PA/600/8-87-044

United States
Environmental Protection
Agency

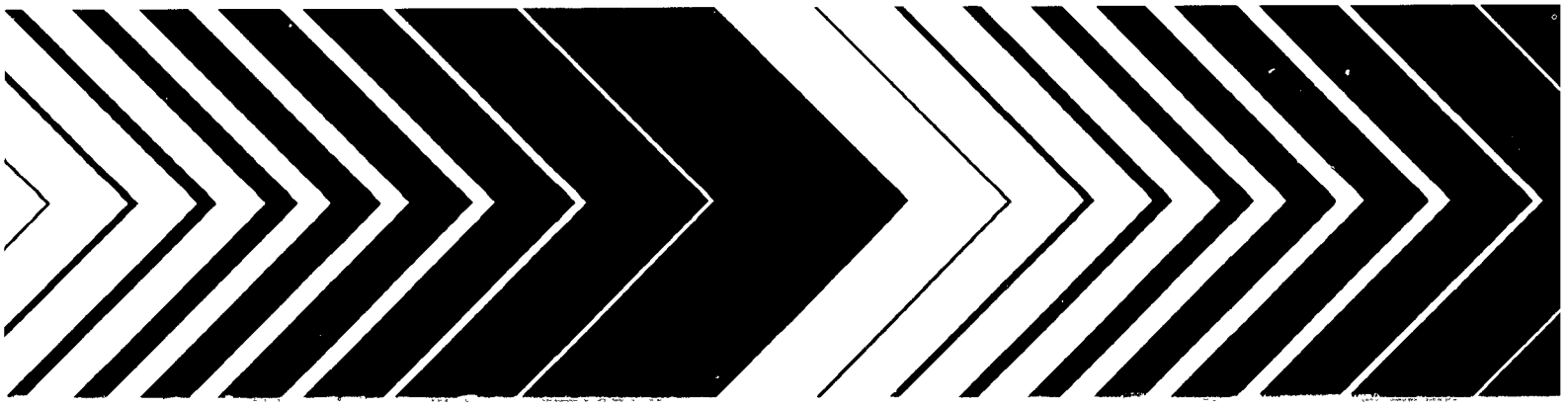
Environmental
Research Laboratory
Corvallis OR 97333

EPA/600/8-87/044
August 1987

Research and Development

EPA

Role of Acute Toxicity Bioassays in the Remedial Action Process at Hazardous Waste Sites



EPA/600/8-87/044
August 1987

ROLE OF ACUTE TOXICITY BIOASSAYS IN THE REMEDIAL ACTION PROCESS AT HAZARDOUS WASTE SITES

by

L. A. Athey
J. M. Thomas
J. R. Skalski

Pacific Northwest Laboratory
Richland, Washington 99352

W. E. Miller
Corvallis Environmental Research Laboratory
Corvallis, Oregon 97333

Project Manager

D. A. Neitzel
Earth and Environmental Sciences Center
Pacific Northwest Laboratory
Richland, Washington 99352

Pacific Northwest Laboratory
Richland, Washington 99352

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

SUMMARY

This document is written to aid in making decisions regarding the use of standardized aquatic and terrestrial acute toxicity bioassays at hazardous chemical waste sites. Other types of bioassays, including those for use with receiving waters and in situ testing, are not discussed. The use of acute bioassays at hazardous chemical sites is explained and illustrated for use in the remedial action process. Step-by-step guidelines are presented whereby decisions can be made concerning the design of site-specific bioassay studies.

The use of acute laboratory bioassays as a method of assessing toxicity of samples from hazardous chemical sites is a relatively recent development. Therefore, we have included currently available bioassay results from three actual hazardous chemical waste site studies to illustrate the ability of standardized bioassays to define toxicity at hazardous waste sites:

- Soils from the Rocky Mountain Arsenal were bioassayed and the results used to prepare a cleanup map based on phytotoxicity.
- Bioassay results from sediment samples taken from a wood treatment site were compared to chemical analysis results to determine whether bioassays can be used effectively to guide remedial actions at creosote-contaminanted sites.
- Bioassay results from soil, sediment, and surface-water samples at two hazardous chemical waste sites were used to evaluate site toxicity, determine sources of chemicals, and illustrate the use of staged sampling and compositing.

References to major U.S. Environmental Protection Agency documents and other papers that explain standard methods for sampling soil, sediment, surface water, and ground water are included.

With the development of a suitable rationale for regulatory action based on bioassay results, bioassays offer great promise as a standard site-specific method of environmental risk assessment at hazardous chemical waste sites.

ACKNOWLEDGMENTS

The technical help of Dr. Dave Thorne, Dr. Bill Trautman, and Mr. Brian Anderson of the Rocky Mountain Arsenal (Colorado) as well as the cooperation of Dr. Royal Nadeau at EPA-Edison, New Jersey were essential in accomplishing the work in Sections 8.1, 8.2, and 8.3. In addition, Dr. David Charters and members of EPA's Emergency Response Team, Edison, New Jersey, collected the samples used in Section 8.2. At the Pacific Northwest Laboratory, Stacy Freeman typed the original manuscript, Linda Parker finished the final report, and Kathy Borgeson, Steve Weiss, and Susan Kreml supplied editorial expertise.

The information in this document has been funded wholly or in part by the U.S. Environmental Protection Agency, Environmental Research Laboratory in Corvallis, Oregon, under Related Services Agreement TD 1598 with the U.S. Department of Energy, Contract DE-AC06-76RLO 1830, to Pacific Northwest Laboratory. It has been subject to the Agency's peer and administrative review, and it has been approved for publication as an EPA document.

CONTENTS

FIGURES

TABLES

1.0 INTRODUCTION

1.1 OBJECTIVES OF THIS DOCUMENT

This document provides guidance for deciding how bioassays may be incorporated into the remedial action process at a hazardous chemical waste site. It also provides aids for designing, executing, and interpreting the results of bioassay studies. Alternative bioassay study designs are discussed, and illustrations showing their use are presented. Because of the focus on hazardous chemical waste sites, information on bioassessment for other purposes is generally excluded (e.g., in situ bioassay, bioassay of receiving waters). The intent of this document is not to prescribe the way to use bioassays to help assess the toxicity of hazardous waste sites. Because specific site problems vary to such a great extent, application at any particular location will require a site-specific design depending on the waste(s), their location, and the intended use of the bioassay information. Thus, the purpose of this document is to present a description of acute bioassay studies, the background for their use at chemical waste sites, and field designs and sampling methods as well as case studies.

1.2 AUDIENCE FOR WHICH THIS DOCUMENT IS INTENDED

This document is intended for site managers, state agency personnel, and other individuals responsible for making decisions about remedial action at hazardous chemical waste sites. It can be used by readers with little experience in performing bioassay studies. The document presents an overview of the decisions that must be made to determine how bioassay studies should be included in the remedial action process and information for choosing among alternative field study designs. References are provided to guide readers to sources of additional technical detail.

1.3 WHAT IS A BIOASSAY?

A bioassay is a technique by which organisms (i.e., whole plants or animals), biological systems (e.g., tissues), or biological processes (e.g., enzymatic activity) are used to measure the biological effects of a

substance. In the context of hazardous chemical waste site management, bioassays may be defined as the exposure of biological indicators to field-collected environmental samples in order to detect the presence of toxicity and/or to identify potential for toxic effects on resident species. Typically, a hazardous waste site bioassay involves laboratory testing of soil, soil leachates, water, or sediment samples using a standard array of test organisms under controlled laboratory conditions.

1.4 ORGANIZATION OF THIS DOCUMENT

Section 2.0 summarizes the conclusions of this document.

Sections 3.1 through 3.3 present information to be used in deciding how bioassays may be used for a particular chemical waste site problem. Section 3.1 presents an overview of where and how bioassays can be used in the remedial action process. Questions that bioassays can or cannot answer are presented in Section 3.2, followed by a discussion in Section 3.3 of the advantages and disadvantages of using bioassays instead of chemical analysis to define site problems.

Section 3.4 contains a list of steps to follow in planning, executing, and interpreting the results of a bioassay study. This section also includes a discussion of the decisions to be made in designing and executing the field sampling, laboratory analyses, and data evaluation.

Section 4.0 contains a detailed discussion of the development and use of bioassays. This section is followed by explanations in Sections 5.0, 6.0, and 7.0 of the basis for choosing among alternative study designs.

Section 8.0 contains examples of bioassay studies that illustrate the information presented in the previous sections, and Section 9.0 presents a discussion of enhancements that could improve bioassay technology and directions for future research.

1.5 MEASUREMENT EQUIVALENTS

Metric units are used in this report. A conversion chart for appropriate English measurements is provided as Table 1.1.

TABLE 1.1. Measurement Conversion Chart

Measurement Used	Multiply By	For
Square mile	2.60	Square kilometers
Acres	0.40	Hectares
Meters	3.28	Feet
Centimeters	0.39	Inches
Feet	0.305	Meters
Inches	2.54	Centimeters
Kilograms	2.21	Pounds
Grams	0.035	Ounces
Liters	1.06	Quarts
Milliliters	0.03	Fluid ounces

2.0 CONCLUSIONS

Based on our review of specific bioassessment procedures and their use at four different sites, we concluded that acute toxicity bioassays are a valuable and cost-effective method for use in the remedial action process at hazardous chemical waste sites. They have two important advantages over chemical analyses: 1) they can directly measure the effects of a known or suspected contaminant on plants and animals, and 2) they are, in general, inexpensive compared to complete chemical analyses.

With the development of a suitable rationale for regulatory action based on bioassay results, bioassays offer great promise as a standard site-specific method of environmental risk assessment at hazardous chemical waste facilities.

3.0 DESCRIPTION AND USE OF STANDARDIZED ACUTE BIOASSAY STUDIES AT WASTE SITES

3.1 WHERE BIOASSAYS FIT IN THE REMEDIAL ACTION PROCESS

Bioassay studies are appropriate before, during, and after remedial action as a cost-effective way to detect the presence of toxic wastes and/or to determine their biological availability at known or suspected hazardous chemical waste sites. The diagram of the remedial action process shown in Figure 3.1 indicates the many ways in which bioassay data are useful. A detailed explanation of this process is found in Ford and Turina (1985).

Bioassay studies may be used before remedial action to detect the presence of hazardous materials and to determine if immediate remedial action is needed. Because bioassays directly evaluate the effect of chemical wastes on biota, they are powerful and efficient tools for ranking sites requiring remedial attention. Bioassay data are also useful for gauging the areal extent of needed remedial action, evaluating remedial action alternatives, and assessing and characterizing waste sites.

During remedial action, bioassay studies may be used both to monitor the cleanup process and to evaluate cleanup impacts on the site. Bioassays are also useful after remedial action has been taken to evaluate the efficacy of the cleanup activities.

3.2 QUESTIONS BIOASSAYS CAN (OR CANNOT) ANSWER

The questions that often need to be addressed in support of remedial action decisions may be grouped into four major areas: 1) Where are the contaminants? 2) Are the contaminants toxic? 3) What quantities of the contaminants are present? and 4) What are the contaminants? Specific questions under each of the four categories are listed in Table 3.1.

Bioassay studies are a cost-effective method for evaluating "where" questions because they can detect contamination, determine contaminant distributions, and define contaminant migration beyond site boundaries.

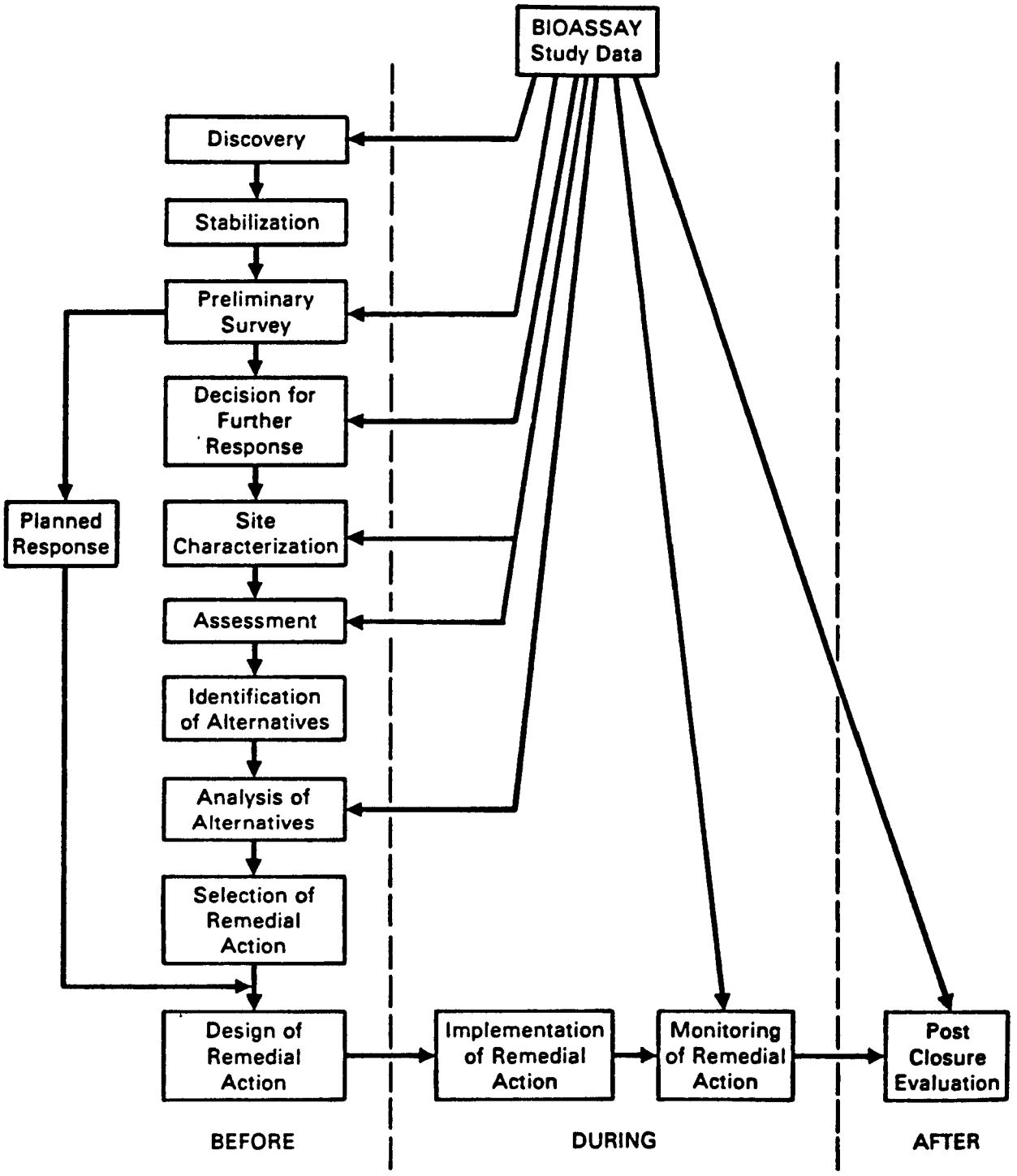


FIGURE 3.1. Bioassay Data Implementation in the Remedial Action Process

TABLE 3.1. Questions That Need to Be Addressed to Support Remedial Action Decisions at Hazardous Chemical Waste Sites

Category	Specific Questions
WHERE?	Is the site contaminated?
	Are specific areas contaminated?
	How much of the total site is contaminated?
	What is the distribution of the contaminants?
	Have the contaminants migrated off site?
TOXICIITY?	How toxic are the contaminants separately or together?
WHAT QUANTITY?	What quantities of the contaminants are present on the site (inventory)?
	Is the contaminant concentration above a prescribed limit [in parts per million (ppm) or a related measure]?
WHAT?	What is the chemical composition of the contamination?
	Is a specific chemical present on the site?
	Is a particular class of toxic chemicals (e.g., organics, metals) present on the site?

While bioassays currently are the only reliable means of evaluating the integrated bioactivity of complex chemical wastes, they usually cannot identify specific chemical contaminants present. However, they provide a quantitative indication (EC50 or LC50) of total toxicity relative to the percentage of sample water, soil or leachate that is required to produce the EC50 or LD50. In addition, they may give some indication of the class of chemicals to which the contaminants belong (e.g., organics, metals). Some examples in which the identification of specific chemicals have been attempted are given in Sections 8.1 and 8.3.

3.3 ADVANTAGES AND LIMITATIONS OF BIOASSAYS COMPARED TO CHEMICAL ANALYSES

Bioassays have two important advantages over chemical analyses. They can directly measure the effects of a known or suspected contaminant on biota, and they are, in general, inexpensive compared to complete chemical analyses.

Complete chemical analysis of a waste site does not provide information as to whether the waste will cause harm to biological organisms. For example, heavy metals may be present at a particular site in high concentrations, but may be chelated by organic compounds in soils or sediments and thus be almost completely unavailable to the biota (Thomas et al. 1984a). Conversely, presumably harmless chemicals may have toxic effects. Results of studies by Miller et al. (1985) have shown that "inert" compounds in a herbicide formulation were, in fact, active toxicants or synergistic components. Also, the toxic effects of chemical mixtures are not readily predictable from knowledge of the toxic effects of the individual chemicals (Miller et al. 1985). Chemical waste sites almost always contain a mixture of chemicals. Therefore, bioassays are valuable tools for measuring the integrated toxicity potential of waste mixtures (Roop and Hunsaker, 1985) whether chemical concentrations have been measured or in cases where chemistry of the mixture is uncharacterized. Thus, bioassays are capable of providing guidance on toxicity limitations, even in situations where federal or state environmental chemical concentration limits do not exist.

Acute toxicity bioassays are generally less expensive than conventional priority pollutant chemical analyses. Schaeffer et al. (1982) reported average analytical costs as high as $2000-$5000 per single soil sample for the U.S. Environmental Protection Agency (EPA) priority pollutants. Costs have declined to $1500-$2000 per sample,[(a)] which are closer to the average $1000-$1500 cost of conducting acute toxicity tests with algae and Daphnia. However, cost alone should not be the determinant in choosing between the use of priority pollutant chemical analyses and bioassays to define the hazard at

---

(a) Personal communication from Dr. G. B. Weirsma of Idaho National Engineering Laboratory, August 15, 1986.

waste sites. All available resources must be allocated to attain the best balance of both chemical and biological data. The concept and use of bioassays to support and/or direct chemical analyses of environmental samples is discussed by Miller et al.[a] They indicate this integrated approach, in addition to being cost effective, is the most feasible current way to define the effects of environmental variables such as solubility, pH, antagonism, synergism, and time of exposure upon the toxicity of complex chemical mixtures.

Acute toxicity bioassays are inferior to chemical analysis for evaluating the danger presented by a hazardous chemical waste when 1) no known bioassay exists for a suspected or known chemical toxicant, 2) there is a need to identify a specific pollutant (e.g., an EPA Priority Pollutant), 3) there is a need to conform to an established regulatory standard for a particular pollutant in the environment (i.e., pollutant concentration or an amount), or 4) there is a need to identify sublethal or chronic effects for a specific chemical waste.

3.4 DESIGN DECISIONS FOR ACUTE BIOASSAY STUDIES AT CHEMICAL WASTE SITES

Design of bioassay studies includes planning the collection of field samples and the laboratory analyses of those samples. It is imperative that the entire project, from objectives to expected results, be thoroughly planned before the actual fieldwork is started. Without proper planning, the study will waste both time and resources. Further, all individuals who will contribute to the project should be involved as early as possible in project planning. Personnel who should be involved at the initial stages of the project include the project manager, a statistician, a field biologist, a chemist, the scientist who will oversee the laboratory work, possibly a hydrologist, meteorologist, or modeler when appropriate, and risk assessment and Quality Assurance/Quality Control experts.

---

[a] Miller, W. E., J. C. Greene, and S. A. Peterson. 1987. Protocol For Bioassessment of Hazardous Waste Sites. 2nd Edition. Corvallis Environmental Research Laboratory, Corvallis, Oregon. Final draft.

The steps to be used in designing and executing a bioassay study are listed below. Each step is explained further in the following sections.

1. Assemble information relevant to the problem.
2. Prepare a statement of the study objectives.
3. Define the evaluation criteria and reliability requirements for the results.
4. Determine what is to be sampled in the field.
5. Choose test organisms for the bioassays.
6. Define the data analysis techniques.
7. Design the field sampling and laboratory studies.
8. Determine the sample collection methods.
9. Define the operational procedures.
10. Review the design.
11. Periodically evaluate progress in the field and laboratory.
12. Analyze and evaluate the results.

