoring program. Without consideration in this natural variability, it may not be possible to discern the contribution of the variability in the measured response of the variable. A reference site provides endpoint data that are identical to those collected for the biomonitoring program, but this data is from an area that is similar to but unrelated

Reference Site

A relatively undisturbed site that is ecologically and environmentally as similar as possible to the site or area of interest. The reference site is used to help discern the contribution of natural variability and other confounding factors, such as non-Navy anthropogenic influences, on the observed biomonitoring data.

to and unaffected by the site or area of interest.

By comparing the biomonitoring data from the project site to the data from the reference site, it may be possible to identify the influence of natural variability on the measured biomonitoring data. For example, suppose that plant productivity is one of the biomonitoring endpoint being used to evaluate a habitat restoration project. Plant biomass data collected in summer shows low plant productivity (below expected levels) at the site, suggesting that habitat restoration is not as successful as desired. However, if a similar endpoint response is observed at the reference site, then the conclusion might be that the low productivity is due to natural environmental conditions (such as a dry spring during the critical growing period) and not poor restoration success. In contrast, if productivity was found to be higher (at or near expected levels) at the reference site, then there may be a problem with the restoration.

The use of a reference site is critical for interpreting biomonitoring results, especially those involving naturally-occurring biota. Reference sites are areas that are ecologically and environmentally similar to the site or area of interest, with the exception of the stressor or environmental condition of interest (USEPA 1997; Reece and Richardson 2000; Sutter et al., 2000; Willis et al., 2003). A reference site is used to differentiate endpoint responses due to the stressor or condition of interest from similar responses resulting from natural variability or other anthropogenic stressors unrelated to the site of interest.

While the use of a reference site is strongly recommended, the identification of a suitable reference site may be difficult. Because of other anthropogenic activities unrelated to the Navy (such as urban development and industrial production) it may be difficult to find a location that is not being affected by these anthropogenic activities. The identification of a suitable reference site typically involves considerable interaction with regulators. The identification of suitable reference sites should include identification of the chemical background of the site or area of interest and the presence of non-Navy sources of potential environmental degradation. The Navy has issued policy and guidance for determining background chemical concentrations and for identifying potential non-Navy sources of environmental degradation within watersheds where Navy site occur (NAVFAC 2002, 2003, 2004b; CNO 2003, 2004).

4.5.2 Baseline Conditions

Baseline differs from a reference site in that the baseline is based on conditions at the site or area of interest under current conditions, before an activity or action has occurred. Comparing biomonitoring results to the baseline conditions provides information on the effectiveness of an activity (a contaminant cleanup or habitat restoration) relative to conditions that required the activity. For example, suppose a remediation project is to be implemented to reduce

Baseline Conditions

Baseline conditions represent conditions at the site or area of interest before an activity is initiated. Comparing the biomonitoring data to the baseline data provides information on how the endpoint is responding during or after project activities.

sediment contaminant concentrations which are believed to be responsible for elevated contaminant levels in fish that are consumed by recreational and subsistence fishermen. As part of the overall project, a biomonitoring program is to be implemented that measures contaminant concentrations in fish from the site. To provide a baseline data set, contaminant concentrations in fish are measured prior to remediation. During and following remediation, the biomonitoring program continues to measure tissue concentrations in fish, and these data are compared to the baseline concentrations. If a decrease in tissue concentrations is observed, then the data may be interpreted to indicate successful remediation. If baseline conditions are to be used for evaluating the biomonitoring data, the biomonitoring program will require selection of species and endpoints, and collection of the biomonitoring data, prior to implementation of the site activity. In order to plan and design a cost-effective biomonitoring program one must know enough about the site, the biomonitoring species, and the potential effects of the contaminant.

4.6 Sample Location and Data Analysis

Sampling locations for biomonitoring can be targeted to focus on specific environmental conditions at specific locations, such as a cleanup or habitat restoration site, or can be random in order to provide information on the overall status of a watershed, basin, or region (Barbour et al., 1999). In a targeted sampling regime, sampling sites are located on the basis of known existing environmental conditions, such as a contaminant source, receptor of concern (such as a water body or a residential development) or a restoration site, and data from those locations are used to provide information for a specific location or receptor. In contrast, random sampling regimes provide information regarding environmental conditions over a spatial scale much larger than the site.

Development of a biomonitoring program must consider how the data will be evaluated and interpreted with regards to the biomonitoring and management objectives. For example, a biomonitoring program evaluating habitat restoration success will require data collection over an extended period of time, and interpretation of the data will likely employ some form of time-series (trend) analysis. In contrast, a biomonitoring program evaluating effluent from a water treatment system may only need to indicate an exceedance of a threshold concentration, and may not require trend analysis.

To ensure that valid statistical analyses can be performed, an adequate sample size must be collected. However, there will invariably be a balance between the number of samples that can actually be collected and the cost of sampling. The biomonitoring program should always strive to attain the most meaningful results within the constraints of time and money. It may be necessary to reduce the sample size to fit the funding constraints. However, if the sample size is reduced to a level below which defensible statistical evaluations are possible, it would be better to not sample at all.

4.7 Quality Assurance and Quality Control

Quality assurance and quality control (QA/QC) is important in all environmental sampling programs. QA/QC ensures that data are being collected as specified in the Quality Assurance Project Plan (QAPP) for the sampling activity. Furthermore, it ensures that the data are sufficient to support a decision. A sound QA/QC program will be especially important for a biomonitoring compliance program. False-positive results can result in unnecessary actions and public concerns, while false negatives could result in the persistence of unacceptable conditions.

This page intentionally blank.

5 REFERENCES

- Angerer, J., C. Mannschreck, and J. Gündel 1997. Biological Monitoring and Biochemical Effect Monitoring Exposure to Polycyclic Aromatic Hydrocarbons. *International Archives of Occupational Environmental Health* 70: 365-377.
- Applied Biomonitoring 2004. 2003 Kennebec River Caged Mussel Study, Final Report. Applied Biomonitoring, Kirkland, WA.
- Balch, G.C., C.D. Metcalfe, W.L. Reichert, and J.E. Stein, 1995. Chapter 18 Biomarkers of Exposure of Brown Bullheads to Contaminants in Hamilton Harbor, Ontario. *In: F.M. Butterworth, L.D. Corkum, and J.Guzman-Rincon, eds., Biomonitoring and Biomarkers as Indicators of Environmental Change. A Handbook.* Environmental Science Research Vol. 50. Plenum Press, New York, NY.
- Barbour, M.T., J. Gerristen, B.D. Snyder, and J.B. Stribling 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
- Bargagli, R., D.H. Brown, and L. Nelli 1995. Metal Biomonitoring with Mosses: Procedures for Correcting for Soil Contamination. *Environmental Pollution* 89: 169-175.
- Beeby, A., 2001. What Do Sentinels Stand For? *Environmental Pollution* 112: 285-298.
- Bickham, J.W., S. Sandhu, P.D.N. Hebert, L. Chikhi, and R. Athwal 2000. Effects of Chemical Contaminants on Genetic Diversity in Natural Populations: Implications for Biomonitoring and Ecotoxicology. *Mutation Research* 463: 33-51.
- Blazer, V.S. 2000. The Necropsy-Based Fish Health Assessment. Pages 18-22 *in: C.J. Schmitt and G.M. Dethloff, eds., Biomonitoring of Environmental Status and Trends (BEST) Program: Selected methods for Monitoring Chemical Contaminants and their Effects in Aquatic Ecosystems*. Information and Technology Report USGS/BRD-2000-0005. U.S. Geologic Survey, Biological Resources Division, Columbia, MO.
- Bramblett, R.G. and K.D. Fausch 1991. Variable Fish Communities and Index of Biotic Integrity in a Western Great Plains River. *Transaction of the American Fisheries Society* 120: 752-769.
- Buikema, A.L. Jr. and J.R. Voshell Jr. 1993. Toxicity Studies Using Freshwater Benthic Invertebrates. Pages 344-398 *in: D.M. Rosenberg and V.H. Resh, eds., Freshwater Biomonitoring and Benthic Invertebrates.* Chapman and Hall, New York, NY.

Bundy, J.G., D.J. Spurgeon, C. Svendsen, P.K. Hankard, J.M. Weeks, D. Osborn, J.C. Lindon, and J.K. Nicholson 2004. Environmental Metabolics: Applying Combination Biomarker Analysis in Earthworms at a Metal Contaminated Site. *Ecotoxicology* 13: 797-806.

Burton, G.A. Jr., M.S. Greenberg, C.D. Rowland, C.A. Irvine, D.R. Lavoie, J.A. Brooker, L. Moore, D.F.N. Rayner, and R.A. McWilliam. 2005. In Situ Exposures Using Caged Organisms: A Multi-Compartment Approach to Detect Aquatic Toxicity and Bioaccumulation. *Environmental Pollution* 134: 133-144.

Chief of Naval Operations. 2003. Watershed Contaminated Source Document (WCSD). Fact Sheet. Chief of Naval Operations N45, Washington, D.C.

Chief of Naval Operations 2004. Navy Policy on the Use of Background Chemical Levels. 5090, Ser N45C/N4U732212. Chief of Naval Operations, Washington, D.C.

CNO. See Chief of Naval Operations.

Custer, T.W., C.M. Custer, R.K. Hines, K.L. Stromberg, P.D. Allen, M.J. Melancon, and D.S. Henshel 2001. Organochlorine Contaminants and Biomarker Response in Double-Crested Cormorants Nesting In Green Bay and Lake Michigan, Wisconsin, USA. *Archives of Environmental Contamination and Toxicology* 40: 89-100.

Daly, H.B., D.M. Sargent, and L.L. Lunkenheimer 1995. Behavioral Measures are Excellent Biomarkers of Toxic Chemicals – A Case History. Chapter 19 in: Butterworth, F.M., L.D. Corkum, and J. Guzmán-Rincon, eds., *Biomonitoring and Biomarkers as Indicators of Environmental Change*. Plenum Press, NY.

DeVault, D.S., W.A. Wallford, and R.J. Hesselberg, 1985. Contaminant trends in lake trout (*Salvelinus namaycush*) from the upper Great Lakes. U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, IL, EPA 905/3-85-001. 22 pp.

Donaldson, E.M., 1990. Reproductive Indices as Measures of Environmental Degradation. Pages 109-122 in: Adams, S.M., ed., *Biological Indicators of Stress in Fish*. American Fisheries Society Symposium 8, American Fisheries Society, Bethesda, MD.

Elizinga, C., D. Salzer, and J. Willoughby 1998. *Measuring & Monitoring Plant Populations*. BLM Technical Reference 1730-1, BLM/RS/ST-98/005+1730, Bureau of Land Management, Natural Science and Technology Center, Lakewood, CO.

Everaarts, J.M., L.R. Shugart, M.K. Gustin, W.E. Hawkins, and W.W. Walker 1993. Biological Markers in Fish: DNA Integrity, Hematological Parameters and Liver Somatic Index. *Marine Environmental Research* 35: 101-107.

Gagnon, M.M., D. Bussieres, J.J. Dodson, and P.V. Hodson 1995. White Sucker (*Catostomus commersoni*) Growth and Sexual Maturation in Pulp Mill-Contaminated and Reference Rivers. *Environmental Toxicology and Chemistry* 14: 317-327.

Goede, R.W. and B.A. Barton 1990. Organismic Indices and an Autopsy-Based Assessment as Indicators of Health and Condition of Fish. Pages 93-108 *in*: Adams, S.M., ed., *Biological Indicators of Stress in Fish*. American Fisheries Society Symposium 8, American Fisheries Society, Bethesda, MD.

Golden, N.H. and B.A. Rattner 2003. Ranking Terrestrial Vertebrate Species for Utility in Biomonitoring and Vulnerability to Environmental Contaminants. Review in *Environmental Contamination and Toxicology* 176: 67-136.

Gómez-Arroyo, S. and R. Villalobos 1995. Chromosomal Aberrations and Sister Chromatid Exchanges in *Vicia faba* as Genetic Monitors of Environmental Pollutants. Chapter 8 *in*: Butterworth, F.M., L.D. Corkum, and J. Guzmán-Rincon, eds., *Biomonitoring and Biomarkers as Indicators of Environmental Change*. Plenum Press, NY.

Handy, R.D. and M.H. Depledge 1999. Physiological Responses: Their Measurement and Use as Environmental Biomarkers in Ecotoxicology. *Ecotoxicology* 8: 329-349.

Hellawell, J.M. 1986. *Biological Indicators of Freshwater Pollution and Environmental Management*. Elsevier Science Publishing Co., New York, NY.

Hicks, B.B. and T.G. Brydges 1994. A Strategy for Integrated Monitoring. *Environmental Management* 18: 1-12.

Hilsenhoff, W.L. 1987. An Improved Biotic Index of Organic Stream Pollution. *Great Lakes Entomologist* 20: 31-39.

Hinton, D.E., et al. 1992. Histopathic Biomarkers. *In*: R.J. Hugget, R.A. Kimerle, P.M. Merhle, Jr., and H. Bergman, eds., *Biomarkers. Biochemical, Physical, and Histological Markers of Anthropogenic Stress*. The SETAC Special Publication Series. Lewis Publishers, Chelsea, MI, pp. 155-209.

Hinton, D.E. 1993. Toxicologic Histopathology of Fishes: A Systemic Approach and Overview. Pages 177-216 *in*: Couch, J.A., and J.W. Fournie, eds., *Pathobiology of Marine and Estuarine Organisms*. CRC Press, Boca Raton, FL.

Hoque, M.T., F.M. Yusoff, and A.T. Law 1998. Effect of Hydrogen Sulfide on Liver-Somatic Index and Fulton's Condition Factor in *Mystus nemurus*. *Journal of Fish Biology* 52: 23-30.

Hugget, R.J., R.A. Kimerle, P.M. Mehrle, Jr., and H.L. Bergman (eds.) 1992. *BIOMARKERS: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*. Lewis Publishers, Boca Raton, FL.

IDQTF. See Intergovernmental Data Quality Task Force.

Intergovernmental Data Quality Task Force 2005. Uniform Federal Policy for Quality Assurance Project Plans: Evaluating, Assessing, and Documenting Environmental Data Collection and Use Programs, Part 1: UFP-QAPP Manual. March 2005.

Jebali, J., M. Banni, H. Guerbej, E.A. Almeida, A. Bannaoui, and H. Boussetta 2006. Effects of Malathion and Cadmium on Acetylcholinesterase Activity and Metallothionein Levels in the Fish *Seriola dumerilli*. *Fish Physiology and Biochemistry* 32: 93-98.

Johnson, R.K., T. Wiederholm, and D. M. Rosenberg 1993. *Freshwater Biomonitoring Using Individual Organisms, Populations, and Species Assemblages of Benthic Invertebrates*. Pages 40 – 158 in: D.M. Rosenberg and V.H. Resh, eds., *Freshwater Biomonitoring and Benthic Invertebrates*. Chapman and Hall, New York, NY.

Kahn, R.A. 2000. Comparison of Tissue Lesions in Four Species of Benthic Fish Sampled in 1972-1973 and 1997-1998 on the Grand Banks off Newfoundland. *Bulletin of Environmental Contamination and Toxicology* 65: 78-83.

Karr, J.R. 1981. Assessment of Biotic Integrity Using Fish Communities. *Fisheries* 6:21-7.

Kim, E.Y., K. Saeki, S. Tanabe, H. Tanaka, and R. Tatsukawa 1996. Specific Accumulation of Mercury and Selenium in Seabirds. *Environmental Pollution* 94: 261-265.

Krebs, C.J., 1978. *Ecology: The Experimental Analysis of Distribution and Abundance*, 2nd Edition. Harper & Row, Publishers, New York, NY.

Lower, W.R. and R.J. Kendall. 1990. *Sentinel Species and Sentinel Bioassays*. Pages 309-331 in: J.F. McCarthy and L.R. Shugart (eds.) *Biomarkers of Environmental Contamination*. Lewis Publishers, Boca Raton, FL.

Mac, M.J. and C.C. Edsall 1991. Environmental Contaminants and the Reproductive Success of Lake Trout in the Great Lakes: an Epidemiological Approach. *Journal of Toxicology and Environmental Health* 33:375-394.

Malins, D.C., J.J. Stegeman, J.W. Anderson, P.M. Johnson, J. Gold, and K.M. Anderson 2004. Structural Changes in Gill DNA Reveal the Effects of Contaminants on Puget Sound Fish. *Environmental Health Perspectives* 112 (5): 511-515.

Maltby, L., S.A. Clayton, R.M. Wood, and N. McLoughlin 2002. Evaluation of the *Gammarus pulex* In Situ Feeding Assay as a Biomonitor of Water Quality: Robustness, Responsiveness, and Relevance. *Environmental Toxicology and Chemistry* 21: 361-368.

Martin, R.C., C.W. Beck, and S. Baker 2005. Comparison of Two Bioassessment Protocols in Five Georgia Streams. *Journal of Freshwater Biology* 20: 2005.

Mayer, F.L., D.J. Versteeg, M.J. McKee, L.C. Folmer, R.L. Graney, D.C. McCume, and B.A. Rattner 1992. Physiological and Nonspecific Biomarkers. Pages 5-86 in: Huggett, R.J., R.A. Kimerle, P.M. Mehrle, Jr., and H.L. Bergman, eds., *Biomarkers. Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*. SETAC Special Publication Series, Lewis Publishers, Boca Raton FL.

Moore, J.N., S.N. Luoma, and D. Peters 1991. Downstream Effects of Mine Effluent on an Intermontane Riparian System. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 222-232.

Naval Facilities Engineering Command 2002. *Guidance for Environmental Background Analysis, Vol I: Soil*. NFESC User's Guide UG-2054-ENV. Naval Facilities Engineering Service Center, Port Hueneme, CA.

Naval Facilities Engineering Command 2003. *Guidance for Environmental Background Analysis, Vol II: Sediment*. NFESC User's Guide UG-2054-ENV. Naval Facilities Engineering Service Center, Port Hueneme, CA.

Naval Facilities Engineering Command 2004a. *Guidance for Habitat Restoration Monitoring: Framework for Monitoring Plan Development and Implementation*, User's Guide UG-2061-ENV. Naval Facilities Engineering Service Center, Port Hueneme, CA.

Naval Facilities Engineering Command 2004b. *Guidance for Environmental Background Analysis, Vol III: Groundwater*. NFESC User's Guide UG-2059-ENV. Naval Facilities Engineering Service Center, Port Hueneme, CA.

NAVFAC. See Naval Facilities Engineering Command.

NJDEP. See New Jersey Department of Environmental Protection.

New Jersey Department of Environmental Protection 2004. Routine Monitoring Program for Toxics in Fish: Estuarine and Marine Waters. Final Work Plan. New Jersey Department of Environmental Protection, Trenton, NJ

NRC (National Research Council) 1991. *Animals as Sentinels of Environmental Health Hazards*. National Academy Press, Washington, D.C.

Oikari, A., B. Holmbom, E. Anas, M. Millunpalo, G. Kruzynski, and M. Castren 1985. Ecotoxicological Aspects of Pulp and Paper Mill Effluent Discharged to an Inland Water System: Distribution in Water, and Toxicant Residues, and Physiological Effects in Caged Fish (*Salmo gairdneri*). *Aquatic Toxicology* 6: 219-239.

Rabinowitz, P.M., M.R. Cullen, and H. Lake 1999. Wildlife as Sentinels for Human Health Hazards: A Review of Study Designs. *Journal of Environmental Medicine* 1: 217-223.

Reece, P.F. and J.S. Richardson 2000. Biomonitoring with the Reference Condition Approach for the Detection of Aquatic Ecosystems at Risk. Pages 549-552 in: L.M. Darling, ed., *Proceedings of a Conference on the Biology and Management of Species and Habitats at Risk*, Kamloops, British Columbia, 15-19 February, 1999. Volume 2. British Columbia Ministry of Environment: Lands and Parks, Victoria, B.C., and University College of the Cariboo, Kamloops, B.C.

Rockwood, J.P., D.S. Jones, and R.A. Coler 1990. The Effect of Aluminum in Soft Water at Low pH on Oxygen Consumption by the Dragonfly *Libellula julia* Uhler. *Hydrobiologia* 190: 55-59.

Rosenberg, D.M. and A.P. Wiens 1976. Community and Species Responses of Chironomidae (Diptera) to Contamination of Freshwaters by Crude Oil and Petroleum Products, with Special Reference to the Trail River, Northwest Territories. *Journal of the Fisheries Research Board of Canada* 33: 1955-1963.

Rushton, S.P. 1986. Development of Earthworm Populations on Pasture Land Reclaimed from Open-cast Coal Mining. *Pedobiologia* 29: 27-32.

Sakai, S. and H. Takigami 2003. Integrated Biomonitoring of Dioxin-like Compounds for Waste Management and Environment. *Industrial Health* 41: 205-214.

Salazar, M.H. and S.M. Salazar 1997a. Using Bioaccumulation and Growth in Caged Intertidal Oysters to Assess Oil Exposure and Effects in Delaware Bay. Pages 661-675 in Volume 1, Proceedings, 20th Arctic Marine Oilspill Program (AMOP) Technical Seminar, Environment Canada, June 11-13, 1997, Vancouver, B.C.

Salazar, M.H. and S.M. Salazar 1997b. Using Caged Bivalves to Characterize Exposure and Effects Associated with Pulp and Paper Mill Effluents. *Water Science Technology* 35: 213-220.

Seiders, K. and B. Yake 2002. Washington State Toxics Monitoring Program: Exploratory Monitoring of Toxic Contaminants in Edible Fish Tissue and Freshwater Environments of Washington State. Quality Assurance Project Plan. Washington State Department of Ecology, Environmental Assessment Program, Olympia, WA.

Sheffield, S.R., J.M. Matter, B.A. Rattner, and P.D. Guiney 1998. Fish and Wildlife Species as Sentinels of Environmental Endocrine Disruptors. Pages 369-430 in: R. Kendall, R. Dickerson, J. Geisy, and W. Suk, eds., *Principles and Processes for Evaluating Endocrine Disruptors in Wildlife*. Technical Publication Series SETAC, Pensacola, FL.

