APPENDIX B

REPRESENTATIVE SAMPLING GUIDANCE DOCUMENT, VOLUME 3: BIOLOGICAL

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Representative Sampling Guidance

Volume 3: Biological

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Environmental Response Team Center
Office of Emergency and Remedial Response
Office of Solid Waste and Emergency Response

U.S. Environmental Protection Agency
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For more information on Biological Sampling procedures, refer to the *Compendium of ERT Toxicity Testing Procedures*, OSWER Directive 9360-4-08, EPA/540/P-91/009 (U.S. EPA 1991a). Topics covered in this compendium include: toxicity testing; and surface water and sediment sampling.

Please note that the procedures in this document should only be used by individuals properly trained and certified under a 40 Hour Hazardous Waste Site Training Course that meets the requirements set forth in 29 CFR 1910.120(e)(3). It should not be used to replace or supersede any information obtained in a 40 Hour Hazardous Waste Site Training Course.

Questions, comments, and recommendations are welcomed regarding the *Superfund Program Representative Sampling Guidance, Volume 3 -- Biological*. Send remarks to:

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Preface

This document is third in a series of guidance documents designed to assist Superfund Program Site Managers such as On-Scene Coordinators (OSCs), Site Assessment Managers (SAMs), and other field staff in obtaining representative samples at Superfund sites. It is intended to assist Superfund Program personnel in evaluating and documenting environmental threat in support of management decisions, including whether or not to pursue a response action. This document provides general guidance for collecting representative biological samples (i.e., measurement endpoints) once it has been determined by the Site Manager that additional sampling will assist in evaluating the potential for ecological risk. In addition, this document will:

- Assist field personnel in representative biological sampling within the objectives and scope of the Superfund Program
- Facilitate the use of ecological assessments as an integral part of the overall site evaluation process
- Assist the Site Manager in determining whether an environmental threat exists and what methods are available to assess that threat

This document is intended to be used in conjunction with other existing guidance documents, most notably, *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments*, OSWER, EPA 540-R-97/006.

The objective of representative sampling is to ensure that a sample or a group of samples accurately characterizes site conditions. Biological information collected in this manner complements existing ecological assessment methods. Representative sampling within the objectives of the Superfund Program is used to:

- promote awareness of biological and ecological issues
- define the parameters of concern and the data quality objectives (DQOs)
- develop a biological sampling plan
- define biological sampling methods and equipment
- identify and collect suitable quality assurance/quality control (QA/QC) samples
- interpret and present the analytical and biological data

The National Contingency Plan (NCP) requires that short-term response (removal) actions contribute to the efficient performance of any long-term site remediation, to the extent applicable. Use of this document will help determine if biological sampling should be conducted at a site, and if so, what samples will assist program personnel in the collection of information required to make such a determination.

Identification and assessment of potential environmental threats are important elements for the Site Manager to understand. These activities can be accomplished through ecological assessments such as biological sampling. This document focuses on the performance of ecological assessment screening approaches, more detailed ecological assessment approaches, and biological sampling methods.

1.0 INTRODUCTION

1.1 OBJECTIVE AND SCOPE

This document is intended to assist Superfund Program personnel in evaluating and documenting environmental threat in support of management decisions. It presents ecological assessment and sampling as tools in meeting the objectives of the Superfund Program, which include:

- Determine threat to public health, welfare, and the environment
- Determine the need for long-term action
- Develop containment and control strategies
- Determine appropriate treatment and disposal options
- Document attainment of clean-up goals

This document is intended to assist Superfund Program personnel in obtaining scientifically valid and defensible environmental data for the overall decision-making process of site actions. Both the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [§104(a)(1)], as amended by the Superfund Amendments and Reauthorization Act (SARA), and the NCP [§300.400(a)(2)], require that the United States Environmental Protection Agency (U.S. EPA) "protect human health and the environment."

Environmental threats may be independent of human health threats, whether they co-exist at a site or are the result of the same causative agents. It is therefore important to determine and document potential, substantial, and/or imminent threats to the environment separately from threats to human health.

Representative sampling ensures that a sample or a group of sample accurately characterizes site conditions.

Representative biological sampling and ecological risk assessment include, but are not limited to, the collection of site information and the collection of samples for chemical or toxicological analyses. Biological sampling is dependent upon specific site requirements during limited response actions or in emergency response situations. Applying the methods of collecting environmental information, as outlined in this document, can facilitate the decision-making process (e.g., during chemical spill incidents).

The collection of representative samples is critical to the site evaluation process since all data interpretation assumes proper sample collection. Samples collected which inadvertently or intentionally direct the generated data toward a conclusion are biased and therefore not representative.

This document provides Superfund Program personnel with general guidance for collecting representative biological samples (i.e., measurement endpoints, [see Section 1.2 for the definition of measurement endpoint]). Representative biological sampling is conducted once the Site Manager has determined that additional sampling may assist in evaluating the potential for ecological risk. This determination should be made in consultation with a trained ecologist or biologist. The topics covered in this document include sampling methods and equipment, QA/QC, and data analysis and interpretation.

The appendices in this document provide several types of assistance. Appendix A provides a checklist for initial ecological assessment and sampling. Appendix B provides an example flow diagram for the development of a conceptual site model. Appendix C provides examples of how the checklist for ecological assessment/sampling is used to formulate a conceptual site model that leads up to the design of a site investigation.

This document is intended to be used in conjunction with other existing guidance documents, most notably, *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments*, EPA 540-R-97/006 (U.S. EPA 1997).

1.2 RISK ASSESSMENT OVERVIEW

The term ecological risk assessment (ERA), as used in this document, and as defined in *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments*, OSWER, EPA 540-R-97/006 (U.S. EPA 1997) refers to:

> "... a qualitative and/or quantitative appraisal of the actual or potential impacts of a hazardous waste site on plants and animals other than humans and domesticated species."

Risk assessments are an integral part of the Superfund process and are conducted as part of the baseline risk assessment for the remedial investigation and feasibility

study (RI/FS). The RI is defined by a characterization of the nature and extent of contamination, and ecological and human health risk assessments. The nature and extent of contamination determines the chemicals present on the site. The ecological and human health risk assessments determine if the concentrations threaten the environment and human health.

An ecological risk assessment is a formal process that integrates knowledge about an environmental contaminant (i.e., exposure assessment) and its potential effects to ecological receptors (i.e., hazard assessment). The process evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to a stressor. As defined by U.S. EPA (1992), a stressor is any physical, chemical or biological entity that can induce an adverse ecological response. Adverse responses can range from sublethal chronic effects in an individual organism to a loss of ecosystem function.

Although stressors can be biological (e.g., introduced species), in the Superfund Program substances designated as hazardous under CERCLA are usually the stressors of concern. A risk does not exist unless (1) the stressor has the ability to cause one or more adverse effects, and (2) it co-occurs with or contacts an ecological component long enough and at sufficient intensity to elicit the identified adverse effect.

The risk assessment process also involves the identification of assessment and measurement endpoints. Assessment endpoints are explicit expressions of the actual environmental values (e.g., ecological resources) that are to be protected. A measurement endpoint is a measurable biological response to a stressor that can be related to the valued characteristic chosen as the assessment endpoint (U.S. EPA 1997). Biological samples are collected from a site to represent these measurement endpoints. See Section 2.2 for a detailed discussion of assessment and measurement endpoints.

Except where required under other regulations, issues such as restoration, mitigation, and replacement are important to the program but are reserved for investigations that may or may not be included in the RI phase. During the management decision process of selecting the preferred remedial option leading to the Record of Decision (ROD), mitigation and restoration issues should be addressed. Note that these issues are not necessarily issues within the baseline ecological risk assessment.

Guidelines for human health risk assessment have been established; however, comparable protocols for ecological risk assessment do not currently exist. *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments."* (U.S. EPA 1997) provides conceptual guidance and explains how to design and conduct ecological risk assessments for a CERCLA RI/FS. The *Framework for Ecological Risk Assessment* (U.S. EPA 1992) provides an Agency-wide structure for conducting ecological risk assessments and describes the basic elements for evaluating site-specific adverse effects of stressors on the environment. These documents should be referred to for specific information regarding the risk assessment process.

While the ecological risk assessment is a necessary first step in a "natural resource damage assessment" to provide a causal link, it is not a damage evaluation. A natural resource damage assessment may be conducted at any Superfund site at the discretion of the Natural Resource Trustees. The portion of the damage assessment beyond the risk assessment is the responsibility of the Natural Resource Trustees, not of the U.S. EPA. Therefore, natural resource damage assessment is not addressed in this guidance.

1.3 CONCEPTUAL SITE MODEL

A conceptual site model is an integral part of a site investigation and/or ecological risk assessment as it provides the framework from which the study design is structured. The conceptual site model follows contaminants from their sources, through transport and fate pathways (air, soil, surface water, groundwater), to the ecological receptors. The conceptual model is a strong tool in the development of a representative sampling plan and is a requirement when conducting an ecological risk assessment. It assists the Site Manager in evaluating the interaction of different site features (e.g., drainage systems and the surrounding topography), thereby ensuring that contaminant sources, pathways, and ecological or human receptors throughout the site have been considered before sampling locations, techniques, and media are chosen.

Frequently, a conceptual model is created as a site map (Figure 1) or flow diagram that describes the potential movement of contaminants to site receptors (see Appendix B). Important considerations when creating a conceptual model are:

- The state(s) (or chemical form) of each contaminant and its potential mobility through various media

- Site topographical features
- Meteorological conditions (e.g., climate, precipitation, humidity, wind direction/speed)
- Wildlife area utilization.

Preliminary and historical site information may provide the identification of the contaminant(s) of concern and the level(s) of the contamination. A sampling plan should be developed from the conceptual model based on the selected assessment endpoints.

The conceptual site model (Figure 1) is applied to this document, *Representative Sampling Guidance Volume 3: Biological*. Based on the model, you can approximate:

- Potential Sources
  hazardous waste site (waste pile, lagoon, emissions), drum dump (runoff, leachate), agricultural (runoff, dust, and particulates)

- Potential Exposure Pathways
  - *ingestion*
    waste contained in the pile on the hazardous waste site; soil particles near the waste pile; drum dump; or area of agricultural activity
  - *inhalation*
    dust and particulates from waste pile, drum dump, or area of agricultural activity
  - *absorption/direct contact*
    soil near waste pile, drum dump, or area of agricultural activity and surface water downstream of sources

- Potential Migration Pathways
  - *air (particulates and gases) from drum dump and area of agricultural activity*
  - *soil (runoff) from the hazardous waste site, drum dump, and agricultural runoff*
  - *surface water (river & lake) from hazardous waste site and agricultural runoff*
  - *groundwater (aquifer) from drum dump leachate.*

- Potential Receptors of Concern (and associated potential routes)
  - *wetland vegetation/mammals/invertebrates if suspected to be in contact with potentially contaminated soil and surface water*
  - *riverine vegetation/aquatic organisms if suspected to be in contact with potentially contaminated surface water and soil*
  - *lake vegetation/mammals/aquatic organisms if suspected to be in contact with potentially contaminated surface water and leachate.*

1.4 DATA QUALITY OBJECTIVES

Data quality objectives (DQOs) state the level of uncertainty that is acceptable from data collection activities. DQOs also define the data quality necessary to make a certain decision. Consider the following when establishing DQOs for a particular project:

- Decision(s) to be made or question(s) to be answered;
- Why environmental data are needed and how the results will be used;
- Time and resource constraints on data collection;
- Descriptions of the environmental data to be collected;
- Applicable model or data interpretation method used to arrive at a conclusion;
- Detection limits for analytes of concern; and
- Sampling and analytical error.

In addition to these considerations, the quality assurance components of precision, accuracy (bias), completeness, representativeness, and comparability should also be considered. Quality assurance components are defined as follows:

- Precision -- measurement of variability in the data collection process.
- Accuracy (bias) -- measurement of bias in the analytical process. The term "bias" throughout this document refers to the QA/QC accuracy component.
- Completeness -- percentage of sampling measurements which are judged to be valid.
- Representativeness -- degree to which sample data accurately and precisely represent the characteristics of the site contaminants and their concentrations.
- Comparability -- evaluation of the similarity of conditions (e.g., sample depth, sample

homogeneity) under which separate sets of data are produced.

Many of the DQOs and quality assurance considerations for soil, sediment, and water sampling are also applicable to biological sampling. However, there are also additional considerations that are specific to biological sampling.

- Is biological data needed to answer the question(s) and, if so, how will the data be used;
- Seasonal, logistical, resource, and legal constraints on biological specimen collection;
- What component of the biological system will be collected or evaluated (i.e., tissue samples, whole organisms, population data, community data, habitat data);
- The specific model or interpretation scheme to be utilized on the data set;
- The temporal, spatial, and behavioral variability inherent in natural systems.

Quality assurance/quality control (QA/QC) objectives are discussed further in Chapter 4.

1.5 TECHNICAL ASSISTANCE

In this document, it is assumed that technical specialists are available to assist Site Managers and other site personnel in determining the best approach to ecological assessment. This assistance ensures that all approaches are up-to-date and that best professional judgment is exercised. Refer to Appendix A for more information.

Support in designing and evaluating ecological assessments is currently available from regional technical assistance groups such as Biological Technical Assistance Groups (BTAGs). Support is also available from the Environmental Response Team Center (ERTC) as well as from other sources within each region.