These steps are listed in the order in which they should be applied by the planning team. However, study design is an iterative process, and decisions made at later steps in the process may require the review and revision of decisions made at previous steps.

These 12 steps should be considered whether the study is an initial site investigation, a feasibility study, or a full remedial action. The amount of effort and degree of technical sophistication required for each step will vary depending on the objectives and cost of the study and the reliability required of the results. For example, in a full and potentially expensive remedial action investigation, the entire team should be involved from the beginning. During preliminary investigations, the objectives, uses of the results, and methods should be carefully planned before sampling; however, the advice of team members from each area of expertise may not be necessary. Depending on the quality of information gathered during the preliminary investigations, the results of the initial studies may be used to design the remedial action program.

3.4.1 Assemble Information Relevant to the Problem

The first step in the design of a bioassay study is to assemble and review all available information relative to the hazardous waste site. This includes the history of waste disposal to the site, regulations regarding the waste, requirements for conducting a site investigation in support of remedial action, and resources available for conducting the bioassay studies.

Information that should be obtained at each hazardous waste site includes:

1. the type of waste likely to be found on the site
2. the likely chemical and physical properties of the waste
3. the environmental media in which the waste is most likely to be found (e.g., does the waste adhere to soil particles or is it more likely to be found in the interstitial soil water?)
4. the location on the site where the waste is most likely to be found
5. the known toxicity of the waste to particular organisms or groups of organisms. Sources of the needed information include records of waste disposal, manufacturer's records, expert chemists, the chemical literature, environmental impact statements (both for the site and the chemicals), and local experts.

Each hazardous chemical waste site is unique in some respect; therefore, each site should be evaluated as a separate problem. Several site characteristics will influence the design, execution, and interpretation of the results of the bioassay study. A more thorough site study may be required if the hazardous waste poses an increased risk because of:

1. human activity on the site (e.g., farming)
2. proximity of the site to human communities
3. the possibility of contaminant transport outside and/or inside the site boundary (e.g., streams, ground water, or wind)
4. the presence of threatened or endangered species, or
5. the presence of commercially or recreationally valuable species on the site.

In addition, the presence of human activity or endangered or valuable species on the site may limit the areas from which samples may be collected. Other site characteristics (e.g., topographical) may also limit the type, number, or location of samples that can be taken during the study.

The study planning should not proceed to the field study design (Step 7) without an initial site visit. Preliminary studies may also be necessary to gather some of the information.

Documentation of the history of waste disposal to the site may provide valuable information about the types of contaminants that will be found and their probable location and distribution. Possible sources of information regarding the site disposal history include waste site records, manufacturer's records, and local experts.

It is also necessary to review federal and state regulations regarding the known or suspected waste materials. The texts of the Toxic Substances Control Act (TSCA), the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), and the Resource Conservation and Recovery Act (RCRA) should be consulted for guidance to the federal regulations regarding individual hazardous substances. The EPA hotline (1-800-424-9346), a good source for the most recent information concerning federally regulated waste materials, is operated during normal business hours (Eastern Standard Time). Some, although not all, states have their own regulations regarding waste substances in the environment. The EPA hotline, state environmental agencies, and regional EPA offices may provide information about or access to documents containing state regulations.

While this document provides guidance for deciding whether bioassays are appropriate for a waste site problem and for planning and carrying out appropriate field and laboratory studies, it does not cover all aspects of conducting a site study in support of remedial action. The reader should consult Ford and Turina (1985) for this information.

Finally, a realistic appraisal must be made of available study resources in order to preclude designing a study that is too expensive to complete within the available budget or manpower. An elaborate study poorly or incompletely done will yield less reliable information than a completed but

smaller scale study. Further, most of the statistical analyses that are used to describe bioassay results will either be questionable or invalid if significant amounts of data are missing. The relationship between anticipated costs and the available resources should be considered in every stage of design planning.

3.4.2 Prepare a Statement of the Study Objectives

It is critical that an unambiguous statement of the study objectives be prepared and understood by all of the project staff before the study begins. This will minimize confusion and wasted effort during the design and execution of the field and laboratory work.

Each objective must be phrased as a specific question. The advantage of specific questions is that they can generally be studied as testable, statistical hypotheses. A question such as "Does the waste site pose a threat to the adjacent community?" is not acceptable because there are many different ways of evaluating the impact of the hazardous waste on the community. Examples of questions that can be answered or tested are "What is the spatial distribution of the contaminant in the soil?" or "Is the concentration of the contaminant in the ground water measured in onsite wells significantly different from that measured in offsite wells?" The reader is referred to Table 3.1 for additional examples of specific questions that can be answered or easily modified into testable hypotheses (depending on site problems).

3.4.3 Define the Evaluation Criteria and Reliability Requirements for the Results

Bioassay results are expressed as the percentage of test organisms affected at various dilutions of soil, water, or sediments sampled at the site. An EC50 or LC50 is often calculated from a series of such dilutions. This parameter represents the concentration of field-collected material at which 50% of the organisms are affected. Either the EC50 (percent affected; e.g., percent inhibition of lettuce roots) or, alternatively, LC50 (percent mortality) resulting from testing the dilutions of the site samples may be used as a measure of toxicity of a substance. Depending on the study objectives, either measure of toxicity may be used for: 1) comparison to a

critical value which, if exceeded, indicates the need for further action; 2) comparison to the toxicity of samples from a "clean" site; or 3) mapping the contaminant distribution and/or bounding the areas of the site at which the toxicity of the samples is greater than some critical value, indicating that further action may be necessary. At this point in the study planning, the project manager must define the critical value, or determine the magnitude of the difference in toxicity between "clean" and possibly contaminated sites, that will indicate further analysis or remedial action is necessary. Porcella (1983) states that an EC50 caused by a 1:5 dilution (i.e., 20%) of the bioassayed sample would generally be cause for concern. However, his choice of this value was arbitrary and may be too high in some situations (see Section 9.3). The critical value should be evaluated separately for each problem, based on site circumstances. Thomas et al. (1984a) found results from tests of undiluted material useful for preparing site "toxicity maps."

The project manager must also decide on the reliability requirements for the results. For this purpose, consultation with the project statistician is strongly recommended. The statistician can aid in defining the required precision and accuracy. A major factor in determining needed precision and accuracy is the use that will be made of the study results. At one extreme, the reliability (precision) and accuracy of data must be well known if it is to be used in litigation. In contrast, the reliability requirements for results of preliminary or pilot studies are not as stringent. The field and laboratory work must be designed to meet the necessary requirements.

The results from bioassays contain a certain amount of "inherent" variability, or "noise." The "noise" is a result of the natural variability of similar field samples, variability in field conditions and sampling techniques when the samples are taken, and variability in sample handling and analysis in the laboratory. These sources of variation are discussed further in Section 6.1. Qualified laboratories routinely calibrate their analytical instruments, to achieve precise and accurate results. Field and laboratory handling procedures can be standardized. Therefore, most of the "noise" is introduced by the natural variation between field samples. Thus, improvements in the field sampling design have the largest impact on increasing the precision and accuracy of the results.

Unless the entire site is sampled, there is some probability that the results based on the samples collected will lead to an under- or overestimation of the extent of the chemical contamination. To determine the desired level of accuracy, the project manager must 1) evaluate the risk posed by underestimating the amount or distribution of the contaminant and thus failing to take appropriate remedial action, and on the basis of this, decide on an acceptable possibility of missing a "hot spot"; and 2) evaluate the cost of performing unnecessary remedial action, and on that basis, decide on an acceptable probability of failing to detect "clean areas."

Finally, the project manager must determine the desired level of precision for the bioassay results. Precision is a function of the "noise" in the data and the number of samples analyzed, and is usually expressed as a standard deviation or confidence interval around the EC50 or LC50. When the objective of the study is to compare the site toxicity to a critical value or samples from a "clean area," the level of precision should be such that uncertainty about the toxicity measure does not impair the ability of the test results to clearly establish the relationship between the site toxicity and the critical value or toxicity of "clean" soil.

When mapping contaminant distributions, the precision of concentration contours estimated by kriging (described in Section 5.3) should be such that the contours are located unambiguously with respect to critical site areas (such as the waste site boundaries or the habitats of endangered or valuable species), or do not significantly under- or overestimate the size of areas that may require further attention. Precision can usually be increased by collecting and/or analyzing a larger number of samples. If there is a preliminary estimate of expected variability in the data, a qualified statistician can calculate the number of samples required to achieve the desired level of precision and power for discriminating between clean and contaminated areas. Precision may also be increased by reducing the "noise" in the data by exerting more careful control over the field and laboratory handling procedures.

3.4.4 Determine What Is to Be Sampled in the Field

The choice of which medium or media to sample in the field (i.e., soil, soil water, surface waters, ground water, or air) depends on the objectives of the study. The medium might be chosen to maximize the capability of detecting contamination on the site or because it is the medium that poses the greatest threat to humans or other organisms. The medium or media chosen for sampling should be either: 1) the ones to which the contaminants were known or suspected to have been disposed, 2) the one(s) to which the contaminant(s) are likely to migrate or adhere to as a result of their chemical or physical properties, 3) the one(s) that are most likely to be in contact with humans or rare, endangered, or commercially or recreationally valuable species, or 4) the one(s) that are most likely to transport the contaminant(s) within or off the site. Thus, the decision should be made only after consultation with the project biologist, chemist, hydrologist and/or meteorologist, and risk assessment expert.

3.4.5 Choose the Test Organisms for the Bioassays

Several species should be used to test each sample because of the differences in tolerance to certain substances between the different organisms (Thomas et al. 1984a). Porcella (1983) outlined a set of standard procedures for conducting bioassay tests on terrestrial and aquatic samples. He recommended using a freshwater algae (Selenastrum capricornutum), a daphnid (Daphnia magna), and a freshwater fish (the fathead minnow, Pimephales promelas) to assess toxicity in soil elutriates and water samples. Soil and sediment samples are tested using soil microorganisms, common plant seeds, and earthworms. Kenaga (1978) compared the acute toxicity test responses of a variety of terrestrial and aquatic organisms to 75 insecticides and herbicides, and recommended that one species of fish (including the fathead minnow), one species of aquatic arthropod (including Daphnia magna), and laboratory rats (Rattus norvegicus) be tested to give the range of responses representative of a variety of aquatic and terrestrial species, including mammals.

Bioassay tests should be conducted using a suite of organisms. The organisms listed in Porcella (1983) and Kenaga (1978) are generally easy to

obtain and culture, represent important levels in the ecological food web, and are widely used in testing, so that the test results may be comparable to those obtained from tests using other substances and these same organisms. In addition, these organisms are common and thus the tests are likely to be less expensive.

Nonstandard organisms may be appropriate for bioassays if 1) standard organisms have previously shown no response to the known or suspected contaminants, 2) the response of a particular organism, not included in the standard list, will be more specific for the suspected contaminants, or 3) the response of a specific organism, not included in the standard list, is needed. However, tests using nonstandard organisms are likely to be more expensive because of difficulties in obtaining, culturing, and standardizing the new bioassay (including quality assurance procedures). In addition, extensive preliminary testing using the nonstandard organism may be required to establish reliability. Some of the problems and additional costs can be identified in consultation with the field biologist and the scientist supervising laboratory bioassay tests.

3.4.6 Define the Data Analysis Techniques

The choice of data analysis techniques depends on the study objectives and will determine the number and location of the field samples collected for laboratory analysis. This decision should be made in consultation with the project statistician. Methods of data analysis applicable to chemical waste site problems are discussed in Section 5.0.

3.4.7 Design the Field Sampling and Laboratory Studies

The approach to the bioassay study and the design of the field sampling and laboratory analysis depend on the study objectives and reliability requirements. These choices may also influence the data analysis technique. Decisions regarding the study design should be made in concert with the project statistician, the field biologist, and the scientist supervising the laboratory work. Section 6.0 provides some guidance for developing an overall strategy for allocating available resources to the field and laboratory work to maximize the reliability of the results and minimize the study costs. Alternative field sampling designs are discussed in Sections

5.0 and 6.0. Procedures for conducting bioassays are in Porcella (1983), and are not repeated here. Finney (1978) presented alternatives to the statistical estimation of EC50s and LC50s included in Porcella (1983). These alternative methods may be needed if a standard deviation or confidence interval is required for the EC50/LC50.

3.4.8 Determine the Sample Collection Methods

Methods for collecting soil, groundwater, surface water, and air samples are presented and discussed in Ford et al. (1984) and are not repeated here. Some modification to the sampling devices and procedures described in Ford et al. (1984) may be necessary because of particular characteristics of the waste site. In addition, it should be noted that all sample collection methods are in some way selective and operate with varying efficiencies under different conditions. Therefore, the collection devices should be thoroughly tested, before the fieldwork is begun, under conditions that approximate those at the site.

The amount of sample collected should be at least threefold (where possible and not cost prohibitive) that required for the laboratory analysis to allow for repeated analysis of samples with unusual results or the loss or damage of the samples. In addition, it may be prudent to archive a portion of the sample to answer additional questions that may be raised after study completion. Finally, extra samples allow additional compositing decisions to be made long after field samples are collected and screening assays have been interpreted (see Section 8.3).

3.4.9 Define the Operational Procedures

Several operational considerations must be taken into account when designing and executing the field and laboratory work for a bioassay study. Ford and Turina (1985) provide guidance for establishing operating procedures for hazardous waste site investigations. Appropriate quality assurance and quality control procedures need to be established for field sampling and laboratory work before the study begins. Chain-of-custody procedures for samples need to be established. In addition, worker safety must be ensured both on the field site and in the laboratory.

Finally, the project manager must consider the possible impacts of the field sampling procedures to the hazardous site and surrounding areas. Extensive field sampling may interfere with physical or biotic features and thereby the functioning of the site, or may mobilize certain toxins. Consultations with chemists, biologists, waste site management personnel, and public officials in surrounding communities are recommended.

3.4.10 Review the Design

Before field sampling is started, the study design should be reviewed again and the following questions should be addressed:

- Is there sufficient information about the problem to design a cost-effective study?
- Is a preliminary study needed to obtain sufficient information?
- Will the final results meet study objectives?
- What constraints do the statistical, sampling, and laboratory methods impose on the interpretation of the results?
- Are there sufficient resources available for the study to obtain the desired precision?

Depending on the answers to these questions, portions of the study may need to be redesigned. This process should continue until a satisfactory design is achieved.

3.4.11 Periodically Evaluate Progress in the Field and Laboratory

No matter how carefully the study was planned or how thoroughly the methods were tested in advance, it is likely that adjustments will be necessary in both field and laboratory procedures during the course of the study. Some procedures may turn out to be impractical or otherwise unsatisfactory, while additional sampling or bioassay tests may be suggested by preliminary results. Therefore, it is important to continually monitor and evaluate current results from each phase of the study. However, a statistician should be consulted before changes are made in either the field and/or laboratory procedures to be sure that the results from the entire study can still be interpreted as planned.

3.4.12 Analyze and Evaluate the Results

The quality and completeness of the information obtained from the study should be examined. The following questions should be answered on the basis of final study results:

- Do the data meet the precision requirements established at the beginning of the study?
- Do the results satisfy the objectives of the study?
- Are there indications that further experimental work is needed? In addition, the information obtained during the study should be assessed to determine its usefulness for design of additional studies at the same site and at other sites.

Finally, the data from the study will need to be interpreted in the context of risk assessment and waste management decisions. To accomplish this, consultation with experts in the area of risk assessment is recommended. A discussion of risk assessment is beyond the scope of this document, but is discussed in Douglas (1985). If done correctly, the study should provide valuable input into decisions about possible remedial measures.

4.0 BACKGROUND FOR ACUTE BIOASSAY USE AT CHEMICAL WASTE SITES

4.1 INTRODUCTION

Historically, bioassays have been used to establish chemical criteria values for specific purposes (i.e., to determine freshwater, drinking water, and air quality standards). These criteria, for the most part, were developed with single pure chemical dose-response bioassays conducted in a well-defined medium under controlled laboratory conditions. A major criticism of these tests is that generally organism response to environmentally derived samples that contain complex chemical mixtures cannot be predicted from individual bioassays using the specific chemical components. However, the problems are more severe when the toxicity potential of a waste is estimated from Priority Pollutant content instead of bioassay results. Reliance on laboratory-derived chemical criteria to predict toxicity of field samples introduces the following potential problems and concerns:

- The data bases for most chemicals are not complete, so reliable criteria are unavailable.
- Most chemicals for which reliable criteria have been developed are not commonly found at chemical waste sites.
- The application of laboratory-derived criteria to complex field samples usually results in conservative and, therefore, overly restrictive toxicity estimates and sometimes misinterpretation because of the lack of knowledge about cause-and-effect relationships.
- The Water Quality Criteria Documents (Federal Register, 1980) cautioned against additive use of a single chemical criterion by stating "It is impossible in these documents to quantify the combined effects of these pollutants and persons using criteria should be aware that site-specific analyses of actual combinations of pollutants may be necessary to give more precise indications of the actual environmental impacts of a discharge."
- There are no criteria for contaminated soils and sediments on which to base decisions about environmental hazards.

Even though about 19,000 uncontrolled waste sites have been inventoried, it is unrealistic to assume that each of these sites can be thoroughly investigated. Costs to identify specific individual priority pollutant chemicals and associated sampling efforts become prohibitive. Bioassays, on the other hand, can be used to screen for potential hazard within and between waste sites. Early research established that standard test organisms could define the toxicity potential of complex chemical mixtures in waste site samples. The response of a living organism to a complex chemical mixture integrates the effects of environmental variables such as solubility, pH, antagonism, synergism, and time of exposure, all of which affect test-organism toxicity. In effect, the bioassay produces a direct estimate of the environmental toxicity of a sample regardless of the causal factors. Bioassays can also be performed on composited samples in order to reduce per sample costs.

4.2 DEVELOPMENT OF BIOASSAYS FOR WASTE SITE STUDIES

Concern about the pitfalls of chemically derived criteria led to the development of a biological assessment protocol for evaluating the environmental hazard potential of waste site contaminants. In October 1981 a workshop was conducted to discuss the conceptual basis, ecological factors, and regulatory requirements that would influence the development of a hazardous waste biological assessment protocol. The attendees also considered the National Contingency Plan prioritization, cleanup, field application, and evaluation procedures. The resulting protocol (Porcella 1983) contains standardized aquatic and terrestrial bioassays that have been used to define the toxicological properties of inorganic and organic chemicals. The bioassays included in Porcella (1983) are an alga (Selenastrum capricornutum), a macroinvertebrate (Daphnia magna), seed germination/root elongation (in lettuce, Lactuca sativa), an earthworm (Eisenia foetida), and fathead minnow larvae (Pimephales promelas). In addition, the Microtox (Photobacterium phosphoreum) microbial (Beckman 1982) and Neubauer seed germination (Thomas and Cline 1985) bioassays are often used. Ongoing research studies have been designed to: 1) ascertain the ability of bioassays to define the areal extent of contamination at waste

sites (Thomas et al. 1984b); 2) determine the predictive capability of bioassays in ide waste site ecological impact zones (Thomas et al. 1984c); and 3) define the ability of sensitive laboratory test organisms in measuring and/or monitoring the effectiveness of cleanup operations (research in progress).

4.3 SENSITIVITY, APPLICATION, AND INTERPRETATION OF WASTE SITE BIOASSAY RESULTS

4.3.1 Bioassays Using Pure Chemicals

Available research using pure chemicals suggests that the algal (Selenastrum) and Daphnia bioassays are the most sensitive to heavy metals, followed in order of decreasing sensitivity, by Microtox, lettuce root, and earthworm bioassays (Miller et al. 1985; Thomas et al. 1986). Root elongation (lettuce root bioassay) is the most affected by the herbicide 2,4-D, followed in increasing order of tolerance by algae, Microtox, Daphnia, and earthworms. Herbicides other than 2,4-D were not tested. Algae and Daphnia are the only organisms sensitive to the insecticides aldrin, dieldrin, chlordane, and heptachlor. These results indicate that algae might be the most broadly sensitive test organism for assays using site soil or sediment elutriates and surface-water or ground-water samples, since they were inhibited by water solutions of all the major chemical subgroups that have been studied. A larger group of chemicals must be tested to ensure the generality of this finding.