Shugart, L. 1988. An Alkaline Unwinding Assay for the Detection of DNA Damage in Aquatic Organisms. *Marine Environmental Research* 24: 321-325.

Stansley, W. 1993. Field Results Using Cholinesterase Reactivation Techniques to Diagnose Acute Anticholinesterase Poisoning in Birds and Fish. *Archives of Environmental Contamination and Toxicology* 25:315-321.

Sutter II, G.W., R.A. Efroymsen, B. Sample, and D.S. Jones. 2000. *Ecological Risk Assessment for Contaminated Sites*. CRC Press, Boca Raton, FL.

Taylor P, Brown JH. 1994. Acetylcholine. Pages 231-260 in: Siegel GJ, ed, *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*. Raven Press, New York, NY.

United States Environmental Protection Agency 1993a. *Wildlife Exposure Factors Handbook, Volume 1*, EPA/600/R-93/187a, Office of Research and Development, Washington, D.C.

United States Environmental Protection Agency 1993b. *Wildlife Exposure Factors Handbook, Volume 2*, EPA/600/R-93/187b, Office of Research and Development, Washington, D.C.

United States Environmental Protection Agency 1997. *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments, Interim Final*, OSWER Directive No. 9285.7-25, PB97-963211, Office of Solid Waste and Emergency Response, Washington, D.C.

United States Environmental Protection Agency 2004. *Guidance for Monitoring at Hazardous Waste Sites: Framework for Monitoring Plan Development and Implementation*, OSWER Directive No. 9355.4-28, Office of Solid Waste and Emergency Response, Washington, D.C.

USEPA. See United States Environmental Protection Agency.

van der Oost, R., J. Beyer, and N.P.E. Vermeulen 2003. Fish Bioaccumulation and Biomarkers in Environmental Risk Assessment: a Review. *Environmental Toxicology and Pharmacology* 13: 57-149.

van der Schalie, W.H., H.S. Gardner Jr., J.A. Bantle, C.T. DeRosa, R.A. Finch, J.S. Reif, R.H. Reuter, L.C. Backer, J. Burger, L.C. Folmer, and W.S. Stokes 1999. Animals as Sentinels for Human Health Hazards of Environmental Chemicals. *Environmental Health Perspectives* 107: 309-315.

van der Schalie, W.H., T.R. Shedd, P.L. Knechtges, and M.W. Widder 2001. Using Higher Organisms in Biological Early warning Systems for Real-Time Toxicity Detection. *Biosensors and Bioelectronics* 16: 457-465.

van der Schalie, W.H., T.R. Shedd, M.W. Widder, and L.M. Brennan 2004. Response Characteristics of an Aquatic Biomonitor used for Rapid Toxicity Detection. *Journal of Applied Toxicology* 24: 387-394.

VDEQ. See Virginia Department of Environmental Quality.

Virginia Department of Environmental Quality 2003. 2003 Fish Tissue and Sediment Monitoring Plan, Water Quality Standards and Biological Monitoring Programs. Virginia Department of Environmental Quality, Richmond, VA.

Weeks, B.A., D.P. Anderson, A.P. DeFour, A. Fairbrother, A.J. Govern, G.P. Lahvis, and G. Peters 1992. Immunological Biomarkers to Assess Environmental Stress. Pages 211-234 in: Hugget, R.J., R.A. Kimerle, R.A. Mehrle Jr, and H.L. Bergman, eds., *Biomarkers, Physiological, and Histological Markers of Anthropogenic Stress*. SETAC Special Publication Series, Lewis Publishers, Boca Raton, FL.

Welsh, H.H. and L.S. Ollivier 1998. Stream Amphibians as Indicators of Ecosystem Stress: A Case Study from California's Redwoods. *Ecological Applications* 8: 1118-1132.

White, D.C. and S.J. MacNaughton 1997. Chemical and Molecular Approaches for Rapid Assessment of the Biological Status of Soils. Pages 371-396 in: Pankhurst, C., B.M. Doube, and V.V.S.R. Gupta, eds., *Biological Indicators of Soil Health*. CAB International, New York, NY.

Whitlock, A.L., N.M. Jarman, J.A. Medina, and J.S. Larson 1994. *WEThings: Wetland Habitat Indicators for Nongame Species. Volume I*. TEI Publication 94-1. The Environmental Institute, University of Massachusetts, Amherst, MA. 45 pp.

Whyte, J.J., C. Schmitt, and D. Tillitt 2004. The H4IIE Cell Bioassay as an Indicator of Dioxin-like Chemicals in Wildlife and the Environment. *Critical Reviews in Toxicology* 34(1): 1-83.

Wilke, T.S. 1991. Evaluation of the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) as a Model System for Mixture Toxicity Hazard Assessment. *Environmental Toxicology and Chemistry* 10: 941-948.

Willis, R.D., R.N. Hull, and L.J. Marshall 2003. Considerations Regarding the Use of Reference Area and Baseline Information in Ecological Risk Assessments. *Human and Ecological Risk Assessment* 9: 1645-1653.

Xavier, S., P. Percis, M. Gnassia-Barelli, M. Romero, and M. Lafaurie 1998. Evaluation of Biomarkers in Caged Fishes and Mussels to Assess the Quality of Waters in a Bay of the NW Mediterranean Sea. *Environmental Pollution* 99: 339-345.

Zweig, L.D. and C.F. Rabeni 2001. Biomonitoring for Deposited Sediment Using Benthic Invertebrates: A Test on 4 Missouri Streams. *Journal of the North American Benthological Society* 20: 643-657.

This page intentionally blank.

Appendix A:

**Example of Biomonitoring for the
Protection of a Sensitive Ecological Resource**

**Rare Turtle Oversight Monitoring Program
Naval Air Station South Weymouth
Weymouth, MA
(Abridged)**

This page intentionally blank.

DRAFT

Rubble Disposal Area Site Closure Rare Turtle Oversight Monitoring Program

Naval Air Station South Weymouth
Weymouth, Massachusetts



**Engineering Field Activity Northeast
Naval Facilities Engineering Command**

Contract Number N62467-94-D-0888

Contract Task Order 0834

February 2005

1.0 INTRODUCTION

This report presents the results of Rubble Disposal Area (RDA) landfill closure construction oversight monitoring activities at the former Naval Air Station (NAS) South Weymouth, Massachusetts (Figure 1). Specifically, this report focuses on the spotted turtle (*Clemmys guttata*) and eastern box turtle (*Terrapene carolina*) monitoring program that was conducted between the fall of 2003 and the fall of 2004, throughout the duration of the RDA landfill closure. This monitoring program was conducted to ensure that the Navy could successfully close this CERCLA landfill in accordance with the December 2003 Record of Decision for the RDA, while ensuring that the box turtle and spotted turtle populations in the vicinity of the RDA were protected. Both turtle species are state-listed Species of Special Concern in Massachusetts and are afforded protection under the Massachusetts Wetlands Protection Act (M.G.L. c. 131, s.40) and the Massachusetts Endangered Species Act (M.G.L. c. 131A). Neither species is a federally threatened or endangered species.

According to the Massachusetts Endangered Species Act (MESA; M.G.L. c.131A and regulations 321 CMR 10.00), the taking of any rare plant or animal species listed as Endangered, Threatened, or of Special Concern by the Massachusetts Division of Fisheries & Wildlife is prohibited. A “taking” is defined under the act as to harass, harm, pursue, hunt, shoot, hound, kill, trap, capture, collect, process, disrupt the nesting, breeding, feeding or migratory activity of an animal or to collect, pick, kill, transplant, cut or process a plant. Permits granted through the Division of Fisheries & Wildlife are required for any kind of “taking”, including management activities that may impact or alter rare species habitat.

The Massachusetts Wetlands Protection Act (MWPA; M.G.L. c.131, s40 and regulations 310 CMR 10.00) requires that the Massachusetts Natural Heritage & Endangered Species Program (MNHESP) review proposed alterations to the wetland habitats of rare wildlife. In addition, alterations causing short or long term adverse effects on the wetland habitats of rare wildlife species are prohibited. The Massachusetts Natural Heritage and Endangered Species Program (MANHESP) are currently in the process of updating the Massachusetts Endangered Species Act regulations (321 CMR 10.00) to set forth procedures, rules and regulations, relative to the delineation of priority habitat and the review of activities and projects within priority habitat.

1.1 RDA Monitoring

Both box turtles and spotted turtles currently use portions of the former NAS South Weymouth for hibernating, breeding, nesting, foraging and aestivating. A high density of both species of turtles occurs in the Old Swamp River corridor (Figure 2), which includes the RDA, Old Swamp River, its floodplain, and abutting terrestrial habitats. Construction activities associated with the landfill closure at the RDA were conducted between 8 April 2004 and 10 November 2004, under US Environmental Protection Agency (U.S. EPA) and Massachusetts Department of Environmental Protection (MADEP) oversight. Because landfill construction occurred within the known habitat of these rare species, the

Navy (in conjunction with the MANHESP program) developed a monitoring program to minimize the potential for short or long-term impacts to state listed turtle species. Under this program, the Navy's designated turtle ecologist conducted the following activities:

- Provided the MANHEHSP with a detailed work plan outlining provisions for protection of rare species during proposed construction activities entitled "*Turtle Monitoring Work Plan – Rubble Disposal Area, August 2003*";
- Communicated with the MANHESP program throughout the duration of the program;
- Implemented a pre-construction (calendar year 2003) focused radiotelemetry field effort (based on the August 2003 Work Plan) to survey and monitor spotted turtles and box turtles that were utilizing habitats on or adjacent to the RDA and Old Swamp River (OSR);
- Provided construction personnel with focused training to ensure that turtles were properly managed should they be encountered during construction;
- Implemented a construction oversight field program in the spring through fall of 2004 to monitor turtle occurrences and movements during construction;
- Re-located turtles from the designated construction area on an as needed basis; and
- Provided a technical review of the TtFW 7/30/2003 Design-Build *Construction Closure Plans* and the 10/2003 *Wetland Restoration Plans* for the RDA, focusing on measures to enhance habitat for state listed turtles within the limits of work (post-construction)

Prior to the recently completed closure, the RDA was an inactive landfill located at the northeastern portion of the NAS South Weymouth property (Figure 2), which was used for four years between 1959 and 1962, and again for a short period in 1978. Its purpose during the first period was for the disposal of fill material dredged from Old Swamp River during construction of a bridge. Its purpose during 1978 was for the disposal of building debris from Building 21, which was destroyed by fire. Its size is approximately 3.83 acres in area, and 8 feet thick at its deepest depth.

The remedial closure of the RDA landfill (included as Alternative "RDA-5" in the Final December 2003 ROD) included the following activities (as outlined in the Final ROD), all of which were conducted (or are currently being conducted) under U.S. EPA and MADEP supervision:

- Clearing and grubbing of vegetation along the perimeter of the RDA;
- Installation of erosion control / turtle exclusion barrier around the RDA perimeter;
- Clearing and grubbing of the remainder of the RDA;
- Excavation and removal of PCB-Impacted material;
- Disposal of PCB-Impacted material;
- Removal of construction debris / landfill material from wetlands associated with OSR;
- Sub-grading, installation of landfill cap, and final grading;
- Wetland restoration;

- Physical (engineering) controls; and
- Institutional controls;
- Post-closure monitoring/maintenance; and
- Five-year reviews.

**Turtle Monitoring and Protection
During Investigation or
Construction Activities at NAS
South Weymouth, MA**

Date: September 16, 2003
Revision Number: 1.0
Authors: J. Bleiler
 S. Egan
 M. Kauffman
Discipline: Turtle Ecology

1.0 Introduction

Two state-listed species of Special Concern, the spotted turtle (*Clemmys guttata*) and the box turtle (*Terrapene carolina*), currently use portions of the former Naval Air Station (NAS) property in South Weymouth, MA for hibernating, breeding, nesting, foraging and aestivating. Investigation and construction activities associated with the U.S. Navy’s environmental programs, including excavation, landfill closure, or other actions are occurring and/or planned within the habitat of these rare species. This Environmental Standard Operating Procedure (ESOP) is written to describe the approach of monitoring and protecting these rare species prior to, and during, these investigation and/or construction activities.

The overall goal of this ESOP is to provide an organized, concise, effective, and implemental approach that ensures future remediation activities conducted by the Navy are conducted in a manner that minimizes impacts to endangered turtles species at NAS South Weymouth. In addition, this ESOP presents a general approach that can be employed at multiple sites at NAS South Weymouth. Depending upon the needs of any particular project, project-specific work sheets (PSWS) must be developed to support specific investigative and/or remedial program needs. These PSWS must be used to amend this ESOP on a project-by-project basis. Depending upon the project-specific needs, the NAVY shall designate a TURTLE ECOLOGIST to implement the activities described in this ESOP and any attached PSWS. The TURTLE ECOLOGIST addressing rare species issues should be a certified ecologist or wildlife biologist, herpetologist, or person with a minimum of 5 years experience studying the life-history requirements of rare freshwater turtles

2.0 Background

According to the Massachusetts Endangered Species Act (MESA; M.G.L. c.131A and regulations 321 CMR 10.00), the taking of any rare plant or animal species listed as Endangered, Threatened, or of Special Concern by the Massachusetts Division of Fisheries & Wildlife is prohibited. A “taking” is defined under the act as to harass, harm, pursue, hunt, shoot, hound, kill, trap, capture, collect, process, disrupt the nesting, breeding, feeding or migratory activity of an animal or to collect, pick, kill, transplant, cut or process a plant. Permits granted through the Division of Fisheries & Wildlife are required for any kind of “taking”, including management activities that may impact or alter rare species habitat.

The Massachusetts Wetlands Protection Act (MWPA; M.G.L. c.131, s40 and regulations 310 CMR 10.00) requires that the Massachusetts Natural Heritage & Endangered Species Program (MNHESP) review proposed alterations to the wetland habitats of rare wildlife. In addition, alterations causing short or long-term adverse effects on the wetland habitats of rare wildlife species are prohibited.

Upon first identifying the presence of rare turtle species at NAS South Weymouth on April 23, 1999, the Navy contracted a TURTLE ECOLOGIST to develop and lead a comprehensive assessment to better define, understand, and protect the turtles from adverse impacts of investigation and construction. This ESOP provides the protocols that the Navy and its contractor have followed to date, and provides direction on continuing the Navy’s high level of turtle monitoring and protection for upcoming and expanded construction activities within the Naval Air Station property.

3.0 Sequencing of Monitoring and Protection Program

In order to provide for the protection of rare species and rare species habitat arising from construction activities, the contractor should coordinate with the Navy Environmental Field Activity North East (EFANE, hereinafter referred to as the "Navy") and the Navy's currently designated TURTLE ECOLOGIST at least one year prior to the onset of construction activities. The primary goal of this coordination effort should be to develop a construction schedule that will account for needs of the contractor and the life-history requirements of rare turtles (e.g., appropriate timing of construction activities). At least 3 months prior to the onset of construction activities, the Navy's TURTLE ECOLOGIST should provide a Work Plan to the MNHESP, outlining provisions for protection of rare species during construction activities and for mitigation of rare species hibernating, breeding, nesting, foraging and aestivating habitats.

As appropriate and subject to the approval and direction of the MNHESP, the Work Plan provisions to be prepared by the TURTLE ECOLOGIST may include, but may not be limited to the following items:

- (1) Trapping efforts during periods of high turtle activity (i.e., April through June) to capture resident turtles.
- (2) Placement of radio-transmitters on adult turtles captured.
- (3) Provisions for handling and care of juvenile or hatchling turtles during construction activities.
- (4) Identification of pre-construction areas used as rare species nesting, foraging, aestivating, or hibernating.
- (5) Provisions for constructing and maintaining turtle exclusion zones to prevent rare turtles from entering construction areas.
- (6) Mitigation provisions for rare species nesting, foraging, aestivating, or hibernating habitat altered during construction activities.

4.0 Spotted Turtle Trapping Procedures

Spotted turtle trapping methods should be similar to those used in a Massachusetts study conducted by Graham (1995). A successful technique at the Naval Air Station property involved the traps baited with sardines in soybean oil, which were deployed overnight and checked every 24 hours. All traps should be deployed with the trap entrances sufficiently submerged to allow for turtle entry into the trap; however, the top of the trap should be exposed above the water line to prevent accidental drowning of the turtles. Areas for trapping should be selected following a review of geospatial data maps of the NAS South Weymouth area. Trap sites should be selected based on: (1) their proximity to key sites under investigation by the Navy, (2) water depth (typical traps should be set in 15 to 30 cm of standing water), and (3) habitat type. Trapping efforts should continue in areas previously characterized during the prior turtle studies (such as the ENSR 2000, 2001, 2002, and 2003 assessments) in an attempt to identify additional individuals. Aquatic habitats identified at the property include palustrine habitats available such as vernal pools, forested swamp, scrub-shrub swamp, and emergent marsh.

5.0 Nesting Survey Procedures

Depending upon project-specific needs, nesting surveys may need to be conducted by the TURTLE ECOLOGIST in terrestrial and wetland habitats prior to, and throughout, the duration of the turtle nesting season (typically June and July). Nesting surveys may involve palpating gravid females for the presence of eggs, dusk and dawn surveys and radio-tracking of female turtles, outfitting gravid female turtles with thread bobbins, covering nests with hardware cloth screen enclosures to deter predation, moving turtle nests off of work areas, and monitoring hatchling success and egg viability. In the event that remedial response actions are expected to overlap temporally or spatially with turtle nesting activity, a detailed PSWS must be developed by the TURTLE ECOLOGIST to provide the site-specific standard operating procedures. Whenever possible, response actions should be scheduled to avoid or minimize impacts on turtle nesting areas.

6.0 Meander Survey Procedures

Meander surveys should be conducted in terrestrial and wetland habitats prior to, and throughout, the duration of the field activities. It is recommended that the surveys involve two individuals walking parallel to one-another in a modified belt transect. These surveys should be conducted to: (a) evaluate areas where turtles have not been

that exhibits a minimum accuracy of within 10 meters. Surveyed point locations should be verified by comparing them to locations sketched on maps while in the field.

9.0 Investigation and Construction Oversight Requirements

A Navy-designated TURTLE ECOLOGIST should perform a minimum of part-time oversight during investigation or construction activities within the NAS property that are in the vicinity of potential rare turtle habitat. The primary objective of the oversight will be to prevent direct impacts to individual spotted turtles and box turtles on or adjacent to the work areas. This should be accomplished through a series of field efforts occurring before and during investigation or construction activities, described below.

Prior to the onset of investigation or construction activities, a turtle exclusion zone encompassing the work area must be established. This should consist of a 3-foot tall silt-fence (or similar material) staked into place and “toed in” at the ground level (the placement of these materials may tie into silt fence and/or hay bale lines already required for construction and erosion control purposes). Silt fence is recommended over the use of hay bales because silt-fence can be toed into place at ground level and the Navy’s TURTLE ECOLOGISTS have observed eastern box turtles to climb over hay bales at the NAS South Weymouth property. This area should then be cleared of turtles in a methodical manner, with several biologists walking shoulder-to-shoulder in transects under the direction of the Navy’s TURTLE ECOLOGIST. Any captured turtles should be measured, weighed, notched, and outfitted with a radio-transmitter, and then released outside of the exclusion zone into similar habitats immediately adjacent to the work area. Releasing turtles adjacent to the immediate work areas is the most sensible approach because:

- there are many uncertainties associated with relocation – repatriation programs (see Burke, 1991; Griffith et al., 1989),
- the majority of sites at NAS South Weymouth are discrete pieces of landscape encompassed by a large system of terrestrial and wetland habitats similar to those found on the sites,
- individual turtles would not be removed from their known home ranges, and
- the pre-established exclusion zone should prohibit individual turtles from inadvertently entering or re-entering the work zone.

Other efforts to ensure the protection of state-listed turtles may include training of field and construction personnel, including professional project engineers, to recognize and capture spotted turtles and box turtles. Turtles encountered during field activities should be captured and temporarily maintained in 20-gallon tubs onsite, or equivalent. The tubs or similar collection devices should be staged with vegetation cover and water, and placed out of direct sunlight for duration of no longer than 4 hours. The TURTLE ECOLOGIST should be immediately notified of any captures. Only the TURTLE ECOLOGIST or other trained and experienced biologists should conduct marking and relocating of turtles. In addition, captured turtles may be outfitted with radio-transmitters to monitor their movements relative to the work area.

The TURTLE ECOLOGIST should closely monitor the movement of turtles and their habitat utilization in the vicinity of the work areas for the duration of proposed activities. Turtle locations should be marked in the field and GPS surveyed, by the TURTLE ECOLOGIST using the aforementioned techniques.

10.0 Completion of Turtle Monitoring Program

Upon completion of site-specific remedial response actions, the TURTLE ECOLOGIST shall conduct the following activities in conjunction with the construction engineer:

- Remove all surveyor’s flags from the field following GPS survey.
- Remove all traps upon completion of trapping efforts.
- Locate and remove all radio-transmitters from turtles using standard operating procedures to ensure minimal damage to carapace scutes.
- Fulfill site-specific short-term and long-term monitoring requirements.

- Remove and/or breach erosion control barriers to ensure the appropriate level of turtle access and repatriation is achieved.
- Prepare turtle monitoring program summary technical memorandum.

11.0 References

Burke, R. L. 1991. Relocations, repatriations, and translocations of amphibians and reptiles: taking a broader view. *Herpetologica*, 47(3): 350-357.

ENSR 2000. Summary of naval air station South Weymouth turtle investigation program. ENSR Progress Report.

ENSR 2001. Summary of naval air station South Weymouth turtle investigation program. ENSR Progress Report.

ENSR 2002. Summary of naval air station South Weymouth turtle investigation program. ENSR Progress Report.

Ernst C. H., M. F. Hershey, and R. W. Barbour. 1974. A new coding system for hardshelled turtles. *Trans. Kentucky Academy of Science*. 35(1-2): 27-28.

Graham, T.E. (1995). Habitat Use and Population Parameters of the Spotted Turtle, *Clemmys guttata*, a Species of Special Concern in Massachusetts. *Chelonian Conservation and Biology*, 1995, Vol 1, No. 3, 207-214.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. *Science* 245:477-480.