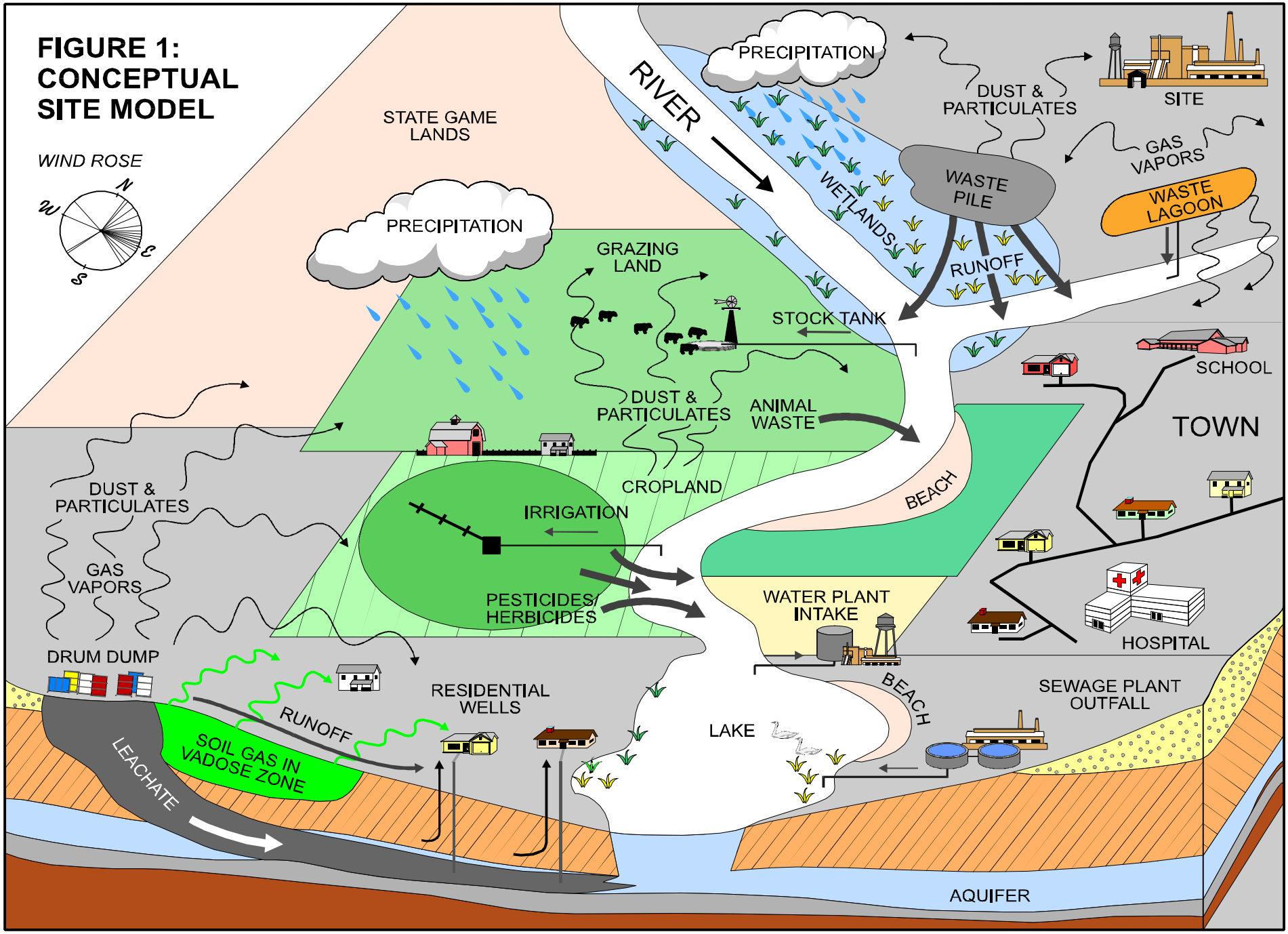


FIGURE 1:
CONCEPTUAL
SITE MODEL
WIND ROSE
N
W
E
S
STATE GAME
LANDS
RIVER
PRECIPITATION
DUST &
PARTICULATES
SITE
GAS
VAPORS
WASTE
PILE
WASTE
LAGOON
WETLANDS
RUNOFF
PRECIPITATION
GRAZING
LAND
STOCK TANK
SCHOOL
TOWN
DUST &
PARTICULATES
ANIMAL
WASTE
CROPLAND
BEACH
DUST &
PARTICULATES
IRRIGATION
GAS
VAPORS
PESTICIDES/
HERBICIDES
WATER PLANT
INTAKE
HOSPITAL
DRUM DUMP
RESIDENTIAL
WELLS
RUNOFF
BEACH
SEWAGE PLANT
OUTFALL
SOIL GAS IN
VADOSE ZONE
LEACHATE
LAKE
AQUIFER

2.0 BIOLOGICAL/ECOLOGICAL ASSESSMENT APPROACHES

2.1 INTRODUCTION

Biological assessments vary in their level of effort, components, and complexity, depending upon the objectives of the study and specific site conditions. An assessment may consist of literature-based risk evaluations and/or site-specific studies (e.g., population/community studies, toxicity tests/bioassays, and tissue residue analyses).

Superfund Program personnel (RPMs and OSCs) may be limited to completing the ecological checklist (Appendix A) during the Preliminary Site Evaluations and to consulting an ecological specialist if it is determined that additional field data are required. The checklist is designed to be completed by one person during an initial site visit. The checklist provides baseline data, is useful in designing sampling objectives, and requires a few hours to complete in the field.

When the Site Manager determines that additional data collection is needed at a response site, the personnel and other resources required depends on the selected approach and the site complexity.

To determine which biological assessment approach or combination of approaches is appropriate for a given site or situation, several factors must be considered. These include what management decisions will ultimately need to be made based on the data; what are the study objectives; and what should be the appropriate level of effort to obtain knowledge of contaminant fate/ transport and ecotoxicity.

2.2 RISK EVALUATION

Three common approaches to evaluating environmental risk to ecological receptors are (1) the use of literature screening values (e.g., literature toxicity values) for comparison to site-specific contaminant levels, (2) a "desk-top" risk assessment which can model existing site-specific contaminant data to ecological receptors for subsequent comparison to literature toxicity values, and (3) field investigation/laboratory analysis that involves a site investigation (which may utilize existing contaminant data for support) and laboratory analysis of contaminant levels in media and/or experimentation using bioassay procedures. These three approaches are described in further detail next.

2.2.1 Literature Screening Values

To determine the environmental effects of contaminants at a hazardous waste site, the levels of contaminants found may be compared to literature toxicity screening values or established screening criteria. These values should be derived from studies that involve testing of the same matrix and a similar organism of concern. Most simply stated, if the contaminant levels on the site are above the established criteria, further evaluation of the site may be necessary to determine the presence of risk. Site contaminant levels that are lower than established criteria may indicate that no further evaluation is necessary at the site for that contaminant.

2.2.2 Risk Calculations

The "desk-top" risk calculation approach compares site contaminants to information from studies found in technical literature. This type of evaluation can serve as a screening assessment or as a tier in a more complex evaluation. Since many assumptions must be made due to limited site-specific information, risk calculations are necessarily conservative. The collection and inclusion of site-specific field data can reduce the number and/or the magnitude of these "conservative" assumptions, thereby generating a more realistic calculation of potential risk. (See Chapter 5.0 for a complete discussion on risk calculations.)

2.2.3 Standard Field Studies

Two important aspects of conducting a field study that warrant discussion are the selection of a reference area and the selection of the receptors of concern. These are important to establish prior to conducting a field study.

2.2.3.1 Reference Area Selection

A reference area is defined in this document as an area that is outside the chemical influence of the site but possesses similar characteristics (e.g., habitat, substrate type) that allows for the comparison of data between the impacted area (i.e., the site) and the unimpacted area (i.e., the reference area). Reference areas can provide information regarding naturally occurring compounds and the existence of any regional contamination independent of the site. They can help determine if contaminants are ubiquitous in the area and can separate site-related issues from non-site related issues.

The reference area must be of similar habitat type and support a species composition similar to the study area. The collection and analysis of samples from a reference area can support site-specific decisions regarding uptake, body burden, and accumulation of chemicals and toxicity.

The reference area should be outside the area of influence of the site and if possible, in an area of minimal contamination or disturbance. Location of reference areas in urban or industrial areas is frequently difficult, but an acceptable reference area is usually critical to the successful use of ecological assessment methods.

2.2.3.2 Receptor Selection

The selection of a receptor is dependent upon the objectives of the study and the contaminants present. The first step is to determine the toxicity characteristics of the contaminants (i.e., acute, chronic, bioaccumulative, or non-persistent). The next step is to determine the exposure route of the chemical (i.e., dermal, ingestion, inhalation).

Selection of the receptor or group of receptors is a component of establishing the measurement endpoint in the study design. When discussing the term measurement endpoint, it is useful to first define a related concept, the assessment endpoint. An assessment endpoint is defined as "an explicit expression of the environmental value that is to be protected." For example, "maintaining aquatic community composition and structure downstream of a site similar to that upstream of the site" is an explicit assessment endpoint. Inherent in this assessment endpoint is the process of receptor selection that would most appropriately answer the question that the endpoint raises. Related to this assessment endpoint is the measurement endpoint which is defined as "a measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint." For example, measurements of biological effects such as mortality, reproduction, or growth of an invertebrate community are measurement endpoints. Establishing these endpoints will ensure (1) that the proper receptor will be selected to best answer the questions raised by the assessment and measurement endpoints, and (2) that the focus of the study remains on the component of the environment that may be used as the basis for decision.

There are a number of factors that must be considered when selecting a target species. The behavioral habits and lifestyle of the species must be consistent with the environmental fate and transport of the contaminants of interest as well as pathways of exposure to receptor species. For example, if the contaminants of concern at the site are PCBs that are bioaccumulative, a mammal such as a mink could be selected for the study since this species is documented to be sensitive to the bioaccumulation of PCBs. The mink in this case has been selected to be used for establishing the measurement endpoint that is representative of piscivorous mammals. However, it may not be feasible to collect mink for study due to their low availability in a given area. Therefore, the food items of the mink (e.g., small mammals, aquatic vertebrates and invertebrates) may be collected and analyzed for PCBs as an alternative means of evaluating the risk to mink. The resulting residue data may be utilized to produce a dose model. From this model, a reference dose value may be determined from which the probable effects to mink calculated.

The movement patterns of a measurement endpoint are also important during the receptor selection process. Species that are migratory or that have large feeding ranges are more difficult to link to site exposure than those which are sessile, territorial, or have limited movement patterns.
Ecological field studies offer direct or corroborative evidence of a link between contamination and ecological effects. Such evidence includes:

- Reduction in population sizes of species that can not be otherwise explained by naturally occurring population cycles
- Absence of species normally occurring in the habitat and geographical distribution
- Dominance of species associated primarily with stressed habitat
- Changes in community diversity or trophic structure relative to a reference location
- High incidence of lesions, tumors, or other pathologies
- Development of exposure response relationships.

Ecologists usually compare data of observed adverse effects to information obtained from a reference area not affected by site contamination. To accomplish this, chemical and biological data should be collected simultaneously and then compared to determine if a correlation exists between contaminant concentrations and ecological effects (U.S. EPA 1991b). The simultaneous collection of the data is important in reducing the effect of temporal variability as a factor in the correlation analysis.

The type of field study selected is directed by the contaminants present linked to the assessment endpoint. Prior to choosing a specific study approach, the site contaminant must be determined using information about

known or suspected site contaminants and how the nature of these contaminants may be modified by several environmental and ecotoxicological factors. In addition, evaluation of chemical fate and transport information is necessary to determine the appropriate matrix and technique.

Contaminants can be a food chain threat, a lethal threat, a direct non-lethal toxicant, indirect toxicant, or some combination of the four. Chemical residue studies are appropriate if the contaminant of concern (COC) will bioaccumulate. Ecotoxicological information can provide insight about contaminants that are expected to accumulate in organisms. It can also provide information about which organisms provide the best data for the study objectives. For example, the species-specific bioaccumulation rate must be considered along with analytical detection limits; the bioaccumulated levels need to be above the analytical detection limits. In contrast, population/ community studies or toxicity testing may be more appropriate if the contaminants cause direct lethality.

2.2.3.3 Exposure - Response Relationships

The relationship between the exposure (or dose) of a contaminant and the response that it elicits is a fundamental concept in toxicology (Timbrell 1989). The simplest response to observe is death. Some examples of other responses that vary in terms of ease of measurement include pathological lesions, cell necrosis, biochemical changes, and behavioral changes. It is this foundation of exposure-response relationships upon which the concept of chemical residue studies, population/community studies, and toxicity testing/bioassays are built upon.

2.2.3.4 Chemical Residue Studies

Residue studies are appropriate to use when there is concern about the accumulation of contaminants in the tissues of indigenous species. Residue studies are conducted by collecting organisms of one or more species and comparing the contaminant bioaccumulation data to those organisms collected from a reference area.

Chemical residue studies require field collection of biota and subsequent tissue analysis. A representative organism for collection and analysis is selected based on the study objectives and the site habitat. Generally the organism should be abundant, sessile (or with limited home range), and easy to capture. These attributes help to provide a sufficient number of samples for analysis thereby strengthening the linkage to the site. A number of organism- and contaminant-specific factors should also be considered when designing residue studies (see Philips [1977] and [1978] for additional information). The subsequent chemical analysis may be conducted on specific target tissues or the whole body. In most cases, whole-body analysis is the method of choice to support biological assessments. This is because most prey species are eaten in entirety by the predator.

In designing residue analysis studies, it is important to evaluate the exposure pathway carefully. If the organisms analyzed are not within the site-specific exposure pathway, the information generated will not relate to the environmental threat. Evaluation of the exposure pathway may suggest that a species other than the one of direct concern might provide a better evaluation of potential threat or bioaccumulation.

Because there are different data needs for each objective, the study objective needs to be determined prior to the collection of organisms. In these studies the actual accumulation (dependent upon the bioavailability) of the contaminants is evaluated rather than assumed from literature values. The information collected then allows for site-specific evaluation of the threat and reduces the uncertainty associated with the use of literature bioavailability values. These factors may be applied for specific areas of uncertainty inherent from the extrapolation of available data (e.g., assumptions of 100 percent bioaccumulation, variations in sensitive populations).

As stated previously, because site conditions as well as the bioavailability can change over time, it is important that exposure medium (soil, sediment, or water) samples and biological samples are collected simultaneously and analyzed for the same parameters to allow for the comparison of environmental contaminant levels in the tissue and the exposure medium. This is critical in establishing a site-specific linkage that must be determined on a case-by-case basis.

2.2.3.5 Population/Community Response Studies

The fundamental approach to population or community response studies is to systematically sample an area, documenting the organisms of the population or community. Individuals are typically identified and enumerated, and calculations are made with respect to the number, and species present. These calculated values (e.g., indices or metrics) are used to compare sampling locations and reference conditions. Some population and

community metrics include the number of individuals, species composition, density, diversity, and community structure.

2.2.3.6 Toxicity Testing/Bioassays

A third common assessment approach is to utilize toxicity tests or bioassays. A toxicity test may be designed to measure the effects from acute (short-term) or chronic (long-term) exposure to a contaminant. An acute test attempts to expose the organism to a stimulus that is severe enough to produce a response rapidly. The duration of an acute toxicity test is short relative to the organism's life cycle and mortality is the most common response measured. In contrast, a chronic test attempts to induce a biological response of relatively slow progress through continuous, long-term exposure to a contaminant.

In designing a toxicity test, it is critical to understand the fate, transport, and mechanisms of toxicity of the contaminants to select the test type and conditions. The toxicity test must be selected to match the site and its conditions rather than modify the site matrix for the use of a particular test. Factors to consider are the test species, physical/chemical factors of the contaminated media, acclimation of test organisms, necessity for laboratory versus field testing, test duration, and selection of test endpoints (e.g., mortality or growth). A thorough understanding of the interaction of these and other factors is necessary to determine if a toxicity test meets the study objectives.

The selection of the best toxicity test, including the choice of test organism, depends on several factors:

- The decisions that will be based on the results of the study
- The ecological setting of the site
- The contaminant(s) of concern

Toxicity testing can be conducted on a variety of sample matrices, including water (or an aqueous effluent), sediment, and soil. Soil and sediment toxicity tests can be conducted on the parent material (solid-phase tests) or on the elutriate (a water extract of the soil or sediment). Solid-phase sediment and soil tests are currently the preferred tests since they evaluate the toxicity of the matrix of interest to the test organisms, thereby providing more of a realistic site-specific exposure scenario.

As stated previously, one of the most frequently used endpoints in acute toxicity testing is mortality (also referred to as lethality) because it is one of the most easily measured parameters.

In contrast, some contaminants do not cause mortality in test organisms but rather they affect the rate or success of reproduction or growth in test organisms. In this case, the environmental effect of a contaminant may be that it causes reproductive failure but does not cause mortality in the existing population. In either case, the population will either be eliminated or drastically reduced.