Thomas et al. (1986) found that a commercial formulation of the herbicide 2,4-D was more toxic than the pure 2,4-D acid. This differential response shows that bioassays can identify additional (or perhaps synergistic) toxicity in chemical mixtures. In addition, these results indicate that a potential problem may exist if toxic effects for pure chemicals are used to estimate the toxicity of commercial formulations containing "other inert ingredients." Based on its 2,4-D content, the commercial product should have had minimal effects on aquatic organisms. However, laboratory bioassays of this commercial chemical formulation (as it would be used in the field) indicate the material was more toxic than its active ingredient. Decreased algal and Daphnia EC50s obtained with commercial aldrin and endrin also suggested the presence of a toxic

constituent not present or bioactive in the chemically pure reference standards (Miller et al. 1985). These results demonstrate the ability of algae and Daphnia to define differences in chemical toxicity caused by formulation differences and/or other impurities. This supports speculation that the "inert components" might themselves be toxic or synergistic. Based on the foregoing studies using pure chemicals, it appears that a multimedia bioassessment protocol can distinguish among a variety of relatively subtle differences in the chemical composition of complex mixtures. Extension of this observation to environmentally derived samples permits the use of the bioassay procedure to identify the presence of a toxicant, regardless of the specific chemical content of the sample.

4.3.2 Bioassays Using Waste Site Samples with Known Chemistry

The responses of selected test organisms to hazardous waste site soils, soil elutriates, and ground water in which major contaminants have been chemically identified are shown in Table 4.1.

An evaluation of the EC50 values in this table indicates how each of the waste sites can be ranked in order of their relative toxicity. However, caution should be exercised when using these values as primary decision guidelines. For example, it is apparent that the oil slop, drilling fluid, and wood preservative wastes (waste samples from Ada, Oklahoma; see Table 4.1) are highly toxic to all the organisms tested and, because they are toxic at very low levels, may constitute an environmental hazard. Both the United Chrome ground-water and Olin soil elutriate samples are also obviously hazardous. When potential aquatic impacts are of primary concern, the algae, Daphnia, and Microtox tests are the most applicable bioassays because these tests use aqueous soil elutriates or water. The earthworm bioassay (and also the Neubauer bioassay; Thomas et al. 1984c) can be used to define the environmental hazard of non-water-soluble contaminants. For instance, both the Thiokol and LaSalle earthworm soil contact results, in concert with the other bioassays, show a potential for environmental damage but little apparent potential for transport via water.

Differential responses to the same major chemical components in various waste site samples are also shown in Table 4.1. For example, the response of

TABLE 4.1. EC50 Response in Percent Soil for Earthworms and Percent Elutriate for Other Organisms Exposed to Chemical Contaminants in Hazardous Waste (Ada Samples Only), Waste Site Soil, Soil Elutriate, and Ground-Water Samples

		Bioassay Response (EC50)				
Waste Site	Major Contaminants	Algae	Daphnia	Microtox	RE[a]	Earthworm
Western Processing						
No. 1	Heavy metals	28	69	NE[b]	NE	NE
No. 11	Heavy metals, solvents, phthalates	1.8	10	29	41	77[c]
No. 17	Heavy metals, phenols, solvents, pesticides	0.2	5.6	2.2	37	55
No. 22	Heavy metals, phthalates, solvents	22	80	11	NE	NE
Hollywood	Pesticides	24	22	90	NE	25
Holder Chemical	Pesticides, herbicides	2.1	3.6	18	3.6	70
Big John Houldt	PAH[d], other organics	5.4	87	28	NE	10
Sapp Battery	Heavy metals	41	70	NE	NE	NE
Thiokol	Diphenylamine	NE	NE	NE	NE	35
Sharon Steel	Heavy metals, tar, PAH	0.6	30	99	NE	75
Rocky Mountain Arsenal No. 92	Heavy metals, insecticides, organosulfur compounds	6.4	25	3	61	<5
Eddystone Arsenal	Heavy metals	12.8	58	NE	NE	NE
Time Oil Well 12A	Trichloroethane solvents	20	85	91	NE	NP[e]

TABLE 4.1. (contd)

Waste Site	Major Contaminants	Bioassay Response (EC50) Algae	Daphnia	Microtox	RE[a]	Earthworm
Ada (waste samples)						
Oil Slop	2,4-dimethyl phenol, phthalates	0.03	0.02	0.13	4.3	NP
Drilling Fluid	Heavy metals, phthalates	0.07	0.51	0.46	2.5	NP
Wood Preservative	Phenol, creosol, PCP	0.04	0.22	0.05	0.59	NP
Olin	Pesticides	0.10	14	15	4.5	10.2
Nease Chemical	Pesticides	99	NE	13	NE	NE
United Chrome Shallow Well No. 1	Chromium	0.03	0.05	8.5	0.6	NP
Hogtown Creek	Phenol, 2,4-dimethyl phenol	11	24	11	-(47)[f]	24
LaSalle	PCB	NE	NE	NE	NE	5-10
Number of Tests Where an EC50 Was Obtained		19	18	16	8	11
Total Number of Tests		21	21	21	21	16
% of Total Tests Where an EC50 Was Obtained		90	86	76	38	69

(a) RE = lettuce seed root elongation test.

(b) NE = no observable toxic effect at 100% concentrations of soil, ground water, wastes, or soil elutriates.

(c) Earthworm 14-day soil test EC50 values.

(d) PAH = polynuclear aromatic hydrocarbons.

(e) NP = test not performed on this sample.

(f) -( ) = % inhibition in 100% test sample when an EC50 value was not obtained.

algae to the heavy metals in the Western Processing No. 1 sample is about midway between those of the Sapp Battery and Eddystone Arsenal samples (other bioassay results contributed little additional information). All of these samples were predominantly contaminated with metals. However, differences in solubility, pH, ionic strength, organic content, etc. may have affected sample toxicity to different degrees, in addition to actual differences in metal concentrations. Based on algal toxicity, the rank order of these metal-contaminated sites is Eddystone > Western Processing No. 1, which is > Sapp Battery (when the statistical error of the EC50s is ignored). The bioassays of Western Processing No. 11 and No. 22 soils indicate that earthworms may not be greatly affected by phthalates or the metals and solvents present. However, as stated previously, earthworms were the only test organism to exhibit a toxic response to diphenylamine and PCBs in the Thiokol and LaSalle soil samples. Thus, bioassay response can be used to define toxicity potential even when specific chemical information is not available. The results from the pesticide-contaminated Hollywood, Olin, and Nease chemical waste sites reinforce this observation.

The results shown in Table 4.1 indicate that a suite of test organisms can be used to define the toxicity of complex waste mixtures so that sites containing chemicals that are most toxic can be easily ranked for remedial action investigations. Moreover, differences in bioassay response among test organisms could also aid in the prioritization of additional tests to provide the most information at the least cost. A planned sequence of preliminary bioassay analyses at single or multiple sites could be used to define the most probable impact areas and identify those waste sites that require extensive chemical and biological investigation.

Algal toxicity caused by water-soluble contaminants was observed in 90% of the waste site samples (see Table 4.1). The lettuce root elongation test only exhibited a toxic response in 38% or 8 of 21 samples assayed, whereas Daphnia, Microtox, and the earthworm soil contact tests showed toxicity in 86%, 70%, and 69% of the waste site samples, respectively. In addition to detecting a large fraction of toxic samples, algal bioassays were usually also the most sensitive (i.e., their respective EC50s were generally lower) Thus, this assay may be the most useful to screen sites. Based on the sar

criteria, Daphnia and Microtox could also be good candidates to screen for environmental hazards.

4.3.3 Bioassays Using Waste Site Samples with Inferred Chemical Constituents

A field study was conducted by Thomas et al. (1984a) at the Rocky Mountain Arsenal near Denver, Colorado. The site had been used for the manufacture of antipersonnel gases, herbicides, and insecticides and as an ordnance testing area. Over the years, myriad organic and inorganic compounds were carried through ditches to a series of interconnecting holding basins for disposal.

Grab samples of soils and water from the arsenal were used to ascertain whether bioassays (including the Neubauer lettuce seed germination test; Thomas and Cline 1985) would respond to the unknown chemical mixtures contained in these samples. The Neubauer test was added to the bioassay series because the earthworm soil contact test was not very sensitive to pure chemicals (see Section 4.3.1). Addition of this assay allowed a better comparison of the toxic properties of soil elutriates and the soils themselves. Because of the known high salt content in the water at one site basin, preliminary wheat and lettuce seed germination bioassays were conducted (Thomas and Cline 1985). These assays showed that copper, sodium, nickel, or arsenic, singly or in combination, were not toxic at levels found in the basin water.

Bioassays of soil elutriates from site 085 indicated that the algal assay was approximately 10 times more sensitive than the Daphnia assay (Table 4.2). No response was observed for Microtox and lettuce root elongation, while the earthworm soil test for sample 085 had an EC50 value >25%. Such results are typical of those for low levels of heavy metals (see Section 4.3.1). In contrast, soil sample 092 (from a lime pit) showed a different response pattern of increased toxic sensitivity to Daphnia, Microtox, lettuce root elongation, and earthworms, suggesting a stronger influence from the organic components in this sample. Both the basin waste water (a holding basin for toxic wastes) and a sample of well water (located in another part of the same area) were toxic to most organisms tested. Basin

TABLE 4.2. EC50 Response or Percent Inhibition Caused by Chemical Contaminants in Rocky Mountain Arsenal Soil, Soil Elutriate, Waste Water, and Ground-Water Samples

Rocky Mountain Arsenal Sample Number	Algae[a]	Daphnia[a]	Modified Microtox[a]	RE[b]	Neubauer	Earthworm
085	8.3	86	NE[c]	NE		>25[d]
092	6.4	25	3.0	61		>5
Basin Water	0.002	0.003	0.006	1	0.5[e]	
Basin Well Water	27	21	NE	12		
1-5	S[f]	72	NE	72[g]	91[g]	62
6	S	94	NE		100[g]	55
7	NE	NE	NE	32	100[g]	<25
8	S	NE	NE	19	92[g]	58
9	S	NE	NE	26	13[g]	NE

(a) EC50, % elutriate or % water.

(b) RE = lettuce root elongation test, EC50 % elutriate or % water.

(c) NE = no biologically significant toxicity was observed.

(d) Earthworm 14-day soil test EC50 values, % soil.

(e) LC50 value in % basin F water.

(f) S = growth stimulation.

(g) 72/100 = 72% inhibition of lettuce root elongation in 100% soil elutriate or seed germination in 100% soil (Neubauer test). Neubauer results are the mean of three replicates of 40 seeds each.

water was toxic to all organisms tested, with EC50 values of <1.0%, while basin well water was much less toxic to algae, Daphnia, and lettuce root elongation, and was not toxic at all in the Microtox assay. The assay results for soil elutriates from samples 1 to 9 are unique in that, except for site 7, these soil elutriates stimulated algal growth rather than depressing it. This lack of an algal toxic response was partially corroborated by low toxicity obtained with Daphnia assays, which showed an EC50 of 72% elutriate for samples 1 through 5 (subsamples of a single large sample collected adjacent to 085, but 1 year later) and site 6 (EC50 of 94%). In contrast, lettuce root elongation results based on 100% soil elutriates (samples 1 through 8) were highly toxic as assessed by percent inhibition (footnote g, Table 4.2).

The earthworm and modified Neubauer soil contact bioassays for the same samples confirm some of the lettuce root elongation results and, unlike the other soil elutriate tests, show the presence of toxicity at sites 1-8 and low toxicity at site 9. Earthworm EC50s ranged from <25% to 62% soil for the same samples. The sample from site 7 was the most toxic (Table 4.2).

A comparison of the soil elutriate and earthworm and lettuce seed soil contact bioassay results based on the Rocky Mountain Arsenal samples suggests the following: 1) These soils contain very low levels of water-soluble heavy metal and pesticide contaminants; 2) if the heavy metals are soluble, they bind to organic compounds so that they are not available and thus are not toxic to algae, Daphnia, and Microtox; and 3) the water- soluble toxic components in these soils are leached as a function of time or are strongly adsorbed by the clay and organic fractions, but are available to earthworms and lettuce seeds.

Thus, in bioassays of the Rocky Mountain Arsenal samples of which the contaminant history was unknown, soil contact bioassays showed that earthworms and lettuce seeds were the most negatively affected, while elutriates of the same soil stimulated algal growth. The lettuce root elongation test, using undiluted soil elutriate, also indicated the presence of toxic components, but was less sensitive than the lettuce seed solid-phase soil contact test. These results suggest (in the absence of chemical

analyses) the presence of low levels of water-soluble metals and possibly pesticides.

5.0 STATISTICAL TECHNIQUES FOR WASTE SITE BIOASSAY STUDIES

This section contains descriptions and discussions of statistical techniques useful for estimating the location, distribution, and toxicity of known or suspected contaminants on hazardous chemical waste sites. These techniques address the "Where?" and "Toxicity?" questions for which bioassays are well suited (Table 3.1). Section 5.1 contains a short discussion of the role of prior information in the design and analysis of bioassay studies, followed in Section 5.2 by a description of techniques for detecting contamination and methods for comparing site toxicity to a regulatory "critical" value or, alternatively, to that of a "clean" area. Finally, a discussion of techniques for mapping the distribution of toxic materials, on or off the waste site, is presented in Section 5.3.

5.1 THE ROLE OF PRIOR WASTE SITE INFORMATION IN BIOASSAY STUDY DESIGN

Any prior information on the source or location of contamination can greatly improve the efficiency of the field sampling strategy and thereby increase the precision and accuracy of the results. When no reliable estimates can be made of the waste source(s) or boundaries, generalized field sampling schemes must be employed. Although a well-designed general sampling plan should be capable of detecting and estimating the distribution of toxic substances, it may not yield sufficiently precise estimates for some important sites. Also, sample analyses may show that a large sampling effort was unnecessarily directed to areas of little or no interest. With some previous knowledge about the expected contaminant distribution, the allocation of sampling effort can be optimized to focus on those areas in which precise estimates of toxicity are needed.

5.2 ANALYSIS TECHNIQUES FOR DETECTING CONTAMINATION AND ESTIMATING ACUTE TOXICITY

Bioassay studies designed to answer "Where?" and "Toxicity?" questions usually involve determining if toxicity in specific hazardous waste site areas exceeds prescribed limits. For this purpose, samples may be collected according to the most appropriate sampling design discussed in Section 6.3,

based on the study objectives and the amount of prior information available about the source or distribution of the contaminant. Confidence interval techniques and compositing are two different approaches that may be used to statistically evaluate the results.

In order to use confidence interval techniques, individual samples must be analyzed and an estimate of the mean toxicity, usually an EC50 or LC50 or the average mortality resulting from diluted material, must be calculated for the whole site or separately for subareas within the site. The variance and 95% confidence intervals are then calculated for these means. Methods for calculating the variance and confidence intervals around several mortality estimates are described in most standard statistical texts (e.g., Snedecor and Cochran 1967). The estimated mean and variance may be used to compare the site toxicity either to a regulatory "critical" value or to the estimated mean toxicity of samples taken from a "clean" site.

If, when comparing the mean site or subsite toxicity to a critical value, the 95% confidence interval around this mean toxicity does not include the critical value, the site or subsite toxicity is clearly distinguished from the critical value and further analyses may be unnecessary. On the other hand, when the critical value is contained within the confidence interval, more samples are needed to obtain a narrower interval or the site and critical values cannot be differentiated. This is equivalent to performing a statistical t-test to decide whether the site or subsite mean is significantly different compared to the critical value. For example, a mean EC50 of 40.99, with 95% confidence intervals extending from 19.65 to 63.40, is clearly distinguished from a critical value of 80. However, an EC50 of 59.99, with 95% confidence intervals extending from 38.95 to 82.40, cannot be declared significantly lower than a critical value of 80. Thus, further sampling and or analysis will be necessary to more clearly define the relationship between the measured and critical values.

The variance estimates are required for the statistical comparison of the mean sample toxicity between "clean" sites and those sites suspected of contamination. For example, it may be appropriate to take remedial action if an EC50 from a suspected contaminated site is significantly lower than an EC50 from a clean site. Moreover, the same data can be used to estimate the

power of the statistical test to discriminate between clean and contaminated sites. Additional sample analyses might be required to increase the power of the test if the results do not meet the established reliability criteria for the study.

Analysis of variance techniques can be used to statistically compare the means from two or more sites. Formulas for calculating the power of the F-tests embedded in the analysis of variance can be found in most standard statistical texts (e.g., Snedecor and Cochran 1967). In some cases, the statistical significance of differences in toxicity between samples from "clean" sites and possibly contaminated sites is not as important as obtaining estimates of the magnitude of the difference between the sites. For example, it may be appropriate to take remedial action if an EC50 from the suspected contaminated site is one-quarter or one-half that from a control site or some other such site-specific relationship. The variances for the two calculated mean values may be used to construct confidence intervals around the differences between the two means as a way to gauge the estimated precision of the toxicity estimates.

Compositing involves combining portions of several field samples into a single or composite sample. Samples from the entire site may be composited, or separate composites can be made from samples from different areas of the site. The component samples of any composite whose toxicity exceeds the critical value (generally relaxed to account for dilution of toxicity of single-composite components) may be analyzed separately, while the constituents of the composites not exceeding the critical value will be ignored in further analyses. In this way, whole areas without appreciable toxicity can be identified with a minimum number of analyses.

In order to use composited samples, several assumptions are needed. Among these are 1) complete mixing of components occurs; 2) there is a linear bioassay response to increasing waste concentration; and 3) no chemical or physical interaction occurs during the mixing of the composite components. Adequacy of mixing procedures can be evaluated by using replicate bioassays on the same composite, while linearity can be tested using several components that contain known, but graded, amounts of toxicants. Unfortunately, known

compounds may not be the ones of greatest interest because the chemical composition of waste site samples is often unknown. However, for those samples with a few known contaminants (based on information obtained as suggested in Section 3.4.1), the likelihood of interactions can be estimated using experimental results from the aquatic toxicology literature. Compositing dilutes sample contaminates, and this can cause failure to detect one or more toxic samples mixed with "clean" samples. The following "rule-of-thumb" for determining the maximum number of samples that should be combined into a composite is adapted from that proposed by Skalski and Thomas (1984) for chemical analyses. The maximum number of samples that should be grouped into a single composite (n) is

$$n \leq \frac{MAL}{MDL}$$

where MDL is the Minimum Detection Limit for the bioassay tests, usually estimated from the effect rate measure among control samples, and MAL is the Maximum Acceptable Limit for the contaminant, or the percentage of bioassay effect that indicates further or remedial action may be necessary.

Ordinarily, composite samples are subsampled for laboratory analysis. The toxicity value obtained from the composite subsamples is a good estimate of the mean toxicity of the component samples, so long as the composite sample was thoroughly mixed before the subsamples were removed. However, the analysis of the composite sample supplies no information about the variability among component samples, some of which may have extreme concentration values. Therefore, variances and confidence intervals cannot be calculated for the average toxicity value estimated from results based on a single composite sample. The individual samples must be analyzed if these variance estimates are needed.

In normal analytical chemistry work, detection limits present problems, especially for cancer-causing chemicals or materials that must be completely removed from the environment (e.g., no lower limit). Thus, statistical questions can and do arise about very low environmental concentrations (e.g., 1.05 ppm when the detection limit for the instrument or procedure is 1 ppm). Frequently, a particular chemical procedure calls for a control blank (all

chemicals and procedures used as if an actual sample were present) to be subtracted from actual sample results. The result of the subtraction can be a negative number [2 ppm (sample) - 2.5 ppm (control) = -0.5 ppm] or below the detection limit [below 1 ppm (sample) - 1.5 ppm (control) = -0.5 ppm or more] in an uninterpretable way. Gilbert and Kinnison (1981) addressed these problems for low levels of radioactivity.

At this point in the development of bioassays for assessing hazardous chemical waste sites, detection limits per se have not been a problem for two reasons. First, the criteria for toxicity as developed by Porcella (1983) are based on LC50 or EC50 values and are not point estimates from a single sample. Second, even if point estimates of mortality (or reduction compared to control values) were used for a 100% sample, mortalities very near 10% would be unlikely to elicit any toxicological interest (see Section 8.1.4). Clearly, the lack of toxicological interest is because observations near 10% mortality (or reduction) are close to the likely upper limit for samples containing no toxic chemicals. Even though the below-detection-limit problem does not directly affect bioassays as currently conducted, the distribution of control-sample results markedly influences compositing schemes (see Sections 8.2 and 8.3), and has some implications for decision rules for action, such as Porcella (1983) proposed. Both these topics are addressed in Section 9.0.