Zug, G. R. 1991. Age determination in turtles. *Society for the Study of Amphibians and Reptiles. Herpetological Circular No. 20*:29pp.

| | | |
|---|---|--------------------------------------|
| PROJECT SPECIFIC WORK SHEET (PSWS) | | PSWS Date: September 17, 2003 |
| Brief Description of Work Subject to SOP and PSWS: Navy-authorized scope of work includes the monitoring and protection of spotted and box turtles at the Rubble Disposal Area at NAS South Weymouth, MA, during hydric soil excavation and landfill capping activities associated with the selected remedial action under CERCLA | Relevant SOP Title: Turtle Monitoring and Protection During Investigation or Construction Activities at NAS South Weymouth, MA | |
| | Relevant SOP Date: September 10, 2003 | |
| | Period of Performance: Oct. 2003 – Oct. 2004 | |
| | Project Lead for Subject Work: J. Bleiler | |

SITE INFORMATION

| | |
|---|--|
| Site: Rubble Disposal Area (RDA), at eastern end of the NAS South Weymouth Air Base, adjacent to Old Swamp River. | |
| Documented Habitat Present (species): | |
| <ul style="list-style-type: none"> • Old Swamp River (riverine corridor) (Cg) • Upland field (landfill) (Cg, Tc) • Vernal pool south of RDA (Cg) | <ul style="list-style-type: none"> • Access roads (Tc, Cg) • Upland field south of runway lights (Tc) • Palustrine wetlands (marsh, swamp) (Cg, Tc) |

PROJECT ACTIVITIES

| | |
|---|--|
| Remedial Response Action Activities: <ul style="list-style-type: none"> • Installation of erosion control; • Excavation and removal of materials; • Grading; • Installation of soil landfill cap; and • Ecological restoration | Duration: RDA CERCLA closure expected to have 6-month duration, from approx. 15 Sept 2003 to 15 Mar 2004 |
| General Turtle Mitigation Activities: <ul style="list-style-type: none"> • Identification of pre-construction areas used as rare species breeding, nesting, foraging, aestivating, or hibernating habitat; • Minimization of disturbance to habitat areas; • Construction and maintenance of turtle exclusion zones to prevent rare turtles from entering construction areas; • No machinery outside of turtle exclusion zone except on designated areas; • Mitigation of rare species nesting, foraging, aestivating, or hibernating habitat altered during construction activities. | |

TURTLE MONITORING AND PROTECTION MEASURES

| <i>Natural History</i> | <i>Approximate Duration (months)</i> | | <i>Turtle Monitoring/Protection Measures</i> | <i>Frequency</i> | <i>Comments</i> |
|------------------------|--------------------------------------|---------------|---|---|--|
| | <i>Cg</i> | <i>Tc</i> | | | |
| Breeding | March/April to May | April to June | <ul style="list-style-type: none"> • Trapping (Cg). • Meander surveys. • Radio-tracking of adult turtles. • Relocating turtles outside of work zone. • Monitoring all turtles within 200-meters of limit of work line. | 2/week; EXCEPT trapping, which will be deployed overnight and checked every 24 hours. | Monitoring breeding activity likely will be needed if RDA activities extend beyond 15 Mar 04. Cg and Tc breeding likely at and in vicinity of RDA. |
| Nesting | June/July | June/July | <ul style="list-style-type: none"> • Palpation of gravid females for the presence of eggs. • Radio-tracking of female turtles. • Outfitting gravid female turtles with thread bobbins. • Covering of nests with hardware | 2/week; EXCEPT during peak nesting activity when daily dusk | Nesting surveys may not be needed pending actual project duration. Whenever possible, response actions |

| <i>Natural History</i> | <i>Approximate Duration (months)</i> | | <i>Turtle Monitoring/Protection Measures</i> | <i>Frequency</i> | <i>Comments</i> |
|------------------------|--------------------------------------|---------------|---|---|---|
| | <i>Cg</i> | <i>Tc</i> | | | |
| | | | cloth screen enclosures to deter predation. <ul style="list-style-type: none"> • Moving turtle nests off of work areas. • Monitoring hatchling success and egg viability (September). • Monitoring all turtle nests within 200-meters of limit of work line. | and dawn surveys should be implemented. | should be scheduled to avoid or minimize impacts on turtle nesting areas. Exclusion zone will eliminate nesting at RDA. Perimeter nests to be monitored/protected. No known nests at RDA, but confirmed nest sites within 100-meters. |
| Foraging | March to July Sept. to Nov. | April to Nov. | <ul style="list-style-type: none"> • Trapping (<i>Cg</i>). • Meander surveys. • Radio-tracking of adult turtles. • Relocating turtles outside of work zone. • Monitoring all turtles within 200-meters of limit of work line | 2/week; EXCEPT trapping, which will be deployed overnight and checked every 24 hours. | Foraging surveys likely will be needed if RDA activities extend beyond 15 Mar 04. Both <i>Cg</i> and <i>Tc</i> known to forage at and in vicinity of RDA |
| Aestivating | July-Oct. | July to Oct. | <ul style="list-style-type: none"> • Meander surveys. • Radio-tracking of adult turtles. • Relocating turtles outside of work zone. | 2/month | Aestivation monitoring may be needed at project commencement. <i>Cg</i> known to aestivate at and in vicinity of RDA |
| Hibernating | Nov. to March/April | Nov. to April | <ul style="list-style-type: none"> • Radio-tracking of adult turtles. • Relocating turtles outside of work zone. | 2/month | Hibernation monitoring likely will be required during the winter of 2003/2004. Both <i>Cg</i> and <i>Tc</i> known to hibernate in vicinity of RDA. |

CONTACTS

| | | | |
|--|--|--|--|
| | | John Bleiler TURTLE ECOLOGIST ENSR International Westford, MA 978/589-3056 | |
|--|--|--|--|

NOTES

- All turtle locations shall be recorded in the field using surveyors flagging tape, and their approximate coordinates shall be sketched on field maps using landmarks. Each flag shall be labeled with the date and the channel number and/or notch pattern of the turtle that was located in that position. Flags shall also be surveyed with a hand-held global positioning system unit.
- Only the TURTLE ECOLOGIST or other trained and experienced biologists will conduct marking and relocating of turtles.

Cg = *Clemmys guttata* (spotted turtle)

Tc = *Terrepenne carolina* (eastern box turtle)

This page intentionally blank.

Appendix B.

Example of Biomonitoring Habitat Restoration Success

**Habitat Mitigation Work Plan for McAllister Point Dredging at Naval Station
Newport, Middletown, RI.
(abridged)**

This page intentionally blank.

FINAL REPORT

HABITAT MITIGATION WORK PLAN

FOR

McALLISTER POINT DREDGING

AT

NAVAL STATION NEWPORT

MIDDLETOWN, RHODE ISLAND

Prepared for

US NAVY NORTHERN DIVISION

Prepared By

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION

221 Third Street

Newport, RI 02840

Under Contract To:

FWENC ENVIRONMENTAL CORPORATION

REMEDIAL ACTION CONTRACT (RAC)

CONTRACT NO. N62472-99-R-0032

CONTRACT TASK ORDER NO. 0002

January 2001

1. INTRODUCTION

Foster Wheeler Environmental Corporation (FWENC) will dredge the nearshore and elevated risk offshore areas along the McAllister Point Landfill at the Naval Station Newport (NAVSTA Newport) and dispose of the dredged material at approved off site disposal facilities. A Feasibility Study (FS) completed in 1999 (Tetra Tech NUS 1999) concluded that remedial actions were needed to address contaminated marine sediment and landfill materials at the site, because they pose potential risks to humans and environment. Based on the evaluation in the FS, the Navy has proposed dredging of sediment and landfill materials that exceed Preliminary Remediation Goals (PRGs) in the nearshore and elevated risk offshore areas, and long-term monitoring of sediment in the remaining offshore area.

1.1 BACKGROUND

The McAllister Point Landfill site is approximately 11.5 acres and is situated between Defense Highway and Narragansett Bay (see Figure 1.1-1). Much of the nearshore area contains contaminated marine sediments interspersed with landfill materials (scrap metal debris, submarine netting, ash, concrete, etc.). The sediment and landfill materials appear to range in thickness from approximately 1 to 15 feet immediately adjacent to the revetment at the toe of the landfill and to extend westward approximately 60 to 140 feet before reducing in thickness to zero. Remedial alternatives were developed separately for the nearshore and offshore areas.

1.1.1 Nearshore Remediation

The Human Health Risk Assessment (HHRA) and marine Ecological Risk Assessment (ERA) (SAIC and URI 1997) evaluations identified the marine sediment in the nearshore area as posing risks to both human health and the environment because of concentrations of polynuclear aromatic hydrocarbons (PAHs), pesticides/polychlorinated biphenyls (PCBs) and select metals. Additionally, landfill debris in the intertidal zone poses a physical hazard to people walking or wading in the intertidal zone. The nearshore area requiring remediation consists of all areas within the -3 ft Mean Low Water (MLW) line that have sediment contamination exceeding recommended PRGs, as well as areas outside the -3 ft MLW line that contain landfill debris beneath the surface and have sediment contamination exceeding recommended PRGs. The nearshore elevated-risk areas exceeding recommended PRGs are bounded by the dredging-limit line shown on Figure 1.1-1.

This selected remedial action requires sediment to be removed from the nearshore and elevated-risk offshore area using a combination of appropriate excavation and dredging techniques. The elements of the remedial action include:

1. Pre-design investigation.
2. Sedimentation controls.
3. Contaminated sediment and debris excavation/dredging (all sediment exceeding PRGs).
4. Excavated sediment and debris dewatering and processing for disposal or reuse.
5. Sediment and debris disposal in a RCRA Subtitle C Landfill, RCRA Subtitle D Landfill, and a facility permitted to receive non-TSCA PCB material, as stated in Section 4.8 of the 85% Design Submittal (FWENC 2000).
6. Excavated/dredged areas backfilling with natural fill.

7. Dewatering fluids treatment and discharge.
8. Monitoring (years 1, 2, and 5 only).
9. Five-year review (year 5 only).

The pre-dredging investigation was completed and included a series of soil borings as well as sediment and elutriate samples to confirm the nature and extent of contamination and determine the treatment requirements for fluids to be generated during dredging and dewatering (Tetra Tech NUS 2000).

Extent of Dredging. The extent of the dredged area will be determined by the extent of visible landfill debris (ash, glass, pottery, brick, wire, large metal pieces, and submarine netting), as well as sediment-associated concentrations of PAHs, PCBs, and metals that exceed the PRGs established for the project and are referenced in the Feasibility Study for Marine Sediment/Management of Migration Report (Tetra Tech NUS 1999). Upon completion of the dredging activities in a given sub-area, and receipt of sample results indicating that the clean-up criteria have been achieved, the dredging area will be backfilled. Size R-3 stone used during the ocean haul road construction will be the primary source of deep fill as the road is removed. An additional source of backfill will be used to supplement the R-3 stone in various excavation areas in order to achieve the original grade and substrate type as discussed in Section 3.

Long Term Monitoring. For the Record of Decision (ROD) it was assumed that long-term monitoring would include sediment, pore water and biota chemistry as well as amphipod and sea urchin toxicity testing during the first 5 years after the remedial action is completed. Since nearly all of the contaminated sediment exceeding recommended PRGs would be removed as part of the selected alternative and any remaining contaminated sediment would be covered by clean fill, it was assumed that sampling would be conducted only in the first 5 years and only one five-year review would be conducted. The specific details of the long-term monitoring plan, including media to be sampled, analytical methods, sampling locations, sampling methods, and sampling frequency, will be developed by the Navy during the remedial design, with input from the Environmental Protection Agency (EPA) and the Rhode Island Department of Environmental Management (RIDEM).

1.1.2 Offshore Remediation

The offshore alternatives were developed to address sediment contamination exceeding baseline PRGs in the subtidal zone adjacent to the McAllister Point Landfill. This area was designated by the ecological risk assessment as posing risks to marine biota due to concentrations of PAHs, pesticides/PCBs and metals in the marine sediments. The contamination in the offshore area is associated with contaminated landfill materials such as ash that migrated into the area from the nearshore, and erosion and migration of contaminants from the landfill materials located in the nearshore. Offshore sediment contamination exceeding baseline PRGs was estimated to cover an area of approximately 40.9 acres to an average depth of 1 foot below the surface. No risks to human health or migratory birds were identified for this offshore area, due to limited access associated with the location of the contaminated sediment in deep water.

The limited action alternative was selected involving no direct remedial response activities for contaminated marine sediment offshore of the McAllister Point Landfill. No institutional controls or access restrictions will prohibit use of the area. However, this alternative will require a long-term monitoring program to allow evaluation of changing conditions at the site. The specific

details of the long-term monitoring plan, including media to be sampled, sampling and analytical methods, sampling locations and sampling frequency, will be developed by the Navy during the remedial design, with input from EPA and RIDEM.

1.2 OBJECTIVES

The goal of this habitat Mitigation work plan is to restore the habitats that have been impacted by the excavation and removal of sediments and debris from the nearshore and elevated risk offshore areas of the McAllister Point Landfill. For purposes of this plan, the habitats have been functionally categorized as “benthic infaunal/epifaunal” and eelgrass based on the mitigation approaches. Specific objectives are:

Benthic infauna/epifauna:

- Restore nearshore substrate to natural slope and sediment texture of intertidal/shallow subtidal benthic environment to optimize for passive recolonization;
- Construct offshore fish/invertebrate habitat beds to provide assisted mitigation of impacted high relief habitat; and
- Monitor for long-term habitat mitigation success (i.e., compare to baseline conditions).

Eelgrass mitigation:

- Relocate eelgrass from within dredged area to serve as potential donor material during mitigation;
- Restore eelgrass substrate to natural slope, depth and sediment to optimize for assisted recolonization (seeding);
- Perform eelgrass seeding/transplantation mitigation activities; and
- Monitor for long-term habitat mitigation success and compare to baseline conditions.

1.3 ANTICIPATED TASKS

The following major activities, not necessarily in the order listed, are assumed to be required for habitat mitigation of benthic infauna/epifauna and eelgrass habitats:

Task 1: Benthic infauna/epifauna:

1. Complete nearshore substrate mitigation (i.e., return to natural slope and sediment texture of intertidal/shallow subtidal benthic environment has been achieved to optimize for passive recolonization);
2. Complete offshore substrate mitigation (i.e., construct fish/invertebrate habitat beds (e.g. rock mounds to provide assisted mitigation of impacted offshore substrate); and
3. Monitor for long-term habitat mitigation success (i.e., compare to baseline conditions).

Task 2: Eelgrass mitigation:

1. Harvest existing habitat (e.g., eelgrass) to serve as potential donor material during mitigation;
2. Document effectiveness of substrate replacement in eelgrass zone (i.e., natural slope, grain size and organic content of benthic environment has been obtained to optimize for assisted recolonization);
3. Perform active (e.g. eelgrass seeding/transplantation) mitigation activities; and
4. Monitor for long-term habitat Mitigation success and compare to baseline conditions.

3. EELGRASS MITIGATION

In this section, the plan for mitigation of eelgrass habitat resulting from impacts due to the dredging activity at McAllister Point are discussed.

3.1 IDENTIFICATION OF GOALS

Eelgrass habitat mitigation establishes a viable eelgrass community that provides resources and habitat equal to that lost. The goal is to provide mitigation for loss of eelgrass habitat due to a permitted dredging activity. The estimated area of eelgrass habitat to be impacted is 0.2 acres (Figure 3.1-1). A final replacement ratio of 3:1 will be the goal for this project. Thus the restoration of 0.2 acres of eelgrass in the dredge zone and the creation of 0.4 acres off-site at the transplant areas, for a total of 0.6 acres of restored plus created eelgrass habitat is planned. We will rely on recent advances in restoration techniques, including the use of seeds and planting grids, to provide greatest likelihood of achieving this goal while simultaneously avoiding the significant financial cost and labor associated with past restoration efforts.

3.2 DESCRIPTION OF IMPACT SITE SURVEY METHODOLOGY

3.2.1 Plant Community

Prior to dredging, baseline density and coverage measurements of the existing eelgrass bed will be made using planview photography. A pre-dredging survey will be conducted along transects that will cross the portion of the bed that will not be impacted by dredging as well as the portion that will be removed during dredging so that a representative baseline of the entire bed is obtained. The transects will extend from the shallow edge of the eelgrass bed into deeper water. A limited confirmatory survey will also be conducted with SCUBA divers in order to ground-truth the results of the planview photography. The divers will use a modified Braun-Blanquet technique in which a 1 m² quadrat is subdivided into squares measuring 20 centimeters by 20 centimeters. Divers will deploy the quadrat at 5 points along several of the transects occupied in the planview survey. The number of grid squares in the quadrat that contain eelgrass plants will be recorded in one of four coverage categories (<10%, 10%-39%, 40%-69% and 70%-100%), to establish densities that will be compared with results from the planview survey. The baseline habitat survey previously conducted by SAIC in July 2000 will provide additional information about the area of impacted eelgrass habitat (SAIC 2000).

3.2.2 Sediment

The development of seedlings and the rate they propagate by lateral branching is quite sensitive to the organic content of the sediment (Olesen and Sand-Jensen 1994; Granger et al. 2000). As part of the site assessment a pole-type corer will be used to collect replicate cores from four depths along a transect line from the shallow edge of the bed to its deep-water edge. Sediment core stations will be located throughout the bed in areas of densest eelgrass, as identified with planview photography. This information will be helpful in determining the necessity for organic enrichment of fill material once a source for this material has been identified. The cores will be sieved, analyzed for grain size, and the percent carbon, as weight

loss on ignition, determined. This information will be relied upon to select the replacement sediment mixture that will prove most beneficial for mitigation.

3.3 SITE SELECTION CRITERIA AND LIST OF SITES

Eelgrass habitat will be restored to the impacted area shown in as well as two areas proximal to the site (Figure 3.3-1). The two sites will be used as a holding area for plants harvested from the impact area during dredging. The sites were chosen because they possess several features that will promote eelgrass growth: 1) intermediate water depth (6-15 feet) occurring over a gradually sloping bottom provides a large surface area at an optimal water depth, a feature dependent largely on water clarity; 2) the observed presence of eelgrass in nearby waters (McAllister Point, Derecktor Shipyard, Coasters Harbor) indicates that general water quality characteristics in this portion of the East Passage of Narragansett Bay are favorable to eelgrass growth; and 3) lastly, the sites' proximity to McAllister Point facilitates transport of eelgrass plants, thus minimizing the time between harvest and transplant and maximizing the environmental benefit closest to the area being impacted.

3.4 LOCATION AND AVAILABILITY OF DONOR MATERIAL/DEMONSTRATION OF APPROPRIATE COLLECTION PERMITS

The primary source material for eelgrass habitat replacement will be from seed. Seeds will be collected from beds near the dredging site in order to propagate seedlings that are phenotypically suited to local environmental conditions. Ample supplies of seeds become available during June and July, when eelgrass plants flower, develop seeds, and ultimately die. Flowering plants are easily harvested with low or no impact to the donor bed. With only a modest effort (approximately 55 person-hours), some 500,000 seeds can be harvested. During the past five years, seed has been routinely harvested seed for restoration and experimental efforts. As a result, equipment and a number of unique techniques have been developed to facilitate the processes of collecting the seed-bearing spathe, separating the seed from attendant shoots, winnowing and screening to remove detrital material, and holding seeds until planting. If necessary, eelgrass harvested from the impacted area prior to dredging can be used as plantings to augment our seeding. The Rhode Island Coastal Resources Management Program, Section 300.18; SAV and Aquatic Habitats of Particular Concern regulate the protection of eelgrass beds and permits will be required prior to mitigation activities.

3.5 PLANTING METHODOLOGY/PLACEMENT

3.5.1 Harvesting and transplanting eelgrass from the dredge site

Immediately before the removal of contaminated sediment, a long-reach excavator working from the haul road will be used to harvest the eelgrass bed. The eelgrass will be placed in fiberglass trays and will be transported by truck via the haul road to the processing site. The processing site will be located at the Derecktor Shipyard staging area in Middletown and will consist of an asphalt staging area, a recirculating seawater system, and a temporary tent-style shelter in which eelgrass sorting will take place. Upon arrival at the processing site, the fiberglass trays containing sediment and eelgrass will be offloaded using a forklift. Seawater from the

recirculating system will be flushed over the eelgrass/sediment matrix to remove the bulk of sediment from the eelgrass root structure. The resulting wash water will be captured and returned to a holding tank where suspended sediments will be removed. After sediment removal, the water will return to the eelgrass flushing area for re-use.

Sections of eelgrass plants that have been washed of bulk sediment will be transferred to a tray tank (located inside the tent shelter) where individual plants will be separated and rinsed again to remove any remaining sediment. Workers trained by eelgrass scientists from the University of Rhode Island will sort individual plants, retaining only healthy eelgrass plants possessing sufficient root structure for transplantation. The selected plants will be bundled in groups of approximately 200.

The plants in each bundle will be attached (2 plants spaced 10 centimeters on center) to a 1 m² piece of vinyl-covered wire mesh by means of a paper tie. This planting frame method was developed by Dr. F. Short, UNH and described in *Guidelines for the Conservation and Restoration of Eelgrasses in the United States and Adjacent Waters* (Fonseca et al. 1998). Each bundle will provide approximately the number of plants needed for each planting frame. Approximately 20,000 mature healthy plants will be fitted to 100 planting frames in this manner. Eelgrass plants awaiting transplant will be kept moist by the periodic spraying of a seawater mist over the frames.

Prepared planting frames will be transferred to a small boat docked at Derecktor Shipyard. The boat will transport the frames to the transplant sites where the frames will be deployed (Figure 3.3-1). Four stand-off buoys will locate the corners of each 0.2-acre rectangular transplant site (Figure 3.5-1). The planting frames will be deployed in clusters of four, with each cluster spaced 3 meters apart. Additional buoys will mark the center of each cluster. Upon arrival at the transplant site, the work boat will maneuver adjacent to a cluster buoy. A planting frame will be lowered by a tethered rope approximately 0.5 meters from the north, south, east and west sides of the buoy. This placement method should minimize the potential for one planting frame landing on another. Buoys fitted to the transplant frame tethers will confirm their placement in relation to the cluster buoy, and will facilitate retrieval of the frames. The frames will be retrieved after six to eight weeks; this is sufficient time for the plants to become rooted and the paper ties to dissolve. After allowing the plants to become established, the planting grids are collected by a small boat and can be used again.