The use of control as well as reference groups is normally required. Laboratory toxicity tests include a control that evaluates the laboratory conditions, and the health and response of the test organisms. Laboratory controls are required for all valid toxicity tests. A reference provides information on how the test organisms respond to the exposure medium without the site contaminants. Therefore, the reference is necessary for interpretation of the test results in the context of the site (i.e., sample data is compared to the reference data). It is not uncommon for conditions other than contamination to induce a response in a toxicity test. With proper reference and control tests, toxicity tests can be used to establish a link between contaminants results and adverse effects.

Within the Superfund Program, conducting toxicity tests typically involves collecting field samples (water, sediment, soil) and transferring the materials to a laboratory. *In situ* (field conducted) tests can be run if field conditions permit. There are benefits and limitations associated with each approach. The most notable benefit of laboratory testing is that exposure conditions are controlled, but this leads to its most notable limitation, a reduction of realism. With *in situ* tests, the reality of the exposure situation is increased, but there is a reduction of test controls. See U.S. EPA's *Compendium of ERT Toxicity Testing Procedures*, OSWER Directive 9360.4-08, EPA/540/P-91/009 (U.S. EPA 1991a), for descriptions of nine common toxicity tests and *Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates*, ASTM Standard E1383, October 1990.

Species Selection for Toxicity Testing

Selection of the test organism is critical in designing a study using toxicity testing. The species selected should be representative relative to the assessment endpoint, typically an organism found within the exposure pathway expected in the field. To be useful in evaluating risk, the test organism must respond to the contaminant(s) of concern. This can be difficult to achieve since the species and tests available are limited. Difficult choices and balancing of factors are frequently necessary.

3.0 BIOLOGICAL SAMPLING METHODS

Once a decision has been made that additional data are required to assess the biological threat posed by a site, an appropriate sampling plan must be developed. The selection of ecological sampling methods and equipment is dependent upon the field assessment approach, as discussed in Chapters 1 and 2. Thus, the selection of an assessment approach is the initial step in the collection process. This chapter does not present step-by-step instructions for a particular method, nor does it present an exhaustive list of methods or equipment. Rather, it presents specific examples of the most commonly used methods and associated equipment. Table 4.1 (at the end of this chapter) lists some of the standard operating procedures (SOPs) used by the U.S. EPA's Environmental Response Team Center (ERTC).

Because of the complex process required for selecting the proper assessment approach for a particular site, consultation with an ecologist/biologist experienced in conducting ecological risk assessments is strongly recommended.

3.1 CHEMICAL RESIDUE STUDIES

Chemical residue studies are a commonly used approach that can address the bioavailability of contaminants in media (e.g., soil, sediment, water). They are often called tissue residue studies because they measure the contaminant body burden in site organisms.

When collecting organisms for tissue analyses, it is critical that the measured levels of contaminants in the organism are attributable to a particular location and contaminant level within the site. Collection techniques must be evaluated for their potential to bias the generated data. Collection methods can result in some form of biased data either by the size, sex, or individual health of the organism. Collection techniques are chosen based on the habitat present and the species of interest. When representative approaches are not practical, the potential bias must be identified and considered when drawing conclusions from the data. The use of a particular collection technique should not be confused with the need to target a "class" of individuals within a population for collection. For example, in a specific study it may be desirable to collect only males of the species or to collect fish of consumable size.

Some receptors of concern (ROCs) cannot be collected and analyzed directly because of low numbers of individuals in the study area, or other technical or logistical reasons. Exposure levels for these receptors can be estimated by collecting organisms that are preyed upon by the ROC. For example, if the ROC is a predatory bird, the species collected for contaminant level measurements may be one of several small mammals or fish that the ROC is known to eat.

As noted previously, it is critical to link the accumulated contaminants both to the site and to an exposure medium. Subsequently, the collection and analysis of representative soil, sediment, or water samples from the same location are critical. A realistic site-specific Bioaccumulation Factor (BAF) or Bioconcentration Factor (BCF) may then be calculated for use in the site exposure models.

"Bioconcentration is usually considered to be that process by which toxic substances enter aquatic organisms, by gill or epithelial tissue from the water. Bioaccumulation is a broader term in the sense that it usually includes not only bioconcentration but also any uptake of toxic substances through the consumption of one organism." (Brungs and Mount 1978).

3.1.1 Collection Methods

It should be noted that any applicable state permits should be acquired before any biological sampling event. States requirements on organism, method, sampling location, and data usage differ widely and may change from year to year.

The techniques used to collect different organisms are specific to the study objectives. All techniques are selective to some extent for certain species, sizes, habitat, or sexes of animals. Therefore, the potential biases associated with each technique should be determined prior to the study. If the biases are recognized prior to collection, the sampling may be designed to minimize effect of the bias. For example, large traps are not effective for trapping small animals since small mammals are not heavy enough to trigger the trap or may escape through minute trap openings.

In determining environmental threat, the target species generally consist of prey species such as earthworms, small mammals, or fish. Residue data from these organisms can be used to evaluate the risk to higher trophic level organisms, which may be difficult to capture or analyze.

3.1.1.1 Comparability Considerations

There are two issues that directly affect field collection. First, organisms such as benthic macroinvertebrates tend to have a patchy or non-uniform distribution in the environment due to micro habitats and other factors. Therefore, professional evaluation in matching habitat for sampling is critical in the collection of a truly representative sample of the community. Second, variability in sampling effort and effectiveness needs to be considered.

3.1.1.2 Mammals

Trapping is the most common method for the collection of mammals. The selection of traps is determined by the species targeted and the habitat present. Both live trap or kill trap methods may be acceptable for residue studies, but consideration of other data uses (e.g., histopathology) or concern for injury or death of non-target species can influence the use of certain trap types.
Several trap methods are available for collecting small mammals. Commonly used traps include Museum Special, Havahart, Longworth, and Sherman traps (Figure 3). Although somewhat labor-intensive, pitfall trap arrays may also be established to include mammals that are not regularly trapped using other techniques (e.g., shrews).

Trap placement is a key element when collecting samples. Various methods of trap placement can be utilized. These include, but are not limited to:

- Sign method/Best set method
- Paceline method
- Grid method

When using the sign/best set method, an experienced field technical specialist searches for fresh mammal signs (e.g., tacks, scat, feeding debris) to determine where the trap should be positioned. This method typically produces higher trapping success than other methods, however, this method is biased and is therefore generally used to determine what species are present at the site.

The paceline method involves placement of traps at regular intervals along a transect. A starting point is selected and marked, a landmark is identified to indicate the direction of the transect, and as the field member walks the transect, the traps are placed at regular intervals along it.

The grid method is similar to the paceline method but involves a group of evenly spaced parallel transects of equal lengths to create a grid. Traps are placed at each grid node. The size of the grid is dependent on the species to be captured and the type of study. Grids of between 500 to 1,000 square meters containing approximately 100 traps are common. If a grid is established in a forest interior, additional parallel trapping lines may be established to cover the edge habitat.

Regardless of the type of trapping used, habitat disturbance should be kept to a minimum to achieve maximum trapping success. In most areas, a trapping success of 10 percent is considered maximum but is oftentimes significantly lower (e.g., 2 to 5 percent). Part of this reduced trapping success is due to habitat disturbance. Therefore, abiotic media samples (e.g., soil, sediment, water) should be collected well in advance of trapping efforts or after all trapping is completed. Trapping success also varies with time but may increase over time with diminishing returns. In other words, extending the trapping period over several days may produce higher trapping success by allowing mammals that were once peripheral to the trapping area to immigrate into the now mammal-depauperate area. These immigrants would not be representative of the trapping area. Therefore, a trapping period of 3 days is typically used to minimize this situation.

Trapping success will also vary widely based on the available habitat, targeted species, season, and geographical location of the site. When determining trap success objectives, it is important to keep in mind the minimum sample mass/volume requirements for chemical residue studies.

3.1.1.3 Fish

Electrofishing, gill nets, trawl nets, seine nets, and minnow traps are common methods used for the collection of fish. The selection of which technique to use is dependent on the species targeted for collection and the system being sampled. In addition, there are other available fish netting and trapping techniques that may be more appropriate in specific areas. As with mammal trapping, disturbance in the area being sampled should be kept to a minimum to ensure collection success.

Electrofishing uses electrical currents to gather, slow down, or immobilize fish for capture. An electrical field is created between and around two submerged electrodes that stuns the fish or alters their swimming within or

around the field. Depending on the electrical voltage, the electrical pulse frequency, and the fish species, the fish may swim towards one of the electrodes, swim slowly enough to capture, or may be stunned to the point of immobilization. This technique is most effective on fish with swimbladders and/or shallow water since these fish will float to the surface for easy capture.

Electrofishing can be done using a backpack-mounted electroshocker unit, a shore-based unit, or from a boat using either type. Electrofishing does not work in saline waters and can be ineffective in very soft water. Electrofishing is less effective in deep water where the fish can avoid the current. In turbid waters, it may be difficult to see the stunned fish.

Gill netting is a highly effective passive collection technique for a wide range of habitats. Because of its low visibility under water, a gill net captures fish by entangling their gill plates as they attempt to swim through the area in which the gill net has been placed in. Unfortunately, this may result in fish to be injured or killed due to further entanglement, predation, or fatigue.

The size and shape of fish captured is relative to the size and kind of mesh used in the net thus creating bias towards a certain sized fish. These nets are typically used in shallow waters, but may extend to depths exceeding 50 meters. The sampling area should be free of obstructions and floating debris, and provide little to no current. (Hurbert 1983)

Otter trawl netting is an active collection technique that utilizes the motion of a powered boat to drag a pocket-shaped net through a body of water. The net is secured to the rear of a boat and pulled to gather any organisms that are within the opening of the pocket. This pocket is kept open through the use of underwater plates on either side of the net that act as keels, spreading the mouth of the net open.

Seining is another active netting technique that traps fish by encircling them with a long wall of netting. The top of the net is buoyed by floats and the bottom of the net is weighed down by lead weights or chains. Seine nets are effective in open or shallow waters with unobstructed bottoms. Beach or haul seines are used in shallow water situations where the net extends to the bottom. Purse seines are designed for applications in open water and do not touch the bottom (Hayes 1983).

The use of minnow traps is a passive collection technique for minnow-sized fish. The trap itself is a metal or plastic cage that is secured to a stationary point and baited to attract fish. Small funnel-shaped openings on either end of the trap allow fish to swim easily into it, but are difficult to locate for exit. Cage “extenders” or “spacers” that are inserted to lengthen the cage, allow larger organisms such as eels, or for a larger mass of fish to be collected.

3.1.1.4 Vegetation

Under certain conditions, the analysis of the chemical residue in plants may be a highly effective method of assessing the impacts of a site. The bioaccumulative potential of plants varies greatly however, among contaminants, contaminant species, soil/sediment texture and chemistry, plant condition, and genetic composition of the plant. In addition to this variability, plants can translocate specific contaminants to different parts of the plant. For example, one contaminant may tend to accumulate in the roots of a plant, whereas a second contaminant may tend to accumulate in the fruit of the same plant. In this scenario, the collection and analysis of a plant part that normally does not receive translocated materials would not result in a useful sample. Therefore, it is crucial to conduct a literature review prior to establishing a sampling protocol.

Sampling of herbaceous plants should be conducted during the growing season of the species of interest. Sampling of woody plants may be conducted during the growing or dormant season, however, most plants translocate materials from the aboveground portions of the plant to the roots prior to dormancy.

Collection methods and sampling specifics may be found in U.S. EPA/ERT SOP #2037, *Terrestrial Plant Community Sampling*; others are provided in Table 4.1.

3.1.2 Sample Handling and Preparation

The animals or plants collected should be identified to species level or the lowest practical taxonomic level. Appropriate metrics (e.g., weight, animal body length, plant height) and the presence of any external anomalies, parasites, and external pathologies should be recorded. If compositing of the sample material is necessary, it should be performed in accordance with the study design.

Depending upon the study objectives, it may be necessary to isolate the contaminant levels in animal tissue from the

contaminant levels in the food or abiotic matrices (e.g., sediment) entrained in the digestive tract of the organism. This is an important process in that it separates the contribution of two distinct sources of contaminants to the next trophic level, thereby allowing the data user to recognize the relative importance of the two sources.

Clearing of the digestive tract (i.e., depuration) of the organism must then be accomplished prior to the chemical analysis. The specific depuration procedures will vary with each type of organism but all involve allowing the organism to excrete waste products in a manner in which the products may not be reingested, absorbed, or deposited back onto the organism.

Biological samples should be handled with caution to avoid personal injury, exposure to disease, parasites, or sample contamination. Personal protection such as gloves should be worn when handling animals and traps to reduce the transfer of scents or oils from the hand to the trap, which could cause an avoidance reaction in the targeted animals.

Samples collected for biological evaluation must be treated in the same manner as abiotic samples (i.e., the same health and safety guidelines, decontamination protocols, and procedures for preventing cross-contamination must be adhered to). Biological samples do require some extra caution in handling to avoid personal injury and exposure to disease, parasites, and venoms/resins. The selection of sample containers and storage conditions (e.g., wet ice) should follow the same protocols as abiotic samples. Refer to Chapter 4.0 for determination of holding times and additional quality assurance/quality control (QA/QC) handling procedures.

3.1.3 Analytical Methods

Chemical analytical methods for tissue analysis are similar to those for abiotic matrices (e.g., soil and water), however, the required sample preparation procedures (e.g., homogenization and subsampling) of biological samples are frequently problematic. For example, large bones, abundant hair, or high cellulose fiber content may result in difficult homogenization of mammals and plants. Extra steps may be required during sample cleanup due to high lipid (fat) levels in animals tissue or high resin content in plant tissue.

Most tissue samples can be placed in a laboratory blender with dry ice and homogenized at high speeds. The sample material is then left to sit to allow for the sublimation of the dry ice. Aliquots of the homogenate may then be removed for the required analyses.

The requirement for split samples or other QA samples must be determined prior to sampling to ensure a sufficient volume of sample is collected. Chapter 4.0 discusses the selection and use of QA/QC samples.

The detection limits of the analytical parameters should be established prior to the collection of samples. Detection limits are selected based on the level of analytical resolution that is needed to interpret the data against the study objectives. For example, if the detection limit for a compound is 10 mg/kg but the concentration in tissue which causes effects is 1 mg/kg, the detection limit is not adequate to determine if a problem exists. It should be noted that standard laboratory detection limits for abiotic matrices are often not adequate for tissue samples. Chapter 4.0 provides details on detection limits and other QA/QC parameters.

The tissue analysis can consist of whole body residue analysis or analysis of specific tissues (i.e., fish fillets). Although less frequently used in Superfund, tissues such as organs (e.g., kidney or liver) may be analyzed. The study endpoints will determine whether whole body, fillet, or specific organ samples are to be analyzed.

Concurrent analyses should include a determination of percent lipids and percent moisture. Percent lipids may be used to normalize the concentration of non-polar organic contaminant data. In addition, the lipid content of the organisms analyzed can be used to evaluate the organism's health. Percent moisture determinations allow the expression of contaminant levels on the basis of wet or dry weight. Wet weight concentration data are frequently used in food chain accumulation models, and dry weight basis data are frequently reported between sample location comparisons.