5.3 TECHNIQUES FOR ESTIMATING SPATIAL DISTRIBUTION

One way to express the spatial distribution of a contaminant at a hazardous chemical waste site is to construct a contour map of toxicant concentrations. Such maps can be used to estimate the boundaries of zones requiring further attention or remedial action, or to predict toxicity at specific site areas. In either case, a set of samples is collected from various locations on the waste site and analyzed. Toxicity at all other locations is then predicted by interpolation between toxicity values obtained from these sample sites. Several data interpolation methods may be employed to accomplish this, including trend surfaces, spatial splines, and kriging.

Kriging is a potentially powerful and cost-saving method for estimating the spatial distribution of a toxicant. It is a weighted moving-average

technique (i.e., predicted values are weighted averages of the data in surrounding locations) in which the derivation of the weights takes into consideration the distance between the predicted point and the sampling location, the variance structure of the data, and any systematic trend or drift in the observations. Detailed explanations of kriging can be found in Clark (1979), Journal and Huijbregts (1978), and Mason (1983). Kriging has one important advantage over other interpolation techniques for the purpose of defining the areal distribution of contaminants for remedial action decisions: the procedure produces variance estimates for the predicted toxicity values. This variance estimate may be used to 1) evaluate the precision of the toxicity estimates between sampling locations for comparison to the study reliability requirements, and 2) indicate areas in which further sampling and/or sample analyses will improve the toxicity estimate as well as areas in which additional sampling or analytical effort is unnecessary, thereby minimizing sampling and analytic costs. Further, the benefit of additional or more intensive sampling, in terms of the reduction of the kriging variance or error about interpolated values and resulting increase in the contour map precision, can be predicted from the analysis of the original small data set.

The kriging technique is most efficient when field samples are collected according to a systematic sampling design. The results of kriging analysis are less reliable with small data sets or when sampling locations are very unevenly spaced. Examples illustrating the use of kriging to analyze bioassay data may be found in Section 8.0. Other interpolation and contouring techniques do not yield a variance estimate. Trend surface analysis involves the use of toxicity values at sampling points to define a (typically) polynomial "toxicity surface." Toxicity at any point on the site can then be predicted from the mathematical equation describing the surface. Splining is a technique for fitting curves smoothly through the data points and is widely used for curve smoothing in computer software packages. At this time, there are no compelling reasons to choose either trend surfaces or spatial splines to estimate the areal distribution of a contaminant. For purposes of remedial action decisions, the lack of variance estimates for predicted values make these techniques second choices to kriging.

6.0 AN OVERVIEW OF WASTE SITE BIOASSAY STUDY DESIGN

This portion of the document describes the factors to consider when allocating effort to field and laboratory work so that the most cost-effective bioassay study design is achieved. Section 6.1 describes possible sources of variation in bioassay results that may decrease the reliability of data-based conclusions, and discusses the impact of variability on the study design. This is followed in Section 6.2 with a discussion of the use of multistage designs that may maximize the information gained from the study while minimizing the study costs. Finally, Section 6.3 contains descriptions of the different overall field sampling strategies useful for bioassay studies and provides guidance for choosing among them.

6.1 COMPONENTS OF VARIABILITY AND THEIR IMPACTS ON STUDY DESIGN

The results from bioassay studies contain a certain amount of variability, or "noise." One of the objectives in study design is to quantify and minimize this "noise" so the results will meet the reliability requirements established for the study (Section 3.4.3). The variability in the data comes from several sources in both the field and laboratory: 1) the natural variability among similar field samples, 2) variability in field conditions when the samples are taken, 3) variability in the sampling techniques, 4) variability in sample handling in the laboratory, and 5) variability in the bioassays (e.g., variability among test organisms, and in the test conditions). Variability from sources 2 through 5 can often be minimized by careful field and laboratory procedures. The natural variance between samples is beyond investigator control, but some fraction may be anticipated and accounted for in the field sampling design.

Contaminant concentrations in the field usually vary naturally both spatially and temporally. Spatial variation tends to be much higher in soils and sediments because mixing is very slow compared to the much faster mixing in air and water. Conversely, temporal variation in air and water may be much higher than in soils and sediments because of the more rapid transport of material in those media. Temporal variation in soils and sediments is not negligible, however. Concentrations of contaminants in soils and sediments

may change seasonally or after rains or other climatic events. Finally, the variation in contaminant concentrations may be a combination of both spatial and temporal components; that is, the relative concentrations in two different locations may not remain constant over time.

In general, the greater the natural spatial and temporal variation in similar field samples, the greater the sampling effort required to characterize the site. However, both spatial and temporal variation can be anticipated and incorporated into the design in order to increase the efficiency of the field sampling strategy. With respect to spatial variation, stratified sampling designs may be used to obtain separate toxicity estimates from areas on the site that are likely to differ in contaminant concentrations (e.g., near the source of the contamination versus farther from it, or different media in which contaminant concentrations are likely to vary because of the chemical or physical properties of the waste). Stratified sampling designs are discussed more thoroughly in Section 6.3. The sampling effort may be allocated unequally over the site with more emphasis on areas where the reliability of toxicity estimates is more important (e.g., where contaminant concentrations are likely to approach a critical value, or at the site boundaries).

Sampling strategies that account for temporal variation must contain a series of samples obtained at several distinct times. In the usual field study, unless rates of contaminant transport are being measured, sampling usually occurs only at a single time. Therefore, the timing of sampling should be carefully considered in the study design. Depending on the objectives of the study, samples might be taken at times when 1) human, animal, or plant exposure to the waste is likely to be greatest (e.g., during the growing season or during periodic events when humans use the site), or 2) transport of hazardous material is likely to occur (e.g., when ephemeral streams are flowing on the site).

Designs that account for both spatial and temporal variation are only now being addressed by the statistical research community. Additional comments on progress in this area may be found in Section 9.0.

Sample collection, handling, and analysis should be standardized to reduce variation. Sample collection techniques should be the same for all samples that will be compared among one another. All samples should represent the same weight or volume of the medium being sampled. Field technicians should receive the same instructions on what should and should not be included in the sample and actions to be taken when sampling problems are encountered. For example, a decision must be made whether to include surface vegetation and leaf litter in soil samples, and additional instruction is needed to deal with events that prevent a complete sample from being taken (e.g., encountering a rock). Sampling procedures should be kept as simple as possible because complicated protocols are difficult to repeat consistently in the field. Even with a uniform sampling method, results can be influenced by individual technicians; therefore, if the study is sufficiently small, all of the samples should be collected by one person. If more than one technician is needed to collect samples, personnel should be assigned so that one person does not collect all of the samples from a "clean" site while another collects all the samples from contaminated areas (i.e., confounding "clean" and "contaminated" with differences in sampling techniques used by different technicians).

The performance of sampling devices may vary dramatically depending on the field conditions under which the samples are taken. For example, the quality and reliability of soil samples taken with coring devices may be very different at different levels of soil moisture. All samples should be collected on the same day or on consecutive days when field conditions are relatively uniform. If field conditions change during sampling, resampling of some areas may be useful for calibrating results based on samples taken under the different circumstances.

Samples should be transported and stored as uniformly as possible. A random arrangement of samples in storage containers is often advisable for more volatile contaminants. The random arrangement prevents confounding the differences between samples with systematic variations in storage conditions.

In the laboratory, whole samples are usually mixed and subsampled for analysis. Incomplete mixing of the sample will result in a larger variance

for subsamples. When soils and sediments are being analyzed, preliminary tests should be conducted to determine whether the sample mixing process is sufficiently thorough.

Variation among the test organisms will also add to the "noise" in the results. The response to chemicals is often dependent on the taxonomic position, age, size, physiological condition, and genetic strain of an organism. If the objective of the bioassay study involves the comparison of toxicity in different areas or sites, then uniformity among test organisms is desired, and these factors should be controlled as much as possible. However, if the objective of the test is to characterize the response of a particular organism to the suspected toxic material, then test organisms of varying ages, sizes, etc. may be desirable since they more closely represent the population of that organism as a whole. Individual organisms should be assigned randomly to test containers and kept under the same conditions during the test. Growth chambers, in which temperature, light, and moisture are controlled, are often used for this purpose. Test containers should be randomly arranged in the growth chamber so the sample differences are not confounded with systematic differences in various areas of the growth chamber.

6.2 ONE-STAGE VERSUS MULTISTAGE STUDY DESIGNS

In a one-stage study, field samples are collected according to some sampling strategy and the resulting samples are subjected to bioassays at the same time. Using this design, all of the field sampling and laboratory analysis would be completed prior to finding that either the number of samples collected and analyzed far exceeded that needed to satisfy reliability requirements, or that the number of samples collected was insufficient to satisfy those reliability requirements and the results consequently are not useful for remedial action decisions without further work. Either mistake can be costly in terms of both time and resources.

In a multistage study, a smaller amount of work is performed at each stage and the information gained is used to optimize the work performed at subsequent stages. The steps in a multistage design might be as follows:

1. Preliminary investigation. The purpose of a preliminary investigation is to test the field sampling methods and obtain an estimate of the expected variability in the bioassay test data. Analysis of the preliminary samples also provides the opportunity to discover and correct difficulties in laboratory techniques.

2. Collection of field samples. The variability estimates based on the preliminary study can be used to design a field sampling strategy with the appropriate number of samples for the required precision and accuracy. All or a portion of the needed samples might be collected at this stage.

3. Initial laboratory analyses. A subset of the field samples or the composited samples (see Section 5.2) may be analyzed to obtain initial estimates of the location, distribution, or toxicity of known or suspected hazardous materials, depending on the study objectives. If the initial estimates satisfy the reliability requirements for the results, then the study may be terminated. If a shorter confidence interval is needed for estimates at a particular location on the site, or the results from a composite sample indicate that the toxicity of one or more of the component samples may exceed the critical value, then sample analysis should continue.

4. Subsequent laboratory analyses. Either another subset of samples from areas shown to be of interest in the initial analyses or the components of a composite sample exceeding the level of concern may be analyzed. The initial estimates of location, distribution, or toxicity may be revised and tested against the established reliability requirements. Analyses will continue until sufficiently precise and accurate results are achieved.

The allocation of effort to the various design stages depends on the scale, cost, and reliability requirements of the study. For small, less expensive projects, or for projects in which the reliability requirements are lower, an extensive preliminary investigation or use of small sample subsets may not be warranted. For large or expensive studies, extra effort in the preliminary stages may result in considerable savings in cost and effort during subsequent sampling and analyses.

Either sample collection, analysis, or both may be done in phases during a multistage study. When sampling costs are small compared to analytical costs, all of the samples that could possibly be needed for all study stages should be collected at one time. Collecting all samples at a single time minimizes the variation caused by sampling techniques and eliminates the need to account for temporal variation (i.e., between sampling periods). However, samples that are collected in phases are appropriate when the cost of collecting samples is very high (e.g., when wells must be drilled).

Multistage bioassay study designs can lead to appreciable cost reductions for large, expensive projects when 1) little is known about the location, distribution, or toxicity prior to the study; 2) fairly precise and accurate information is needed; and 3) sufficient time exists to evaluate the results from each stage before proceeding to the next. Single-stage studies may be more appropriate if 1) the approximate distribution and field variability of the contaminants are known, 2) the field conditions allow proven sampling techniques to be used, 3) the reliability requirements for the data are low, or 4) there is an emergency response.

6.3 SAMPLING STRATEGIES FOR COLLECTING FIELD SAMPLES

The three basic design strategies for collecting field samples are simple or stratified random, systematic, and judgment sampling. These strategies are described in Ford and Turina (1985) and are discussed here only as they apply to bioassay studies at hazardous chemical waste sites.

All remedial action decisions for the hazardous waste site will be based on the results obtained from samples collected on the site. Therefore, it is important that the samples obtained accurately represent the conditions on the site. The traditional approach to collecting a representative sample is to randomly select the sampling locations over the entire site with the aid of a random number table or similar device. This procedure is called simple random sampling and is an appropriate strategy when no prior information is available on the likely location or distribution of the contaminant.

With stratified random sampling, sampling sites are randomly chosen within several defined site areas or strata. Appropriate strata for a hazardous chemical waste site might be any division of areas in which it is anticipated that toxicity will differ; for example, areas at increasing distance from the known or suspected source of chemical contamination. Stratified random sampling is often a useful technique even if there is insufficient information available to identify distinct strata a priori, and is likely to produce a more widespread distribution of sampling locations on the site than will a simple random sampling strategy. The construction of arbitrary strata can allow the variability in toxicity between different areas of the site to be estimated and may help identify actual strata. For these reasons, stratified random sampling is a particularly useful approach for preliminary studies.

Systematic sampling involves the collection of samples at regular intervals over the site. This method is sometimes preferred to random sampling strategies because it ensures even coverage of the site. However, as Eberhardt and Thomas (1986) warn, a systematically distributed contaminant may not be detected when using this strategy. Systematic sampling (often in grids) is often the preferred method to provide input data for kriging and other mapping techniques.

Judgment sampling relies on the sampler's judgment of what constitutes a representative site sample. The purpose of these samples might be to assess the presence or absence of contaminants in obvious places (e.g., a streambed if transport is a concern), or for use in special studies of a preliminary nature (e.g., are toxic chemicals nearer the spill source?). However, judgment sampling is biased and such results should not be used in a statistical analysis. In addition, judgment samples can be collected along with samples from the designed study in the event that unusual or interesting circumstances arise or are discovered during sampling. A statistical evaluation of the data from the judgment samples can be used to suggest additional sampling or to make statements without accompanying error statements or probablistic assertions. In cases where prior information about a possible spill location becomes available during sampling, that information should be used to advantage in the survey design. In fact, design

modifications can be made onsite (e.g., an extra grid, transect, or stratum).

Locating field sampling sites in order to implement any of the sampling designs discussed is not a trivial matter. At least one-third of the field effort should be devoted to locating and marking the sampling sites. Each sample location should be accurately recorded to aid in the interpretation of the bioassay results, to accurately define areas in which remedial action may be necessary, and to permit return to any sampling site to collect necessary additional material.

7.0 METHODS OF SAMPLING MEDIA FOR WASTE SITE BIOASSAYS

Bioassays may be performed on soil, sediment, ground water, or surface water. These different media present different problems in field sampling and in the performance of laboratory tests that need to be taken into consideration in the planning and execution of bioassay studies. The special considerations for sampling and analyzing samples from each medium are discussed in Sections 7.1 through 7.4. Sampling methods have been presented in several other documents, and that information will not be repeated here. However, references to helpful documents are included in each section.

7.1 SOIL

Soil is usually collected with grab or core samplers. Discussions of sampling techniques and other considerations in soil sampling may be found in Mason (1983), Barth and Mason (1984), DeVera et al. (1980), Ford et al. (1984), and Sisk (1981).

Mixing in soils occurs very slowly so that the concentrations of toxic chemicals will vary substantially from location to location. In fact, as much as half the variation between similar soil samples often occurs within a distance of 1 m (Mausbach et al. 1980). This variation must be taken into account in the field sampling design, and a larger number of samples may need to be collected to adequately characterize the soils on the waste site. The extreme heterogeneity of soils also requires that extra effort be made to ensure that soil samples are thoroughly mixed before being subsampled for compositing (see Section 5.2) or other purposes. Tests should be conducted in the laboratory to ensure that the mixing procedure is adequate.

During some bioassay tests, organisms are exposed to the whole soil sample (e.g., the earthworm bioassay). More commonly, however, the soils are eluted and organisms are exposed to the extracts. Soil samples containing sand or silt are more readily extracted than those containing clay. Special techniques and equipment may be needed to elute clay samples (Miller et al. 1984).

Soil samples are frequently collected with coring devices to obtain a depth profile of the chemical waste. Because contamination of the lower layers may occur during the insertion of the coring tube into the soil, outer edges of the core should be removed, if possible, before the sample is analyzed. With any type of sampling device, a decision must be made whether to include, in the samples, surface vegetation, plant roots, rocks, small organisms, or other material that might be collected along with the soil.

Sampling equipment should always be cleaned between samples to avoid cross-contamination. Samples should be collected and stored in containers made of inert materials that will neither absorb materials from the sediments nor add toxicants. In addition, soil samples should be stored uniformly under conditions that will prevent or retard chemical degradation and microbial metabolism.

7.2 SEDIMENT

Sediment is usually collected with devices similar to those for collecting soils. References that discuss sediment sampling techniques and problems include DeVera et al. (1980), Ford et al. (1984), American Public Health Association (1985), and Sisk (1981).

The major problems encountered when sampling sediment are similar to those for soil sampling. However, sediment samples usually have a higher moisture content compared to soil samples and therefore less integrity. Clean core samples are more difficult to obtain in sediments. Depending on the depth and size of the overlying water body, sediment sampling may present additional problems with sample site location and the use of sampling equipment.

7.3 GROUND WATER

Ground-water samples are usually pumped from wells or collected from seeps. Discussions of ground-water sampling techniques and concerns may be found in Ford et al. (1984), U.S. Environmental Protection Agency (1977), Sisk (1981), Gibb et al. (1980), and Dunlap et al. (1977).

Representative groundwater samples for bioassays are particularly difficult to obtain. Changes in pressure, oxidizing/reducing conditions, and other factors may alter the chemical composition of samples as they are brought to the surface. Certain toxic compounds in seeps may precipitate from ground water when the chemicals encounter the strong oxidizing environments of receiving streams.

Samples pumped from wells may be contaminated with surface soils or drilling fluids inadvertently added to the ground water during the boring of the wells. Well casings and pumps may absorb or add toxic materials to the samples. Gibb et al. (1980) discovered that pumping depth, type of pump used, and sample filter sizes had an effect on the measured chemical composition of ground-water samples, the magnitude of which depended on the yield, depth, diameter and water level of the well, the pumping rate, and the general character of the water being pumped, as well as the specific toxicants. Because of these problems, sampling conditions and techniques should be standardized when collecting ground-water samples. Well casings, pumps, and sampling equipment should be made of inert materials. The type of pump used, the water level of the well, and other measurements should be made at the time of sampling and recorded on data sheets. Since ground water contains many trace constituents, extra care should be taken to avoid contamination of the samples, and storage should be under uniform conditions (4°C) to prevent or slow chemical conversions in the storage containers.

Several characteristics of water, other than the concentration of toxic substances, may affect the outcome of bioassay tests. The pH or hardness of the water sample may render it toxic to the assay organisms. Most of these factors are accounted for in the bioassay procedures.[a]

7.4 SURFACE WATER

Surface water is usually collected by dipping it into sample bottles. Remotely operated devices may be used to collect samples at various depths.

---

[a] Miller, W. E., J. C. Greene, and S. A. Peterson. 1987. Protocol for Bioassessment of Hazardous Waste Sites. 2nd edition. Corvallis Environmental Research Laboratory, Corvallis, Oregon. Final Draft.

Water from very shallow levels is often collected with pumps. References that discuss surface-water sampling methods include Ford et al. (1984), DeVera et al. (1980), Gibb et al. (1980), and American Public Health Association (1985).

Representative surface water samples are relatively easy to collect. Mixing occurs quickly in water so these samples do not have the extreme heterogeneity of soil samples; although stratification and stagnant layers in surface waters do occur, thorough sample mixing in the laboratory is relatively simple to achieve.

Several characteristics of the water, other than the concentration of toxic substances, may affect the outcome of the bioassay tests. The pH or hardness of the water sample alone may render it toxic to the bioassay organisms. Bioassay procedures include methods to account for these factors.[a]

In vivo reactions proceed readily in surface water samples, so samples should be analyzed quickly and/or stored (4°C). The length of time between collection and analysis should be noted for each sample.

(a) Miller, W. E., J. C. Greene, and S. A. Peterson. 1987. Protocol for Bioassessment of Hazardous Waste Sites. 2nd Edition. Corvallis Environmental Research Laboratory, Corvallis, Oregon. Final Draft.

8.0 CASE STUDIES: ILLUSTRATIONS OF BIOASSAY METHODS AND DESIGN DECISIONS

The field studies in this chapter were selected to illustrate the steps in executing a bioassay study (Section 3.4), statistical principles (Sections 5.0 and 6.0), and sampling of various media (Section 7.0). The site bioassay studies evaluated site toxicity and defined areal extent, if toxicity was detected. Because the sites were large and resources were limited, many extra samples were collected and some composited, which illustrates the principles presented in Section 5.2.