In order to maintain optimal health of harvested eelgrass, the eelgrass plants will be transplanted at the designated sites within one day of their harvest. If this is not possible due to severe weather or other circumstances, the plants can be held in flowing seawater tanks for several days without adverse effects.

3.5.2 Seeding of the dredge site

Seeding of the site can occur from September through November after depth contours have been restored and a layer of sediment with the proper grain size and organic content put down. A number of techniques have been developed for applying seed to the sediment at a depth and density that produces the greatest number of seedlings for the fewest seeds (Granger et al. 1996). A recently developed technique involves the placement of eelgrass seed by means of a mechanical planting sled (Granger, pers. comm.). The seeds will be suspended in an agar matrix and pumped from a small boat at the surface to the benthic sled. As the sled moves over the bottom (towed by a small boat), it opens a furrow, deposits the seed suspension at the optimum depth in the sediment, and closes the furrow by means of a trailing roller.

A specially engineered pump located in the surface boat allows for a variable rate of seed delivery. The organic content in the sediment will determine the appropriate seed delivery rate for the site. The seeding machine will be towed along transect lines located ten meters on center and oriented north to south at the site. Seeds will be deposited in furrows 10 centimeters on center over a one-meter wide path along each transect line. The target seeding density will be 700-1,000 seeds m^{-2} , a rate previously determined to result in the greatest seedlings survival and highest production of lateral shoots while still providing the most economic use of seeds (Granger et al., 2000b). The proposed rate of seed application would require 150,000 to 200,000 seeds to plant the 0.2-acre area.

3.5.3 Transplanting of mature plants

The majority of seeds will germinate by March the following year. Increases in plant density after March are the consequence of lateral shoot production. If the monitoring surveys determine that the success criteria has not been met, additional plantings of eelgrass retrieved from the holding sites will be used to increase the coverage and density of the developing seedlings. Divers will collect mature plants from the holding sites, transport the eelgrass to the University of Rhode Island's Bay Campus where they will be washed free of sediment and held in flowing seawater tanks. The planting grids described earlier will be used to reestablish the plants at the dredge site.

3.6 MONITORING SPECIFICATIONS

In the sections below, general aspects of the monitoring requirements for documenting eelgrass mitigation are presented. Complete details for monitoring at McAllister Point Landfill will be presented in the long term monitoring plan for the site.

3.6.1 Identification of variables and methods of collection

Plant density and coverage are the most common monitoring parameters used to quantify the success of a restoration project. The primary verification tool for these parameters will be planview photography that will be applied in a similar manner as in the baseline survey. These data will be used to estimate shoot density and aerial coverage. Limited diver observations will be used to verify and calibrate the photographic assessment of eelgrass coverage and density. In addition, a limited set of plant growth parameters will be measured that are considered to be reliable indicators of eelgrass habitat vitality and persistence. The growth measurements will be compared to measurements taken from the adjacent bed outside the dredging area. The measurements include shoot density as well as the rate of production of rhizomes, leaves, and lateral shoots. These data will allow a determination of the rate of new leaf/root node initiation and the rate of production of new lateral shoots. These plant growth measurements will be used to provide an initial assessment of the potential for long-term success of the planted eelgrass, and therefore aid in evaluating the next steps for mitigation (e.g., re-seeding, transplantation). For example, a low rate of lateral shoot production would suggest diminished long-term survival of the restored bed since increases in the density of eelgrass beds are due largely to the production of lateral shoots (Tomlinson 1980, Olesen and Sand-Jensen 1994). These parameters have been useful both in the field and in experimental mesocosms in assessing the vitality of the eelgrass population and will determine if additional plantings are warranted and likelihood of success of those efforts. In this way, the likelihood of meeting the

success criteria can be assessed early in the growing season, before the optimum time for supplemental plantings passes.

3.6.2 Monitoring and frequency and duration

During the first year, monitoring will occur during March to determine the percentage of seeds that successfully germinated and produced a seedling. A second survey will be conducted in July to assess changes in the areal coverage of eelgrass, and determine the rate of lateral shoot production. During the following years surveys will be conducted twice a year, once in April to document losses from winter storms and ice scouring and again during July when plant biomass and growth is greatest. The original plantings will be monitored for three growing seasons and any remedial plantings made during the second year will also be followed for three growing seasons.

In order to determine a mean plant density and coverage for the bed as a whole, the monitoring surveys will be conducted along transect lines that traverse the entire bed and include comparisons of representative areas of existing and restored eelgrass. Damage to the existing bed from dredging operations may result from deposition of sediment and subsequent eelgrass burial. However, sediment suspended during dredging will be captured by a turbidity curtain installed around the dredging operation (Section 4.2.3 of the 85% Design Submittal, FWENC 2000). An on-site work crew will be responsible for maintaining and verifying the integrity of the turbidity curtain during operations (discussed at Meeting November 2000). In addition, turbidity meters will be deployed as had been conducted during the baseline survey to detect any periodic increases in suspended sediment that may be attributable to the dredging operation. Finally, after the dredging operation is completed, any significant sediment deposition/plant burial should be detected in a post-dredging REMOTS camera survey that will visually verify the presence/absence of a recently deposited layer.

3.6.3 Monitoring interpretation criteria

Interpretation of monitoring data will be performed as described in the long term monitoring plan.

3.7 SPECIFY CRITERIA FOR REMEDIAL PLANTING

Remedial planting will occur if fewer than 50 shoots/m² are obtained over > 50% of the seeded area. Remedial planting will include additional seeding in the unplanted sediment at the site. In addition, mature plants will be transferred from the holding sites to the impacted area and planted using the planting grids described earlier. The holding areas for plants harvested from the dredge site will be used to create eelgrass habitat in addition to the direct mitigation of the dredged area. For this reason, these planting efforts will not be held to the same criteria established for success at the impacted site.

3.8 SPECIFY CRITERIA FOR SUCCESS

Criteria for determination of successful eelgrass habitat mitigation will be the attainment of a total of 75% bottom coverage with eelgrass, that is, 0.45 acres of the cumulative 0.6-acre mitigation area will contain eelgrass. Bottom coverage will be quantified by the presence/absence of eelgrass in 0.3 m² planview photographs collected in a survey performed in a similar manner as the baseline percent cover survey described in Section 3.2.1. Attainment of this level of coverage will indicate that a healthy eelgrass bed has been established and that natural expansion of the bed is likely. The specified seed application rate and planting density for mature plants is expected to produce plant densities similar to those measured before dredging. Unvegetated areas between the seeded rows (path of the seeding machine) and planting grids are expected to occur initially but will be colonized by the coalescence of restored areas within the bed over time.

3.9 DURATION OF RESPONSIBILITY AND CONSEQUENCES OF NON-COMPLIANCE.

The Navy's responsibility for successful eelgrass habitat mitigation will end when the success criteria are met or when concurrence among regulators is reached that despite best efforts, successful mitigation is unlikely. The monitoring data will be reviewed yearly, and after the third growing season the success of the project will be assessed and further actions identified, as practicable. If the Navy, in conjunction with regulators, determines that the causes of failure can be remedied within reason, alternative methods of mitigation at these sites will be presented. If it is determined that these causes cannot be remedied because they are inherent conditions resulting from the physical and biological features of the mitigation sites, it will be agreed upon that the Navy's responsibility to meet the aforementioned success criteria will end. At that time the Navy, in conjunction with regulators, may decide that a separate eelgrass mitigation effort is warranted at an alternate site more favorable to eelgrass growth. This separate effort would be subject to similar percent cover success criteria, but would be limited in overall area to 0.2 acres, an area equal to the proposed eelgrass impact area at McAllister Point.

4. REPORTING

4.1 TIME ZERO REPORT

The Time Zero report will document results of impact site survey and statistical relevance of the survey methodologies. The report will also evaluate the final approach and success of the implementation of benthic and eelgrass mitigation as compared to the work plan objectives and approach. Yearly progress reports will be prepared to present results of monitoring as described in Section 3.7, above. The reports will identify and document any remedial action taken and will offer best professional estimate of likelihood of meeting the success criteria as presented in Section 3.8, above.

4.2 FINAL PROJECT REPORT

A final habitat mitigation report will be written and finalized within 90 days of project completion for submittal. The eelgrass portion of the report will follow the format as outlined in Appendix D of *Guidelines for the Conservation and Restoration of Eelgrasses in the United States and Adjacent Waters* (Fonseca et al. 1998). The final report will describe methodologies, the degree of success each technique achieved and recommendations for additional monitoring. Habitat measures (e.g., benthic cover, fish density, eelgrass density) will be entered into ArcView Spatial Analyst (GIS software) to construct contour maps as a chronology of abundance and coverage before and after dredging and also during the subsequent monitoring surveys. A review of operational errors/shortcomings in the context of the original work statement will be presented to improve future projects and establish standard mitigation protocol. In addition an assessment of compliance with all stated requirements in this plan, will be provided.

6. LITERATURE CITED

Brown and Root Environmental. 1997. Technical Memorandum for Phase III Investigations; McAllister Point Landfill Marine Ecological Risk Assessment, April.

Castro, K., Cobb, J.S., R.A. Wahle and J. Catena. 1998. Role of artificial reefs for lobsters in Rhode Island: Enhancement or Redistribution? Results of Years One and Two. URI Fisheries Center Tech. Rep. 3-98. 14 pp.

Fonseca, M.S., W.J. Kenworthy and G.W. Thayer. 1998. Guidelines for the Conservation and Restoration of Eelgrasses in the United States and Adjacent Waters. National Oceanic and Atmospheric Administration, Beaufort, North Carolina.

FWENC. 2000. 85% Design Submission Remedial Design Work Plan for McAllister Point. Foster Wheeler Environmental Corporation, September.

FWENC. 2000b. 100% Design Submission Remedial Design Work Plan for McAllister Point. Foster Wheeler Environmental Corporation.

Granger, S.L., S.W.Nixon, M.S.Traber and R. Keyes. 1996. The Application of Horticultural Techniques in the Propagation of Eelgrass(*Zostera marina* L) from Seed. Eelgrass Biology: Proceedings of an International Workshop: Rottneest Island, Western Australia, 25-29 January 1996 p377.

Granger, S.L., M.S.Traber and S.W.Nixon. 2000. in press. Observations on the Propagation of *Zostera marina* L. from Seed. In "Seas of the Millenium" edited by Sheppard, C.

Granger, S.L., M.S.Traber and S.W.Nixon. 2000b. The Influence of Planting Depth and Density on Germination and Development of *Zostera marina* L. Seeds. Eelgrass Biology: Proceedings of an International Workshop: Corsica, France, Sept 25-Oct 4, 2000.

Meeting November 2000. Working meeting convened to discuss 85% Design Submittal and Habitat Mitigation Work Plan: Naval Station Newport, November 29. Christie Davis NDIV, coordinator.

Olesen, B. and K. Sand-Jensen. 1994. Demography of shallow eelgrass (*Zostera marina*) populations - shoot dynamics and biomass development. *J. Ecology* 82(2): 379-390.

SAIC (Science Applications International Corporation) and URI (University of Rhode Island). 1997. Marine Ecological Risk Assessment Final Report. Prepared under contract with Brown and Root Environmental for the Navy, March.

SAIC (Science Applications International Corporation). 2000. Draft Report, Baseline Habitat Survey and Essential Fish Habitat Assessment For Offshore Waters of McAllister Point Landfill. Prepared under contract with Foster Wheeler Environmental.

SAIC (Science Applications International Corporation). 2000b. Draft Report, Habitat Mitigation Work Plan for McAllister Point Dredging. Prepared under contract with Foster Wheeler Environmental Corporation, October.

Tetra Tech NUS. 1999. Feasibility Study Report for Marine Sediment/Management of Migration, McAllister Point Landfill. Prepared under contract with Brown and Root Environmental for the Navy.

Tetra Tech NUS. 2000. Phase 1 Draft Predesign Investigation for Off Shore Areas of the McAllister Point Landfill, January.

Tomlinson, P. D. 1980. Leaf morphology and anatomy in eelgrasses, pp. 7-28. In: Phillips, R. C. and C. P. McRoy (eds.), Handbook of Eelgrass Biology, and Ecosystem Perspective. Garland STPM Press, NY.

Appendix C.

Example of Biomonitoring Wetland Restoration Success

**Draft Year 3 Long-Term Monitoring Report for Restored Wetlands at
Naval Submarine Base-New London
Groton, CT
(abridged)**

This page intentionally blank.

**U.S. NAVY ENGINEERING FIELD ACTIVITY NORTHEAST
REMEDIAL ACTION CONTRACT (RAC)
CONTRACT NO. N62472-99-D-0032
CONTRACT TASK ORDER NO. 0063**

**DRAFT
YEAR 3 LONG-TERM MONITORING REPORT
AREA A DOWNSTREAM/OBDA**

**NAVAL SUBMARINE BASE – NEW LONDON
GROTON, CONNECTICUT**

February 13, 2004

Prepared by

Tetra Tech FW, Inc.
133 Federal Street, 6th Floor
Boston, Massachusetts 02110



| <u>Revision</u> | <u>Date</u> | <u>Prepared By</u> | <u>Reviewed By</u> | <u>Approved By</u> | <u>Pages Affected</u> |
|-----------------|-------------|---|--|--------------------|-----------------------|
| 0 | 12/17/03 | J. Fischl P. Gohring | J. Scott | L. Kahrs, P.E. | All |
| 1 | 2/13/04 | R. Cantagallo J. Fischl P. Gohring R. Cantagallo | J. Schaffer J. Scott J. Schaffer | L. Kahrs, P.E. | All |

1.0 INTRODUCTION

This document presents Year-3 results of the long-term monitoring program for restored wetlands at the Area A Downstream Watercourses and Over Bank Disposal Area (Area A Downstream/OBDA) at Naval Submarine Base - New London in Groton, Connecticut. Contaminated soils and sediments were remediated at Area A Downstream/OBDA between July 1999 and August 2000. Remedial activities involved removal, treatment, and discharge of surface waters, excavation of contaminated soil and sediment; onsite dewatering of excavated soil and sediment to remove free water; treatment and discharge of removed water; and offsite disposal of dewatered media at approved landfills. As a result of soil and sediment excavation and removal, 2.90 acres of palustrine wetlands were disturbed. Pursuant to the wetland restoration plan, as outlined in the 100% Design (FWENC, 2000a), compensatory mitigation for this impact required the restoration of 2.43 acres of palustrine wetlands and 0.47 acres of open water.

Restoration of impacted wetlands was completed in two stages:

- Stage 1 Restoration of disturbed wetlands and uplands to final grade and seeding with herbaceous material.
- Stage 2 Planting of woody material.

The wetland restoration was implemented in two stages to allow for groundwater monitoring prior to the planting of woody material, as pre-remediation water table data were not available. Stage 1 seeding of disturbed areas was completed to provide soil erosion/sediment control, establish desirable wetland or upland species, and prevent establishment of weedy invasive species.

1.1 Stage 1 Restoration

Stage 1 Restoration was completed on August 24, 2000 (FWENC, 2000b) and consisted of post-excavation backfilling, rough and final grading, and seeding of all excavated areas. Final regrading entailed placement of a topsoil mix supplemented with organic material and was completed sequentially between April 13, 2000 and July 24, 2000. Final graded areas were re-seeded with native wetland and upland herbaceous species. The following four seed mixes were used to re-seed all restored areas:

- Northeast Wetland Grass Mix — Streams 1, 2, 3, and 4 Wetlands
- Northeast Wetland Diversity Mix — OBDA Pond
- Northeast Wetland Grass/Forb Mix — Upper Pond, Lower Pond, OBDA Pond Wetland
- Northeast Upland Wildlife Seed Mix — Stream 5, Uplands

Seeding was completed between May 1, 2000 and August 24, 2000.

All re-seeded areas were allowed to equilibrate or “settle” through the first winter and early spring post-remediation. Re-seeded areas were monitored during this period for germination and aerial coverage of seeded material, encroachment of invasive species, and ground water levels. Monitoring results were evaluated and hydrological zones were established (FWENC, 2001a). Two hydrology zones were established: an emergent zone where soils were saturated or ponded for 10 consecutive days during the growing season, and a scrub/shrub–forested zone where the ground water table was within 10 inches of the surface for 10 consecutive days during the growing season.

1.2 Stage 2 Restoration

Stage 2 restoration efforts constituted planting of woody material. A total of 235 trees and 420 shrubs were planted in restored wetlands and adjacent uplands at Area A Downstream/OBDA. Species and locations of plantings are provided in Appendix A, Figure 1 and Table 1. Locations were determined using a Trimble ProXRS global positioning system (GPS) unit and plotted on a site map. This site map will be further developed into a site wetlands Geographic Information System (GIS) database. Placement of woody plants was based on hydrological requirements and tolerance of each species. Plantings were completed on April 26, 2001. At the time of planting, each planted tree and shrub was tagged with a metal tag having a unique identification number (1 through 655).

1.3 Monitoring to Date

Monitoring of restored wetlands was initiated in the fall of 2000 and has been conducted twice annually since. Data collected during the 2000 monitoring event included estimates of seeded herbaceous cover, a baseline survey for benthic communities, and piezometer and staff gauge readings. Monitoring events conducted in spring 2001 included percent cover estimates of seeded herbaceous vegetation, baseline height and diameter at breast height (dbh) measurements for planted wood material, and deer/insect browse estimates. In fall of 2001, herbaceous vegetation composition, percent cover, woody plant survival, woody plant height and dbh measurements, benthic community survey, soil sampling and stream flow measurements were collected. Bi-weekly hydrology monitoring was also conducted throughout the 2001 growing season. Monitoring events in 2002 and 2003 duplicated those of 2001. In addition, a post-remedial wetland delineation and function and value assessment was also completed during fall 2003 in accordance with the Final Long-term Monitoring Plan (FWENC, 2001b).

2.0 LONG TERM PERFORMANCE STANDARDS

The long-term goal of the Wetlands Restoration Plan, pursuant to the Long-term Monitoring Plan (FWENC, 2001b), is the successful re-establishment of wetlands disturbed during site remediation. The specific objectives of the Wetland Restoration Plan are fourfold:

1. Restore 2.90 acres of palustrine wetlands and open water (1.26 acres emergent, 1.17 acres scrub-shrub/forested, and 0.47 acres open water) disturbed during removal of contaminated soils and sediments;
2. Establish a self sustaining, functional palustrine wetland system composed of emergent, scrub-shrub, forested, and open water cover classes;
3. Establish a plant community that has a competitive advantage over invasive species; and
4. Restore and enhance pre-remediation wetland functions.

To evaluate the achievement of the above stated objectives, the following standards were selected to determine successful re-establishment of restored wetlands at the Area A Downstream/OBDA site:

Vegetation

- V1. A minimum of 80% area cover, excluding planned open water areas, by noninvasive hydrophytic species for all seeded areas;
- V2. Greater than 50% of dominant plant species that have a wetland indicator status of facultative (FAC), facultative wetland (FACW), or obligate wetland (OBL) with no more than 50% of FAC species;
- V3. For planted woody species, a minimum of 80% survival based on stem count; and
- V4. A 20% increase in tree height and diameter at breast height.

Soils

- S1. Trend towards hydric condition within upper 18 inches of soil profile.

Hydrology

- H1. Emergent zone hydrology that consists of soil saturated to the surface, water on the surface or a combination of surface water and saturated soils for at least 10 consecutive days during the growing season; and
- H2. Scrub/shrub and forested zone hydrology that consists of soil that is saturated to the surface, or the groundwater table that is within 10 inches of the surface, for at least 10 consecutive days of the growing season.

Functions and Values

- F1. All streams and ponds show a trend toward greater biological diversity in the benthic invertebrate community;
- F2. Post-remedial functions and values equal to or greater than pre-remedial functions and values;

- F3. Predicted potential habitat for 27% (16) of all wetland-dependent amphibians, reptiles, and mammals evaluated by the WETHings Method; and
- F4. Restoration of 1.26 acres of emergent wetland, 1.17 acres of scrub/shrub/forested wetland and 0.47 acres open water.

Pursuant to the work plan, the assessment of restored functions and values for performance standards F2 through F4 are scheduled for the end of year three. For the purpose of the vegetation performance standard V1, invasive species will be defined as one of the following:

- Cattails (*Typha latifolia*, *T. angustifolia*, *T. glauca*)
- Common Reed (*Phragmites australis*)
- Purple Loosestrife (*Lythrum salicaria*)
- Reed Canary Grass (*Phalaris arundinacea*)

6.0 PROGRESS TOWARDS ACHIEVING THE PERFORMANCE STANDARDS

To determine progress towards achieving the long term performance standards and hence success of the project objectives, the 2003 monitoring results were evaluated against the long-term performance standards (Appendix J, Table 1). Table 1 provided in Appendix J outlines the performance standards, performance standards achieved for all restored wetlands as of 2003, an explanation of how the performance standard was achieved, and proposed corrective actions, if required. Results of the evaluation, indicate that seven out of the eleven performance standards have been achieved (V2, V4, S1, H1, H2, F2, F3), and three have not been achieved (V1, V3, F1, F4).

Performance Standard V1 requires that a minimum of 80% areal cover, excluding planned open water areas, by noninvasive hydrophytic species for all seeded areas be achieved. Invasive species account for less than five percent of the herbaceous cover in all restored wetlands. Percent herbaceous cover in four of the restored wetlands (Stream 2, Stream 3, Stream 4 and Upper Pond) was greater than 80%. Average percent herbaceous areal cover estimates for all restored wetlands is 77.4%, resulting in a 2.6% difference in performance standard requirements. Since wetland functions and values have increased significantly with the restored wetlands from pre-remedial conditions and the difference in percent cover from the performance standard is negligible (2.6%), there is no recommendations to continue herbaceous plot and transect monitoring.