Histopathological Analysis

Histopathological analysis can be an effective mechanism for establishing causative relationships due to contaminants since some contaminants can cause distinct pathological effects. For example, cadmium causes visible kidney damage providing causal links between contaminants and effects. These analyses may be performed on organisms collected for residue analysis. A partial necropsy performed on the animal tissue may indicate the presence of internal abnormalities or parasites. The time frame and objectives of the study determine if histopathological analysis is warranted.

3.2 POPULATION/COMMUNITY RESPONSE STUDIES

Population/community response studies are a commonly utilized field assessment approach. The decision to conduct a population/community response study is based on the type(s) of contaminants, the time available to conduct the study, the type of communities potentially present at the site, and the time of year of the study. These studies are most commonly conducted on non-time-critical or long-term remediation-type site activities. During limited time frame responses, however, a population/community survey or screening level study may be useful for providing information about potential impacts associated with a site.

3.2.1 Terrestrial Vertebrate Surveys

Methods for determining adverse effects on terrestrial vertebrate communities are as follows: censusing or population estimates, sex-age ratio determinations, natality/mortality estimations, and diversity studies.

True or accurate censuses are usually not feasible for most terrestrial vertebrate populations due to logistical difficulties. Estimations can be derived by counting a subset of organisms or counting and evaluating signs such as burrows, nests, tracks, feces, and carcasses. Capture-recapture studies may be used to estimate population size but are labor-intensive and usually require multiple-season sampling. If conducted improperly, methods for marking captured organisms may cause irritation or injury or interfere with the species' normal activities.

Age ratios provide information on natality and rearing success, age-specific reproductive rates, and mortality and survival rates. Sex ratios indicate whether sexes are present in sufficient numbers and proportions for normal reproductive activity.

Community composition (or diversity) can be assessed by species frequency, species per unit area, spatial distribution of individuals, and numerical abundance of species (Hair 1980).

3.2.2 Benthic Macroinvertebrate Surveys

Benthic macroinvertebrate (BMI) population/community evaluations in small- to medium- sized streams have been successfully used for approximately 100 years to document injury to the aquatic systems. There are many advantages to using BMI populations to determine the potential ecological impact associated with a site. Sampling is relatively easy, and equipment requirements are minimal. An evaluation of the community structure may be used to assess overall water quality, evaluate the integrity of watersheds, or suggest the presence of an influence of the community structure that is independent of water quality and habitat conditions.

Because BMIs are a primary food source for many fish and other organisms, threats beyond the benthic community can be inferred from the evaluation of BMIs. Techniques such as rapid bioassessment protocols may be used as a tool to support this type of finding and inference. A more comprehensive discussion of general benthological surveys may be found in U.S. EPA (1990).

3.2.2.1 Rapid Bioassessment Protocols for Benthic Communities

Rapid bioassessment protocols are an inexpensive screening tool used for determining if a stream is supporting or not supporting a designated aquatic life use. The rapid bioassessment protocols advocate an integrated assessment, comparing habitat and biological measures with empirically defined reference conditions (U.S. EPA 1989a).

The three major components of a rapid bioassessment essential for determining ecological impact are:

- Biological survey
- Habitat assessment
- Physical and chemical measurements

As with all population/community evaluations, the habitat assessment is of particular concern with respect to representative sampling. Care must be taken to prevent bias during collection of the benthic community resulting from sampling dissimilar habitats. Similar habitats must be sampled to make valid comparisons between locations. In addition to habitat similarity, the sampling technique and level of effort at each location must be uniform to achieve an accurate interpretation of results.

In the U.S. EPA Rapid Bioassessment Protocol (RBP), various components of the community and habitat are evaluated, a numerical score is calculated, and the score is compared to predetermined values. A review of the scores, together with habitat assessment and the physical and chemical data, support a determination of impact. U.S. EPA Reference (May, 1989a) presents the calculation and interpretation of scores.

Standard protocols, including the RBP, have been developed to facilitate surveying BMIs to determine impact rapidly. These protocols use a standard approach to reduce the amount of time spent collecting and analyzing samples. Protocols range from a quick survey of the benthos (Protocol I) to a detailed laboratory classification analysis (Protocol III). Protocol I may be conducted in several hours; Protocol II is more intensive and focuses on major taxonomic levels; and Protocol III may require numerous hours to process each sample to a greater level of taxonomic and community assessment resolution. These protocols are used to determine community health and biological condition via tolerance values and matrices. They also create and amend a historical data base that can be used for future site evaluation.

3.2.2.2 General Benthological Surveys

Benthological surveys can be conducted with methods other than those discussed in the RBP protocols utilizing techniques discussed in the literature. The overall concept is generally the same as that used in the RBP, but the specific sampling technique changes depending on the habitat or community sampled.

3.2.2.3 Reference Stations

The use of a reference station is essential to determine population/community effects attributable to a site. The use of a reference station within the study area is preferable (upstream or at a nearby location otherwise outside the area of site influence). In some cases this is not possible due to regional impacts, area-wide habitat degradation, or lack of a similar habitat. In these cases the use of population/community studies should be re-evaluated within the context of the site investigation. If the choice is made to include the population/community study, regional reference or a literature-based evaluation of the community may be options.

3.2.2.4 Equipment for Benthic Surveys

The selection of the most appropriate sampling equipment for a particular site is based primarily on the habitat being sampled. This subsection is a brief overview of the equipment available for the collection of BMIs. Detailed procedures are not discussed in this document. For additional information, refer to the SOPs and methods manuals provided in Table 4.1, or consult an ecologist/biologist experienced in this type of field collection.

Long-handled nets or a Surber sampler with a 0.5-millimeter (mm) size mesh are common sampling nets for the collection of macroinvertebrates from a riffle area of a stream. Samples to be collected from deep water gravel, sand, or soft bottom habitats such as ponds, lakes, or rivers are more often sampled using a small Ponar or Ekman dredge. Artificial substrates are used in varying habitats when habitat matching is problematic and/or native substrate sampling would not be effective. The most common types of artificial substrate samplers are multiple-plate samplers or barbecue basket samplers.

The organisms to be taken to the laboratory for identification or retained for archival purposes may be placed in wide-mouthed plastic or glass jars (for ease in removing contents) and preserved in 70 percent 2-propanol (isopropyl alcohol) or ethyl alcohol (ethanol), 30 percent formalin, or Kahle's solution. Refer to methods manuals for detailed information on sample handling and preservation.

3.2.3 Fish Biosurveys

3.2.3.1 Rapid Bioassessment Protocols for Fish Biosurveys

RBPs IV and V are two levels of fish biosurvey analyses. Protocol IV consists of a questionnaire to be completed with the aid of local and state fisheries experts. Protocol V is a rigorous analysis of the fish community through careful species collection, identification, and enumeration. This level is comparable to the macroinvertebrate Protocol III (see Section 3.2.2.1) in effort. Detailed information on both protocols can be found in *Rapid Bioassessments Protocols for Use In Streams and Rivers* (U.S. EPA 1989a).

3.3 TOXICITY TESTS

Toxicity tests evaluate the relative threat of exposure to contaminated media (e.g., soil, sediment, water) in a controlled setting. These tests are most often conducted in the laboratory, although they may be conducted in the field as well. These tests provide an estimate of the relationship between the contaminated medium, the level of contaminant, and the severity of adverse effects under specific test parameters. Toxicity tests are categorized by several parameters which include duration of the test, test species, life stage of the organism, test end points, and

other variables.

The collection of the actual samples on which the tests are to be conducted follow the same protocols as collection of representative samples for chemical analyses. Typically, a subsample of the media collected for toxicity testing is submitted for chemical analyses. The use of a concentration gradient for toxicity testing is frequently desired to establish a concentration gradient within the test. This also eliminates the need to sample all the locations at a site. The specific methods to be followed for toxicity tests are described in detail in U.S. EPA's *Compendium of ERT Toxicity Testing Procedures*, OSWER Directive 9360.4-08, EPA/540/P-91-009 (U.S. EPA 1991a), as well as existing SOPs listed in Table 4.1. These published procedures address sample preservation, handling and storage, equipment and apparatus, reagents, test procedures, calculations, QA/QC, and data validation. The practical uses of various toxicity tests, including examples of acute and chronic tests, are described next. Each section includes an example toxicity test.

3.3.1 Examples Of Acute Toxicity Tests

Example No. 1 (solid-phase soil)

Laboratory-raised earthworms are placed 30 per replicate into test chambers containing site soil. A laboratory control and a site reference treatment are established to provide a means for comparison of the resulting data set. Depending on the anticipated contaminant concentrations in the site soil, the soil may be used in its entirety or diluted with control or site reference soil. The test chambers are examined daily for an exposure period of 14 days and the number dead organisms is tabulated. When the observed mortality in the site soil treatments is statistically compared to control and site reference treatments, inferences regarding the toxicity of the contaminant concentrations in the site soil treatments may be drawn.

Example No. 2 (surface water)

Fathead minnows (*Pimephales promelas*) are exposed for 96 hours in aerated test vessels containing surface water from sampling locations representing a concentration gradient. The mortality of the organisms is recorded at the end of the exposure period and statistically compared to control and site reference treatments. Statistically significant differences between treatments may be attributed to the varying contaminant concentrations.

3.3.2 Examples of Chronic Toxicity Tests

Example No. 1 (surface water)

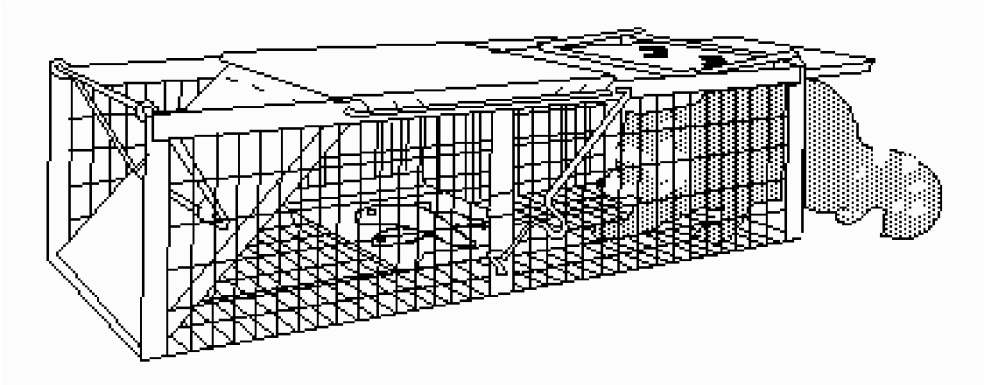
Fathead minnow larvae (*Pimephales promelas*) are exposed for 7 days to surface water collected from sampling locations that represent a concentration gradient. Each replicate consists of 20 individuals of the same maturity level. The test vessels are aerated and the water is replaced daily. The fish, which should have remained alive throughout the exposure period, are harvested and measured for body length and body weight. These results represent growth rates and are statistically compared to the control and site reference treatments to infer the toxicological effects of the contaminant concentrations.

Example No. 2 (sediment)

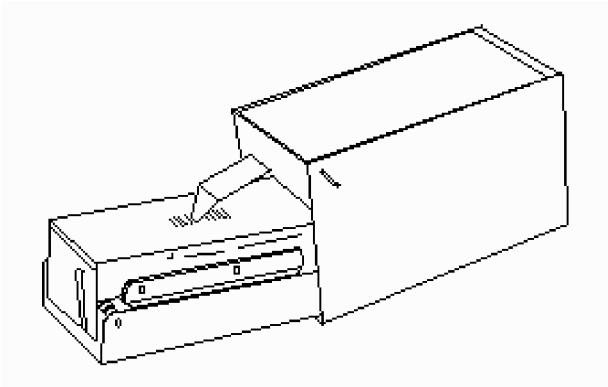
Midge (*Chironomus* sp.) larvae are exposed for 10 days to sediment, overlain with site reference water, and collected from sampling locations that represent a concentration gradient. Each replicate consists of 200 individuals of the same maturity level (1st instar). The test vessels are aerated and the water is replaced daily. At the end of the exposure period, the larvae are removed from the test vessels and measured for body length and body weight.

The organisms are then returned to the test vessels and allowed to mature to the adult stage. An emergence trap is placed over the test vessel and the number of emerging adults is recorded. These results, as well as the length and weight results, are statistically compared to the control and site reference treatments to infer the toxicological effects of the contaminant concentrations.

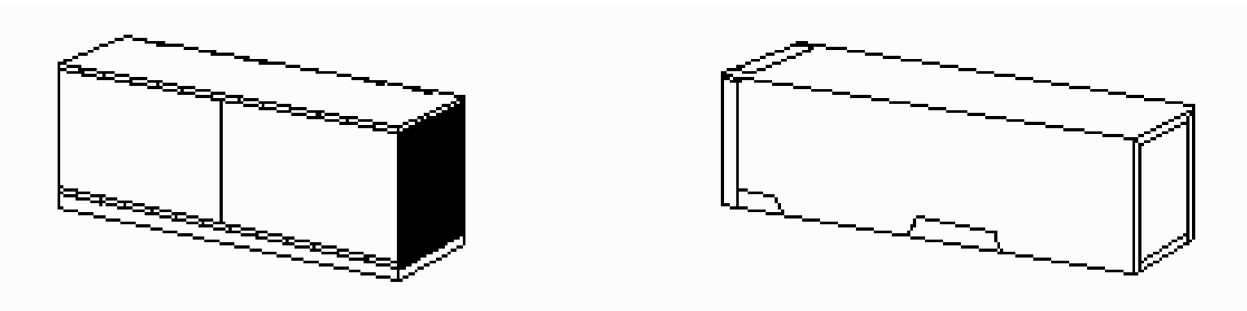
Figure 2: Common Mammal Traps



Havahart Trap



Longworth live trap



(A) (B)

Folding (A) and non-folding (B) Sherman live traps

TABLE 1

Reference List of Standard Operating Procedures -- Ecological Sampling Methods

SOP/Method No.	Source	Procedure/Method Title	Publication No.
SOP No. 1820	ERTC	Tissue Homogenization Procedure	(in development)
SOP No. 1821	ERTC	Semi-Volatiles Analysis of Tissue Samples by GC/MS	(in development)
SOP No. 1822	ERTC	Pesticides/PCB Analysis of Tissue Samples by GC/ECD	(in development)
SOP No. 1823	ERTC	Microwave Digestion and Metals Analysis of Tissue Samples	(in development)
SOP No. 2020	ERTC	7-Day Standard Reference Toxicity Test Using Larval Fathead Minnows *Pimephales promelas*	OSWER EPA/540/P-91/009
SOP No. 2021	ERTC	24-Hour Range Finding Test Using *Daphnia magna* or *Daphnia pulex*	OSWER EPA/540/P-91/009
SOP No. 2022	ERTC	96-Hour Acute Toxicity Test Using Larval *Pimephales promelas*	OSWER EPA/540/P-91/009
SOP No. 2023	ERTC	24-Hour Range Finding Test Using Larval *Pimephales promelas*	OSWER EPA/540/P-91/009
SOP No. 2024	ERTC	48-Hour Acute Toxicity Test Using *Daphnia magna* or *Daphnia pulex*	OSWER EPA/540/P-91/009
SOP No. 2025	ERTC	7-Day Renewal Toxicity Test Using *Ceriodaphnia dubia*	OSWER EPA/540/P-91/009
SOP No. 2026	ERTC	7-Day Static Toxicity Test Using Larval *Pimephales promelas*	OSWER EPA/540/P-91/009
SOP No. 2027	ERTC	96-Hour Static Toxicity Test Using *Selenastrum capricornutum*	OSWER EPA/540/P-91/009
SOP No. 2028	ERTC	10-Day Chronic Toxicity Test Using *Daphnia magna* or *Daphnia pulex*	OSWER EPA/540/P-91/009
SOP No. I-001	ERTC	15-Day Solid Phase Toxicity Test Using *Chironomus tentans*	(in development)
SOP No. I-002	ERTC	28-Day Solid Phase Toxicity Test Using *Hyalella azteca*	(in development)
Greene et al.(1989)	-	14-Day Acute Toxicity Test Using adult *Eisenia andrei* (earthworms)	EPA 600/3-88-029
SOP No. I-005	ERTC	Field Processing of Fish	(in development)
SOP No. 2029	ERTC	Small Mammal Sampling and Processing	(in development)
SOP No. 2032	ERTC	Benthic Sampling	(in development)
SOP No. 2033	ERTC	Plant Protein Determination	(in development)
SOP No. 2034	ERTC	Plant Biomass Determination	(in development)
SOP No. 2035	ERTC	Plant Peroxidase Activity Determination	(in development)
SOP No. 2036	ERTC	Tree Coring and Interpretation	(in development)
SOP No. 2037	ERTC	Terrestrial Plant Community Sampling	(in development)

4.0 QUALITY ASSURANCE/QUALITY CONTROL

4.1 INTRODUCTION

The goal of representative sampling is to yield quantitative data that accurately depict site conditions in a given period of time. QA/QC measures specified in the sampling procedures minimize and quantify the error introduced into the data.