Rocky Mountain Arsenal soil sample bioassay results (Section 8.1) were used to prepare a map that could be used for cleanup decisions. In Section 8.2, bioassay results are compared to results of chemical analyses on sediment samples collected from a stream on a wood treatment plant site in Mississippi. Other objectives of the Canton study were to determine whether standard bioassay organisms can be used to detect creosote contamination and to map contaminant distribution. Section 8.3 includes soil, sediment, and surface-water sampling at a waste site presumed to be clean (Friedman) and at a second site thought to be toxic (Combe). Both waste sites are in New Jersey.

8.1 SOIL SAMPLING AT THE ROCKY MOUNTAIN ARSENAL

The Rocky Mountain Arsenal was used principally by the U.S. Army and the Shell Chemical Company to manufacture, test, and dispose of toxic chemicals. Certain areas of the 26-square-mile (67.6-km^2) arsenal have been contaminated by various spills during waste disposal operations. The site is surrounded by homes, farms, and businesses (e.g., Stapleton Airport, Commerce City, and Denver). In 1974, diisopropylmethylphosphonate (DIMP) and dicyclopentadiene (DCPD) were detected in the surface water draining from a manmade bog at the northern boundary of the arsenal.

8.1.1 Assemble Information Relevant to the Problem

Decontamination wastes and process waste streams containing toxic materials consisting of salts, heavy metals, and pesticides were

deposited in defined areas on the Rocky Mountain Arsenal (Figure 8.1). Basins A and F were the two major sites of waste material storage. Initially, all waste materials were placed in Basin A (Figure 8.2, Section 36). Three basins (C,D,E,) in Section 26 were constructed from 1952 through 1955 to contain overflow from Basin A. In 1955, Basin F was constructed to contain overflow from Basins C, D, and E. Beginning in 1957, all liquid waste from the Rocky Mountain Arsenal and Shell operations was pumped into Basin F. Basin C was also used as a temporary holding pond for Basin F.

Certain portions of the arsenal were leased to private industry for chemical manufacturing. Shell Chemical Company, the major lessee, has leased a considerable portion of the manufacturing facilities at the Rocky Mountain Arsenal since 1952. Shell has made major alterations and additions to the facilities for the manufacture of various pesticides. The Rocky Mountain Arsenal has been used for the manufacture and disposal of waste residuals of GB, a chemical warfare agent, TX, a biological anticrop agent, and cyanogen chloride and phosgene. Since 1970, several major chemical demilitarization actions have been conducted. These actions include the incineration of both the anticrop agent TX and a mustard agent.

In May 1974, DIMP and DCPD were detected in surface water draining from the manmade bog on the northern boundary of the arsenal. DIMP is a persistent compound produced in small quantities (<3%) during the manufacture of GB. DCPD is a chemical used in the production of pesticides. Detection of these two compounds resulted in the construction of a dike north of the manmade bog to eliminate surface drainage from the arsenal.

In December 1974, the Colorado Department of Health detected small concentrations of DIMP [0.57 parts per billion (ppb)] in a well near the city of Brighton. This discovery indicated that DIMP was also present in the ground water traveling in a northerly direction from the Rocky Mountain Arsenal boundary. A 2-year study by the U.S. Geological Survey confirmed the direction of ground-water flow.

Because of the foregoing manufacturing and disposal actions, various analyses of chemicals in air, water, soil, and certain organisms have been conducted over the years. Table 8.1 contains a partial list of some of the chemicals identified.

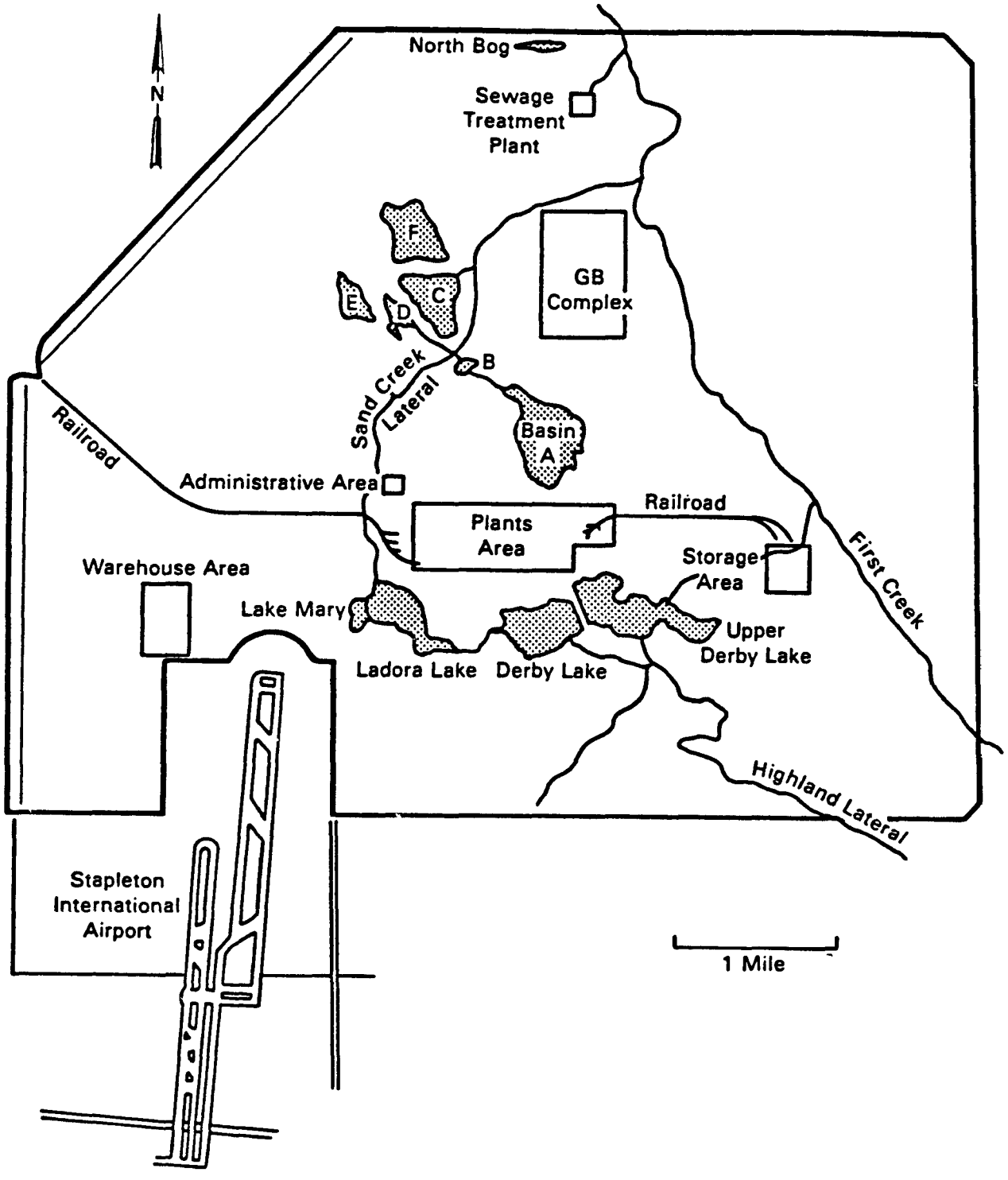


FIGURE 8.1. Principal Physical Features of the Rocky Mountain Arsenal, Commerce City, Colorado

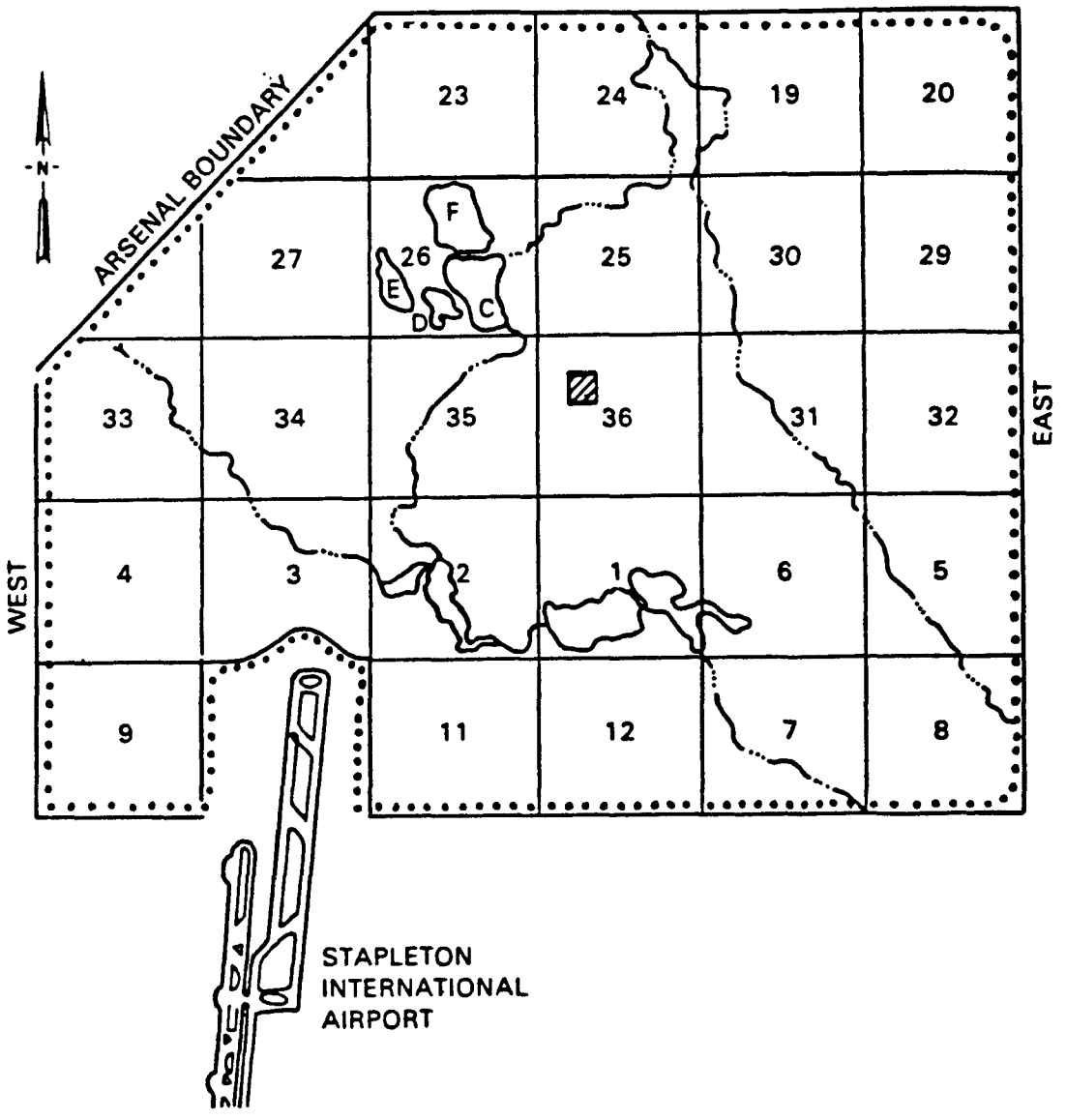


FIGURE 8.2. Location of the Study Site in Basin A (which includes most of Section 36) at the Rocky Mountain Arsenal. The areas in Section 26 labeled C, D, E, and F are or were waste ponds.

TABLE 8.1. Examples of Some Chemicals Found In Soils, Air, Water, Animals, and Plants at the Rocky Mountain Arsenal

Aldrin	Methylphosphonic acid
Arsenic compounds	Isopropyl methylphosphonate
Benzene	Diisopropyl methylphosphonate
Chlordane	Dicyclopentadiene
Chloroform	p-Chlorophenyl methyl sulfone
Dieldrin	Hexachloronorboradiene
Endrin	Tetrachloroethylene
Lewisite	1,4,-Thioxane
Lewisite oxide	Methylene chloride
Mercury salts	Toluene
Mustard gas (HD)	Xylene
Thiodiglycol	

8.1.2 Prepare a Statement of Study Objectives

Objective 1 was to assess the toxicity of a trench site in Basin A. If one or more bioassays identified toxic components, a field study would be designed to supply data needed for kriging to devise a contour map useful for cleanup decisions (objective 2). Finally, an assessment of contaminant mobility was needed (objective 3). To meet these objectives, a toxic site at the Rocky Mountain Arsenal had to be located and the most sensitive bioassay selected.

8.1.3 Design the Field Sampling and Laboratory Studies

Previous results (see Section 4.3.3; Table 4.2) indicated that an area in Basin A was toxic (objective 1) and would be useful for addressing the second study objective. Soils from the Basin A location caused a major reduction in lettuce seed germination (insoluble compounds were likely causative) and had a variable effect on other bioassays (little likelihood of soluble compounds; objective 3). Because of these results and, in part, because it appeared that plant growth diminished with distance from the trench, a sampling location was established near the trench in Basin A (to meet objective 2).

The results also suggested a possible gradient of contamination on the west side of Section 36, extending north-south from a trench that drains Basin A and runs to the west. This possibility of a toxicity gradient offered good prospects for the kriging method, which generally requires a physical mechanism of toxicant dispersal (Journal 1984) for valid error predictions. Thus, four parallel transects were established on the west side of Basin A, each beginning on the north bank of the trench and running south for 90 m (Figure 8.3). A logarithmic scale was used beyond the south trench edge because it was assumed that contamination might have been moved by some physical means (e.g., wind or water). The transects were 15 m apart and labeled L, M, N, and P. The first three sample points of each transect fell within the trench and the fourth was on top of the south bank. Sample numbers 5 through 9 were 15, 20, 30, 50, and 90 m, respectively, south of the north trench edge (Figure 8.3). Each of the 36 sampling points was marked with a stake.

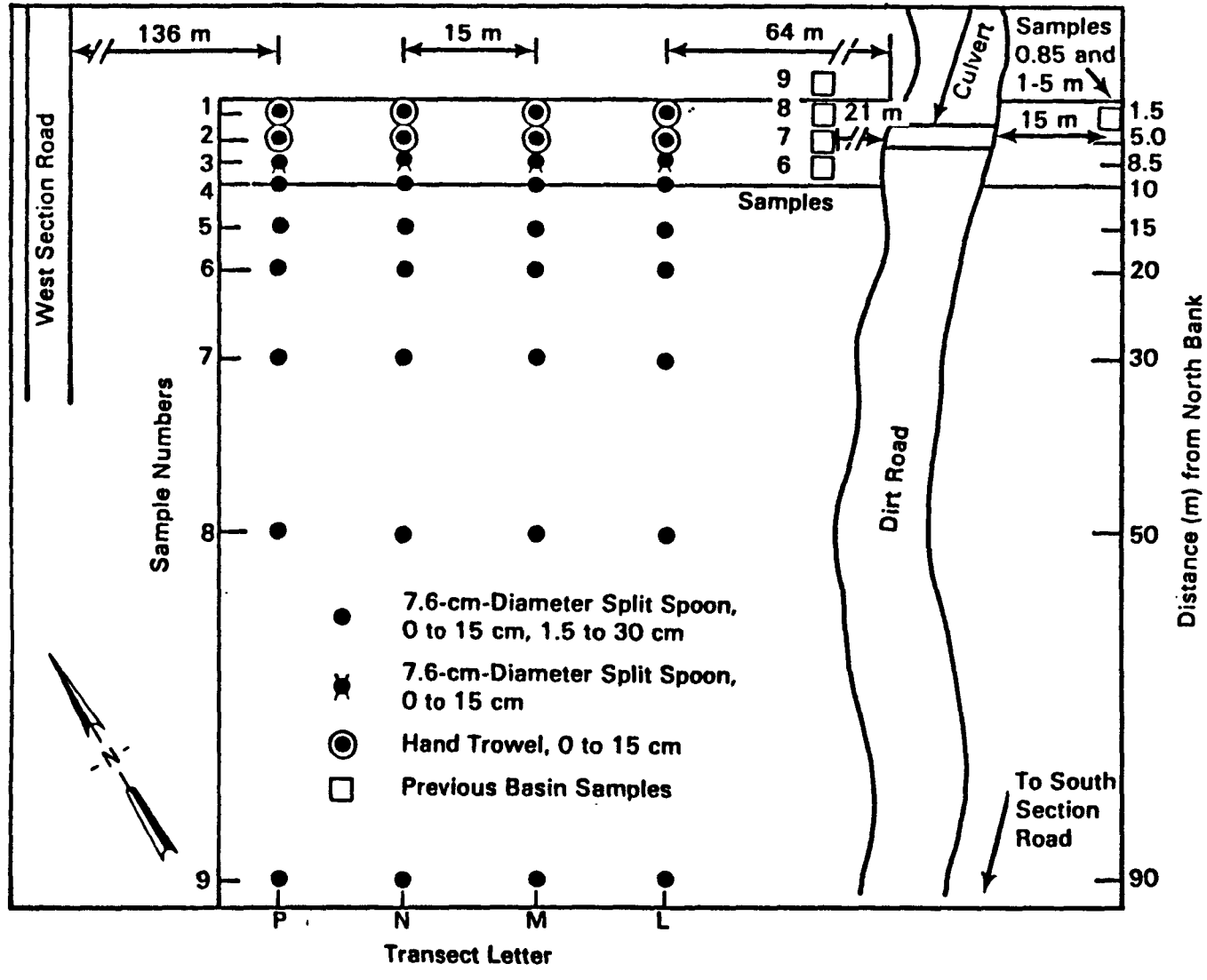


FIGURE 8.3. Location of Logarithmic Sampling Points in Basin A (Section 36) at the Rocky Mountain Arsenal

A drilling company was hired to do most of the soil sampling. At each sampling point a split spoon was used to take two 7.6-cm-diameter soil cores. One core was taken from a 0- to 15-cm depth, and the second was taken from a 15- to 30-cm depth. Together, these cores weighed approximately 4 kg. Between sampling points the split spoon and drill bit were decontaminated by washing with methanol and rinsing with distilled water. All samples were put in plastic bags, sealed, and labeled. The area being sampled and any problems encountered (e.g., mud, accessibility) dictated exactly how the cores were taken and the variations on the basic sampling scheme (see below).

In Basin A, the first two points in each transect were in the trench, which was very wet and soft. The samples from these points were difficult to obtain. It was impossible to sample by depth, so a hand trowel was used to take two surface samples (to approximately 15 cm deep) from these points. The split spoon was used to take most of the other samples in Basin A (points 4 through 9 in transects L, M, N, and P). Sample points L, N, and P-3 were just over the south bank of the trench and could not be reached from the drill rig. At these points, the split spoon was hammered into the ground and extracted by hand. Only a 0- to 15-cm sample was obtained from each of these points; the soil from 15 to 30 cm deep was too wet to stay in the split spoon. A surface sample of undefined depth was taken from the M-3 transect because the entire profile was very wet.

8.1.4 Analyze and Evaluate the Results

Bioassays of Field Study Soils

Lettuce seeds were used in the bioassays of the Rocky Mountain Arsenal field study soils, because they are more sensitive than other seeds (Thomas et al. 1986) and previous work has shown these soils to be phytotoxic (Table 4.2). The mortalities (mean from three subsamples from each core fraction; Figures 8.4 and 8.5) indicate that four samples in transect M and three in transect P showed large differences as a function of depth, suggesting that the contaminants had either migrated below 15 cm or were purposely placed there. We found no records to support the latter argument and concluded that the toxic material had migrated.