Performance Standard V3 requires that for planted woody species, a minimum of 80% survival based on stem count should be achieved. Survival of shrubs planted in wetlands exceeded the 80% criteria at the end of the third growing season, however wetland tree survival did not. Despite the success of the deer spraying program, it is likely that additional planted trees and shrubs would continue to be severely damaged and browsed by deer, therefore additional replanting to meet the 80% success criteria for trees is not recommended for 2004. There is strong documented evidence of volunteer seedlings of woody species (see Section 4.1.1). It is anticipated that natural recruitment will continue from seed sources provided by 2001 and 2002 planted woody material and the existing undisturbed tree and shrub canopies.

Performance Standard F1 requires that all streams and ponds show a trend toward greater biological diversity in the benthic invertebrate community. The results also indicate that all waterbodies except for Stream 2 and OBDA Pond show a trend toward greater biological diversity. This is most likely due to the hydrology of these two water bodies, as Stream 2 has little or no standing water during each of the benthic surveys, and the extent of open water at the OBDA Pond has decreased steadily during the monitoring period. Since 2003 data show that all of the sampling stations supported a benthic community, indicating recolonization of remediated areas, and no pre-remedial baseline benthic data was established to compare post- and pre-remedial benthic communities, there are no recommendations for future corrective activities to increase benthic diversity within Stream 2 and OBDA Pond.

Performance Standard F4 requires that 0.92 acres of emergent wetland, 1.10 acres of scrub-shrub/forested wetland and 0.46 acres of open water be restored (refer to Section 4.6). Emergent wetland acreage exceeds the success criteria, however scrub-shrub/forested wetland and open water acreage estimates were approximately 0.41 acres less than pre-remedial wetland acreage. Based upon the findings presented in the Post-Remedial Functions and Values Assessment (Appendix K), the Area A Downstream/OBDA site wetland functions and values have increased significantly following remedial activities conducted between 1999 and 2000. For this reason, there is no recommendation to restore additional wetland acreage at the Area A Downstream/OBDA site.

7.0 CORRECTIVE ACTIONS COMPLETED THIS YEAR

Corrective actions implemented during the 2003 monitoring effort included:

- Manual removal of single stems of cattails (*Typha latifolia*, *T. angustifolia*, *T. glauca*.) from Lower Pond, OBDA Pond, Stream 1 and Stream 2;
- Application of herbicide on single stems of common reed (*Phragmites australis*) at Upper Pond, OBDA Pond, Stream 1 and Stream 2; and
- Application of deer repellent on all planted trees.

Cattails were removed on a bi-weekly basis throughout the 2003 monitoring effort. The manual removal of cattails has been successful in limiting the seed source and subsequent spreading of these invasive species. Total percent cover of cattails has stabilized at an estimated <3 percent aerial cover in 2003.

The common reed stands that were treated with glyphosate in 2001 and 2002 show very little to no growth during the 2003 monitoring period. Native vegetation (i.e. rice cutgrass (*Leersia oryzoides*)) has naturally colonized a majority of these areas. A topical application of glyphosate herbicide was applied on the single stems of common reed in September of 2003.

A program was instituted in late spring of 2001 to control deer browse/damage consisting of an application of non-toxic, commercially available deer repellent, Deer-Off and Plantskydd Animal Repellent, to the woody-planted trees. A total of three applications were made in 2003: one during May, August and October.

8.0 RECOMMENDATIONS FOR FUTURE CORRECTIVE ACTIONS AND CONTINUED WETLAND MONITORING

Results of the 2003 monitoring events indicate that overall, the wetlands at the Area A Downstream/OBDA have been restored. Continued limited monitoring and corrective actions proposed for 2004 will provide continued success and natural development of the restored wetland communities, and ensure that the intent of the Long-term Monitoring Plan is met.

Recommendations include:

- limited hydrology monitoring;
- control of remnant common reed single stems;
- removal of remnant cattail single stems;
- 2002 woody planted material monitoring; and
- deer repellent application to planted trees and shrubs greater than 3 feet in height;

Limited hydrology monitoring consisting of monthly visits to the site during the 2004 growing season are recommended to assess water levels within restored wetlands and may include removal or replacement of weir boards as necessary. Based on 2003 monitoring results it is recommended that the continuation of the successful invasive species control program and the deer spraying program resume throughout the 2004-growing season. If significant spread of invasive species is observed within the onsite wetland communities in 2004, remnant common reed stands and single stems should be treated with a topical application of a glyphosate herbicide. In addition, continued application of deer repellent on 2001 and 2002 newly planted trees and shrubs and monitoring of 2002 planted woody material is recommended.

9.0 REFERENCES

- Atlantic Environmental Services, Inc., 1995. "Wetland Delineation, Area A. Naval Submarine Base – New London, Groton, CT", Atlantic Environmental Services, Inc., Colchester, CT.
- Cowardin, L.M., V. Carter, F.C. Golet and L.T. Edward. 1979. Classifications of Wetlands and Deepwater Habitats of the United States, pp 1-31. FWS/OBS-79/31.
- Dittmar, L. A. and R. K. Neely. 1999. Seed Bank Response to Sedimentation Varying in Loading Rate and Texture. *Wetlands* 19(2):
- Dunne, Tim, 2004, Biologist and Resource Conservationist, Natural Resources Conservation Service, North Jersey Regional Office, Clinton, NJ, Personal communications
- Environmental Laboratory. 1987. Corps of Engineers Wetlands Delineation Manual. Technical Report Y-87-1. U. S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Foster Wheeler Environmental Corporation, 2000a. "100% Design Area A/OBDA, Naval Submarine Base - New London, Groton, CT," Foster Wheeler Environmental Corporation, Boston, MA.
- Foster Wheeler Environmental Corporation, 2000b. "Removal Action Report for Area A/OBDA, Naval Submarine Base - New London, Groton, CT," Foster Wheeler Environmental Corporation, Boston, MA.
- Foster Wheeler Environmental Corporation, 2001a. "Draft Post Construction Monitoring Report, Area A/OBDA, Naval Submarine Base - New London, Groton, CT," Foster Wheeler Environmental Corporation, Boston, MA.
- Foster Wheeler Environmental Corporation, 2001b. "Final Long-term Wetland Monitoring Report Plan, Area A/ OBDA, Naval Submarine Base - New London, Groton, CT," Foster Wheeler Environmental Corporation, Boston, MA.
- Hicks, A. L., 1997. "New England Freshwater Wetlands Invertebrate Biomonitoring Protocol." United States Department of Agriculture.
- Reed, P. B., Jr., 1988, National List of Plant Species that Occur in the Northeast (Region 1), U.S. Fish and Wildlife Service, Biological Report 88(26.1), 111 pp.
- U.S. Army Corps of Engineers (USACE), New England Division. 1995. The Highway Methodology Workbook Supplement: Wetlands Functions and Values: A Descriptive Approach. NEDEP-360-1-30a.
- U.S. Army Corps of Engineers (USACE), New England Division. 2000. New England District Wetland Delineation Datasheet and Supplemental Information.
- Wetzel, R.G., 1983. "Limnology, Second Edition," Saunders College Publishing, New York, New York.
- Whitlock, A. L., N. M. Jarman, J. A. Medina, and J. S. Larson. 1994. WETHings: Wetland Habitat Indicators for Non Game Species. The Environmental Institute, University of Massachusetts. TEI Publication 94-1.

Appendix J
Table 1
Comparison of 2003 Monitoring Results and the Long-Term Performance Standards

| Performance Standard | | Achieved (yes/no) | Comments | Corrective Actions (if applicable) |
|-----------------------------|--|-------------------|---|---|
| Vegetation | | | | |
| V1 | A minimum of 80% areal cover, excluding planned open water areas, by noninvasive hydrophytic species for all seeded areas. | No | Percent herbaceous cover in Stream 2, Stream 3, Stream 4 and Upper Pond wetlands was greater than 80. Additional percent cover estimates included Stream 1 (72%), OBDA Pond wetlands (76%), Stream 4 (60%), and Lower Pond (38%). Average herbaceous cover for all transects and plots is 77.4% Invasive species account for less than five percent of herbaceous cover in all restored wetland and have been reduced by 70% from their fall 2002 percent coverage as a result of topical application of glyphosate. | Continue control of remnant common reed single stems and removal of remnant cattail single stems. |
| V2 | Greater than 50% of dominant plant species that have a wetland indicator status of facultative (FAC), facultative wetland (FACW), or obligate wetland (OBL) with no more than 50% of FAC species. | Yes | Dominant plant species having a wetland indicator status of facultative, facultative wetland, or obligate wetland was greater than 50% for all wetlands and averaged 83.7%. Facultative species accounted for less than 50% of dominant species in all cases and averaged 16.5% of their total with a range of 0% to 43%. | |
| V3 | For planted woody species, a minimum of 80% survival based on stem count. | No | Survival of planted woody species in all wetlands averaged 74.6%. Survival of shrubs planted in wetlands (83.2%) exceeded the 80% criteria at the end of the third growing season, but survival of tree did not (57.8%). | Recommended corrective actions for the 2004 growing season include continued application of the deer repellent to deter future deer browse on planted trees and shrubs, and continued monitoring of trees and shrubs planted in 2002. |
| V4 | A 20% increase in tree height and diameter at breast height (dbh). | Yes | Average height of trees planted in wetlands during 2001 increased by 11.6% from 2001 mean height. When severely deer damaged (main stem snapped) trees are taken in to account, average increase in height for wetland trees was 60.7% which exceeds the 20% increase growth standard. Diameter at breast height increased by 107.2% for all wetland trees planted in 2001 and by 187.6% when adjusted for severely deer damaged trees, both exceeding the 20% growth standard. | |
| Soils | | | | |
| S1 | Trend towards hydric condition within upper 18 inches of soil profile. | Yes | Refer to Sections 4.2 and 5.2 for a discussion on the trend towards hydric soil conditions. | |
| Hydrology | | | | |
| H1 | Emergent zone hydrology that consists of soil saturated to the surface, water on the surface or a combination of surface water and saturated soils for at least 10 consecutive days during the growing season. | Yes | Refer to section 4.3 and 5.3 for a discussion on restored site hydrology. | |
| H2 | Scrub/shrub and forested zone hydrology that consists of soil that is saturated to the surface, or the groundwater table that is within 10 inches of the surface, for at least 10 consecutive days of the growing season. | Yes | Refer to section 4.3 and 5.3 for a discussion on restored site hydrology. | |
| Functions and Values | | | | |
| F1 | All streams and ponds show a trend toward greater biological diversity in the benthic invertebrate community. | No | 2003 monitoring data show a trend toward greater biological diversity in all of the water bodies except two, Stream 2 and the OBDA Pond. This is likely related to the hydrology and physical characteristics of these water bodies. Refer to Sections 4.5.2.3 and 5.5 for discussion on trends in the benthic community. | It appears that, overall, the water bodies in the remediated areas display robust benthic communities. No additional monitoring is proposed. Refer to sections 4.5.2.3 and 5.5 for a discussion on benthic community metrics. |
| F2 | Post-remedial functions and values equal to or greater than pre-remedial functions and values. | Yes | Principal functions increased for six of the seven restored wetlands. Post-remedial functions and values were greater than pre-remedial functions and values, thereby meeting the performance standard. | |
| F3 | Predicted potential habitat for 27% (16) of all wetland-dependent amphibians, reptiles, and mammals evaluated by the WETHings Method. | Yes | The WETHings Method predicted potential habitat for a total of 36 species of wetland-dependent amphibians, reptiles, and mammals well in excess of the 16 species criteria. | |
| F4 | Restoration of 0.92 acres of emergent wetland, 1.10 acres of scrub/shrub/forested wetland and 0.46 acres open water. Note: This performance standard has been revised to adjust for inaccuracies in pre-remedial wetland acreage estimates. Refer to Section 4.6 for a detailed description of the acreage adjustments. | No | Approximately 0.99 acres of emergent wetland, 0.73 acres of scrub-shrub/forested wetland and 0.35 acres of open water were restored within the Area A Downstream/OBDA. Emergent wetland acreage exceeds the success criteria by 0.07 acres. Scrub-shrub/forested wetland and open water acreage were approximately 0.37 and 0.11 acres less than pre-remedial acreage and do not meet the success criteria. | However, despite slight acreage differences (0.41 acres) between pre-remedial and post-remedial wetland delineations, the restored wetlands have significantly increased in wetland functions and values. As a result of the increase in wetland functions and values, at this time, there is no recommendation to restore additional acreage at the Area A Downstream/OBDA site. |

Appendix D.

Example of a Fish Tissue Biomonitoring Program

**Washington State Toxics Monitoring Program
Quality Assurance Project Plan 2002
(abridged)**

This page intentionally blank.

**Washington State Toxics Monitoring
Program:
Exploratory Monitoring of Toxic
Contaminants in
Edible Fish Tissue and Freshwater
Environments of Washington State**

Quality Assurance Project Plan

Prepared by
Keith Seiders and Bill Yake

March 25, 2002

Publication No. 02-03-065

Washington State Department of Ecology
Environmental Assessment Program
Olympia, Washington

Approvals:

Approved by: Will Kendra _____ Date: 3-28-02
Section Manager
Watershed Ecology Section

Approved by: Stuart Magoon _____ Date: 3-29-02
Laboratory Director
Ecology Manchester Environmental Laboratory

Approved by: Cliff Kirchmer _____ Date: 4-10-02
Ecology Quality Assurance Officer
Environmental Assessment Program

Approved by: Dale Norton _____ Date: 3-27-02
Unit Supervisor
Toxics Studies Unit

Approved by: Bill Yake _____ Date: 3-27-02
Senior Scientist
Watershed Ecology Section

Approved by: Keith Seiders _____ Date: 3-27-02
Toxics Studies Unit

Table of Contents

| | |
|---|----|
| Table of Contents | 2 |
| Background | 3 |
| Problem Statement | 4 |
| Project Description | 5 |
| Goal | 5 |
| Objectives..... | 5 |
| Project Organization and Schedule | 5 |
| Organization | 5 |
| Schedule | 6 |
| Sampling Design | 7 |
| Sampling Strategy | 7 |
| Representativeness | 8 |
| Comparability..... | 12 |
| Data Quality Objectives | 12 |
| Field Procedures | 12 |
| Sampling Procedures..... | 12 |
| Decontamination Procedures..... | 14 |
| Field Records..... | 15 |
| Sample Handling..... | 15 |
| Laboratory Procedures | 16 |
| Quality Control..... | 17 |
| Laboratory Quality Control..... | 17 |
| Field Quality Control | 17 |
| Data Management | 18 |
| Audits and Reports | 18 |
| Data Review, Verification, and Validation | 19 |
| Data Quality Assessment | 19 |
| References | 20 |

Background

Toxic contamination of our air, water, and soil is a concern for the health of the public and provides some of the greatest challenges to environmental managers. These contaminants include polychlorinated biphenyls (PCBs), chlorinated pesticides, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDD/Fs), and mercury. Many of these chemicals are persistent, they do not break down easily and remain in the environment for decades. Many toxic contaminants also bioaccumulate; their concentrations in organisms increasing at the higher trophic levels because the contaminant is not broken down or excreted by metabolic processes. The accumulation of these chemicals can have a variety of health effects on humans and wildlife such as reproductive abnormalities, neurological problems, and behavioral changes.

Monitoring efforts in Washington State have detected toxic contaminants in surface water, sediment, and aquatic animal tissues. In many studies, concentrations of toxic contaminants in water, sediment, and tissue have been high enough to threaten the health of humans, wildlife, and fish. Resource management decisions resulting from toxic contamination have included establishing fish consumption advisories, Clean Water Act Section 303(d) listings of contaminated waterways, the regulation of fertilizers, and contaminant source identification and control. The Washington State Department of Health (Health) currently has ten fish consumption advisories in Washington State due to contamination by mercury, PCBs, PCDD/Fs, chlorinated pesticides, and /or other metals and organic chemicals. Three consumption advisories exist for shellfish due to similar contaminants (Health, 2001).

Ecology has conducted or participated in studies that characterized toxic contaminants in Washington. The Washington State Pesticide Monitoring Program (WSPMP) monitored surface water, fish, shellfish, and sediments throughout the state from 1992 to 1997 in areas suspected of contamination (Davis, 2000). The Puget Sound Ambient Monitoring Program (PSAMP) monitors toxic contaminants in sediments and fish throughout Puget Sound with Ecology participating in several components of this program (Llanso, et al., 1998). Ecology has conducted numerous studies for site-specific concerns in freshwater environments (such as those associated with point source discharges) as well as for streams and lakes on a statewide basis (Johnson and Norton, 1990; Hopkins, 1991; and Serdar, et al., 1994). Fish tissue and sediment from several areas throughout Washington are contaminated with PCDD/Fs and Yake, et al. (1988) characterized sources of these contaminants in Washington. Johnson and Olson (2001) described the occurrence of PBDEs in Washington fish; these compounds were previously unreported in Pacific Northwest fish.

A number of agencies other than Ecology have contributed to our knowledge of toxic contaminants in Washington. The U. S. Geological Survey (USGS) has monitored pesticides in several Washington basins as part of their National Water-Quality Assessment Program (NAWQA). Watersheds that are NAWQA projects include: Central Columbia Plateau, Yakima River, and Puget Sound (Williamson, et al., 1998; Rinella, et al., 1993; MacCoy and Black, 1998; Bortleson and Ebbert, 2000). The U.S. Environmental Protection Agency (EPA)

monitored fish tissue during the mid-1990s for toxic contaminants as part of the Columbia River Basin Fish Contaminant Survey (CRITFC) (EVS, 2000). EPA is also conducting a National Lakes Study which characterizes toxic contaminants in lakes throughout the nation; Ecology participates by conducting field collection of fish (Tetra Tech, 2000). The U.S. Fish and Wildlife Service (USFWS) sampled for toxic compounds in the 1980's during the National Contaminant Biomonitoring Program (Schmitt and Brumbaugh, 1990; Schmitt, et al., 1990).

While site-specific monitoring has occurred and continues to occur in Washington State for specific concerns, a broad and consistent statewide toxics monitoring effort has not been developed. Efforts to monitor toxic contamination in fish tissue, sediments, water, and wildlife in Washington on a programmatic basis have declined during the last decade since funding for the WSPMP ended. Interest in toxic contamination of our water, fish, and wildlife was rekindled in 2000 and Ecology management directed resources to the development of a Washington State Toxics Monitoring Program (WSTMP). A technical committee of Ecology staff reviewed issues surrounding toxics contamination in Washington and developed a conceptual base for toxics monitoring (Yake, 2001). While a range of concerns and issues were discussed, limited resources resulted in the selection of four components for the initial Washington State Toxics Monitoring Program:

- Conduct exploratory monitoring to identify new instances and locations of toxics contamination in freshwater environments and freshwater fish tissue.
- Conduct trend monitoring for persistent toxic contaminants using residues in edible fish tissue.
- Establish an Internet Web page featuring toxics monitoring efforts in Washington to disseminate and inform citizens about toxics contamination.
- Develop other toxics monitoring efforts to address particular issues and establish cooperative programs with other agencies.

This Quality Assurance Project Plan (QAPP) addresses the first component (exploratory monitoring) listed above. This QAPP was prepared following guidance developed by Lombard and Kirchmer (2001).

Problem Statement

Humans and wildlife face a variety of risks due to toxic chemicals in the environment. For many areas of Washington, information is lacking about the levels of toxic contamination in freshwater fish and surface water.

Project Description

Goal

The goal of this project is to investigate the occurrence and concentrations of toxic contaminants in edible fish tissue and surface waters from freshwater environments in Washington where contamination is suspected yet recent data are absent.

Objectives

- Provide information about the level of toxic contamination in surface water and edible fish tissue from freshwater lakes, rivers, and streams that have not yet been monitored or where relevant data are greater than ten years old.
- Provide a screening level assessment of the potential for adverse effects of toxic chemicals on aquatic biota and other wildlife.
- Provide screening level information to the Washington State Department of Health that could be used to trigger additional studies for evaluating health risks associated with the consumption of fish.
- Provide information for resource managers and the public about the status of toxics contamination in water and edible fish from freshwater environments in Washington.

Project Organization and Schedule

Organization

All persons listed below work within Ecology's Environmental Assessment Program at Olympia, Washington.

Keith Seiders

Overall project manager on the exploratory monitoring component. Develops QAPP, organizes and conducts field sampling efforts, arranges laboratory analysis, and develops annual report. Phone 360-407-6689.

Bill Yake

Provides conceptual and technical guidance on development of the exploratory monitoring component, reviews QAPP, assists with field sampling, and assists in report development and review. Phone 360-407-6778.

Dale Norton

Oversees component management and budget, provides conceptual and technical guidance, reviews/approves QAPP, and reviews reports. Phone 360-407-6765.

Cliff Kirchmer

Reviews/approves QAPP and provides guidance on analytical methodology and data quality. Phone 360-407-6455.

Stuart Magoon

Coordinates laboratory services (i.e. sample analyses, data quality documentation, data submittal to EIM), data quality reviews, and provides guidance on analytical methodology and data quality. Phone 360-871-8801.

Will Kendra

Reviews/approves QAPP and reviews reports. Phone 360-407-6698.

Morgan Roose, Randy Coots, and Dave Serdar

Assists with field sampling. Phone 360-407-6458, 360-407-6690, and 360-407-6772, respectively.

Art Johnson and Mike Gallagher

Reviews and comments on QAPP. Phone 360-407-6766 and 360-407-6868.

Schedule

This schedule is for the initial year. The completion of tasks in subsequent years would occur during the indicated month for a given year.