Many QA/QC measures are dependant on QA/QC samples submitted with regular field samples. QA/QC samples evaluate the three following types of information: (1) the degree of site variation; (2) whether samples were cross-contaminated during sampling and sample handling procedures; and (3) whether a discrepancy in sample results is attributable to field handling, laboratory handling, or analysis. For additional information on QA objectives, refer to U.S. EPA *Quality Assurance/Quality Control (QA/QC) Guidance for Removal Activities*, EPA/540/G-90/004, April 1990.

4.2 DATA CATEGORIES

The U.S. EPA has established a process of data quality objectives (DQOs) which establish what type, quantity, and quality of environmental data are appropriate for their intended application. In its DQO process, U.S. EPA has defined two broad categories of data: screening and definitive.

Screening data are generated by rapid, less precise methods of analysis with less rigorous sample preparation. Sample preparation steps may be restricted to simple procedures such as dilution with a solvent, rather than an elaborate extraction/digestion and cleanup. At least 10 percent of the screening data are confirmed using the analytical methods and QA/QC procedures and criteria associated with definitive data. Screening data without associated confirmation data are not considered to be data of known quality. To be acceptable, screening data must include the following:

- chain of custody
- initial and continuing calibration
- analyte identification
- analyte quantification

Streamlined QC requirements are the defining characteristic of screening data.

Definitive data are generated using rigorous analytical methods (e.g., approved U.S. EPA reference methods). These data are analyte-specific, with confirmation of analyte identity and concentration. Methods produce tangible raw data (e.g., chromatograms, spectra, digital values) in the form of hard-copy printouts or computer-generated electronic files. Data may be generated at the site or at an off-site location as long as the QA/QC requirements are satisfied. For the data to be definitive, either analytical or total measurement error must be determined. QC measures for definitive data contain all the elements associated with screening data, but also include trip, method, and rinsate blanks; matrix spikes; performance evaluation samples; and replicate analyses for error determination.

For more details on these data categories, refer to *U.S. EPA Data Quality Objectives Process For Superfund*, EPA/540/R-93/071, Sept 1993.

4.3 SOURCES OF ERROR

The four most common potential sources of data error in biological sampling:

- Sampling design
- Sampling methodology
- Sample heterogeneity
- Sample analysis

4.3.1 Sampling Design

The initial selection of a habitat is a potential source of bias in biological sampling, which might either exaggerate or mask the effects of hazardous substances in the environment. In a representative sampling scheme, habitat characteristics such as plant and animal species composition, substrates, and degree of shading should be similar at all locations, including the reference location. The same individual should select both the test site and the control and background site to minimize error in comparing site conditions.

Standardized procedures for habitat assessment and selection also help minimize design error. The selection of an inappropriate species may introduce an error into the representative sampling design. This error can be minimized by selecting a species that is representative of the habitat and whose life-cycle is compatible with the timing of the study. In addition, migratory or transient species should be avoided.

4.3.2 Sampling Methodology

Sampling methodology and sample handling procedures may contain possible sources of error such as unclean sample containers, improper sample handling, and improper shipment procedures. Procedures for sample collection and handling should be standardized to allow easier identification of potential error. Follow SOPs or established procedures to ensure that all sampling techniques are performed consistently despite different sampling teams, dates, or locations. Use QA/QC samples (Section 4.4) to evaluate errors due to improper sampling methodology and sample handling procedures. These guidelines should apply to biological as well as soil, sediment, and water sampling.

During fishing operations, the sampling crew can prevent habitat disturbance by staying out of the water body near the sampling locations. The use of any particular technique may introduce judgment error into the sampling regimen if done improperly. For all techniques, sampling should be conducted from the downstream location to the upstream location to avoid contamination of the upstream stations. Data comparability is maintained by using similar collection methods and sampling efforts at all stations.

Rapid bioassessments in the field should include two QA/QC procedures: 1) collection of replicate samples at stations to check on the accuracy of the collection effort, and 2) repeat a portion (typically 10%) recount and reidentification for accuracy.

For tissue analyses, tools and other sampling equipment should be dedicated to each sample, or must be decontaminated between uses. To avoid contamination, sample containers must be compatible with the intended tissue matrix and analysis.

4.3.3 Sample Heterogeneity

Tissues destined for chemical analysis should be homogenized. Ideally, tissue sample homogenates should consist of organisms of the same species, sex, and development stage and size since these variables all affect chemical uptake. There is no universal SOP for tissue homogenization; specific procedures depend on the size and type of the organism. For example, tissues must be cut from fur and shell-bearing organisms as they cannot be practically homogenized as a whole. Homogenization procedures may vary by site objective. Tissue homogenates should be stored away from light and kept frozen at -20° C. Tissue homogenates are prepared in the laboratory and could be subject to cross-contamination.

Refer to U.S. EPA/ERT SOP #1820, *Tissue Homogenization Procedures* for further details on tissue homogenization procedures.

4.3.4 Sample Analysis

Analytical procedures may introduce errors from laboratory cross-contamination, extraction difficulties, and inappropriate methodology. Fats naturally present in tissues may interfere with sample analysis or extraction and elevate detection limits. Detection limits in the tissue samples must be the same as in the background tissue samples if a meaningful comparison is to be made. To minimize this interference, select an extraction or digestion procedure applicable to tissue samples.

Because many compounds (e.g., chlorinated hydrocarbons) concentrate in fatty tissues, a percent lipid analysis is necessary to normalize results among samples. Lipid recoveries vary among different analytical methods; percent lipid results for samples to be normalized and compared must be generated by the same analytical method. Select a lipid analysis based on the objective of the study (see references Herbes and Allen [1983] and Bligh and Dyer 1959). Sample results may be normalized on a wet-weight basis. If sample results are to be reported on a dry-weight basis, instruct the analytical laboratory to report the percent moisture content for each sample.

Appropriate sample preservation prevents loss of compounds and decomposition of tissues before analysis. Consult the appropriate SOP, analytical method, or designated laboratory contact to confirm holding times for tissue samples.

Tissue samples destined for sorting and identification (e.g., benthic macroinvertebrates, voucher fish) should be preserved in isopropyl or ethyl alcohol, formalin, or Kahle's solution. Preservation in these solvents precludes any chemical analysis.

4.4 QA/QC SAMPLES

QA/QC samples are collected at the site as prepared by the laboratory. Analysis of the QA/QC samples provides information on the variability and usability of biological sampling data, indicates possible field sampling or laboratory error, and provides a basis for future validation and usability of the analytical data. The most common field QA/QC samples are field replicates, reference, and rinsate blank samples. The most common laboratory

QA/QC samples are performance evaluation (PE), matrix spike (MS), and matrix spike duplicate (MSD) samples. QA/QC results may suggest the need for modifying sample collection, preparation, handling, or analytical procedures if the resultant data do not meet site-specific quality assurance objectives.

Refer to data validation procedures in *U.S. EPA Quality Assurance/Quality Control (QA/QC) Guidance for Removal Activities*, EPA/540/G-90/004, April 1990, for guidelines on utilizing QA/QC samples.

4.4.1 Replicate Samples

Field Replicates

Field replicates for solid media are samples obtained from one sampling point that are homogenized, divided into separate containers, and treated as separate samples throughout the remaining sample handling and analytical processes. Field replicates for aqueous samples are samples obtained from one location that are homogenized and divided into separate containers. There are no "true" field replicates for biological samples, however, biological samples collected from the same station are typically referred to as replicates. In this case, the biological replicates are used to determine the variability associated with heterogeneity within a biological population. Field replicates may be sent to two or more laboratories or to the same laboratory as unique samples.

Field replicates may be used to determine total error for critical samples with contaminant concentrations near the level that determines environmental impact. To determine error, a minimum of eight replicate samples is recommended for valid statistical analysis. For total error determination, samples should be analyzed by the same laboratory. The higher detection limit associated with composite samples may limit the usefulness of error determination.

NOTE: A replicate biological sample may consist of more than a single organism in those cases where the species mass is less than the mass required by the analytical procedure to attain required detection limits. This variability in replicate biological samples is independent of the variability in analytical procedures.

Toxicity Testing Replicates

For sediment samples, at least 3 replicate treatments should be conducted to determine variability between tests. The function of these replicates is to determine the variability of the test organism population within each treatment. This assumes the sample matrix exhibits a uniform concentration of the contaminants of concern within each treatment. Large variability may indicate a problem with the test procedures or organisms or lack of contaminant homogeneity within the sample matrix.

Site-Specific Examples of the Use of Replicates

Example No. 1

Two contaminant sources were identified at an active copper smelting facility. The first area was a slag pile containing high levels of copper suspected of migrating into the surrounding surface runoff pathways, subsequently leaching into the surface water of a surrounding stream system. The second area was the contaminated creek sediment that was present in the drainage pathway of the slag pile.

Whole-phase sediment toxicity tests were selected to evaluate the toxicity associated with the copper levels in the stream sediments. Sediment was collected at each sampling location (six locations total) to provide the testing laboratory with sufficient sample volume to perform these evaluations. Ten-day static renewal tests using the amphipod, *Hyalella azteca*, and the midge, *Chironomus tentans*, were chosen. The toxicity test utilized four “replicates” per sampling location (or treatment), each replicate containing fifteen organisms. The purpose of these replicates was to determine the variability within the test organism population within each treatment.

The results reported mean survival for *Hyalella azteca* in the contaminated sediment (8 to 50 percent) to be significantly lower than survival in the uncontaminated reference sediment (85 percent). Similarly, mean survival for *Chironomus tentans* in the contaminated sediment (0 to 63 percent) was significantly lower than survival in the uncontaminated reference sediment (83 percent).

Example No. 2

An inactive manufacturing facility had stored its stock compounds in unprotected piles for a number of years, resulting in DDT contamination of the adjacent watershed. DDT contamination in a stream located adjacent to the site extended from the manufacturing facility to approximately 27 miles downstream.

A field study was designed to quantitatively determine if the levels of DDT in the water and sediment in this stream were resulting in an adverse ecological impact.

This was accomplished through the examination of several in situ environmental variables in conjunction with laboratory analyses. Water, sediment, and resident biota were collected and submitted for various physical and chemical determinations. Additional sediments were secured and utilized for toxicity testing with three surrogate species. Finally, the benthic invertebrate community was sampled and the structure and function of this segment of the aquatic ecosystem evaluated.

Benthic invertebrates were collected from three areas at each sampling location (i.e., three "replicates" per location) and evaluated for various quantitative community metrics. The purpose of these replicates were to determine the spatial variability in the stream among the three areas within each sampling location. Community structure, diversity indices, taxonomic evenness, an evaluation of the function feeding groups, and statistical analyses were performed on the data set.

Qualitative and statistical comparison of the results between the contaminated areas and the uncontaminated reference indicated that the benthic invertebrate community was adversely affected downstream of the site compared to the upstream reference. Taxonomic and functional diversity varied inversely with DDT levels in sediment and water. These results were further substantiated by the toxicity evaluation results.

Example No. 3

Phase I and II Remedial Investigation and Feasibility Studies (RIFS) have indicated that the soils surrounding an industrial and municipal waste disposal site were contaminated with PCBs. A preliminary site survey revealed the presence of small mammal habitat and mammal signs in the natural areas adjacent to the site as well as an area that appeared to be outside of the site's influence (i.e., a potential reference area). A site investigation was subsequently conducted to determine the levels of PCBs accumulating into the resident mammal community from contact with the PCB-contaminated soil.

Three small mammal trapping areas were identified for this site. Two areas were located in PCB-contaminated areas, the third area was a reference. Trapping grids were established in each area consisting of 100 traps of various design. Six soil samples were also collected from each trapping area to characterize the levels of PCBs associated with the anticipated captured mammals.

A total of 32 mammals were collected at this site. Twelve were collected from each on-site area and six were collected from the reference area. All captured mammals were submitted for whole body analysis of PCBs. Mean PCB concentrations in the mammals were as follows: on-site areas (1250 and 1340 μg/kg, wet weight); reference area (490 μg/kg, wet weight). A one-way analysis of variance was conducted on the data set treating each animal in an area as a "replicate" (i.e., 12 replicates from each on-site area and 6 replicates from the reference). The results of the statistical analyses indicated that there was a statistically significant difference between on-site and reference area PCB levels in the mammals ($p<0.10$). Therefore, in this example, there were no analytical replicates since each individual mammal was analyzed. However, each mammal represented a statistical replicate within each trapping area.

4.4.2 Collocated Samples

A collocated sample is collected from an area adjoining a field sample to determine variability of the matrix and contaminants within a small area of the site. For example, collocated samples for chemistry analysis split from the sample collected for the toxicity test are collected about one-half to three feet away from the field sample location. Plants collected from within the same sampling plot may be considered collocated. Collocated samples are appropriate for assessing variability only in a small area, and should not be used to assess variability across the entire site or for assessing error.

4.4.3 Reference Samples

Reference biological samples may be taken from a reference area outside the influence of the site. Comparison of results from actual samples and samples from the reference area may indicate uptake, body burden, or accumulation of chemicals on the site. The reference area should be close to the site. It should have habitats, size and terrain similar to the site under investigation. The reference site need not be pristine. Biological reference samples should be of the same species, sex, and developmental stage as the field site sample.

4.4.4 Rinsate Blank Samples

A rinsate blank is used to assess cross-contamination from improper equipment decontamination procedures. Rinsate blanks are samples obtained by running analyte-free water over decontaminated sampling equipment. Any residual contamination should appear in the rinsate

data. Analyze the rinsate blank for the same analytical parameters as the field samples collected that day. When dedicated cutting tools or other sampling equipment are not used, collect one rinsate blank per device per day.