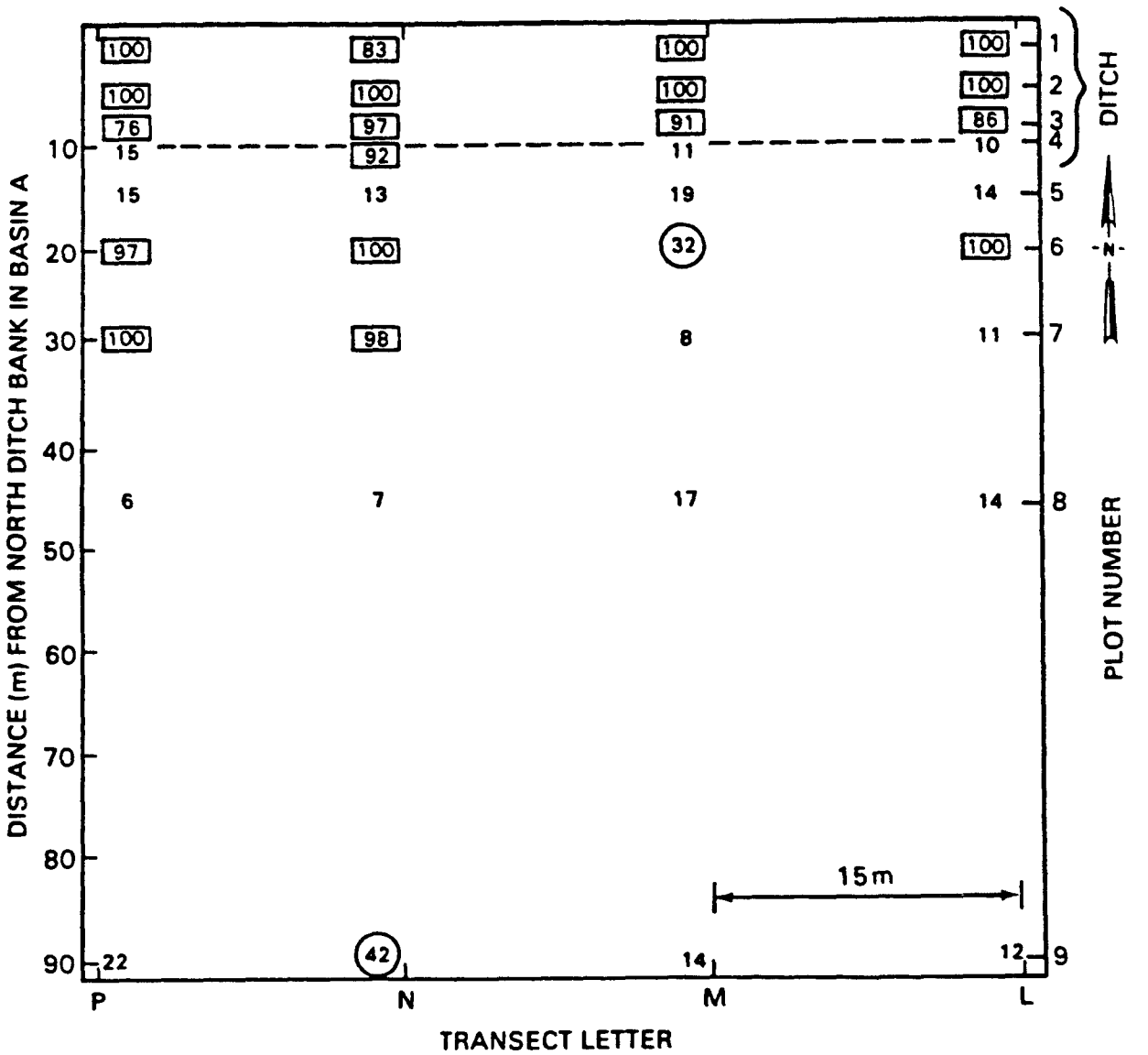


FIGURE 8.4 Observed Mean Lettuce Seed Mortality at Each Basin A Plot, 0- to 15-cm Soil Fraction. Means enclosed with a box are >75%, an enclosing circle indicates means from 30% to 75%, and numbers with no symbol are means <30%.

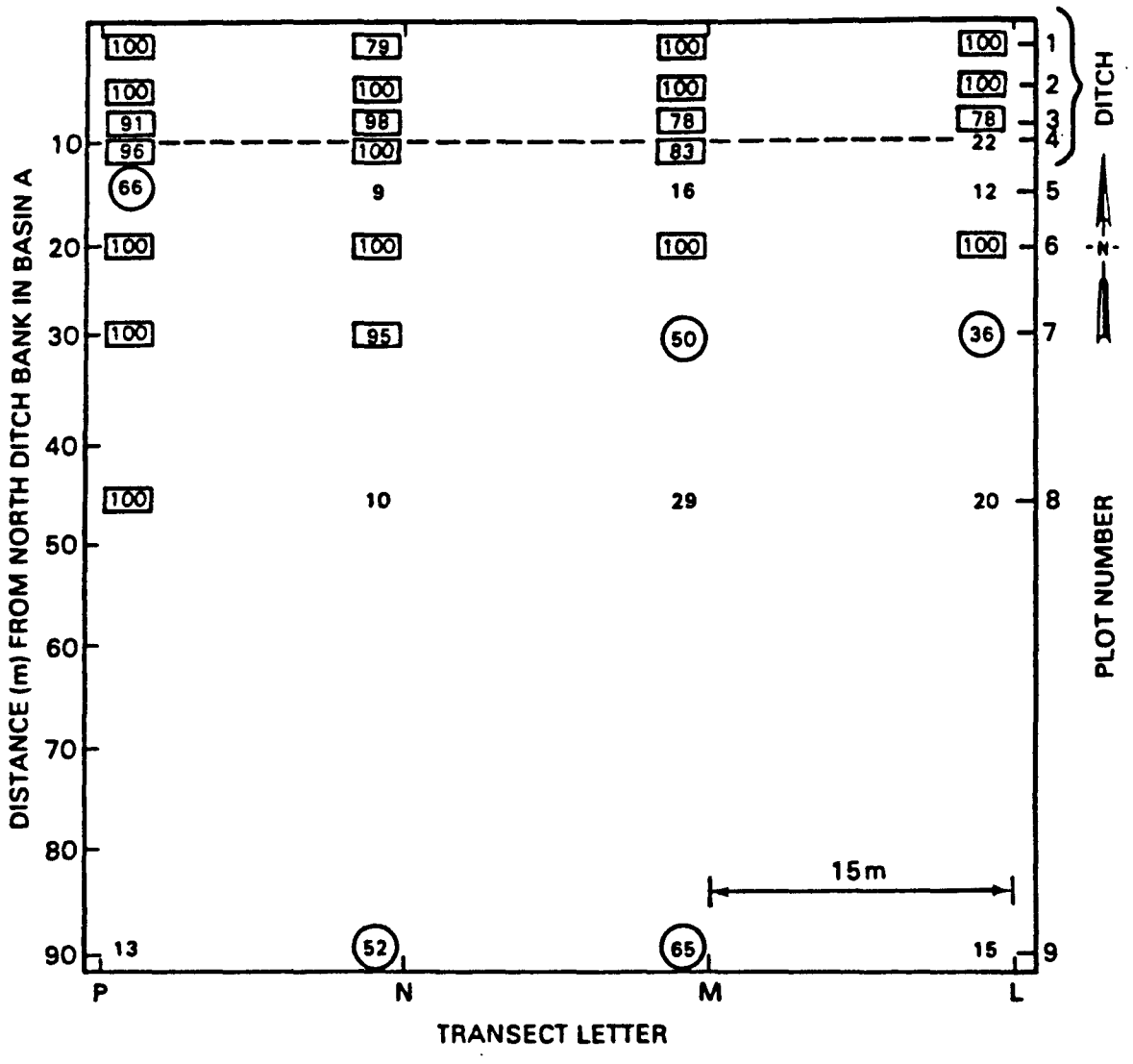


FIGURE 8.5. Observed Mean Lettuce Seed Mortality at Each Basin A Plot, 15- to 30-cm Soil Fraction. Means enclosed with a box are >75%, an enclosing circle indicates means from 30% to 75%, and numbers with no symbol are means <30%.

The results for all other bioassays (Porcella 1983) conducted using Basin A field study soils are shown in Table 8.2. Sample unavailability either precluded analyses or curtailed the number of assays that could be conducted. Daphnia or Microtox bioassay results are not included because no mortality was observed.

Results from the algal bioassay revealed that small quantities of elutriate from all but four samples were stimulatory. The four elutriate samples that inhibited algae were obtained on or very near the waste trench on transects L and N. According to the criteria outlined in Porcella (1983), these sites would be classified as moderately toxic. Interestingly, only a 1% to 14% elutriate from transect M samples was needed to stimulate algal growth. Because there was little effect on the algal bioassay, it appears that the toxic components detected using lettuce seeds (see Figures 8.4 and 8.5) were not water-soluble heavy metals, herbicides, or insecticides (except perhaps at sites L-2, L-4, and N-2), since results using pure chemicals (Section 4.3) showed depressed algal growth in the presence of these contaminants. This evidence suggests the presence of water-insoluble contaminants that are not likely to migrate (objective 3).

Results of the earthworm bioassay are presented as a function of lettuce seed mortality in Figure 8.6. Except for plot P-3, earthworms were only affected by Basin A soil when lettuce seed mortalities were greater than 70%. In contrast, the five soil samples that caused 20% to 70% lettuce seed mortality resulted in no earthworm deaths. Thus, for these Basin A samples, it appears that lettuce seeds are more sensitive to lower levels of an insoluble toxic component than are earthworms (objective 3).

Results from the lettuce root elongation (based on elutriates) and the lettuce seed mortality (based on intact soil) bioassays are compared in Figure 8.7. There was a slight correspondence, based on samples from transect L. It appears that the phytotoxic component that impairs lettuce seed germination may not be water soluble (objective 3) or does not affect root elongation.

Cleanup Decisions Based on Bioassays and Kriging

One way to depict the lettuce seed mortality patterns observed at each depth (see Figures 8.4 and 8.5) is to prepare a contour map based on the

TABLE 8.2. Intercomparison of Lettuce Seed Mortality (MN), Lettuce Root Elongation (RE), Earthworm Mortality (EW), and Algal Inhibition (S) for Two Fractions (0-15 cm and 15-30 cm depth) of Basin A Soils Obtained from the Rocky Mountain Arsenal

	Transect											
	L (15-30 cm)				M (15-30 cm)				N (15-30 cm)			
PLOT	MN[a]	RE[b]	EW[c]	S[d]	MN	RE	EW	S	MN	RE	EW	S
1	100	53		11	100	58	100	4	83	20	100	17
2	100	56	100	39[-]	100		100	9	100	31	100	69[-]
3	86	42		50[-]	78				97	44		12
4	22	0		49[-]	83	14		14	100	4		26
5	12	21[+]		25	16	45[+]	0	1	9	35[+]	0[*]	25
6	100	9	0[*]	27	100	10	100	13	100	8		30
7	36	18		23	50	21	0	7	95	30	100	5
8	20	14		23	29	33	0	10	10	17		21
9	15	33	0[*]	40	65	20	0		52	15	0	39

TABLE 8.2. (cont'd)

	Transect							
	P (15-30 cm)				L (0-15 cm)			
PLOT	MN	RE	EW	S	MN	RE	EW	S
1	100	25	100	15	100	53		11
2	100		100		100	56	100	39^{-}
3	91	2	0	24	86	42		50^{-}
4	96	10^{+}	50	4	10	21		10
5	66	23	0	17	14	29		9
6	97	15	100	14	100	40		25
7	100	8	100	8	11	27		20
8	100	0	100	19	14	11		35
9	13	1	0	30	12	20		27

(a) Percent lettuce seed mortality. Each value is the mean mortality of three replicate plates of 40 seeds grown in each soil.

(b) Percent of control lettuce root length (based on soil elutriate). Values accompanied by a superscripted plus (+) were greater than controls.

(c) Earthworm percent mortality using 100% soil (one replicate of 10 earthworms). Values with an asterisk (*) are questionable since soils had been previously used to prepare elutriates.

(d) EC50 values in % elutriates are denoted by a superscripted minus (-), while values with no superscript were stimulatory.

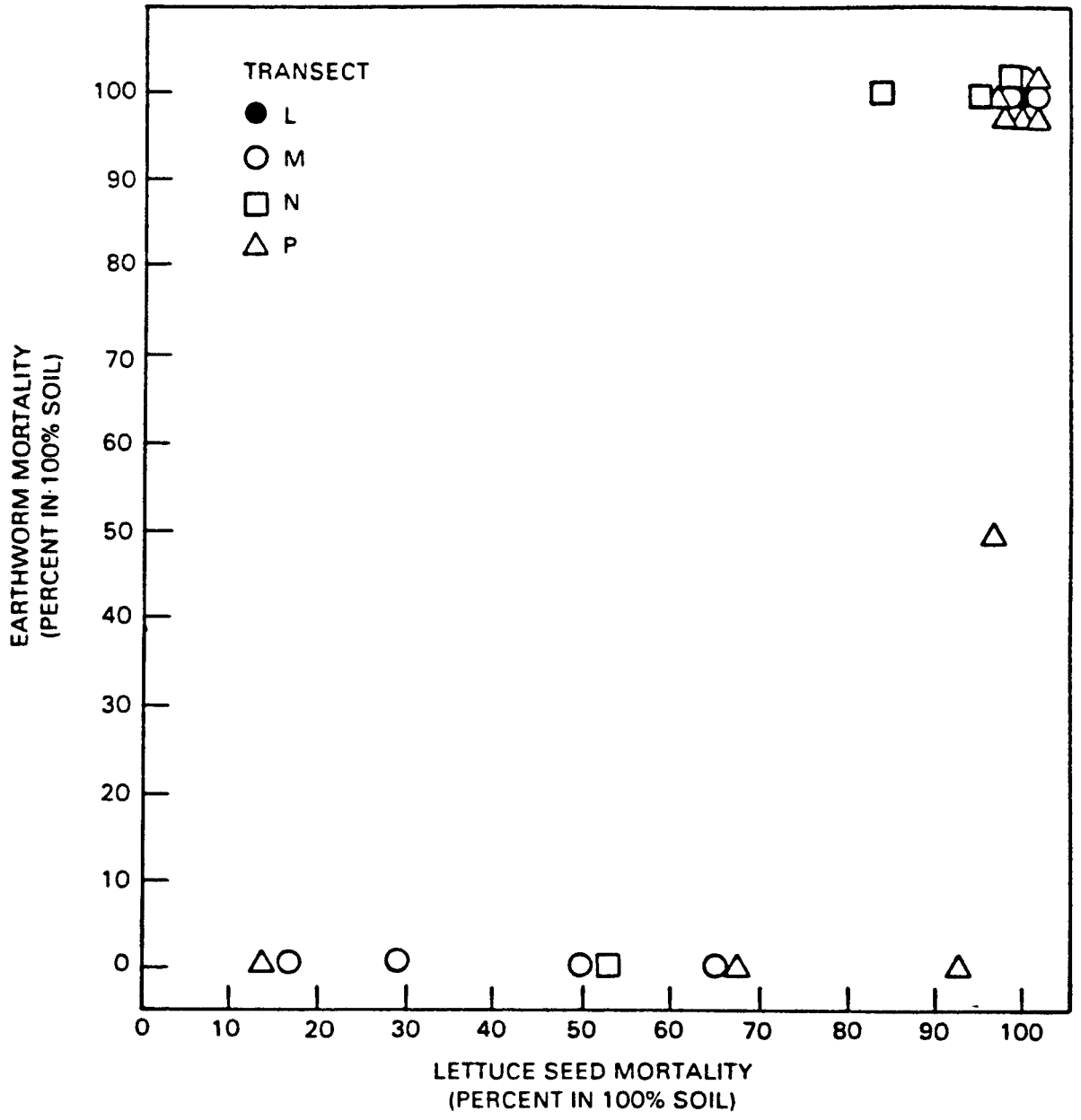


FIGURE 8.6. Comparison of Earthworm and Lettuce Seed Mortality Using Basin A Soils from the Rocky Mountain Arsenal

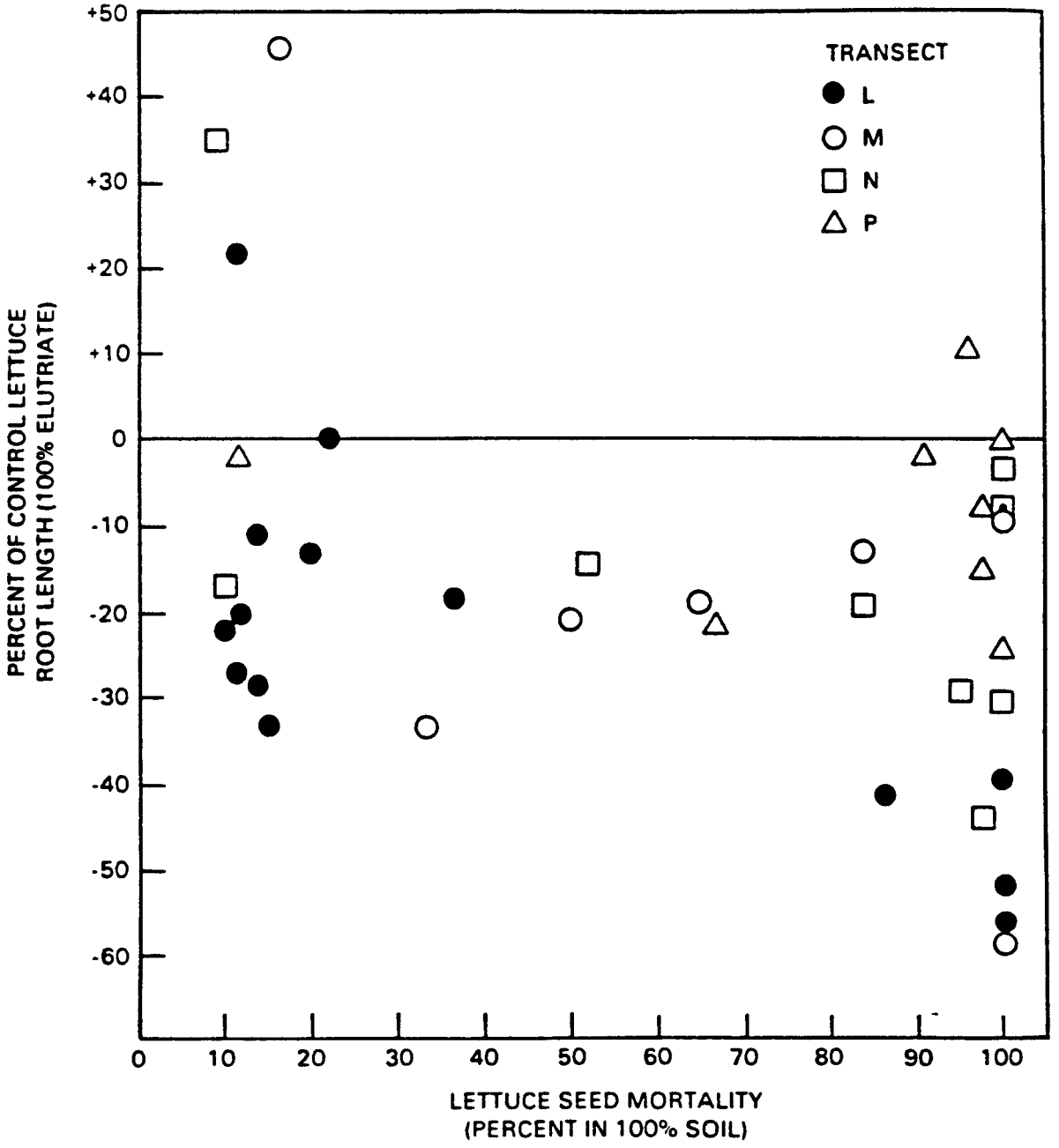


FIGURE 8.7. Comparison of Lettuce Root Elongation and Lettuce Seed Mortality Using Basin A Soils from the Rocky Mountain Arsenal

observations (objective 2). We estimated contours for a map of lettuce seed mortality using a relatively new statistical technique called kriging, which was developed for use in the mining industry and is used principally in Europe and South Africa (see Section 5.3). Kriging provides a variance estimate that can be used to construct a confidence interval for the true value. Results based on block kriging are presented in Figures 8.8 and 8.9. The results clearly show the lettuce seed mortality differences at the two depths. Estimated contamination is greater from 15 to 30 cm deep than from 0 to 15 cm deep. This contamination difference was also indicated by the qualitative analyses of results (see Figures 8.4 and 8.5).

Contour maps are useful for making site cleanup decisions. As a scenario, we selected 30% lettuce mortality as a criterion for cleanup of the Basin A site (see Figures 8.8 and 8.9). In the absence of any other guidance, the cleanup criterion was selected as two standard deviations above the mean control mortality (i.e., 16.7 ± 14.0, $n = 6$). The shaded areas below the heavy solid black lines would be targeted for cleanup. The cleanup decision would be different for the 0- to 15-cm-deep (Figure 8.8) and the 15- to 30-cm-deep fractions (Figure 8.9). While this difference complicates decision making, the available data and the kriging maps show that the field situation is complex, and cleanup decisions based solely on either soil fraction would not result in a "clean" site. Cleanup using the 30% mortality contour of the 15- to 30-cm samples would remove all known contamination, but additional samples taken below this depth would be needed to assure that the site meets the 30% mortality cleanup criterion. The need for additional samples below 30 cm was not evident from preliminary profile data in Thomas et al. (1984c).