Fish Tissue (Includes Water Samples Collected Concurrent with Fish Collection)

| | |
|------------------------------------|--------------------------------|
| Sample Collection | October - November, 2001 |
| Tissue Processing | November - December, 2001 |
| Laboratory Analyses | December, 2001 - January, 2002 |
| Laboratory Data to Project Officer | February - March, 2002 |
| Data Entry in EIM | March, 2002 |
| Draft Annual Report | May, 2002 |
| Final Annual Report | June, 2002 |
| Site Selection for Following Year | July, 2002 |

Water (For Spring/Summer 3x Repetitive Sampling Effort)

| | |
|------------------------------------|------------------------------|
| Sample Collection | April - July, 2002 (3x/site) |
| Laboratory Analyses | May - August, 2002 |
| Laboratory Data to Project Officer | September - October, 2002 |
| Data Entry in EIM | November, 2002 |
| Draft Report | January, 2003 |
| Final Report | March, 2003 |
| Site Selection for Following Year | April, 2003 |

Sampling Design

Sampling Strategy

Fish Tissue

Fish tissue samples will be collected from selected sites throughout the state. Collection of target fish species will occur annually during the late summer to early fall (September-October) since lipids content is usually higher at this time; lipids are where organic contaminants tend to be stored in organisms. Water levels are also lowest at this time, allowing easier and safer access. One to two species of fish will be collected at each site, with five to ten fish of each species forming a composite sample as recommended by EPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (EPA, 2000). Ten fish will be the target number for a composite sample; no less than five fish will be used in a single composite. Individuals used in a composite sample should:

- Satisfy any legal requirements of harvestable size or weight, as defined by the Washington State Department of Fish and Wildlife (WDFW) in their sport fishing rules, or at least be of consumable size if no legal harvest requirements are in effect.
- Be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length of the largest individual.
- Be collected within a two-week period. (It may take more than one day to capture adequate numbers of fish from a site).

Fish will be collected using methods most appropriate for the target species and site characteristics. The primary method will be electrofishing using a boat or backpack units. Other methods may be used where electrofishing is impractical. In these cases, hook and line or the use of nets (gill net, trawl net, fyke net) may be employed. Collection methods will be used that minimize unintended capture of non-target fish. When adequate species or numbers of fish are not available from a preferred site, an alternative site will be selected for sampling.

Water

Water sampling will address two objectives: to characterize pesticides concentrations in water where fish tissue sampling occurs; and to characterize pesticide concentrations during times of pesticide application in urban and agricultural settings. At sites selected for fish tissue sampling, water samples will be collected from a well-mixed area of the waterbody (when available) prior to fish collection. Urban and agricultural sites will be sampled three times during the spring and summer during the pesticide application season. Information about pesticide use in selected basins will be obtained by consulting with persons knowledgeable about local practices, such as Conservation District and Cooperative Extension staff.

Water samples will be composites to better represent the waterbody being sampled. For streams, the composite will consist of subsamples taken from horizontal and vertical transects of the stream. For lakes, depth-integrated subsamples will be taken from one or more locations in the lake and then composited. Detailed descriptions of water sampling methods are described in the section on Field Procedures.

Representativeness

Site Selection for Fish Tissue

Fish tissue data collected in Washington by Ecology, EPA, USGS, and USFWS were reviewed to determine the nature of past monitoring efforts. More than 200 sites have been sampled for analysis of fish tissue since about 1980. The type of tissue, species, and analytes varied among the many monitoring efforts.

About half of the available data was compiled in summary tables that included site locations, tissue types, and parameters analyzed. These sites were then displayed using ArcView GIS to examine the location and nature of current information on fish tissue. Areas that are on the state's 1998 303(d) list for contaminants in fish tissue will not be sampled in this program for listed contaminants because these sites are likely to be sampled during Total Maximum Daily Load (TMDL) studies. Where data used for listing a waterbody are more than ten years old, the waterbody may be considered for sampling during this effort.

Potential sampling sites were selected after considering factors related to the probability of site contamination and the nature of the fish resource. The presence of public fishing opportunities and the nature and age of historical fish tissue sampling efforts guided the initial selection of sites. Locations of many potential pollution sources were identified using Ecology's Facility/Site database (Ecology, 2001) with ArcView GIS. The Washington Atlas and Gazetteer (DeLorme Mapping, 1988) was also used to help evaluate the proximity of potential sources of contamination. Sample site selection will occur each year using a similar process and consider new information and concerns. Factors considered in site selection include:

Probability of Detecting Contamination in Areas Not Previously Monitored

- Suspected contamination due to the proximity of historic or current land uses such as: pesticide handling/storage, pesticide application as in agricultural areas, wood treating facilities, EPA Superfund sites, metal ore processors, pulp mills, refineries, chemical handlers, incinerators, and coal-fired power plants.
- Lack of recent (within the last 10 years) data on levels of toxic contaminants in fish tissue.

Value and Interest of the Fish Resource to Consumers

- Popularity of sites by the fishing public and/or high consumption rates by the public; value or interest of the site as indicated by the experience of various Ecology staff.
- Ability to collect appropriate fish considering: site accessibility for sampling, presence of adequate fish age and size classes, ability to capture fish, and the bioaccumulative characteristics of target analytes among species present.

Several sites that are suspected of having no contamination were also chosen in order to gain some perspective on the results from sites suspected of contamination. The criteria for such “reference” sites are the same as those listed above except the probability of detecting contamination is low. These sites would be streams and lakes far from potential sources or contaminant transport mechanisms.

About 100 sites were initially screened using this process which resulted in candidate sites for the first year of sampling (Figure 1). First-year candidate and alternate sites are listed in Appendix A-1 along with target analyte groups and rationale for selection. A regional distribution of selected sites was desirable in order to address toxic contamination as a statewide concern. Because federal scientific collection permits (discussed below) for this study may not be available until July 2002, this first year’s effort is restricted to sites where federal permits are not required.

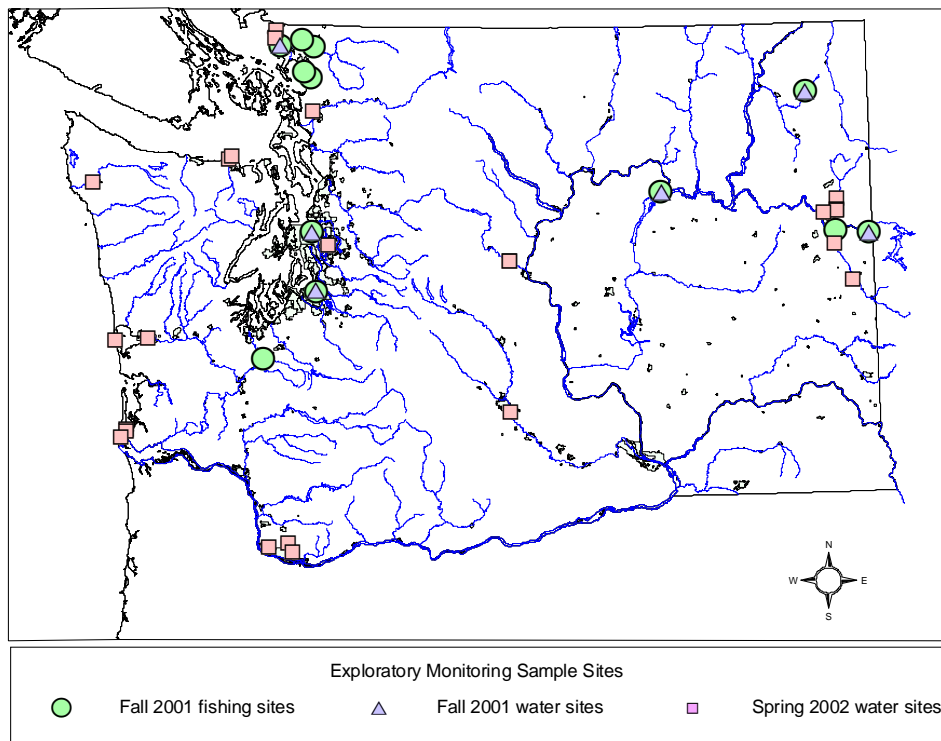


Figure 1. Proposed Sample Sites for 2001-2002

A number of variables were considered in determining the suitability of a site for fish collection such as: types of species present, location and regional distribution, suite of target analytes for that site and species, analytical budget, need for scientific collection permits from federal and state agencies, site accessibility, and likelihood of success in catching target species. The rationale for selecting individual sites is noted in Appendix A-1 and demonstrates that many sites are grouped roughly by geographic location and conditions they may be representative of. For example, five or more sites are located in the Federal Way area, and only one of these sites would be selected. The designation of multiple target sites contributes to flexibility in selecting sites as circumstances change during the progression of field work. Estimates of analytical costs for samples from one or two species at selected sites are listed in Appendix A-1.

Biologists from WDFW will be contacted to better determine the nature of the fishery and fish stocks at candidate sites. Factors needing consideration prior to sampling include: fish size and age classes present, fish stocking practices, length of time target species reside at the candidate site, popularity of the site to the public, and ease of fish capture. Many of Washington's lakes and streams contain fish that originate from natural and hatchery production. There may be sites where hatchery fish of significant size are planted for upcoming fishing seasons. These hatchery fish may bioaccumulate different amounts of contaminants than do naturally produced fish because of differences in the time these fish are exposed to contaminants.

Site Selection for Water

Sites for water sampling will be selected to address two objectives: characterize pesticide concentrations in water where fish tissue sampling occurs and characterize pesticide concentrations during times of pesticide application in urban and agricultural settings. Sites selected for the urban and agricultural characterization will include a mix of sites: those that were monitored in previous studies, those which previous studies recommended for monitoring, and those where no data exist. Site selection for water sampling will use a similar process as that previously described for fish tissue site selection. Information from historical sampling efforts will be compiled, reviewed, and then used to help select sites for monitoring. Criteria for selecting sites for spring-summer monitoring include:

- Presence of potential sources of contamination and contaminant transport mechanisms.
- Probability of detecting target analytes considering factors such as basin size and flow.
- The site is not listed in the State's 1998 303(d) list for analytes of interest (listed waters are anticipated to be studied during the TMDL process).
- The area or site has been recommended for monitoring from previous studies.
- For new sites, previous monitoring data are lacking.
- For sites previously monitored, historical data are sparse (data greater than ten years old).
- The site may be within the drainage basin where fish have been collected for tissue sampling.

As with sites for fish collection, a regional distribution of selected sites is desirable in order to address toxic contamination as a statewide concern. Also, one or more "reference" sites may be selected to help provide perspective on findings from other sites. These sites would be streams and lakes far from potential sources or contaminant transport mechanisms. The number of sites to be monitored will largely be determined by resources available for collection and analysis of

samples. Initially, eight sites from a list of potential sites (Appendix A-2) will be selected for the spring-summer repetitive sampling while approximately five sites will coincide with fish tissue collection (Figure 1).

Target Analytes for Fish Tissue and Water

Target analytes for fish include various persistent, bioaccumulative, and toxic chemicals that have been found in water, sediments, and fish tissue in other monitoring efforts in Washington. Most sites will be sampled for a basic suite of contaminants: chlorinated pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and mercury. The lipid content of tissue will be determined as an ancillary parameter. Other analytes may be added when site characteristics warrant. For example, PCDD/Fs are of interest at some sites due to the proximity of potential sources. A different Ecology study will be looking at levels of total and inorganic arsenic in fish tissue from Washington so this project will not include arsenic as a target analyte.

Target analytes for water include a broad number of pesticides. For sites where fish tissue is collected, about 50 chlorinated pesticides are the target analytes for water samples. For the urban and agriculture repetitive sampling sites, about 140 analytes are targeted and include pesticides from the chlorinated, organophosphorus, nitrogen, and carbamate groups. Ancillary parameters for water samples include lab determination of total organic carbon (TOC) and total suspended solids (TSS). Field measurements will include temperature, conductivity, and pH. Streamflow may be measured or determined from other sources such as USGS. Target analytes for fish tissue and water are listed in Appendix B.

Target Fish Species

Target species were selected based on recommendations from EPA (2000) and previous experience with fish collection efforts in Washington. The following criteria were used to select target species:

- Are commonly captured and likely to be consumed by humans.
- Potentially bioaccumulate high concentrations of chemicals of interest.
- Abundant, easy to identify, and easy to capture.
- Large enough to provide adequate tissue for analysis.
- Resident fish and fish likely to stay relatively close to the sampling site.

Target species for this study are listed in Appendix C. Efforts will focus on collecting the desired species and number of fish, yet the outcome of field sampling will depend on the availability and abundance of fish at the study sites. In some cases, two different species may be sought due to differences among species to bioaccumulate certain types of chemicals. While edible game fish are preferred over bottom-dwelling species, bottom feeders in some areas that are caught and consumed by humans may also be collected. Information about managed species at sites will be obtained from WDFW biologists.

Comparability

Data from this project will be compared to various regulatory and/or biological effects concentrations, as well as findings from historical work. Sample collection, processing, and analytical methods used will be documented and are expected to produce data that are comparable to various criteria and data from other studies. Data regarding the lipid content and tissue type from this study will help to allow appropriate comparisons to be made. Monitoring results may be compared to various standards and previous studies such as:

- Criteria in Washington's water quality standards (Chapter 173-201A WAC) and the National Toxics Rule (40 CFR 131).
- Results from historical work in Washington, such as from WSPMP and NAWQA.
- Risk-based consumption values as described in EPA (2000).

Data Quality Objectives

The quality of analytical data will be evaluated according to MEL's practices described in Ecology's Lab User's Manual (Ecology, 2000). The data review process is an integral part of producing analytical results at MEL. This review addresses: sample preparation, instrument calibration and performance, completeness of the raw data package, checks for errors, holding times, and usefulness of the data. These reviews are summarized in a case narrative that accompanies the reported results.

The case narratives and field data will be reviewed by the project officer to determine how the data compare to the Measurement Quality Objectives (MQOs) for this project. The MQOs were developed using information about data quality from past monitoring efforts of fish tissue and water (Davis, 1998; Davis, et al., 1998; and Serdar, et al., 1999). Appendix D contains MQOs for: practical quantitation limits, bias, precision, and accuracy for target analytes. Data quality assessment will be made using information from laboratory case narratives, laboratory duplicates, field replicates, matrix spike recoveries, and matrix spike duplicate recoveries.

Field Procedures

Sampling Procedures

Scientific Collection Permits

Scientific collection permits will be acquired prior to collecting fish. Washington's Department of Fish and Wildlife issues permits for any collection activities in the state. For areas inhabited by fish listed under the Endangered Species Act (ESA), the appropriate permit will be obtained from National Marine Fisheries Service (for anadromous species) or the U.S. Fish and Wildlife Service (for inland species). Approximately three to six months are needed for these federal

agencies to process applications for scientific collection permits. Permits are expected to be issued in the summer of 2002 and be valid for up to five years.

Permits are needed because ESA-listed species may be encountered during collection activities. The collection methods used (electrofishing primarily) may disturb or harass listed species and is considered “take” under the ESA. There are currently 15 species or stocks of anadromous fish in Washington waters that are listed or are proposed for listing as endangered or threatened. The Bull Trout (*Salvelinius confluentes*) is listed as threatened in Washington and other northwest states. These species or stocks collectively inhabit large areas of Washington so the initial year’s collection efforts will focus on areas where federal collection permits are not needed.

Fish Tissue

Methods for the collection, handling, and processing of fish tissue samples for analysis will be guided by methods described in EPA (2000). Upon capture in the field, fish will be identified to species and target species retained: non-target species will be released. Fish that are retained will be inspected to ensure that they are acceptable for further processing (e.g. proper size, no obvious damage to tissues, skin intact). Fish to be kept will be stunned by a blow to the head with a dull object, rinsed in ambient water to remove foreign material from their exterior, weighed, and their fork and total length measured. Individual fish will then be double-wrapped in foil and placed in a plastic zip-lock bag along with a sample identification tag. The bagged specimens will be placed on ice in the field. Fish may remain on ice for a maximum of 24-48 hours and then they will be frozen to -20 C at Ecology facilities in Lacey, Washington. Sampling instructions for field crews are given in Appendix E.

Up to ten fish will be used to create a composite sample for laboratory analyses. The edible portion of target species will be used for the composite sample. Fish will be removed from the freezer and partially thawed; and then, in most cases, filleted. Whole fish and/or other tissues may be used in cases where people prepare fish using more than muscle tissue. Gamefish fillets will include the skin and belly flap. Skin will be removed from scaleless fishes (e.g., catfishes) and fish to be analyzed for mercury (e.g. largemouth bass) prior to filleting. Appropriate structures used to determine the age of individual fish (scales, otoliths, opercles, spines) will be extracted and sent to a WDFW biologist contracted to determine fish age from these structures.

Fillets for compositing will be cut into small cubes, and equal weights from each individual fed into a grinder or blender in order to yield a composite sample of adequate size for the required analyses. The ground tissue will be homogenized by stirring to a consistent texture and color. Subsamples from the homogenate will be taken and placed into appropriate containers and transported to the laboratory for analyses. Excess homogenate will be placed into an appropriate container, labeled, and archived frozen at -20 C .

Water

Water samples for organic contaminant analyses will be collected following procedures described by Davis (1993) for the WSPMP. For streams, depth-integrating samplers will be used at three points along a stream cross section to obtain a sample representative of the stream.

Samples from each point will be composited to obtain adequate volume for the analyses to be requested. Several models of USGS depth integrating samplers and nozzles will be used depending upon water depth, stream velocity, and ease of handling. These samplers use Teflon™ and glass for the parts of the device that contact sample water. A DH-76 sampler, attached to a cable or rope, is available for use from bridges where the water is swift and deep. A DH-48 sampler, attached to a wading rod, is available for use in waters with velocities less than four feet per second and up to four feet deep. Hand grab samples will be taken where waters are less than one foot deep.

The sample collection methods for lakes and reservoirs will depend upon the characteristics of the waterbody and use techniques described by Rogowski and Davis (1999) when possible. For small lakes, one location will be selected for sampling while two or three locations on larger lakes may be selected for sampling. Sites that are believed to be representative of the lake will be chosen.

For shallow lakes that are not stratified (as determined by a vertical temperature profile) samples will be collected with a DH-76 sampler attached to a depth-marked hand line. The depth of the water will first be determined using the sampling vessel's depth sounding device or a marked and weighted line. The amount of line that the sampler can be lowered to, within several feet of the bottom, will be measured and marked. The sampler will then be lowered at a constant rate to the marked depth and then raised at a constant rate. The rate of lowering and raising will be adjusted in order to allow the sample container to just fill as it is recovered from the water. Upon recovery, the water sample will be transferred to an appropriate sample container. This process will be repeated until the desired sample volume is collected.

For deep lakes, water will be collected from several depths within the epilimnion and the hypolimnion. A sampling device such as General Oceanics' "Go-Flo" will be rinsed in surface water, lowered to desired depths, and triggered to collect a sample. The sampler will be retrieved and the desired volume of water sample will be transferred to a sample container. This process will be repeated until the desired depths have been sampled and an adequate sample composited. The final sample will consist of equal volumes from each of the two to six depths sampled. The parts of the sampling device that contact the sample are made with stainless steel and/or Teflon™; these areas will be decontaminated as described below for each waterbody the sampler is used at.

Decontamination Procedures

All utensils used for processing tissue samples will be cleaned in order to prevent contamination of the sample. Utensils include bowls and knives of stainless steel and tissue grinding appliances having plastic and stainless steel parts. All utensils for fish tissue sampling will be cleaned with the following procedure: soap (Liquinox) and hot water wash; hot tap water rinse; deionized water rinse; and a final rinse with pesticide-grade acetone, hexane, and/or methanol (choice of solvent depending upon the exact materials used in sampling or processing equipment). Utensils will be air-dried and then packaged in aluminum foil until used. Water sampling devices will be

cleaned and packaged in the same way. Fish will be filleted and tissues processed on aluminum foil that covers the workbench.

Field Records

Field notes will be kept for each sampling event. Notes will be entered in a field notebook and include: date and time, sampling personnel, general sampling location and latitude/longitude coordinates of fish collection, general weather conditions, method of sampling, fish species collected, weights and lengths for individual specimens, and results from field measurements. Latitude and longitude coordinates, and their datum, will be obtained with a state-of-the-art, hand-held Global Positioning System (GPS) device. Additional notes will be taken when samples are processed and submitted for laboratory analysis such as: type of tissue, laboratory identification numbers, and laboratory analyses requested. The sex of individual fish will be determined during tissue processing.

Sample Handling

Containers and Preservation

Tissue and water samples will be stored, preserved, and transported following procedures designed to maintain the integrity, quality, and identification of the sample. Appendix F includes requirements for sample containers, preservation, and holding times for each set of analyses required for tissue and water. Pre-cleaned sample containers will be obtained prior to field sampling efforts with containers for metals and organics possessing Quality Assurance Certification from the supplier (e.g. I-Chem 200 series or equivalent).

Identification and Transport

The identification of water and tissue samples will be maintained from the time of collection to the time of reporting of results. For water samples, the sample container will be tagged and labeled with a unique laboratory identifier. A field record form will be created to record information about the sample collected: location, date, time, and the method of collection. Other information may also be recorded on the field form – such as observations about land use.

For tissue samples, a field record form will be created for describing individual fish and their attributes such as: species, length, weight, site location, date of capture, and any other observations. Field record forms will be patterned after examples given by EPA (2000) and clearly identify the laboratory identifier code used for each sample. Whole fish will be transported, on ice, to Ecology headquarters facilities in Lacey, Washington by field crews. Fish will be frozen to -20 C at the Ecology facility until processed at a later date.

Fish samples will be processed (tissue removed, composited, and homogenized) and then put into the appropriate sample containers for transport to the laboratory. Sample containers will be tagged and labeled with unique laboratory identifier. These numeric and alphanumeric

identifiers will be in the format used by Ecology's laboratory and data management systems. Ecology's "Request for Analysis" form will accompany the samples transported to the laboratory. This form includes sample information such as: date and time of collection, numeric and alphanumeric identification codes, sample media, number of containers, analyses requested, and a chain-of-custody record. The laboratory will be notified of the approximate date when samples will arrive for analysis. The type of structures removed for determining the age of individual fish will be noted, assigned an identification number, and packaged for shipment to WDFW for aging. Containers for these structures will be supplied by WDFW.

Laboratory Procedures

The analytical methods for target analytes were selected to achieve a balance of analytical sensitivity, comparability, and cost-effectiveness. Appendix F summarizes the parameter groups to be analyzed for, sample matrix, analytical method, practical quantitation limits (PQL), sample containers, preservations, and holding times for samples. The laboratory procedures to be used by the Ecology/EPA Manchester Environmental Laboratory (MEL) are documented in Ecology (2000).