4.4.5 Field Blank Samples

Field blanks are samples prepared in the field using certified clean water or sand that are then submitted to the laboratory for analysis. A field blank is used to evaluate contamination or error associated with sampling methodology, preservation, handling/shipping, and laboratory procedures. If appropriate for the test, submit one field blank per day.

4.4.6 Trip Blank Samples

Trip blanks are samples prepared prior to going into the field. They consist of certified clean water or sand, and they are not opened until they reach the laboratory. Use trip blanks when samples are being analyzed for volatile organics. Handle, transport, and analyze trip blanks in the same manner as the other volatile organic samples collected that day. Trip blanks are used to evaluate error associated with sampling methodology, shipping and handling, and analytical procedures, since any volatile organic contamination of a trip blank would have to be introduced during one of those procedures.

4.4.7 Performance Evaluation / Laboratory Control Samples

A performance evaluation (PE) sample evaluates the overall error from the analytical laboratory and detects any bias in the analytical method being used. PE samples contain known quantities of target analytes manufactured under strict quality control. They are usually prepared by a third party under a U.S. EPA certification program. The samples are usually submitted "blind" to analytical laboratories (the sampling team knows the contents of the samples, but the laboratory does not). Laboratory analytical error (usually bias) may be evaluated by the percent recoveries and correct identification of the components in the PE sample.

4.4.8 Controls

Analytical Laboratory Control Samples

A chemical analytical laboratory control sample (LCS) contains quantities of target analytes known to the laboratory and are used to monitor "controlled" conditions. LCSs are analyzed under the same sample preparation, reagents, and analytical methods as the field samples. LCS results can show bias and/or variability in analytical results.

Toxicity Testing Control Groups

In toxicity tests, a laboratory reference toxicant treatment and a control treatment are both typically utilized in addition to a site reference treatment. This test involves exposing the test organism population to a standardized reference toxicant at a standardized dose, then comparing the response to historical laboratory records for that culture. The mortality results of the newly conducted reference toxicant test should be similar to the historical results. This is conducted to reveal if the generation(s) in the present culture is viable for use in the toxicity test, or if the culture has grown resistant or intolerant to the toxicant over time. Therefore, a laboratory reference toxicant test should be conducted prior to the testing of the site matrices.

In contrast, a laboratory control test is conducted simultaneously with the testing of the site matrices. This treatment identifies mortality factors that are unrelated to site contaminants. This is accomplished by exposing the test organism population to a clean dilution water and/or a clean laboratory substrate.

4.4.9 Matrix Spike/Matrix Spike Duplicate Samples

Matrix spike and matrix spike duplicate samples (MS/MSDs) are supplemental volumes of field-collected samples that are spiked in the laboratory with a known concentration of a target analyte to determine matrix interference. Matrix interference is determined as a function of the percent analyte recovery in the sample extraction. The percent recovery from MS/MSDs indicates the degree to which matrix interferences will affect the identification and/or quantitation of a substance. MS/MSDs can also be used to monitor laboratory performance. When two or more pairs of MS/MSDs are analyzed, the data obtained may also be used to evaluate error due to laboratory bias and precision. Analyze one MS/MSD pair to assess bias for every 10 samples, and use the average percent recovery for the pair. To assess precision, analyze at least eight matrix spike replicates from the same sample, and determine the standard deviation and the coefficient of variation. See the *U.S. EPA Quality Assurance/ Quality Control (QA/QC) Guidance for Removal Activities* (April 1990) for directions on calculating analytical error.

MS/MSDs are a required QA/QC element of the

definitive data objectives. MS/MSDs should accompany every 10 samples. Since the MS/MSDs are spiked field samples, sufficient volume for three separate analyses must be provided. Organic analysis of tissue samples is frequently subject to matrix interferences which causes biased analytical results. Matrix spike recoveries are often low or show poor precision in tissue samples. The matrix interferences will be evident in the matrix spike results. Although metals analysis of tissue samples is usually not subject to these interferences, MS/MSD samples should be utilized to monitor method and laboratory performance. Some analytical parameters such as percent lipids, organic carbon, and particle-size distribution are exempt from MS/MSD analyses.

4.4.10 Laboratory Duplicate Samples

A laboratory duplicate is a sample that undergoes preparation and analysis twice. The laboratory takes two aliquots of one sample and treats them as if they were separate samples. Comparison of data from the two analyses provides a measure of analytical reproducibility within a sample set. Discrepancies in duplicate analyses may indicate poor homogenization in the field or other sample preparation error, whether in the field or in the laboratory. However, duplicate analyses are not possible with most tissue samples unless a homogenate of the sample is created.

4.5 DATA EVALUATION

4.5.1 Evaluation of Analytical Error

Analytical error becomes significant in decision-making as sample results approach the level of environmental impact. The acceptable level of error is determined by the intended use of the data and litigation concerns. To be definitive, analytical data must have quantitative measurement of analytical error with PE samples and replicates. The QA samples identified in this section can indicate a variety of qualitative and quantitative sampling errors. Due to matrix interferences, causes of error may be difficult to determine in organic analysis of tissue samples.

4.5.2 Data Validation

Data from tissue sample analysis may be validated according to the Contract Laboratory Program National Functional Guidelines (U.S. EPA 1994) and according to *U.S. EPA Quality Assurance/Quality Control (QA/QC) Guidance for Removal Activities*, EPA/540/G-90/004, April 1990. Validation of organic data may require an experienced chemist due to complexity of tissue analysis.

5.0 DATA ANALYSIS AND INTERPRETATION

5.1 INTRODUCTION

The main objective of biological surveys conducted at Superfund sites is the assessment of site-related threat or effect. For many types of biological data (e.g., levels of contaminants in organisms collected on site and from a reference location), hypotheses are tested to determine the presence or absence of an effect. For some biological tests (e.g., benthic macroinvertebrate studies, toxicity tests), the data analysis and interpretation process is outlined in existing documents (U.S. EPA November 1990, U.S. EPA May 1996). For many Superfund ecological assessments, a weight-of-evidence approach is used to interpret the results of different studies or tests conducted at a site.

The statistical tests and methods that will be employed should be based on the objective of the data evaluation. These components should be outlined in the Work Plan or Sampling and Analysis Plan. This process will help focus the study to ensure that the appropriate type and number of samples are collected.

5.2 DATA PRESENTATION AND ANALYSIS

5.2.1 Data Presentation Techniques

In many cases, before descriptive statistics are calculated from a data set, it is useful to try various graphical displays of the raw data. The graphical displays help guide the choice of any necessary transformations of the data set and the selection of appropriate statistics to summarize the data. Since most statistical procedures require summary statistics calculated from a data set, it is important that the summary statistics represent the entire data set. For example, the median may be a more appropriate measure of central tendency than the mean for a data set that contains outliers. Graphical display of a data set could indicate the need to log transform data so that symmetry indicates a normal distribution. Four of the most useful graphical techniques are described next.

A histogram is a bar graph that displays the distribution of a data set, and provides information regarding the location of the center of the sample, amount of dispersion, extent of symmetry, and existence of outliers. Stem and leaf plots are similar to histograms in that they provide information on the distribution of a data set; however they also contain information on the numeric values in the data set. Box and whisker plots can be used to compare two or more samples of the same characteristic (e.g., stream IBI values for two or more years). Scatter plots are a useful method for examining the relationship between two sets of variables. Figure 4 illustrates the four graph techniques described previously.

5.2.2 Descriptive Statistics

Large data sets are often summarized using a few descriptive statistics. Two important features of a set of data are the central tendency and the spread. Statistics used to describe central tendency include the arithmetic mean, median, mode and geometric mean. Spread or dispersion in a data set refers to the variability in the observations about the center of the distribution. Statistics used to describe data dispersion include range and standard deviation. Methods for calculating descriptive statistics can be found in any statistics textbook, and many software programs are available for statistical calculations.

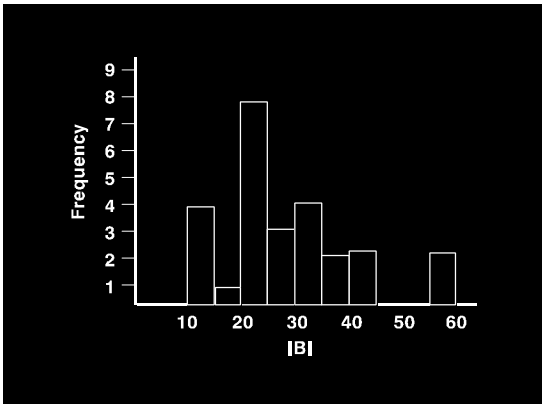
5.2.3 Hypothesis Testing

Biological studies are conducted at Superfund sites to determine adverse effects due to site-related factors. For many types of biological data, hypothesis testing is the statistical procedure used to evaluate data. Hypothesis testing involves statistically evaluating a parameter of concern, such as the mean or median, at a specified probability for incorrectly interpreting the analysis results. In conventional statistical analysis, hypothesis testing for a trend or effect is based on a null hypothesis. Typically, the null hypothesis is presumed when there is no trend or effect present. To test this hypothesis, data are collected to estimate an effect. The data are used to provide a sample estimate of a test statistic, and a table for the test statistic is consulted to determine how unlikely the observed value of the statistic is if the null hypothesis is true. If the observed value of the test statistic is unlikely, the null hypothesis is rejected. In ecological risk assessment, a hypothesis is a question about the relationship among assessment endpoints and their predicted responses when exposed to contaminants. The most basic hypothesis that is applicable to virtually all Superfund sites is that site-related contaminants are causing adverse effects of the assessment endpoint(s).

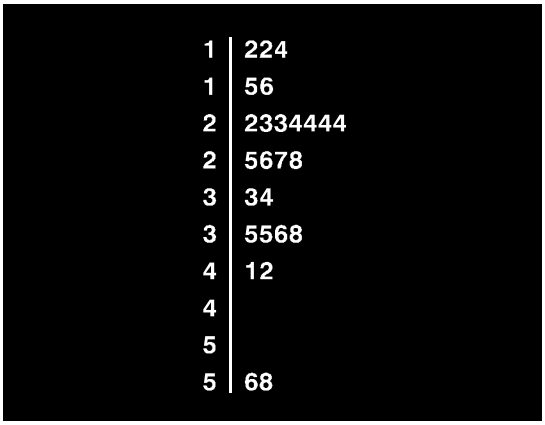
Figure 3 Illustrations of Sample Plots

IBI DATA

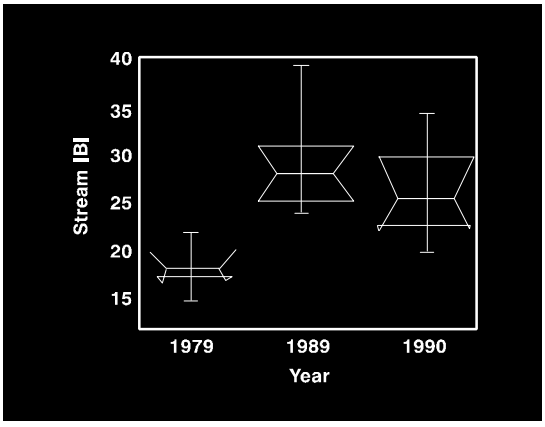
12	25	33	56
12	24	34	58
14	26	35	
15	24	36	
16	24	35	
22	27	38	
24	23	41	
23	28	42	



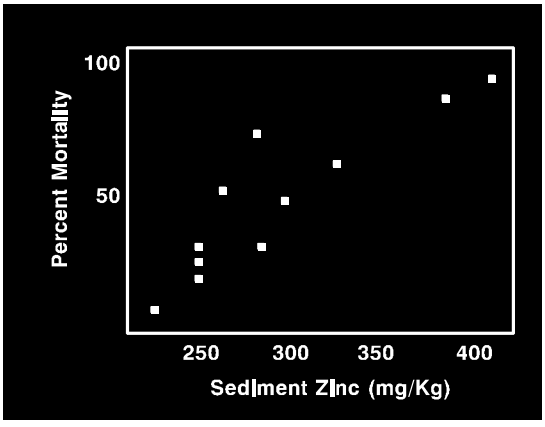
A) Histogram



B) Leaf Plot



C) Whisker Plot



D) Scatter Plot

5.3 DATA INTERPRETATION

5.3.1 Chemical Residue Studies

Chemical residue data may be evaluated in two ways. First, the contaminant concentrations by themselves provide evidence of bioaccumulation and probable food chain transfer of the contaminants, and an overall picture of the distribution of contaminants in the biological community. Second, the residue data may be evaluated against literature residue values that are known to cause no effect or an adverse effect in the organism.

5.3.2 Population/Community Studies

The interpretation of population/community data is extensive, therefore, the reader is referred to a detailed treatment in U.S. EPA (November 1990), U.S. EPA (1989a), Karr et al. (1986), and other literature.

5.3.3 Toxicity Testing

Measurement endpoints obtained in toxicity tests are generally compared to results from a laboratory control and a reference location sample to determine whether statistically significant differences exist. If significant effects (e.g., mortality, decreased reproduction) are observed, additional statistical analyses can be run to determine whether observed effects correlate with measured contaminant levels. The reader is referred to a detailed treatment in ASTM (1992), U.S. EPA (May 1988), U.S. EPA (March 1989b).

5.3.4 Risk Calculation

Preliminary screening value results are interpreted by comparison of historical and/or new site analytical data against literature toxicity values. This comparison will suggest if the probability of risk exists and whether additional evaluation is desired.

If the evaluation is pursued to an ecological risk assessment, mathematical models, such as the Hazard Quotient method, are used to evaluate the site data against literature toxicity values. Based on the type of model used, the results can be extrapolated to suggest the presence of ecological risk.

APPENDIX A - CHECKLIST FOR ECOLOGICAL ASSESSMENT/SAMPLING

Introduction

The checklist that follows provides guidance in making observations for an ecological assessment. It is not intended for limited or emergency response actions (e.g., removal of a few drums) or for purely industrial settings with no discharges. The checklist is a screening tool for preliminary site evaluation and may also be useful in planning more extensive site investigations. It must be completed as thoroughly as time allows. The results of the checklist will serve as a starting point for the collection of appropriate biological data to be used in developing a response action. It is recognized that certain questions in this checklist are not universally applicable and that site-specific conditions will influence interpretation. Therefore, a site synopsis is requested to facilitate final review of the checklist by a trained ecologist.

Checklist

The checklist has been divided into sections that correspond to data collection methods and ecosystem types. These sections are:

I. Site Description

   IA. Summary of Observations and Site Setting

II. Terrestrial Habitat Checklist

   IIA. Wooded
   IIB. Shrub/Scrub
   IIC. Open Field
   IID. Miscellaneous

III. Aquatic Habitat Checklist -- Non-Flowing Systems

IV. Aquatic Habitat Checklist -- Flowing Systems

V. Wetlands Habitat Checklist

Checklist for Ecological Assessment/Sampling

I. SITE DESCRIPTION

1. Site Name: _____

   Location: _____

   _____

   County:_____City:_____State:_____

2. Latitude: _____ Longitude: _____

3. What is the approximate area of the site? _____

4. Is this the first site visit? ☐ yes ☐ no If no, attach trip report of previous site visit(s), if available.

   Date(s) of previous site visit(s):_____.

5. Please attach to the checklist USGS topographic map(s) of the site, if available.

6. Are aerial or other site photographs available? ☐ yes ☐ no If yes, please attach any available photo(s) to the site map at the conclusion of this section.