It appears that bioassays of field samples and subsequent kriging analyses (objective 2) offer a practical method to aid in cleanup decisions based on environmental toxicity, especially when accompanied by error estimates for the mortality isopleths. We did not present confidence limits here because of some questions about the possibility that the observed toxicity was caused by pollutants that were "dumped" rather than spread from the trench by wind or water. Journal (1984) argues that the confidence limits may be invalid unless movement is caused by physical forces. The

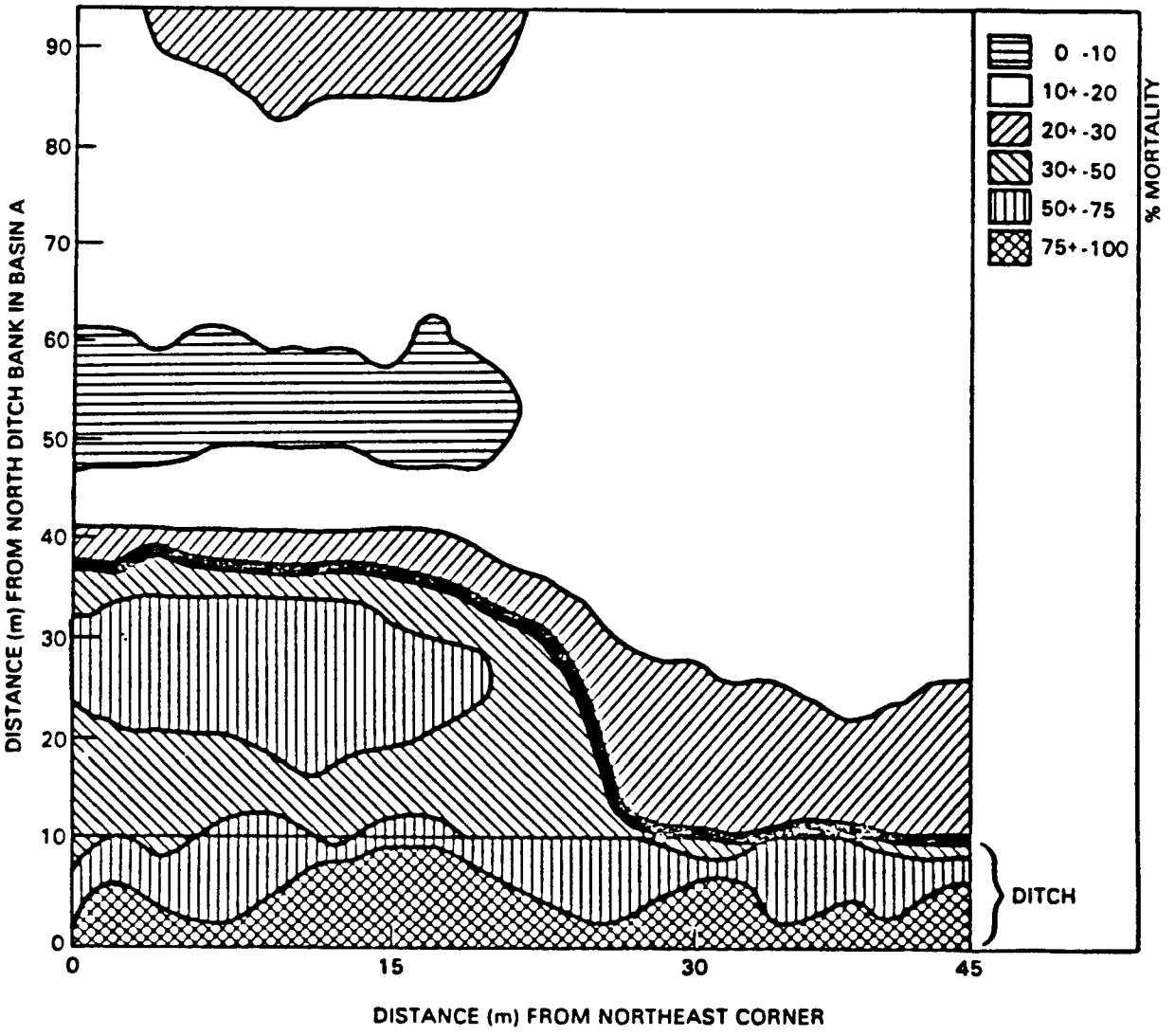


FIGURE 8.8. Estimated Lettuce Seed Mortality (based on kriging) for the 0- to 15-cm Soil Fraction from the Rocky Mountain Arsenal

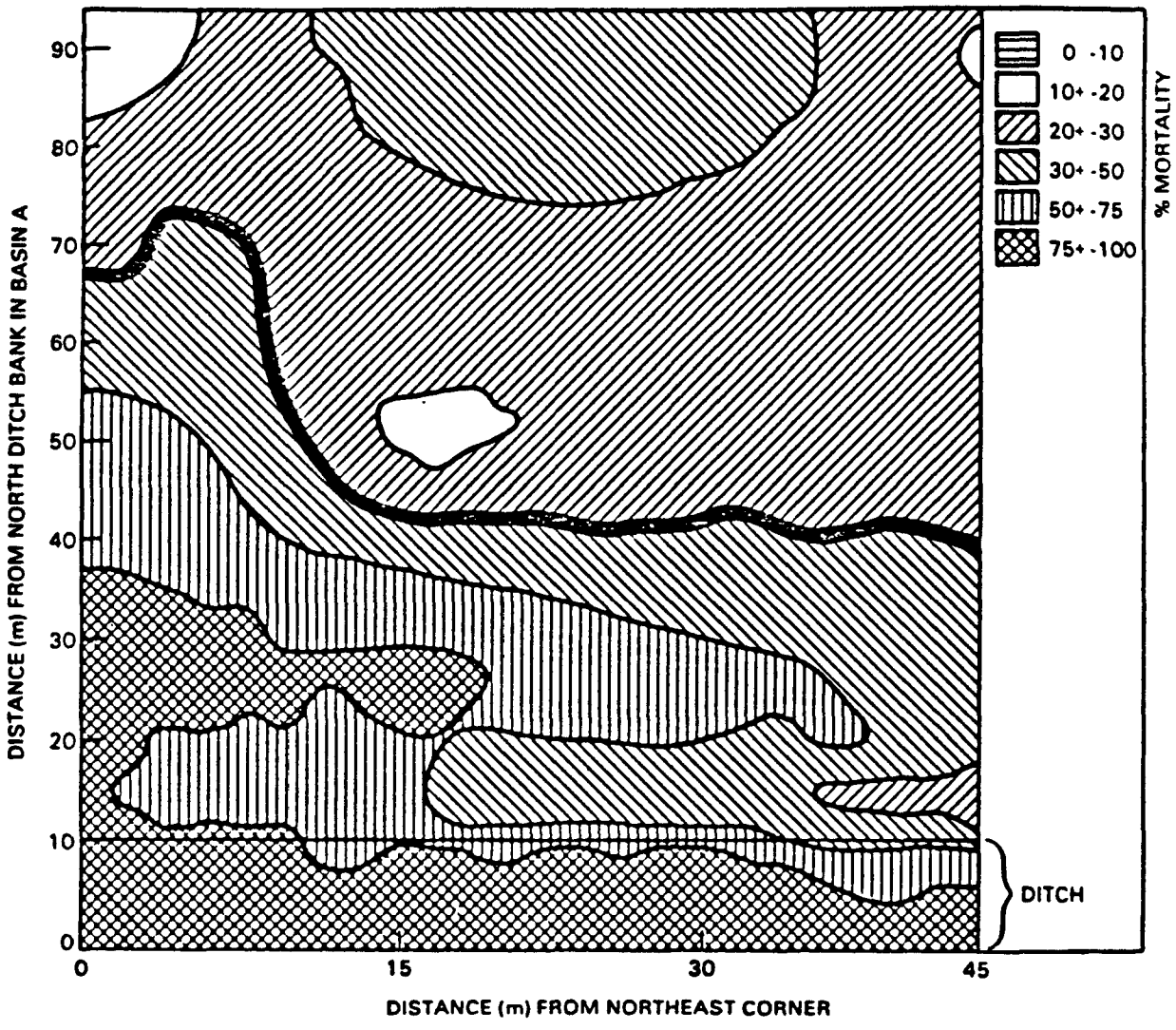


FIGURE 8.9. Estimated Lettuce Seed Mortality (based on kriging) for the 15- to 30-cm Soil Fraction from the Rocky Mountain Arsenal

limits in our study averaged between ±10% and 25%, and depended on data density, whether block or point kriging was used, and the contour of concern.

An arbitrary crosshatching has been superimposed on Figures 8.10 and 8.11 to show where the observed data indicate that mortality was actually 30%. These crosshatched "boxes" were constructed by drawing a line halfway between grid points with observed mortalities >30% and those <30%. Thus, the crosshatched box at the top of Figure 8.10 is a consequence of the observed mortality at plot N-9 (Figure 8.4, 42%) and all other surrounding plots (P-9, N-8, and M-9) exhibiting mortalities <30%. The >30% kriging mortality contour does not include this area or a "hot spot" at L-6 (100% observed mortality, Figure 8.4) because it is a weighted moving-average technique. Figure 8.11 shows that the kriging results mimicked the observed data very well. This observation is not surprising since point-kriging theory requires a correspondence of observed and predicted values. The block-kriging technique used here results in a less than one-to-one correspondence.

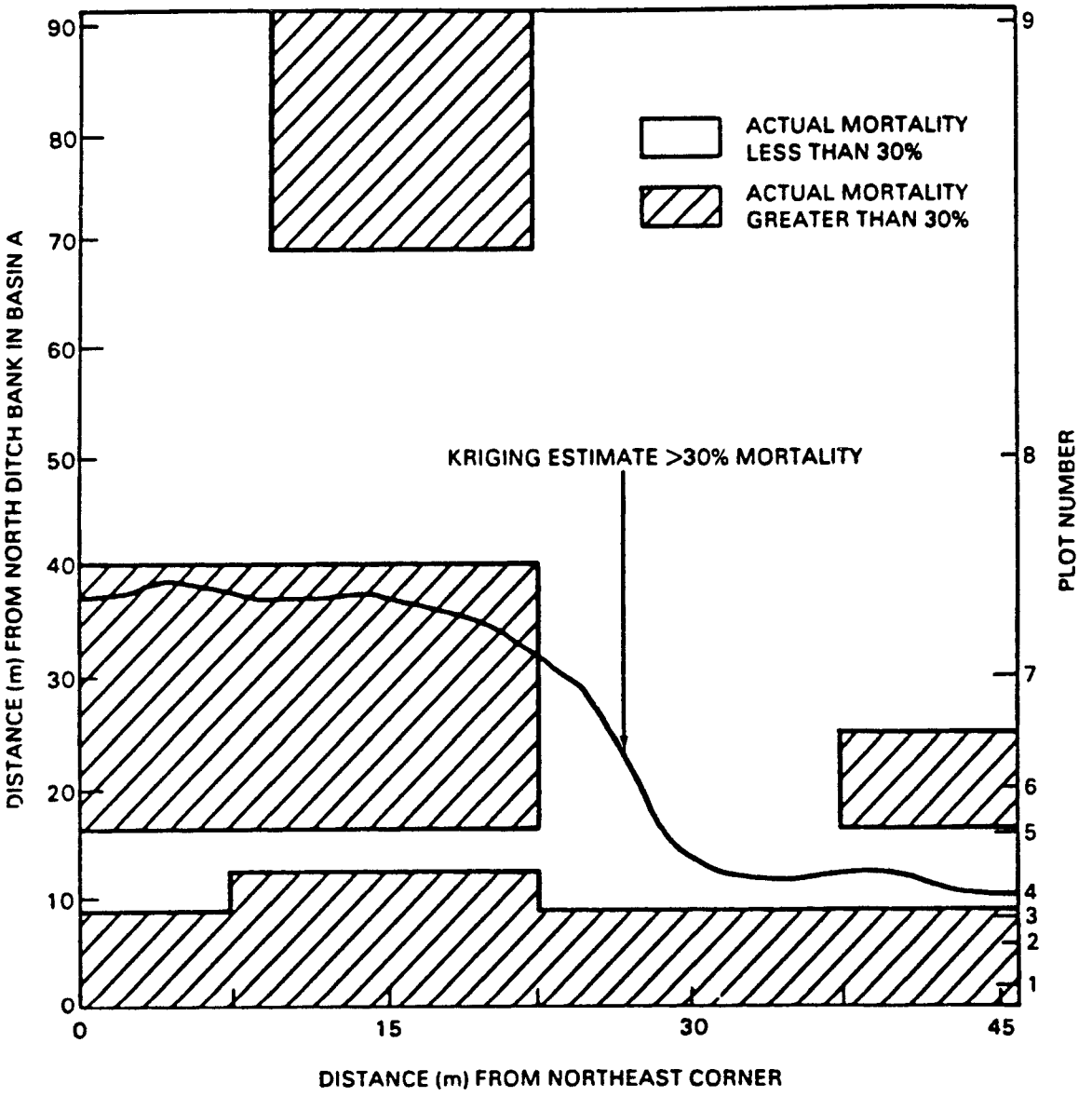


FIGURE 8.10. Comparison of Lettuce Seed Mortality Predicted from Kriging to Actual Lettuce Seed Mortality, 0- to 15-cm Soil Fraction

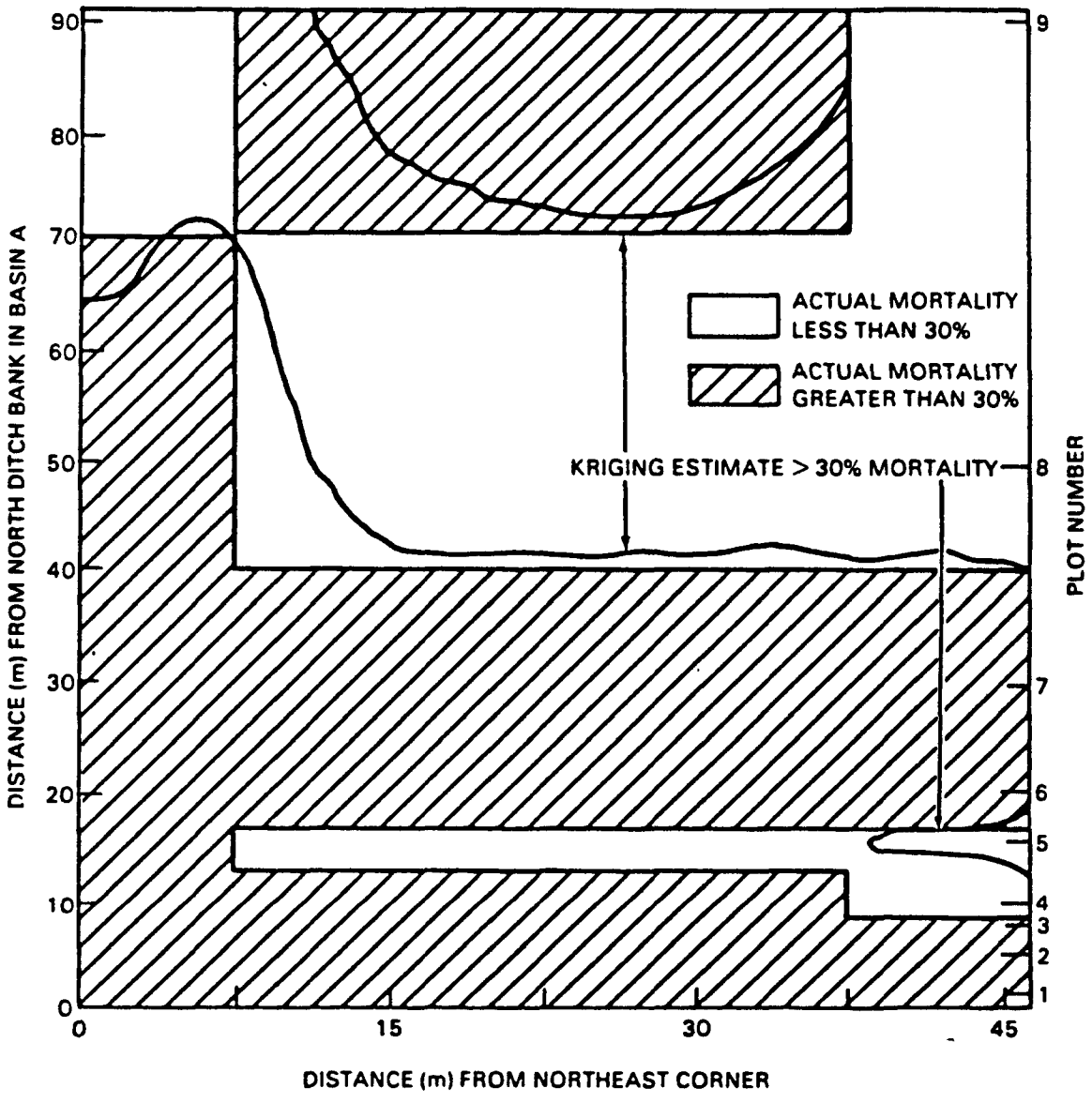


FIGURE 8.11. Comparison of Lettuce Seed Mortality Predicted from Kriging to Actual Seed Mortality, 15- to 30-cm Soil Fraction

8.2 SEDIMENT SAMPLING AT A WOOD TREATMENT SITE

The wood treatment site in Mississippi ceased operations in 1979. The site was known to be contaminated with creosote and other wood-preserving materials. The results discussed below are from an exploratory study to determine whether bioassays can be used to detect creosote contamination in stream sediments and water, and if feasible, to define the boundaries of creosote-contaminated zones on the site. Samples were also subjected to chemical analyses.

8.2.1 Assemble Information Relevant to the Problem

A preliminary site visit was made to obtain background information on the history of creosote disposal, to determine the dimensions of the site, and to define any special sampling problems. The site is bounded on the south by Covington Avenue and on the east and north by a creek (Figure 8.12). The creek is approximately 2 m wide at the widest point, with 2-m-high banks on either side. The creek flows northwest (on the site), enters an open concrete channel at the western site boundary, and then flows into a nearby city.

Records obtained from the Mississippi Department of Natural Resources (Bureau of Pollution Control) indicate that creosote and occasionally pentachlorophenol were used for wood treatment on the site. From 1965 to 1979, the owner of the site permitted wastes from the treatment process to flow overland to the creek, in violation of state pollution laws. Little cleanup had been done when the site closed in 1979. However, it was clear from the site visit that the site had been covered with fill material. Creosote was still being emitted from the bank into the water along some parts of the stream. Piles of creosote-contaminated material, as well as pools of black sludge, were located immediately adjacent to the storage tanks on the south side of the site (Figure 8.12).

8.2.2 Prepare a Statement of Study Objectives

The objectives of the study were to 1) determine if standard bioassays could detect creosote contamination in water and sediments, and, if so, 2) map the distribution of creosote contamination in the creek.