Appendix G shows the desired PQLs for individual analytes. Unfortunately, the PQLs for a number of analytes are higher than criteria found in water quality standards or screening level criteria. For tissue samples, these analytes include toxaphene, total PCBs, and PCDD/Fs. For water samples, the freshwater chronic criteria in Washington's water quality standards (Chapter 170-201A WAC) are lower than the selected method's quantitation limits for: DDT and metabolites, chlordanes and nonachlors, aldrin and dieldren, endrin, heptachlor, heptachlor epoxide, and toxaphene. Values in bold in Appendix G are desired PQLs that may not be met using the selected analytical methods: the bold value approximates the value of the water quality criterion or screening level for fish consumption for the specific analyte.

The EPA (2000) recognizes the unavailability of cost-effective analytical methods that can achieve lower quantitation limits for some analytes. The use of performance-based analytical techniques are encouraged by EPA which may help in developing analytical methods that achieve needed detection limits for particular analytes. This project anticipates that method development will occur in other studies where method development is the focus and that those methods can be incorporated into this study as they become available. For example, MEL is exploring the use of larger sample volumes for use in gas chromatography; the goal is to lower detection limits for some chlorinated pesticides and PCBs for a water quality study in the Walla Walla River basin (Johnson, 2002; Mandjikov, 2002).

Appendix H shows estimated analytical costs for one year's analysis of fish tissue and water samples.

Quality Control

Laboratory Quality Control

Laboratory quality control procedures as described in Ecology (2000) will include various analyses to evaluate data that are generated. For water samples, check standards will be used to estimate analytical accuracy and bias. The standard deviation of the check standard results gives one estimate of analytical precision. Bias can be estimated by finding the difference between the mean of the check standard results and the true value of the check standard. Analytical precision may be estimated using laboratory duplicate analyses by finding the Relative Percent Difference (RPD) or Relative Standard Deviation (RSD) of the results. Method blanks for water sample will be analyzed to assess contamination from laboratory procedures.

For water and tissue samples, matrix spikes will be used to indicate the presence of bias due to the sample matrix while spike duplicate results can help estimate analytical precision. The project officer will indicate which samples should be used for matrix spikes. Analyses of organic compounds will include spikes with surrogate compounds in order to help estimate the accuracy, precision, and bias of the results. For tissue analyses, Standard Reference Materials (SRM) will be obtained from the National Institute of Standards and Technology and submitted “blind” to the laboratory as a regular sample.

Field Quality Control

Field quality control procedures will include blank samples and field replicate samples. About 10% of samples will be blanks or field replicates submitted “blind” to the laboratory. Blank samples will be for water samples only. Water free of organic chemicals will be obtained from MEL, transported to the sample site, transferred to sampling device, then transferred from the sampling device to a sample container.

Field replicates will consist of an additional sample taken from the same location at the same time or within three days of the first sample. For fish tissue, a replicate sample will consist of a separate sample of fish tissue obtained from the same area, number of fish, species, and size range that made up the first sample. Replicate water samples will be taken for about 10% of the sites sampled. A replicate water sample will consist of a separate sample collected within four hours of, and in the same manner as, the first sample. The laboratory will be asked to perform their duplicate analysis (split sample) on the first sample of the replicate pair. This will allow separation of sampling variability from analytical variability.

Data Management

Data management for this project will include written and electronic media generated from field and laboratory activities. Field notes and observations will be recorded by hand onto prepared field forms and/or notebooks. Pertinent data collected in field books will be transferred to electronic media using Microsoft Office products (Word, Excel, Access) and ArcView GIS. After entry into electronic media, the electronic data will be reviewed and compared to handwritten data to check and correct data entry errors. After these reviews, pertinent field data will be entered into Ecology's electronic Environmental Information Management (EIM) system. Hardcopy and electronic data not entered into EIM will be retained in a file system maintained by the project officer.

Laboratory analyses of samples generate data recorded in handwritten and electronic formats. These data will be examined by designated laboratory personnel for: quality control, completeness, accuracy, errors, and usefulness. Analytical data generated by MEL will be entered in the EIM system by MEL. Analytical data generated by contract laboratories will be submitted to MEL electronically to facilitate entry into EIM. For tabular data generated by contract laboratories, comma delimited files are the preferred format with Excel spreadsheet format also acceptable. Information obtained from the analytical procedures other than results will be retained in the laboratory's electronic and hardcopy filing systems.

Audits and Reports

Oversight of project components will occur through established practices within Ecology. The laboratories employed for sample analysis participate in audits that include review of laboratory facilities, capabilities, and analytical performance through various federal and state programs (Ecology, 2000). Laboratories will report the analytical results and data quality through a case narrative, typically provided for each batch of samples analyzed by a specific procedure. Annual reports for the project will be produced which:

- Describe the project and methods used.
- Display locations of sampling sites.
- Assess the quality of the data.
- Summarize the data collected and discuss significant findings.
- Recommend follow-up actions.

Data Review, Verification, and Validation

Hard copy and electronic forms of data will be reviewed and examined for errors, omissions, and legibility. Field data will be examined by the field leader prior to leaving the sampling site. Laboratory data are reviewed by qualified staff at MEL before they are entered into the EIM system and released to the project officer. Where errors or omissions in the data are found, the source of the data (e.g. field sampling personnel, laboratory technician) will be consulted to determine the correct value or form of the data in question. Corrections or qualifications will be made where possible.

Data verification will be determined by examining the quality control information for each set of data. The project officer will examine field data while qualified laboratory staff will examine laboratory data and document findings in a case narrative. Laboratory staff may be consulted in order to review QC data that are normally retained by MEL. The project officer will be responsible for validating all data by examining the complete data record and determining whether the methods and procedures described in this QAPP were used. Results from the quality control procedures used in the laboratory and field will be used to determine how well the data comply with the Measurement Quality Objectives (accuracy, precision, bias) described in Appendix D.

Data Quality Assessment

Data quality assessment is the determination of whether or not the data generated by the project can be used to meet project objectives. The project officer will make this determination by examining the data and quality control information associated with it. The procedures described in the above sections will guide the project officer in making this determination. Others may be consulted where their expertise can be of value (e.g. quality assurance staff, laboratory staff). The project's annual report will discuss data quality and the determination of whether or not the data can be used to meet project objectives. Limitations of the data, where present, will also be described.

References

- Bortleson, G. and J. Ebbert, 2000. *Occurrences of Pesticides in Streams and Ground Water in the Puget Sound Basin, Washington, and British Columbia, 1996-98*. U.S. Geological Survey, Tacoma, WA. Water Resources Investigations Report 00-4118.
- Davis, D., 1993. *Washington State Pesticide Monitoring Program: Reconnaissance Sampling of Surface Water (1992)*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 93-e09.
- Davis, D., 1998. *Washington State Pesticide Monitoring Program: 1995 Surface Water Sampling Report*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 98-300.
- Davis, D., 2000. *Washington State Pesticide Monitoring Program: 1997 Surface Water Sampling Report*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 00-03-003.
- Davis, D., D. Serdar, and A. Johnson, 1998. *Washington State Pesticide Monitoring Program: 1995 Fish Tissue Sampling Report*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 98-312.
- DeLorme Mapping, 1988. *Washington Atlas and Gazetteer, First Edition*. DeLorme Mapping. Freeport, Maine.
- Ecology, 2000. *Manchester Environmental Laboratory: Lab Users Manual, Fifth Edition*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA.
- Ecology, 2001. *Information Integration Project: Facilities/Site Database*. Washington State Department of Ecology, Information Services Program. Environmental Assessment Program. Olympia, WA.
- EPA, 2000. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories - Volume 1: Field Sampling and Analysis, Third Edition*. U.S. EPA Office of Water. Washington, D.C. EPA Publication Number EPA-823-B-00-007.
- EVS Environmental Consultants, Inc., 2000. *Columbia River Basin Fish Contaminant Survey: Volume 2 – Data Appendices*. Prepared for the U.S. Environmental Protection Agency Region 10 by EVS Environmental Consultants, Inc. Seattle, WA. EVS Project Number 2/294-10.
- Health, 2001. *Fish and Shellfish Consumption Advisories in Washington State Due to Chemical Contamination*. http://www.doh.wa.gov/ehp/oehas/EHA_fish_adv.htm. Washington State Department of Health, Olympia, WA.

Hopkins, B., 1991. *Basic Water Monitoring Program Fish Tissue and Sediment Sampling for 1989*. Washington State Department of Ecology, Ecology Publication Number 91-e14.

Johnson, A. and D. Norton, 1990. *1989 Lakes and Reservoir Water Quality Assessment Program: Survey of Chemical Contaminants in Ten Washington Lakes*. Washington State Department of Ecology, Environmental Investigations and Laboratory Services Program. Olympia, WA. Ecology Publication Number 90-e38.

Johnson, A. and N. Olson, 2001. "Analysis and Occurrence of Polybrominated Diphenyl Ethers in Washington State Freshwater Fish." *Archives of Environmental Contamination and Toxicology*, 41:339-344.

Johnson, A., 2002. Personal Communication. March 2001. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA.

Llanso, R., S. Aasen, and K. Welch, 1998. *Marine Sediment Monitoring Program: Chemistry and Toxicity Testing 1989-1995*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 98-323.

Lombard, S. and C. Kirchmer, 2001. *Guidelines for Preparing Quality Assurance Project Plans for Environmental Studies*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 01-03-003.

MacCoy, D. and R. Black, 1998. *Organic Compounds and Trace Elements in Freshwater Streambed Sediment and Fish from the Puget Sound Basin*. . U.S. Geological Survey. Denver, CO. U.S. Geological Survey Fact Sheet 105-98.

Mandjikov, M., 2002. Personal Communication. March 2002. Washington State Department of Ecology, Manchester Environmental Laboratory. Manchester, WA.

Rinella, J., P. Hamilton, and S. McKenzie, 1993. *Persistence of the DDT Pesticide in the Yakima River Basin Washington*. U.S. Geological Survey. Denver, CO. U.S. Geological Survey Circular 1090.

Rogowski, D. and D. Davis, 1999. *Potholes Reservoir Pesticide Survey, 1998*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 99-331.

Schmitt, C. and W. Brumbaugh, 1990. "National Contaminant Biomonitoring Program: Concentrations of: Arsenic, Cadmium, Copper, Lead, Mercury, Selenium, and Zinc in U. S. Freshwater Fish, 1976-1984." *Archives of Environmental Contamination and Toxicology*, 19 731-747.

Schmitt, C., J. Zajicek, and P. Peterman, 1990. "National Contaminant Biomonitoring Program: Residues of Organochlorine Chemicals in U. S. Freshwater Fish, 1976-1984." *Archives of Environmental Contamination and Toxicology*. 19 748-781.

Serdar, D., A. Johnson, and D. Davis, 1994. *Survey of Chemical Contaminants in Ten Washington Lakes*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 94-154.

Serdar, D., D. Davis, and J. Hirsch, 1999. *Lake Whatcom Watershed Cooperative Drinking Water Protection Project: Results of 1998 Water, Sediment, and Fish Tissue Sampling*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 99-337.

Tetra Tech, Inc., 2000. *Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue*. Prepared for the U.S. Environmental Protection Agency Office of Science and Technology by Tetra Tech, Inc., Owings Mills, MD.

Williamson, A., M. Munn, S. Ryker, R. Wagner, J. Ebbert, and A. Vanderpool, 1998. *Water Quality in the Central Columbia Plateau, Washington, and Idaho, 1992-95*. U.S. Geological Survey. Denver, CO. U.S. Geological Survey Circular 1144.

Yake, B., S. Singleton, and K. Erickson, 1998. *Washington State Dioxin Source Assessment*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA. Ecology Publication Number 98-320.

Yake, W., 2001. *Proposed Toxics Monitoring Plan*. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA.

Appendix E.

Example of a Fish Tissue Biomonitoring Program

**New Jersey Department of Environmental Protection
Routine Monitoring for Toxics in Fish
Final Work Plan
(abridged)**

This page intentionally blank.

FINAL

WORK PLAN

**Routine Monitoring Program for Toxics in Fish:
Estuarine and Marine Waters**

JUNE 2004

Co-Project Managers: Gary A. Buchanan, Thomas Belton, and Bruce Ruppel
Division of Science, Research and Technology

New Jersey Department of Environmental Protection



WORK PLAN

Routine Monitoring Program for Toxics in Fish: Estuarine and Marine Waters

I. INTRODUCTION

Fish and shellfish consumption advisories due to toxic chemical contamination were announced in New Jersey in the 1980s and 1990s. Data from Division of Science, Research and Technology (DSRT) studies revealed that unacceptable risks existed for eating certain species of fish and shellfish from certain waters in the State. These advisories particularly apply to pregnant women, nursing mothers and young children because polychlorinated biphenyls (PCBs), dioxin and mercury are known to cause birth defects, developmental problems, neurological problems and/or cancer. However, limited new data has been generated in the past ten years. Therefore, it is not known how appropriate the advisories are today. Current advisories are listed on NJDEP's Website (www.state.nj.us/dep/fgw).

Therefore, new data are needed on a recurring basis to evaluate and adjust advisories as appropriate. Without regular monitoring data, current advisories could be either under or overly protective of human health. The primary objectives of the monitoring program are;

1. To provide current and more comprehensive data on concentrations of toxic contaminants in fish and shellfish in order to assess human health risks and thus update/recommend fish consumption advisories, and
2. To provide data to develop environmental indicators to assess the progress of environmental management actions (See Program Objectives below).

A statewide "Routine Monitoring Program for Toxics in Fish" has been developed. However, the scope of work detailed in this Work Plan covers only 1) the Marine and Estuarine components of the proposed statewide Monitoring Plan (i.e., Year 2 of the plan), and 2) a separate stand-alone investigation of dioxin and other contaminants on the tidal Passaic River and its downstream receiving waters, which will support both the Department's Natural Resource Damage (NRD) claim process as well as its Passaic River Directive.

Note: It is important to add, that a dedicated source of annually renewable funding for the complete "Routine Monitoring Program for Toxics in Fish" (on a rotating five year plan) would be preferable and more protective of public health and natural resources, as well as supplying a continuous means for enforcing State laws affecting the abatement of toxic chemical releases into the waters of the State.

II. OBJECTIVES

The primary goal of the Monitoring Program is to update the human health consumption advisories for certain foodfish species and/or geographic areas. However, NJDEP recognizes the additional potential usages of these data for such important collateral activities as natural

resources management, hazardous site characterization, water quality assessment, natural resource damage claims, and Total Maximum Daily Load (TMDL) development. Where possible, the Monitoring Plan has been designed in cooperation with these Programs and in such a way as to meet the Department's data quality objectives (fish and shellfish collection and analytical techniques) and to maximize benefits for each individual program. The Program Objectives described below outline some of these goals.

Division of Fish and Wildlife

The marine resources in New Jersey support some of the largest recreational and commercial fisheries on the United States Atlantic Coast. On average, about one million recreational anglers spend over 5 million days fishing our marine waters. Each year, recreational marine anglers spend over \$750 million resulting in over 20,000 full time equivalent jobs and \$45 million in sales tax income to New Jersey. The commercial fishery has approximately 1,900 commercial fishing vessels producing dockside sales of fish, shellfish and crustaceans valued at over \$95 million annually. The commercial industry employs approximately 22,000 people in the harvesting, processing and wholesale and retail sales of marine fish and shellfish. The combined value of the commercial and recreational industries to the economy of New Jersey is between \$1.5 and \$2.1 Billion. These numbers do not include the value that our marine environment and fishing opportunities have on New Jersey's tourist industries. Approximately one-third of all marine-angling participants are non-residents.

Consumption advisories have undoubtedly affected and will continue to affect the quality of the fishing experience and therefore the amount of money spent on fishing in New Jersey. A regular and continuing fish tissue-sampling program will aid in issuing up to date and accurate fish advisories as well as support advisory outreach efforts. A consistent positive message on the benefits of eating seafood along with a fish tissue-sampling program will benefit the recreational and commercial fishing interests in New Jersey.

Measurements of contaminants up the food chain can also assist in assessing ecological as well as human health risks in the region. Monitoring of contaminant levels in piscivorous (fish-eating) birds is planned to determine the magnitude and effects of contaminants at higher trophic levels. Cormorants are common in the harbor and a strict piscivore. Cormorants have wide foraging ranges, however birds tied to a nesting colony have a more localized range. Therefore, eggs and/or blood from nesting colony birds will be targeted for sampling. Samples will be collected from Shooters Island (Newark Bay) and Swinburne Island (Lower Bay). Data will be compared to samples collected by New York State Department of Environmental Conservation (NYSDEC) in 1999.

Office of Natural Resource Restoration (ONRR)

ONRR pursues restoration of injured resources for the citizens of New Jersey. Having accurate fish tissue data will enable ONRR to effectively prioritize damage assessment of watershed specific fishery resources. Accurate and up to date sampling results will also aid in producing legally and scientifically defensible damage assessments. This will further the cause for DEP to fully realize its natural resource trustee obligation under existing statutes. ONRR supports the current proposal and would find it beneficial if the scope of the proposal were expanded into additional watersheds, targeted additional fish species and include waterfowl sampling.

Settlement monies recovered due to lost fishery resources will be returned to the public through primary and/or secondary restoration projects. These projects will have a nexus to the injury and will focus on improving the fishery resources that have been damaged.

Water Monitoring and Standards

The Bureau of Water Quality Standards and Assessment is responsible for water quality characterization and assessment of all waters of the State. Section 305(b) of the Federal Clean Water Act requires states to periodically (every two years) assess and report on the overall quality of their waters. These assessments are reported through the New Jersey Integrated Water Quality Monitoring and Assessment Report (305(b) and 303(d)). Section 303(d) requires states to identify “Impaired Waters” where specific designated uses are not fully supported (See Appendix B). For these waters, the state is required to establish total maximum daily loads (TMDLs) in accordance with a priority ranking.

Marine Water Monitoring has indicated a need to collect bivalve (e.g., clam) samples in estuarine waters. Establishment of routine monitoring of bivalve tissues is needed in order to establish that they meet federal guidance levels for metals. Targeted contaminants identified under the National Shellfish Sanitation Program include arsenic, cadmium, chromium, lead, mercury and nickel. Meeting federal guidance values could result in opening additional waters to direct market harvest.

Division of Watershed Management

Approximately 117 TMDLs that are required as a result of listings for contaminated fish tissue will be due starting in 2006. The last group is due in 2011. It is very important that current fish tissue data be available for these TMDLs. Conditions have most likely changed since the available data were collected in 1987. In some cases, TMDLs may no longer be necessary, as environmental conditions have improved. This happened in Strawbridge Lake, one of New Jersey’s first completed TMDLs. Fish tissue contamination with chlordane was the basis for original listing, whereas current data were available to show that chlordane levels had dropped significantly. To develop a TMDL that reflects current conditions and solve existing contamination problems recurrent sampling and data gathering is crucial.

Site Remediation and Waste Management Program (SRWM)

SRWM’s Division of Remediation Management and Response is responsible for the remediation, management and response to environmental impacts associated with hazardous waste sites. For the purpose of evaluating the progress of site characterization and cleanup activities it is important to understand the pathways that contaminants travel through the environmental as well as the risks to both human health and the surrounding ecosystem. Aquatic food chain impacts are common side effects especially from a site contaminated with persistent bioaccumulative toxics (PBTs) such as PCBs, dioxins and mercury. From a risk perspective fish contamination by PBTs can become an important remedial investigative concern in the Remedial Investigation Feasibility Study (RI/FS) process, the first steps in the development of a clean up plan or a claim for natural resource damages. The analysis of fish from this Work Plan will assist SRWM in determining the fate and effects of PBTs from sites into the surrounding environment. Of particular interest to SRWP is the aquatic effects and bioaccumulation of dioxins from the

Diamond-Alkali Facility on the Passaic River, which is the primary source of fish consumption advisories for the surrounding and downstream waters. The Diamond Alkali RI/FS for the Passaic River Area of Concern is ongoing and any additional data from fish contaminant results would be beneficial.

Ecological impacts are also a concern of SRWM. Impacts on the aquatic food chain from contaminants can be significant, and potential risks and impacts can be ascertained from the planned sampling. Levels of contaminants in fish and shellfish (i.e., crabs and lobster), as well as piscivorous birds can be used to approximate the potential ecological risks and impacts to the ecosystem.

III. METHODS

The scope of work is presented below in two tasks. Task I details the baseline status and trends sampling for the Coastal Region of the State. Whereas Task II presents more targeted sampling within the tidal Passaic River and Newark Bay for dioxin/furan analysis to support the Passaic River Directive and the Passaic River/Newark Bay Natural Resource Damage Claim. The latter sampling includes more dioxin analyses of samples, more species per site, and additional down bay sampling of the Lower Passaic-Hackensack Rivers, Newark and Raritan Bays, and Sandy Hook Bay. Sampling will be coordinated with other state or federal agencies' fish/shellfish collection efforts (e.g., NMFS) where appropriate.

Task I. Coastwide Routine Monitoring: Estuarine & Marine Species

Seven species of estuarine-marine fish/shellfish (striped bass, bluefish, white perch, white catfish, American eel, blue crab and lobster) are under consumption advisories on a statewide, regional and waterway specific for PCB and/or dioxin contamination. These species and locations are a starting point for the design of the Fish Monitoring Program (See Table 1). Weakfish and five samples of other fish species of opportunity (snapper bluefish, winter flounder, menhaden or other species) will be collected from select locations. Unless specified, fillets from all coastal estuarine and marine species will be analyzed for PCBs, pesticides and total mercury, as well as lipids (Table 2). Monitoring for dioxins/furans at specific stations (e.g., Newark Bay and Raritan River) will be included in Task II. Sampling includes alongshore-coastal areas, Delaware Bay and Estuary and Barnegat Bay. If supplemental funding becomes available, additional sampling sites, fish species or non-routine contaminants may be included in the monitoring program. A subset of samples will be analyzed for polybrominated diphenyl ethers (PBDEs) under an initial screening assessment to be developed in conjunction with the laboratory.

The results of this monitoring will expand upon the existing contaminant database used to develop fish consumption advisories. The monitoring will identify chemical contaminant levels in Atlantic marine and estuarine species from several waterways throughout the coastal portions of the state.