7. The land use on the site is:

_____% Urban

_____% Rural

_____% Residential

_____% Industrial (☐ light ☐ heavy)

_____% Agricultural

(Crops:_____)

_____% Recreational

(Describe; note if it is a park, etc.)

_____

_____

_____% Undisturbed

_____% Other

The area surrounding the site is:
_____ mile radius

_____% Urban

_____% Rural

_____% Residential

_____% Industrial (☐ light ☐ heavy)

_____% Agricultural

(Crops:_____)

_____% Recreational

(Describe; note if it is a park, etc.)

_____

_____

_____% Undisturbed

_____% Other

8. Has any movement of soil taken place at the site? ☐ yes ☐ no. If yes, please identify the most likely cause of this disturbance:

_____ Agricultural Use _____ Heavy Equipment _____ Mining

_____ Natural Events _____ Erosion _____ Other

Please describe:

9. Do any potentially sensitive environmental areas exist adjacent to or in proximity to the site, e.g., Federal and State parks, National and State monuments, wetlands, prairie potholes? *Remember, flood plains and wetlands are not always obvious; do not answer "no" without confirming information.*

   Please provide the source(s) of information used to identify these sensitive areas, and indicate their general location on the site map.

10. What type of facility is located at the site?

    ☐ Chemical ☐ Manufacturing ☐ Mixing ☐ Waste disposal

    ☐ Other (specify)_____

11. What are the suspected contaminants of concern at the site? If known, what are the maximum concentration levels?

12. Check any potential routes of off-site migration of contaminants observed at the site:

    ☐ Swales ☐ Depressions ☐ Drainage ditches

    ☐ Runoff ☐ Windblown particulates ☐ Vehicular traffic

    ☐ Other (specify)_____

13. If known, what is the approximate depth to the water table?_____

14. Is the direction of surface runoff apparent from site observations? ☐ yes ☐ no If yes, to which of the following does the surface runoff discharge? Indicate all that apply.

    ☐ Surface water ☐ Groundwater ☐ Sewer ☐ Collection impoundment

15. Is there a navigable waterbody or tributary to a navigable waterbody? ☐ yes ☐ no

16. Is there a waterbody anywhere on or in the vicinity of the site? If yes, also complete Section III: Aquatic Habitat Checklist -- Non-Flowing Systems and/or Section IV: Aquatic Habitat Checklist -- Flowing Systems.

    ☐ yes (approx. distance_____) ☐ no

17. Is there evidence of flooding? ☐ yes ☐ no *Wetlands and flood plains are not always obvious; do not answer "no" without confirming information.* If yes, complete Section V: Wetland Habitat Checklist.

18. If a field guide was used to aid any of the identifications, please provide a reference. Also, estimate the time spent identifying fauna. [Use a blank sheet if additional space is needed for text.]

19. Are any threatened and/or endangered species (plant or animal) known to inhabit the area of the site? ☐ yes ☐ no *If yes, you are required to verify this information with the U.S. Fish and Wildlife Service.* If species' identities are known, please list them next.

20. Record weather conditions at the time this checklist was prepared:

    DATE:_____

    _____ Temperature (°C/°F) _____ Normal daily high temperature

    _____ Wind (direction/speed) _____ Precipitation (rain, snow)

    _____ Cloud cover

IA. SUMMARY OF OBSERVATIONS AND SITE SETTING

Completed by_____ Affiliation_____

Additional Preparers_____

Site Manager_____

Date_____

II. TERRESTRIAL HABITAT CHECKLIST

IIA. WOODED

1. Are there any wooded areas at the site? ☐ yes ☐ no If no, go to Section IIB: Shrub/Scrub.

2. What percentage or area of the site is wooded? (_____% _____ acres). Indicate the wooded area on the site map which is attached to a copy of this checklist. Please identify what information was used to determine the wooded area of the site.

3. What is the dominant type of vegetation in the wooded area? (Circle one: Evergreen/Deciduous/ Mixed) Provide a photograph, if available.

   Dominant plant, if known:_____

4. What is the predominant size of the trees at the site? Use diameter at breast height.

   ☐ 0-6 in. ☐ 6-12 in. ☐ > 12 in.

5. Specify type of understory present, if known. Provide a photograph, if available.

IIB. SHRUB/SCRUB

1. Is shrub/scrub vegetation present at the site? ☐ yes ☐ no If no, go to Section IIC: Open Field.

2. What percentage of the site is covered by scrub/shrub vegetation? (_____% _____ acres). Indicate the areas of shrub/scrub on the site map. Please identify what information was used to determine this area.

3. What is the dominant type of scrub/shrub vegetation, if known? Provide a photograph, if available.

4. What is the approximate average height of the scrub/shrub vegetation?

   ☐ 0-2 ft. ☐ 2-5 ft. ☐ > 5 ft.

5. Based on site observations, how dense is the scrub/shrub vegetation?

   ☐ Dense ☐ Patchy ☐ Sparse

IIC. OPEN FIELD

1. Are there open (bare, barren) field areas present at the site? ☐ yes ☐ no If yes, please indicate the type below:

   ☐ Prairie/plains ☐ Savannah ☐ Old field ☐ Other (specify)_____

2. What percentage of the site is open field? (_____% _____ acres). Indicate the open fields on the site map.

3. What is/are the dominant plant(s)? Provide a photograph, if available.

4. What is the approximate average height of the dominant plant?_____

5. Describe the vegetation cover: ☐ Dense ☐ Sparse ☐ Patchy

IID. MISCELLANEOUS

1. Are other types of terrestrial habitats present at the site, other than woods, scrub/shrub, and open field? ☐ yes ☐ no If yes, identify and describe them below.

2. Describe the terrestrial miscellaneous habitat(s) and identify these area(s) on the site map.

3. What observations, if any, were made at the site regarding the presence and/or absence of insects, fish, birds, mammals, etc.?

4. Review the questions in Section I to determine if any additional habitat checklists should be completed for this site.

III. AQUATIC HABITAT CHECKLIST -- NON-FLOWING SYSTEMS

Note: Aquatic systems are often associated with wetland habitats. Please refer to Section V, Wetland Habitat Checklist.

1. What type of open-water, non-flowing system is present at the site?

   ☐ Natural (pond, lake)
   ☐ Artificially created (lagoon, reservoir, canal, impoundment)

2. If known, what is the name(s) of the waterbody(ies) on or adjacent to the site?

   _____

3. If a waterbody is present, what are its known uses (e.g.: recreation, navigation, etc.)?

4. What is the approximate size of the waterbody(ies)? _____ acre(s).

5. Is any aquatic vegetation present? ☐ yes ☐ no If yes, please identify the type of vegetation present if known.

   ☐ Emergent ☐ Submergent ☐ Floating

6. If known, what is the depth of the water? _____

7. What is the general composition of the substrate? Check all that apply.

   ☐ Bedrock ☐ Sand (coarse) ☐ Muck (fine/black)

   ☐ Boulder (>10 in.) ☐ Silt (fine) ☐ Debris

   ☐ Cobble (2.5-10 in.) ☐ Marl (shells) ☐ Detritus

   ☐ Gravel (0.1-2.5 in.) ☐ Clay (slick) ☐ Concrete

   ☐ Other (specify)_____

8. What is the source of water in the waterbody?

   ☐ River/Stream/Creek ☐ Groundwater ☐ Other (specify)_____

   ☐ Industrial discharge ☐ Surface runoff

9. Is there a discharge from the site to the waterbody? ☐ yes ☐ no If yes, please describe this discharge and its path.

10. Is there a discharge from the waterbody? ☐ yes ☐ no If yes, and the information is available, identify from the list below the environment into which the waterbody discharges.

☐ River/Stream/Creek	☐ onsite	☐ offsite	Distance_____
☐ Groundwater	☐ onsite	☐ offsite	
☐ Wetland	☐ onsite	☐ offsite	Distance_____
☐ Impoundment	☐ onsite	☐ offsite	

11. Identify any field measurements and observations of water quality that were made. For those parameters for which data were collected provide the measurement and the units of measure below:

_____ Area

_____ Depth (average)

_____ Temperature (depth of the water at which the reading was taken) _____

_____ pH

_____ Dissolved oxygen

_____ Salinity

_____ Turbidity (clear, slightly turbid, turbid, opaque) (Secchi disk depth _____)

_____ Other (specify)

12. Describe observed color and area of coloration.

13. Mark the open-water, non-flowing system on the site map attached to this checklist.

14. What observations, if any, were made at the waterbody regarding the presence and/or absence of benthic macroinvertebrates, fish, birds, mammals, etc.?

IV. AQUATIC HABITAT CHECKLIST -- FLOWING SYSTEMS

Note: Aquatic systems are often associated with wetland habitats. Please refer to Section V, Wetland Habitat Checklist.

1. What type(s) of flowing water system(s) is (are) present at the site?

☐ River	☐ Stream	☐ Creek
☐ Dry wash	☐ Arroyo	☐ Brook
☐ Artificially created (ditch, etc.)	☐ Intermittent Stream	☐ Channeling
	☐ Other (specify)_____	

2. If known, what is the name of the waterbody?_____

3. For natural systems, are there any indicators of physical alteration (e.g., channeling, debris, etc.)?
☐ yes ☐ no If yes, please describe indicators that were observed.

4. What is the general composition of the substrate? Check all that apply.

☐ Bedrock	☐ Sand (coarse)	☐ Muck (fine/black)
☐ Boulder (>10 in.)	☐ Silt (fine)	☐ Debris
☐ Cobble (2.5-10 in.)	☐ Marl (shells)	☐ Detritus
☐ Gravel (0.1-2.5 in.)	☐ Clay (slick)	☐ Concrete
☐ Other (specify)_____		

5. What is the condition of the bank (e.g., height, slope, extent of vegetative cover)?

6. Is the system influenced by tides? ☐ yes ☐ no What information was used to make this determination?

7. Is the flow intermittent? ☐ yes ☐ no If yes, please note the information that was used in making this determination.

8. Is there a discharge from the site to the waterbody? ☐ yes ☐ no If yes, please describe the discharge and its path.

9. Is there a discharge from the waterbody? ☐ yes ☐ no If yes, and the information is available, please identify what the waterbody discharges to and whether the discharge is on site or off site.

10. Identify any field measurements and observations of water quality that were made. For those parameters for which data were collected, provide the measurement and the units of measure in the appropriate space below:

_____ Width (ft.)

_____ Depth (ft.)

_____ Velocity (specify units):_____

_____ Temperature (depth of the water at which the reading was taken_____)

_____ pH

_____ Dissolved oxygen

_____ Salinity

_____ Turbidity (clear, slightly turbid, turbid, opaque)
(Secchi disk depth _____)

_____ Other (specify)_____

11. Describe observed color and area of coloration.

12. Is any aquatic vegetation present? ☐ yes ☐ no If yes, please identify the type of vegetation present, if known.

    ☐ Emergent ☐ Submergent ☐ Floating

13. Mark the flowing water system on the attached site map.

14. What observations were made at the waterbody regarding the presence and/or absence of benthic macroinvertebrates, fish, birds, mammals, etc.?

V. WETLAND HABITAT CHECKLIST

1. Based on observations and/or available information, are designated or known wetlands definitely present at the site?
   ☐ yes ☐ no

   Please note the sources of observations and information used (e.g., USGS Topographic Maps, National Wetland Inventory, Federal or State Agency, etc.) to make this determination.

2. Based on the location of the site (e.g., along a waterbody, in a floodplain) and site conditions (e.g., standing water; dark, wet soils; mud cracks; debris line; water marks), are wetland habitats suspected?
   ☐ yes ☐ no If yes, proceed with the remainder of the wetland habitat identification checklist.

3. What type(s) of vegetation are present in the wetland?

   ☐ Submergent ☐ Emergent
   ☐ Scrub/Shrub ☐ Wooded

   ☐ Other (specify)_____

4. Provide a general description of the vegetation present in and around the wetland (height, color, etc.). Provide a photograph of the known or suspected wetlands, if available.

5. Is standing water present? ☐ yes ☐ no If yes, is this water: ☐ Fresh ☐ Brackish
   What is the approximate area of the water (sq. ft.)?_____
   Please complete questions 4, 11, 12 in Checklist III - Aquatic Habitat -- Non-Flowing Systems.

6. Is there evidence of flooding at the site? What observations were noted?

   ☐ Buttressing ☐ Water marks ☐ Mud cracks

   ☐ Debris line ☐ Other (describe below)

7. If known, what is the source of the water in the wetland?

   ☐ Stream/River/Creek/Lake/Pond ☐ Groundwater

   ☐ Flooding ☐ Surface Runoff

8. Is there a discharge from the site to a known or suspected wetland? ☐ yes ☐ no If yes, please describe.

9. Is there a discharge from the wetland? ☐ yes ☐ no. If yes, to what waterbody is discharge released?

   ☐ Surface Stream/River ☐ Groundwater ☐ Lake/Pond ☐ Marine

10. If a soil sample was collected, describe the appearance of the soil in the wetland area. Circle or write in the best response.

    Color (blue/gray, brown, black, mottled) _____

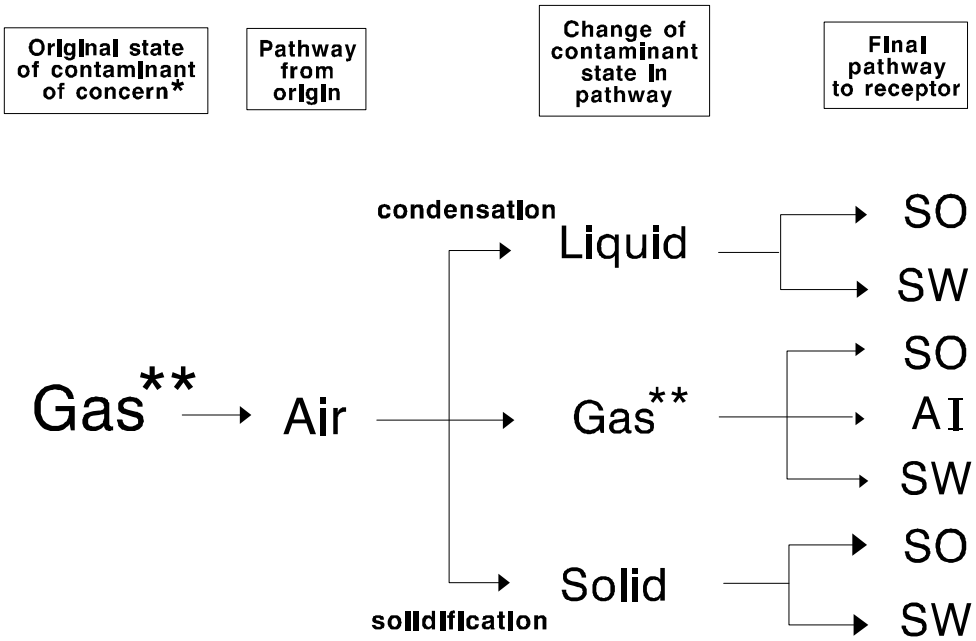
    Water content (dry, wet, saturated/unsaturated) _____

11. Mark the observed wetland area(s) on the attached site map.

APPENDIX B -- Example of Flow Diagram For Conceptual Site Model

Figure B-1

Migration Routes of a Gas Contaminant from Origin to Receptor



Receptor		
Human	Ecological Threat	
	Terrestrial	Aquatic
G,D	G,D	N/A
G,D	G,D	G,D
I,D	I,D	N/A
I,D	I,D	N/A
G,D	I,D	G,D
G,D	G,D	N/A
G,D	G,D	G,D

* May be a transformation product
** Includes vapors

Receptor Key

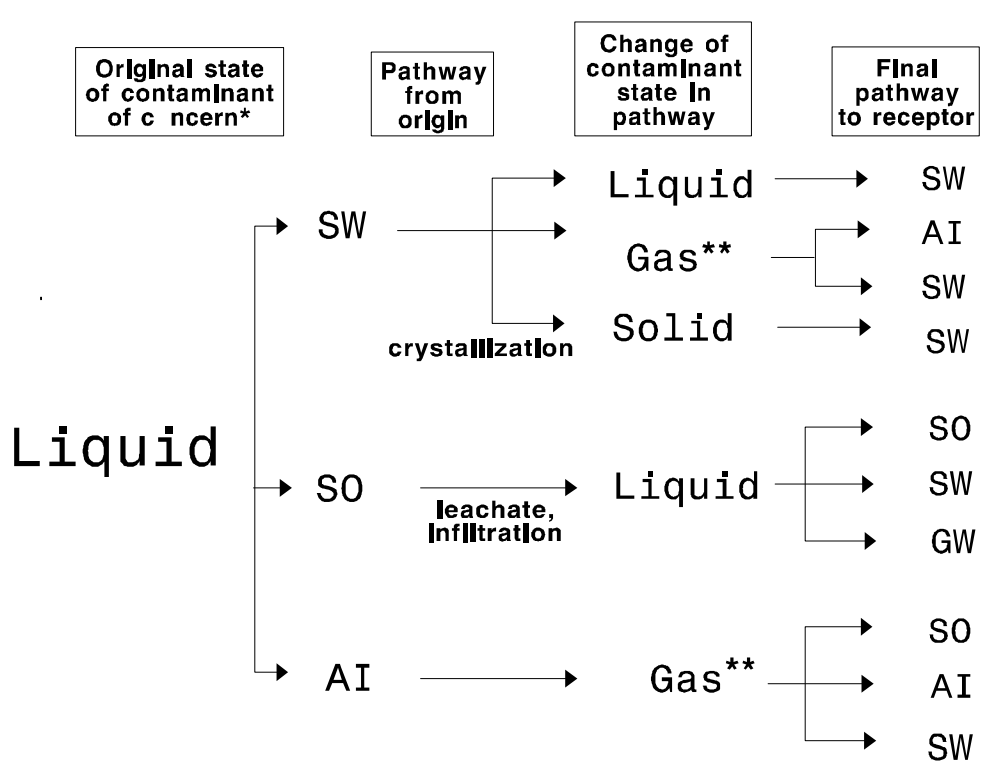
D = Dermal Contact
I = Inhalation
G = Ingestion
N/A = Not Applicable

Pathway Key

AI = Air
SO = Soil
SW = Surface Water (Including sediments)
GW = Ground Water

Figure B-2

Migration Routes of a Liquid Contaminant from Origin to Receptor



Receptor		
Human	Ecological Threat	
	Terrestrial	Aquatic
G,D	G,D	G,D
I,D	I,D	N/A
G,D	G,D	G,A
G,D	G,D	G,D
G,D	G,D	N/A
G,D	G,D	G,D
G,D	N/A	N/A
G,D	G,D	N/A
I,D	I,D	N/A
G,D	G,D	G,D

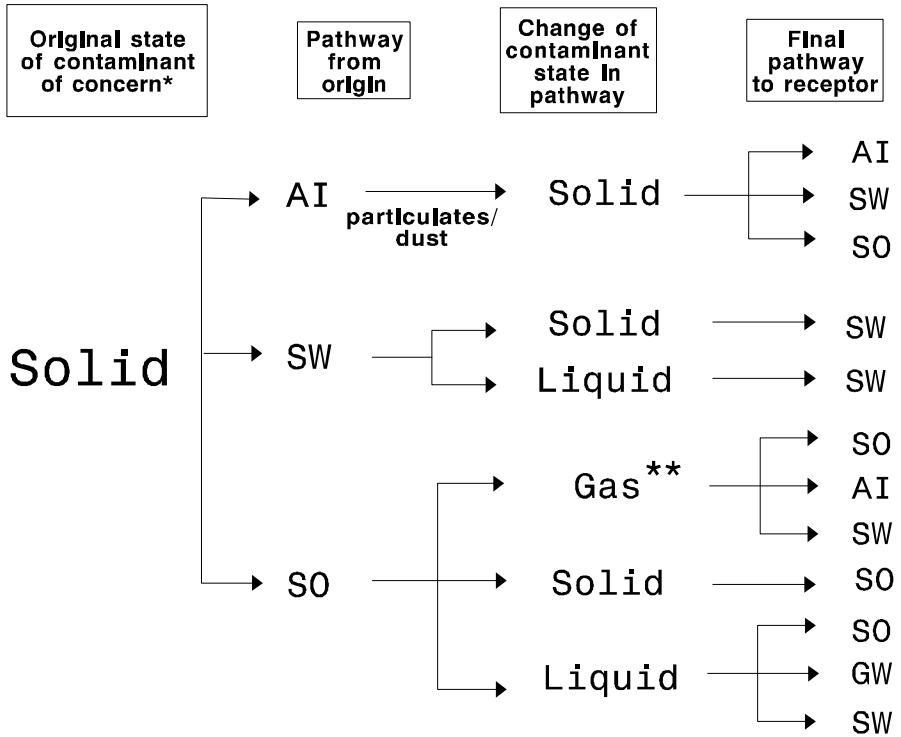
* May be a transformation product
** Includes vapors

Pathway Key
AI = Air
SO = Soil
SW = Surface Water (including sediments)
GW = Ground Water

Receptor Key
D = Dermal Contact
I = Inhalation
G = Ingestion
N/A = Not Applicable

Figure B-3

Migration Routes of a Solid Contaminant from Origin to Receptor



Receptor		
Human	Ecological Threat	
	Terrestrial	Aquatic
I,D	I,D	N/A
G,D	G,D	G,D
G,D	G,D	N/A
G,D	G,D	G,D
G,D	G,D	G,D
G,D	G,D	N/A
I,D	I,D	N/A
G,D	G,D	G,D
G,D	G,D	N/A
G,D	G,D	N/A
G,D	N/A	N/A
G,D	G,D	G,D

* May be a transformation product
** Includes vapors

Receptor Key
D = Dermal Contact
I = Inhalation
G = Ingestion
N/A = Not Applicable

Pathway Key
AI = Air
SO = Soil
SW = Surface Water (including sediments)
GW = Ground Water

APPENDIX C - EXAMPLE SITES

Example sites are presented in this document to demonstrate how information from the checklist for ecological assessment/sampling is used in conjunction with representative biological sampling to meet the study objectives. A general history for each site is presented first, then additional preliminary information

I. SITE HISTORIES

Site A -- Copper Site

This is a former municipal landfill located in an upland area of the mid-Atlantic plain. Residential, commercial, and industrial refuse were disposed at the site from 1961 to 1980. Large amounts of copper wire were also disposed at this site. Minimal grass cover has been placed over the fill. Terrestrial ecosystems in the vicinity of the landfill include upland forest, successional fields, agricultural land, and residential and commercial areas. The surface of the landfill has deteriorated in several locations. Leachate seeps have been noted on the slope of the landfill, several of which discharge to a 5-acre pond down-gradient of the site.

Site B -- Stream DDT Site

This is a former chemical production facility located adjacent to a stream. The facility manufactured and packaged dichlorodiphenyltrichloroethane (DDT). Due to poor storage practices, several DDT spills have occurred.

Site C -- Terrestrial PCB Site

This site is a former waste oil recycling facility located in a remote area. Oils contaminated with polychlorinated biphenyl compounds (PCBs) were disposed in a lagoon. The lagoon is not lined and the substrate is composed mostly of sand. Oils contaminated with PCBs have migrated through the soil and contaminated a wide area adjacent to the site.

II. USE OF THE CHECKLIST FOR ECOLOGICAL ASSESSMENT/SAMPLING

Site A -- Copper Site

A preliminary site visit was conducted, and the checklist indicated the following: 1) the pond has an organic substrate, 2) emergent vegetation including cattail and *Phragmites* occurs along the shore near the leachate seeps, and 3) the pond reaches a depth of five feet toward the middle. Several species of sunfish, minnows, and carp were observed. A diverse benthic macroinvertebrate community also has been noted in the pond. The pond appears to function as a valuable habitat for fish and other wildlife.

Preliminary sampling indicated elevated copper levels in the seep as well as elevated base cations, total organic carbon (TOC), and depressed pH levels (pH 5.7).

Copper can cause toxic effects in both aquatic plants and invertebrates at relatively low water concentrations, thereby affecting the pond's ability to support macroinvertebrate and fish communities, as well as the wildlife that feed at the pond. Terrestrial ecosystems do not need to be evaluated because the overland flow of the seeps is limited to short gullies. Thus, the area of concern has been identified as the 5-acre pond and the associated leachate seeps.

A review of the literature on the ecotoxicity of copper to aquatic biota and plants, both algae and vascular, was conducted. In general it was found that young organisms are more sensitive to copper with decreasing sensitivity as body weight increases. The toxicity of copper in water is influenced by water hardness, alkalinity, and pH.

Site B -- Stream DDT Site

The ecological checklist was completed as part of the preliminary site visit. The information gathered indicates that surface water drainage from the site flows through several drainage swales toward a small unnamed creek. This creek is a second order stream containing riffle-run areas and small pools. The stream substrate is composed of sand and gravel in the pools with some small depositional areas in the backwater areas, and primarily cobble in the riffles. Previous sampling efforts have indicated the presence of DDT and its metabolites in the stream sediments at a concentration of 230 milligrams per kilogram (mg/kg). A variety of wildlife, especially piscivorous birds, utilize this area for feeding. Many species of minnow have been noted in this stream. DDT is well known for its tendency to bioaccumulate and biomagnify in food chains, and available evidence indicates that it can cause reproductive failure in birds due to eggshell thinning.

In freshwater systems, DDT can have direct effects on animals, particularly insects. A literature review of the aquatic toxicity of DDT was conducted, and a no observed adverse effects level (NOAEL) was identified for aquatic insects. Aquatic plants are not affected by DDT. Additional information on the effects of DDT on birds identified decreased reproductive success due to eggshell thinning.

Site C -- Terrestrial PCB Site

During a preliminary site visit, the ecological checklist was completed. Most of the habitat is upland forest, old field, and successional terrestrial areas. Biological surveys at this site have noted a variety of small mammals, and red-tailed hawks were also observed. The area of concern has been identified as the 10-acre area surrounding the site. PCBs have been shown to reduce reproductive success in mammals or target liver functions. PCBs are not highly volatile, so inhalation of PCBs would not be an important exposure pathway. However, PCBs have been shown to biomagnify indicating that the ingestion exposure route needs evaluation. Shrews and/or voles would be appropriate mammalian receptors to evaluate for this exposure route. Potential reproductive effects on predators that feed on small mammals would also be important to evaluate. The literature has indicated that exposure to PCBs through the food chain can cause chronic toxicity to predatory birds.

Limited information was available on the effects of PCBs to red-tailed hawks. Studies on comparable species have indicated decreased sperm concentration that may affect reproductive success.

III. CONCEPTUAL SITE MODEL FORMULATION

Site A -- Copper Site

The assessment endpoint for this site was identified as the maintenance of pond fish and invertebrate community composition similar to that of other ponds in the area of similar size and characteristics. Benthic macroinvertebrate community studies may be relatively labor-intensive and potentially an insensitive measure in this type of system. Measuring the fish community would also be unsuitable due to the limited size of the pond and the expected low diversity of fish species. In addition, copper is not strongly food-chain transferrable. Therefore, direct toxicity testing was selected as an appropriate measurement endpoint. Toxicity was defined as a statistically significant decrease in survival or juvenile growth rates in a population exposed to water or sediments, as compared to a population from the reference sites.

One toxicity test selected was a 10-day solid-phase sediment toxicity test using early life-stage *Hyalella azteca*. The measurement endpoints for the test are mortality and growth rates (measured as length and weight changes). Two water-column toxicity tests were selected: a 7-day test using the alga *Selenastrum capricornutum* (growth test) and a 7-day larval fish test using *Pimephales promelas* (mortality and growth endpoints).

Five sediment samples were collected from the pond bottom at intervals along an identified concentration gradient. Reference sediment was also collected. A laboratory control was utilized in addition to the reference sediment in this toxicity test. The study design specified that sediment for the toxicity tests was collected from the leachate seeps known to be at the pond edge, and from four additional locations transecting the pond at equidistance locations. A pre-sampling visit was required to confirm that the seep was flowing due to the intermittent nature of leachate seeps.

Site B -- Stream DDT Site

A conceptual model was developed to evaluate the environmental pathways for DDT that could result in ecological impacts. DDT in the sediments can be released to the water column during natural resuspension and redistribution of the sediments. Some diffusion of DDT to the water column from the sediment surface may also occur. The benthic macroinvertebrate community would be an initial receptor for the DDT in sediments. Fish that feed on the benthic macroinvertebrates could be exposed to the DDT both in the water column and in their food. Piscivorous birds would be exposed to the DDT that has accumulated in the fish. For example, belted kingfishers are known to feed in the stream. Given the natural history of this species, it is possible that they forage entirely in the contaminated area. From this information, the assessment endpoint was identified to be the protection of piscivorous birds from eggshell thinning due to DDT exposure. From this assessment endpoint, eggshell thinning in the belted kingfisher was selected as the measurement endpoint.

Existing information identified a DDT gradient in the stream sediments. Forage fish (e.g., creek chub) were selected to measure exposure levels for kingfishers. The study design for measuring DDT residue levels specified that 10 creek chub of the same size and sex will be collected at each location for chemical residue analysis. Although analytical data for the stream sediment exists, new co-located sediment samples were specified to be collected to provide a stronger link between the present state of contamination in the sediment and in the fish.

Site C -- Terrestrial PCB Site

A conceptual model was prepared to determine the exposure pathways by which predatory birds could be exposed to PCBs originating in the soil at the site. The prey of red-tailed hawks includes voles, deer mice, and various insects. Voles are herbivorous and prevalent at the site. However, PCBs do not strongly accumulate in plants, thus voles may not represent a strong exposure pathway to hawks. Deer mice are omnivorous and may be more likely than voles to be exposed to PCBs. The assessment endpoint for this site was identified to be the protection of reproductive success in high trophic level species exposed to PCBs via diet.

Initially, a sampling feasibility study was conducted to confirm sufficient numbers of the deer mice. Two survey lines of 10 live traps were set for deer mice in the area believed to contain the desired concentration gradient for the study design. Previous information indicated a gradient of decreasing PCB concentration with increasing distance from the unlined lagoon. Three locations were selected along this gradient to measure PCB concentrations in prey. Co-located soil and water samples were also collected. The analytical results of these matrices were utilized as variables in a food chain accumulation model which predicted the amount of contaminant in the environment that may travel through the food chain, ultimately to the red-tailed hawk.

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