Table 1. Planned Sampling Locations and Samples for Task I

| Sampling Locations | Striped Bass | Bluefish | Amer. Eel | Blue Crab* | White Perch | White Catfish | Weakfish | Other | Totals |
|---|--------------|----------|-----------|------------|-------------|---------------|----------|-------|--------|
| Raritan River at Rt. 35 | | | | | 5 | | | | 5 |
| Raritan Bay at Union Beach | | 5 | | | | # | 5 | ↔ | 20 |
| Delaware River | | | | 5* | | | | | 10 |
| Delaware Bay | 5 | | | | | # | 10 | ↔ | 20 |
| Navesink River | | | 5 | | | | | | 5 |
| Shrewsbury River | | | 5 | | | | | | 5 |
| Shark River | | | 5 | | | | | | 5 |
| Mullica River | | | 5 | | | | | | 5 |
| Atlantic Ocean just N of Sandy Hook | 5 | 5 | | | | | | | 10 |
| Atlantic Ocean at Island Beach State Park | 5 | 5 | | | | | | | 10 |
| Atlantic Ocean off Belmar | 5 | 5 | | | | | | | 10 |
| Atlantic Ocean E of Sea Isle City | 5 | 5 | | | | | | | 10 |
| Barnegat Bay at Toms River | | | 5 | 5* | | | 5 | ↔ | 20 |
| Totals | 25 | 25 | 25 | 20 | 5 | 5 | 20 | 10 | 135 |
| ANALYSES: PCBs (congeners + coplanars), Pesticides & Mercury | | | | | | | | | |

* Each crab sample to be separately analyzed as muscle and hepatopancreas (i.e., total = sample x 2). Each sample will be a composite of approximately 5 individuals.

Indicates potential collection site for indicated species.

↔ Indicates potential collection site. A total of five samples of other fish species of opportunity (snapper bluefish, winter flounder, menhaden or other species) will be collected from one of these locations.

Table 2. Analyte List for Task I

| <u><i>OC Pesticides</i></u> | <u><i>Polychlorinated Biphenyls (congeners)¹</i></u> | | | | | |
|---|---|----------|--------|-------------|---------|---------|
| BHC (alpha, beta, gamma delta) | 1 | 31,28 | 74 | 134,144 | 185 | 207 |
| Heptachlor | 3 | 33,21,53 | 70,76 | 107 | 174 | 194 |
| Heptachlor epoxide | 4,10 | 22 | 66,95 | 149 | 177 | 205 |
| Chlordanes (gamma and alpha) | 6 | 45 | 91 | 118 | 201,171 | 206 |
| Nonachlors (cis ² and trans) | 7 | 46 | 56,60 | 134 | 172,197 | 209 |
| Dieldrin | 8,5 | 52 | 101 | 131 | 180 | 16,32 |
| DDD _s (o,p and p,p) | 14 | 49 | 99 | 146 | 193 | 163,138 |
| DDE _s (o,p ³ and p,p) | 19 | 48,47 | 83 | 132,153,105 | 191 | 25 |
| DDT _s (o,p and p,p) | 12,13 | 44 | 97 | 141 | 199 | 63 |
| Aldrin | 18 | 37,42 | 81,87 | 137,176 | 170,190 | 151 |
| Endosulfan I and II | 17 | 41,71 | 85 | 158 | 198 | 128 |
| Endrin | 24,27 | 64 | 136 | 129,178 | 201 | 208,195 |
| Oxychlordane | 29 | 40 | 77,110 | 187,182 | 203,196 | |
| | 26 | 100 | 82 | 183 | 189 | |
| Total Mercury | | | | | | |
| | <u><i>Co-Planar PCBs</i></u> | | | | | |
| PBDEs | 81 | 77 | 126 | 169 | | |
| Lipids | | | | | | |

1-PCB congeners appearing as pairs or triplets will coelute and will be reported as sum.

2-Evidence for PCB coelution with cis-nonachlor

3-o,p-DDE coelutes with PCB congeners 92,85

Task II. Tidal Passaic River and Downstream Receiving Waters (NRD)

In recognition of the continuing public health advisories associated with dioxin contamination in foodfish and shellfish within the tidal Passaic River, Newark Bay, the two Kills, Raritan and Sandy Hook Bays, as well as the near shore ocean waters of the New York Bight, a separate yet interrelated study will be carried out involving the collection of fish/shellfish/bird samples for dioxin/furan analysis. These locations will be sampled differentially for four species; white perch, striped bass, blue crab and American lobster within their preferred ecological zones or habitats (Table 3). An additional “species of opportunity” will be collected at two locations. Potential species include winter flounder, snapper bluefish, weakfish, and menhaden. Cormorant tissue (i.e., eggs and/or blood) will be collected at two locations. Species and sample location are designed to address multiple program data needs including water quality assessment, contaminated site assessment, natural resource damage claims, TMDLs and resource management concerns.

Each tissue (i.e., fillet, muscle, hepatopancreas, egg, and blood) will undergo analysis for dioxins/furans, PCBs/Pesticides, mercury and lipids (Table 4). Composite samples will be used for crab and lobster tissue. A subset of samples will also be analyzed for PBDEs under an initial screening assessment to be developed in conjunction with the laboratory.

Optional Sampling: DEP programs also identified several additional data needs. If money is available, additional species will be collected at the same or additional locations. Samples will potentially include bivalve clams (e.g., in Raritan/Sandy Hook Bay) and other fish species.

Table 3. Planned Sampling Locations and Samples for Task II

| Sampling Locations | Crab* | Lobster* | White Perch | Striped Bass | Fish Species | Piscy. Bird** | Totals |
|-----------------------|-------|----------|-------------|--------------|--------------|---------------|--------|
| Upper Tidal Passaic | 5 | | | | | | 10 |
| Lower Tidal Passaic | 5 | | 3 | 3 | ← | | 16 |
| Hackensack River | 3 | | | | ← | | 6 |
| Newark Bay | 3 | | | | ← | 6 | 15 |
| Upper Bay | 3 | | | | | | 6 |
| Arthur Kill | 3 | | | | | | 6 |
| Raritan River | 3 | | | | | | 6 |
| Western Raritan Bay | 3 | | | | ← | | 9 |
| Eastern Raritan Bay | 3 | | | | ← | | 6 |
| Mid-Lower Raritan Bay | 3 | 5 | | 2 | ← | 6 | 24 |
| Offshore NY Bight | | 5 | | | | | 10 |
| Totals | 68 | 20 | 3 | 5 | 6 | 12 | 114 |

* Each crab and lobster sample to be separately analyzed as muscle and hepatopancreas (i.e., total = sample x 2). Each sample will be a composite of approximately 5 individuals.

** Piscivorous bird samples will consist of cormorant eggs and/or blood.

← Three samples of other fish species of opportunity will be collected in the Newark Bay/Passaic/Hackensack region, and three samples will be collected in Raritan Bay. Target species will include weakfish, winter flounder, snapper bluefish, and menhaden.

Tissue Processing

Fish tissue samples will be processed according to the planned analysis. The program will follow the procedure used in past monitoring programs (i.e., ANSP Procedure P-14-12 (Rev. 4 (12/00) titled Preparation of Fish Samples For Contaminant Analysis):

3.1.1 Fillet with skin, but with scales removed this is the default type of tissue sample for most scaly fish (i.e. sunfish and shad). This consists of the entire fillet or pairs of fillets (right and left sides), overlaying skin and belly flap meat.

3.1.3 Fillet without skin - (i.e. gar, catfish, sturgeon), This consists of the entire fillet, including the belly flap tissue, with skin and scales removed.

3.1.4 Fillet with skin on (except catfish and eels), scales off and including pelvic fin, rib cage and belly meat (equivalent to USFDA fillet and "New York standard fillet").

Fish prepared using 3.1.1 and 3.1.4 will be analyzed for organic compounds (i.e. PCBs, OCP, and dioxin) and fish prepared using 3.1.3 will be used for mercury analysis.

Table 4. Analyte List for Task II

| <u>OC Pesticides</u> | <u>Individual PCB congeners</u> | | | |
|----------------------------------|--|---------|-----------------------|-----------------------------|
| Alpha HCH | 1 | 41/64 | 118 | 180 |
| Beta HCH | 7/9 | 40 | 114 | 193 |
| Gamma HCH | 8/5 | 67 | 146 | 191 |
| Delta HCH | 30 | 63 | 153/132 | 200 |
| Heptachlor | 18/17 | 74/61 | 105 | 169 |
| Heptachlor Epoxide | 15 | 70 | 141/179 | 170/190 |
| Oxychlordane | 24/27 | 66 | 130 | 199 |
| Alpha Chlordane | 16/32 | 95/80 | 176/137 | 203/196 |
| Gamma Chlordane | 29 | 55/91 | 138 /160 | 189 |
| Cis-Nonachlor | 26 | 56/60 | 58 | 195/208 |
| Trans-Nonachlor | 25 | 92 | 129 | 207 |
| Aldrin | 31 | 84 | 126 | 194 |
| Dieldrin | 28 | 101/90 | 178 | 205 |
| Endrin | 33/20 | 99 | 166 | 206 |
| Pentachloroanisole | 53 | 119 | 175 | 209 |
| Chlorpyrifos | 22/51 | 83 | 187 | |
| Mirex | 45 | 97 | 183 | <u>Coplanar PCBs</u> |
| Endosulfan II | 46 | 81 | 128 | 81 |
| Methoxychlor | 39 | 87/115 | 167 | 77 |
| 2,4' DDE | 69 | 85 | 185 | 126 |
| 4,4' DDE | 52 | 136 | 174 | 169 |
| 2,4' DDD | 49 | 110/77 | 177 | |
| 4,4' DDD | 47/75 | 82 | 171/202 | |
| 2,4' DDT | 48 | 151 | 156 | |
| 4,4' DDT | 44 | 135 | 201/157/173 | |
| <u>Trace Metals</u> | 42/59/37 | 107 | 172 | |
| Mercury | 72 | 149/123 | 197 | Lipids |
| <u>Dioxins and Furans</u> | | | | |
| 2,3,7,8-TCDD | 2,3,7,8-TCDF | | 1,2,3,7,8,9-HxCDF | |
| 1,2,3,7,8-PeCDD | 1,2,3,7,8-PeCDF | | 1,2,3,4,6,7,8-HpCDF | |
| 1,2,3,4,7,8-HxCDD | 2,3,4,7,8-PeCDF | | 1,2,3,4,7,8,9, -HpCDF | |
| 1,2,3,6,7,8-HxCDD | 1,2,3,4,7,8-HxCDF | | OCDF | |
| 1,2,3,7,8,9-HxCDD | 1,2,3,6,7,8-HxCDF | | OCDD | |
| 1,2,3,4,6,7,8-HpCDD | 2,3,4,6,7,8-HxCDF | | | |
| <u>PBDEs</u> | | | | |

Analytical Methods

The Academy of Natural Sciences of Philadelphia (ANSP) and the Geochemical and Environmental Research Group (GERG) at Texas A&M University will be conducting the analysis of all tissue samples. These laboratories will use the following analytical methods as outlined in Table 5 and detection limits are listed in Tables 6 and 7.

Table 5. Analytical Methods for Tasks I and II

| DESCRIPTION OF PROCEDURES USED: | GERG | ANS |
|--|---|---|
| EPA Method | Modified 8082 PCBs by GC-ECD | Modified 8082 PCBs by GC-ECD |
| Approximate amount of fish tissue extracted (wet wt): | ~10 g | ~1 to 5 g |
| Method used for determining percentage water: | drying and weighing | drying and weighing |
| Method used for extractables (lipid): | gravimetric | gravimetric |
| Extraction method: | grinding with sodium sulfate | grinding with sodium sulfate |
| Extraction solvent: | dichloromethane | dichloromethane |
| Extraction time: | 3 x 3 min | 18 h Soxhlet |
| Sample extract cleanup method: | Slica gel/alumina and phenogel | GPC followed by florisil |
| Analytical method used for PCBs and Organochlorine Pesticides (OCPs): | | |
| Analytical Instrument. | GC-ECD | GC-ECD |
| Column Phase | DB 5 | DB 5 |
| Column Length, m | 30m | 60 m |
| Column i.d., mm | 0.25 mm | 0.25 mm |
| Column film thickness, µm | 0.25um | 0.25um |
| Method of quantitation (IS = internal standard, ES = external standard): | IS | IS |
| Identity of internal standards/surrogates used that were: | | |
| Added PRIOR to extraction of sample: | DBOFB, PCB 103, PCB198 | PCB14, PCB65,PCB166 |
| Added after extraction/cleanup and JUST PRIOR to chromatographic analysis: | TCMX | PCB30,PCB204 |
| Number of Points on Calibration Curve | | |
| PCB Congeners | 4 | 5 |
| Pesticides | 4 | 5 |
| Range of Calibration Curve | | |
| PCB Congeners | 5 to 200 ng/ul | 30 to 300 ng/ul |
| Pesticides | 5 to 200 ng/ul | 0.25 to 2.5 ng/ul |
| Coplanar PCBs using HRGC/HRMS | EPA Method 1668 | - |
| Mercury using Cold Vapor AA | | |
| Sample Preparation: Microwave Assisted Digestion of Siliceous and Organically Based Matrices. 1996. | - | EPA, SW-846, modified Method 3052 |
| Sample Analysis: Mercury in Liquid Waste (Manual Cold-Vapor Technique). 1994. Rev. 1. | - | EPA, SW-846, modified Method 7470A |
| Dioxin using HRGC/HRMS | Modified EPA Method 1613 | - |

Table 6. GERG Analytical Detection Limits

| Pesticides | Detection Limit (ng/g wet) | PCB Congeners | Detection Limit (ng/g wet) | PCB Congeners | Detection Limit (ng/g wet) |
|-----------------------------|-----------------------------------|----------------------|-----------------------------------|----------------------|-----------------------------------|
| Alpha HCH | 0.01 | PCB1 | 0.02 | PCB118 | 0.02 |
| Beta HCH | 0.02 | PCB7/9 | 0.02 | PCB119 | 0.02 |
| Gamma HCH | 0.01 | PCB8/5 | 0.02 | PCB126 | 0.02 |
| Delta HCH | 0.01 | PCB22/51 | 0.02 | PCB128 | 0.01 |
| Heptachlor | 0.01 | PCB24/27 | 0.02 | PCB129 | 0.02 |
| Heptachlor Epoxide | 0.02 | PCB25 | 0.02 | PCB130 | 0.02 |
| Oxychlorane | 0.03 | PCB26 | 0.02 | PCB135 | 0.02 |
| Alpha Chlordane | 0.02 | PCB28 | 0.02 | PCB136 | 0.02 |
| Gamma Chlordane | 0.02 | PCB29 | 0.02 | PCB138 /160 | 0.03 |
| Cis-Nonachlor | 0.02 | PCB30 | 0.02 | PCB141/179 | 0.02 |
| Trans-Nonachlor | 0.04 | PCB31 | 0.02 | PCB146 | 0.02 |
| Aldrin | 0.02 | PCB33/20 | 0.02 | PCB149/123 | 0.02 |
| Dieldrin | 0.05 | PCB39 | 0.02 | PCB15 | 0.02 |
| Endrin | 0.04 | PCB40 | 0.02 | PCB151 | 0.02 |
| Pentachloroanisole | 0.01 | PCB41/64 | 0.02 | PCB153/132 | 0.05 |
| Chlorpyrifos | 0.06 | PCB42/59/37 | 0.02 | PCB156 | 0.02 |
| Mirex | 0.01 | PCB44 | 0.02 | PCB158 | 0.02 |
| Endosulfan II | 0.02 | PCB45 | 0.02 | PCB16/32 | 0.02 |
| 2,4' DDE | 0.02 | PCB46 | 0.02 | PCB166 | 0.02 |
| 4,4' DDE | 0.06 | PCB47/75 | 0.02 | PCB167 | 0.02 |
| 2,4' DDD | 0.07 | PCB48 | 0.02 | PCB169 | 0.02 |
| 4,4' DDD | 0.01 | PCB49 | 0.02 | PCB170/190 | 0.02 |
| 2,4' DDT | 0.00 | PCB52 | 0.02 | PCB171/202 | 0.02 |
| 4,4' DDT | 0.01 | PCB53 | 0.02 | PCB172 | 0.01 |
| | | PCB55/91 | 0.02 | PCB174 | 0.02 |
| Dioxins & Furans | pg/sample ¹ | PCB56/60 | 0.02 | PCB175 | 0.02 |
| 2,3,7,8-TCDF | 10.00 | PCB63 | 0.02 | PCB176/137 | 0.02 |
| 1,2,3,7,8-PeCDF | 50.00 | PCB66 | 0.02 | PCB177 | 0.02 |
| 2,3,4,7,8-PeCDF | 50.00 | PCB67 | 0.02 | PCB178 | 0.02 |
| 1,2,3,4,7,8-HxCDF | 50.00 | PCB69 | 0.02 | PCB18/17 | 0.02 |
| 1,2,3,6,7,8-HxCDF | 50.00 | PCB70 | 0.02 | PCB180 | 0.01 |
| 2,3,4,6,7,8-HxCDF | 50.00 | PCB72 | 0.02 | PCB183 | 0.02 |
| 1,2,3,7,8,9-HxCDF | 50.00 | PCB74/61 | 0.02 | PCB185 | 0.02 |
| 1,2,3,4,6,7,8-HpCDF | 50.00 | PCB81 | 0.02 | PCB187 | 0.02 |
| 1,2,3,4,7,8,9-HpCDF | 50.00 | PCB82 | 0.02 | PCB189 | 0.02 |
| OCDF | 100.00 | PCB83 | 0.02 | PCB191 | 0.02 |
| 2,3,7,8-TCDD | 10.00 | PCB84 | 0.02 | PCB193 | 0.02 |
| 1,2,3,7,8-PeCDD | 50.00 | PCB85 | 0.02 | PCB194 | 0.02 |
| 1,2,3,4,7,8-HxCDD | 50.00 | PCB87/115 | 0.03 | PCB195/208 | 0.01 |
| 1,2,3,6,7,8-HxCDD | 50.00 | PCB92 | 0.02 | PCB197 | 0.02 |
| 1,2,3,7,8,9-HxCDD | 50.00 | PCB95/80 | 0.02 | PCB199 | 0.02 |
| 1,2,3,4,6,7,8-HpCDD | 50.00 | PCB97 | 0.02 | PCB200 | 0.02 |
| OCDD | 100.00 | PCB99 | 0.02 | PCB201/157/173 | 0.02 |
| Coplanar PCBs | pg/sample ¹ | PCB101/90 | 0.02 | PCB203/196 | 0.02 |
| PCB77 | 10.20 | PCB105 | 0.01 | PCB205 | 0.02 |
| PCB81 | 10.00 | PCB107 | 0.02 | PCB206 | 0.01 |
| PCB126 | 10.00 | PCB110/77 | 0.02 | PCB207 | 0.02 |
| PCB169 | 10.40 | PCB114 | 0.02 | PCB209 | 0.01 |

1 - Dependent on size of sample extracted

Table 7. ANSP Analytical Detection Limits

| | Detection Limit | | Detection Limit | | Detection Limit |
|--------------------|------------------|----------------------|-----------------|----------------------|-----------------|
| Pesticides | ng/g wet | PCB Congeners | ng/g wet | PCB Congeners | ng/g wet |
| opDDE | 0.04 | 1 | 0.17 | 85 | 0.02 |
| ppDDE | 0.18 | 3 | 0.29 | 136 | 0.01 |
| op ddt | 0.12 | 4+10 | 0.07 | 77+110 | 0.11 |
| pp ddt | 0.51 | 7 | 0.03 | 82 | 0.02 |
| o,p ddd | 0.06 | 6 | 0.02 | 151 | 0.02 |
| p,p ddd | 0.14 | 8+5 | 0.12 | 135+144 | 0.02 |
| | 0.00 | 19 | 0.03 | 107 | 0.01 |
| alpha BHC | 0.17 | 12+13 | 0.01 | 149 | 0.06 |
| beta BHC | 0.16 | 18 | 0.05 | 118 | 0.06 |
| delta BHC | 0.07 | 17 | 0.03 | 131 | 0.00 |
| lindane | 0.12 | 24+27 | 0.13 | 146 | 0.06 |
| | 0.00 | 16+32 | 0.07 | 153+132+105 | 0.21 |
| heptaclor | 0.06 | 29 | 0.08 | 141 | 0.01 |
| heptachlor epoxide | 0.11 | 26 | 0.02 | 137+176 | 0.04 |
| oxychlordan | 0.09 | 25 | 0.23 | 163+138 | 0.14 |
| gamma chlordan | 0.30 | 31+28 | 0.13 | 158 | 0.06 |
| alpha chlordan | 0.12 | 53+33+21 | 0.07 | 129+178 | 0.02 |
| cis nonachlor | 0.10 | 22 | 0.11 | 187+182 | 0.05 |
| trans nonachlor | 0.16 | 45 | 0.02 | 183 | 0.03 |
| | 0.00 | 46 | 0.02 | 128 | 0.02 |
| dieldrin | 0.02 | 52 | 0.09 | 185 | 0.02 |
| endrin | 0.08 | 49 | 0.07 | 174 | 0.02 |
| aldrin | 0.15 | 47 | 1.08 | 177 | 0.02 |
| endosulfan I | 0.10 | 48 | 0.22 | 202+171 | 0.02 |
| endosulfan II | 0.06 | 44 | 0.07 | 157+200 | 0.03 |
| | | 37+42 | 0.06 | 172+197 | 0.08 |
| | | 41+71 | 0.08 | 180 | 0.07 |
| PBDEs | To be determined | 40 | 0.02 | 193 | 0.15 |
| | | 100 | 0.02 | 191 | 0.01 |
| | | 63 | 0.02 | 199 | 0.01 |
| | | 74 | 0.06 | 170+190 | 0.05 |
| | | 70+76 | 0.08 | 201 | 0.03 |
| | | 66+95 | 0.19 | 203+196 | 0.05 |
| | | 91 | 0.02 | 189 | 0.01 |
| | | 56+60 | 0.12 | 208+195 | 0.05 |
| | | 101 | 0.07 | 207 | 0.01 |
| | | 99 | 0.04 | 194 | 0.02 |
| | | 83 | 0.01 | 205 | 0.01 |
| | | 97 | 0.02 | 206 | 0.03 |
| | | 87+81 | 0.24 | 209 | 0.01 |

This page intentionally blank.