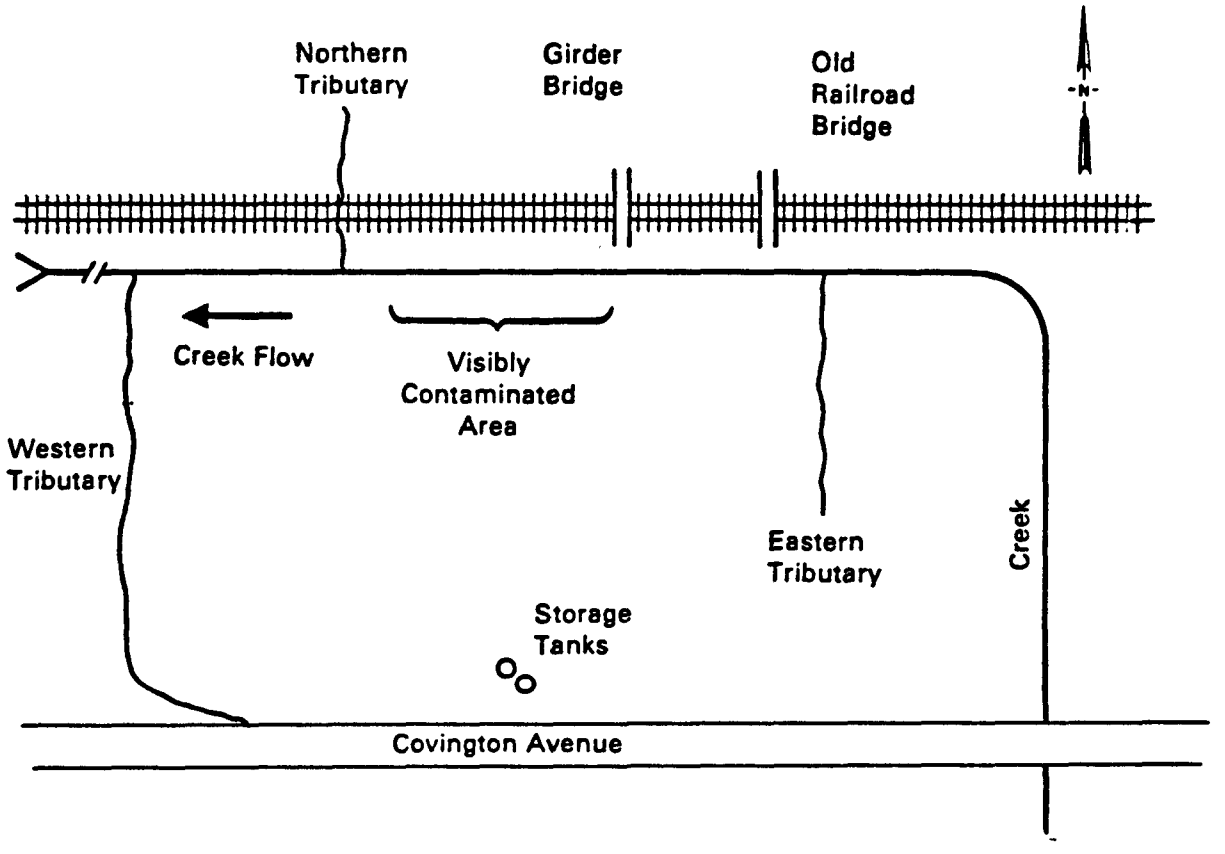


FIGURE 8.12. Wood Treatment Site in Mississippi

8.2.3 Define the Data Evaluation Criteria and Reliability Requirements

Since this was an exploratory study rather than one conducted in support of regulatory activities, the data did not need to meet specific reliability requirements. However, usual Quality Assurance procedures were observed at the Corvallis Environmental Research Laboratory and the Pacific Northwest Laboratory.

8.2.4 Determine What Is to Be Sampled

The primary interest in this study was creosote contamination of creek sediments. Therefore, stream sediment samples comprised the bulk of the samples taken. A small number of water samples were taken, as well as some samples from the bank where creosote appeared to be entering the stream. In addition, samples were taken from an upstream site (negative control) and from the pile of creosote-contaminated sludge (positive control).

8.2.5 Choose Test Organisms for the Bioassay

An array of bioassays were used, including algae, Daphnia, Microtox, earthworm, Neubauer, and root elongation tests (Porcella 1983).

8.2.6 Define the Data Analysis Technique

A description of the spatial distribution of the contamination was one objective of the study. Kriging was the first choice as a data analysis technique because it permits generation of confidence intervals about estimates of areal distribution. However, kriging generally requires a large number of data points, and in the absence of a sufficient amount of data to perform kriging, a simple linear interpolation could be substituted (principally because the area of concern was a narrow stream channel).

8.2.7 Design the Field Sampling and Laboratory Studies

The field sampling scheme is diagrammed in Figure 8.13. The girder bridge was used as the staging area and the starting point from which distances to each sampling location were measured. One sediment sample was collected at the point just before the stream entered the concrete channel at the western end of the site, 660 m west of the initial sampling location at the girder bridge. The next sample was collected at 420 m west of the initial location, then every 40 m to the east until the visibly contaminated

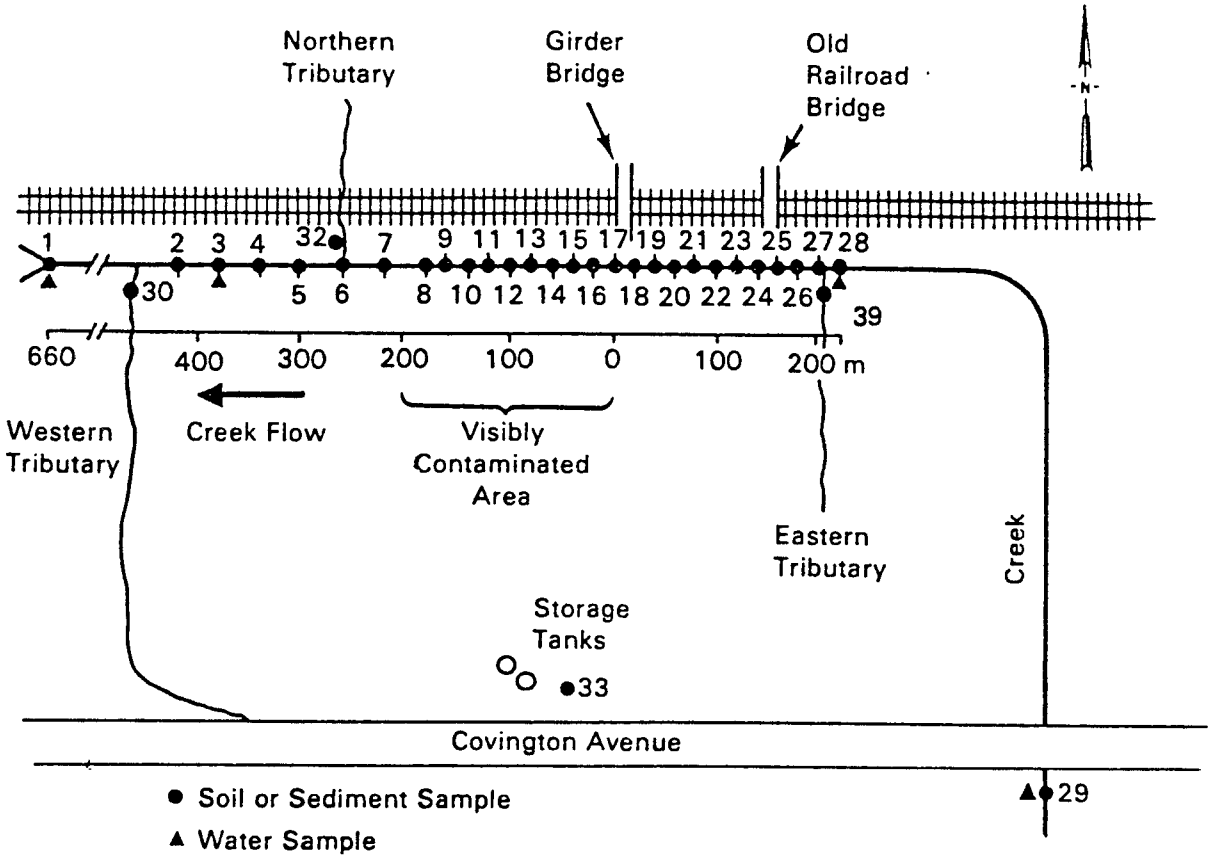


FIGURE 8.13. Location of Samples Collected from the Wood Treatment Site in Mississippi (distances are in meters). Soil or sediment samples are indicated by solid circles and water samples with solid triangles.

zone of the stream was reached. Samples were collected every 20 m in the visibly contaminated zone, and beyond until a location 220 m east of the starting point was reached. Three tributaries (Eastern, Western, and Northern on Figure 8.13) drain into the creek. The last sample (220 m east of the initial point) was collected upstream of the three tributaries. In addition, one composite sample was collected from each of the three tributaries. The negative control sediment sample was taken from the creek south of Covington Avenue, upstream from the site. The positive control sample was taken from the piles of creosote-contaminated sludge near the storage tanks. This sludge was believed to be the same material that was visibly contaminating the stream.

Water samples were collected at 660 m west of the initial point (farthest downstream location), 380 m west of the initial point, 220 m east of the initial point (farthest upstream location), and south of Covington Avenue, where the negative control sediment sample was taken (Figure 8.13). All samples were taken on the same day to maximize the comparability of bioassay results and to minimize the sampling costs [samples were collected from west to east (downstream to upstream) to minimize cross-contamination of samples]. The laboratory analyses were completed in two phases. In phase 1, only sediment samples from 660, 380, and 20 m west of the initial point, 120 and 220 m east of the initial point, the positive and negative controls, the composite sample from the eastern tributary, and the water samples were analyzed. The results of these bioassay tests were used to bracket the contaminated zone. In phase 2, samples from 300, 140, and 60 m west of the initial point were analyzed to aid in defining the contaminant boundaries.

8.2.8 Determine the Sample Collection Methods

The clay sediments lining the creek were very hard and were sampled using a hand coring device. Where possible, surface sediment samples were collected to a depth of 15 cm with hand trowels.

8.2.9 Define the Operational Procedures

Protective clothing and masks were worn by the field sampling personnel during sample collection.

8.2.10 Review the Design

The sampling design was reviewed by the project manager, a statistician, the field sampling crew, and the biologist in charge of the laboratory analysis before the fieldwork began.

8.2.11 Periodically Evaluate Progress

The bioassay results from phase 1 samples were evaluated and used as the basis for selecting the samples to be analyzed in phase 2.

8.2.12 Analyze and Evaluate the Results

The results of bioassay analyses of phase 1 samples are shown in Table 8.3. The only locations where appreciable toxicity occurred were the positive control and locations 660 and 380 m west of the initial sample location. The water sample from the 380-m-west location was highly toxic, while the sediments from that location were less toxic. At 660 m west, however, the sediments were highly toxic to some organisms while the water was not toxic.

Figure 8.14 contains toxicity maps of creek sediment elutriates from 660 m west to 220 m east of the initial point. These maps were based on bioassay and chemical analyses from both phase 1 and phase 2 samples (distances to the west of the initial point on Figure 8.14 are indicated by negative numbers). The respective creosote concentrations determined for each sample by IR (infrared spectroscopy) are expressed as a percentage of the highest creosote value measured (9500 and 25 ppm for sediments and sediment elutriates, respectively). We note that creosote is a complex mixture of organic compounds. Since there were insufficient data to use the kriging technique to devise maps, the maps were prepared using simple linear interpolation of the results between the sampling points. Therefore, the precision of the division locations between different zones of contaminant concentrations cannot be estimated (as in kriging).

The top four bars on Figure 8.14 illustrate EC50s from the algal, Daphnia, Microtox, and root elongation bioassays in which the test materials were sediment elutriates. The results from different elutriate bioassays led to different conclusions regarding the relative toxicities of different areas of stream sediments. Such variable biological responses could result from

TABLE 8.3 Bioassay Results from Phase 1 Samples Collected from the Wood Treatment Plant in Mississippi

		EC50					
Sample Location	Sample Type	Algae[a]	Daphnia[a]	Microtox[a]	Root Elongation[a]	Neubauer[b]	Earthworm[b]
660 m west	Sediment	63.7	73.6	4.0	100	70.3	27.9
	Water	NE[c]	NE	NE	NE	NR[d]	NR
380 m west	Sediment	73.7	NE	29.9	100	100	27.9
	Water	6.6	0.2	9.6	100	NR	NR
20 m west	Sediment	NE	NE	NE	NE	NE	NE
120 m east	Sediment	NE	NE	NE	NE	NE	NE
220 m east	Sediment	NE	NE	NE	NE	NE	NE
	Water	NE	NE	NE	NE	NR	NR
Negative Control	Sediment	NE	NE	NE	NE	NE	NE
	Water	NE	NE	NE	NE	NR	NR
Positive Control	Sediment	0.6	6.9	8.5	8.1	0.9	3.9
Eastern Tributary	Sediment	NE	NE	NE	NE	NE	NE

(a) Tests conducted with sediment elutriates.

(b) Tests conducted with sediment samples.

(c) NE = No effect.

(d) NR = Bioassay not required.

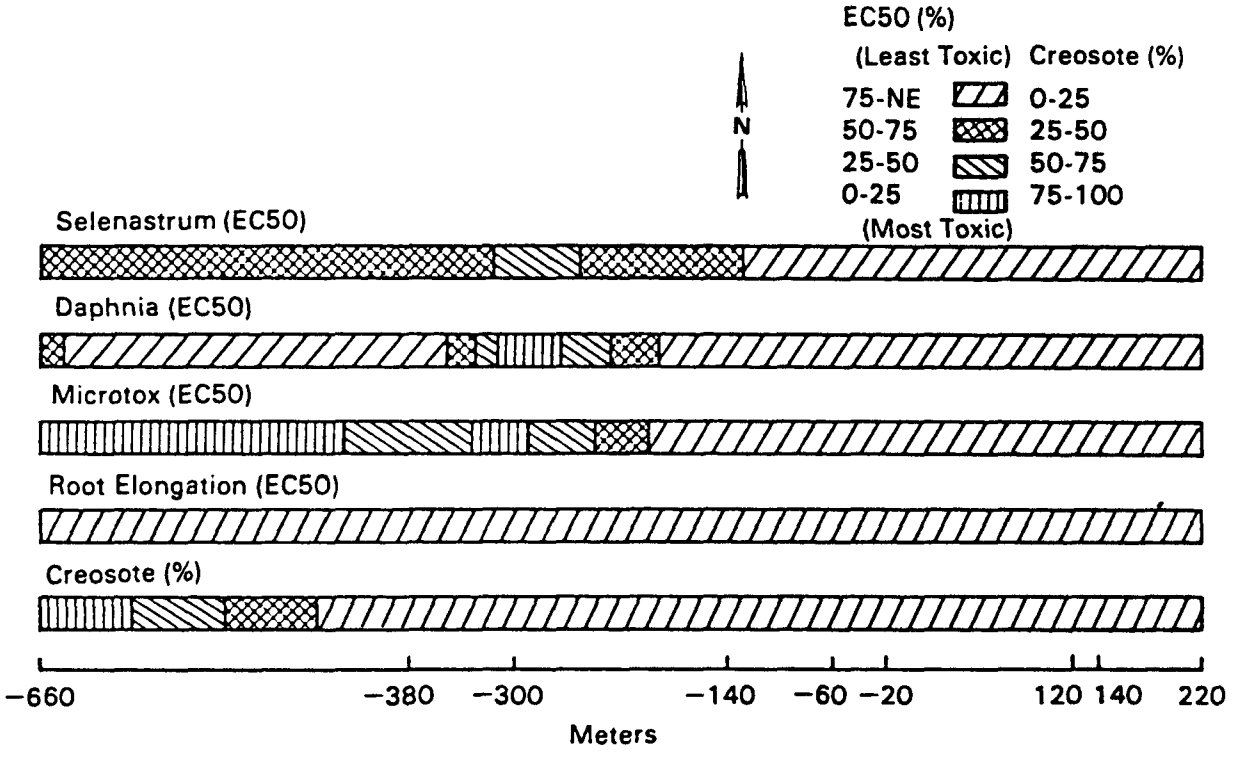


FIGURE 8.14. Bioassay Results from Sediment Elutriates at the Wood Treatment Site in Mississippi. Negative numbers represent samples collected downstream from the site.

different organic compounds in creosote, which may bind differentially in each area of stream sediments, or from in-stream seeps from the waste site. Chemical analyses indicated that the most severe contamination occurred in the extreme downstream portion of the creek study area. The algae, Daphnia, and Microtox bioassays indicated that the most extreme toxicities actually occur about 300 m west of the initial point. The Microtox bioassay was most sensitive to the chemical contaminants in the downstream sediment elutriates. The results from root elongation tests show a complete absence of a detectable phytotoxic component. A comparison between relative creosote amounts and algae, Daphnia, and Microtox response to sediment elutriates collected between -140 and -400 m (Figure 8.14) suggests that contaminants other than creosote caused the toxicity.

Figure 8.15 contains the bioassay results from creek sediments and the respective chemical analyses. The highest creosote concentration measured in sediments was 9500 ppm (two orders of magnitude greater than that in elutriates). Again, the conclusions regarding contaminant distribution differ depending on the bioassay used. Chemical analyses did not directly correspond to toxicity (Figure 8.15). However, earthworm toxicity appeared to track increasing creosote amounts more closely than the Neubauer plant test.

It appears from a comparison of the results in Figures 8.14 and 8.15 that a significant fraction of the toxic compounds in sediments are water soluble. In addition, stream cleanup decisions based on bioassay results are simplified, because the stream is so narrow that extra sediment removal would not involve much extra cost. Based on Porcella's (1983) criteria and the Daphnia and Microtox sediment elutriate bioassay results, cleanup should begin at about 220 m west of the initial point and continue to about the 660-m area.

The major conclusions from this study are 1) standard bioassay organisms are sensitive to contaminants resulting from wood treatment operations, 2) different bioassay organisms have different sensitivities to the mixture of contaminants resulting from wood treatment operations, 3) infrared measurements of sediment contaminants resulting from wood treatment operations are inaccurate predictors of biotoxicity, and 4) Daphnia and Microtox bioassay results can be used to make cleanup decisions.

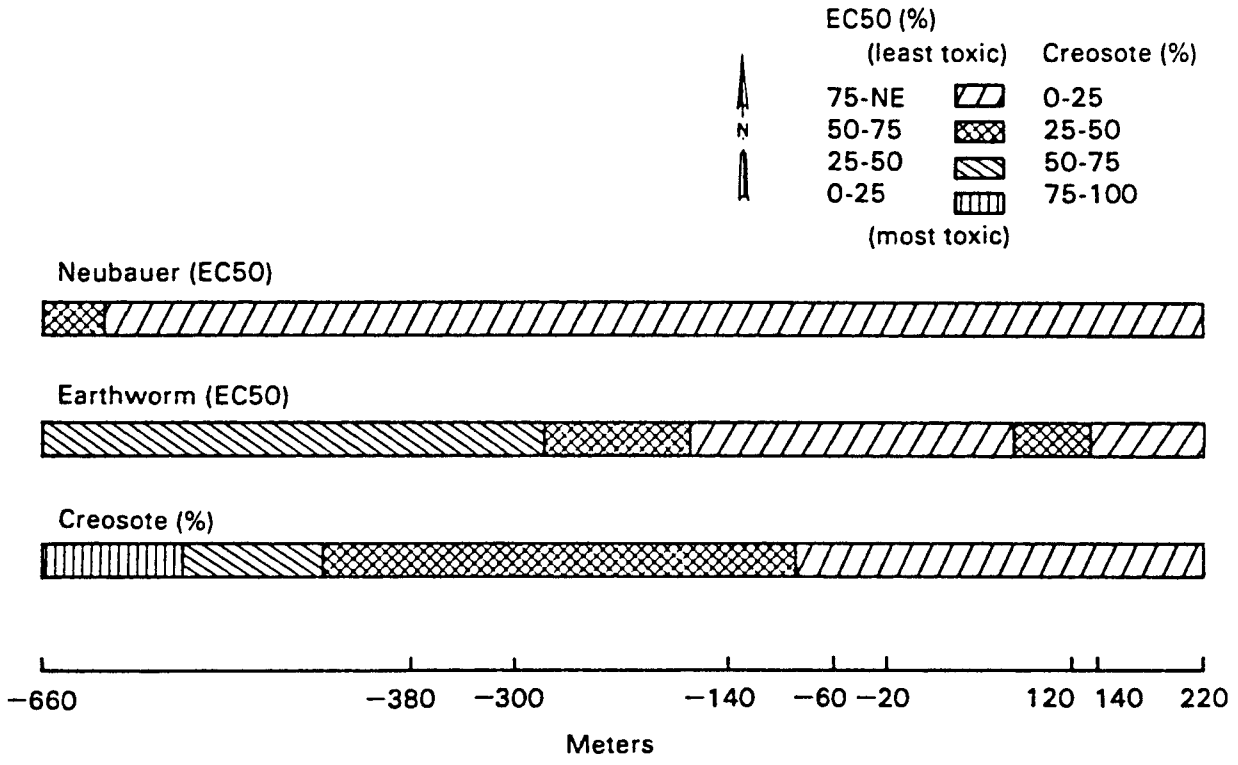


FIGURE 8.15. Bioassay Results from Sediments at the Wood Treatment Site in Mississippi

8.3 SOIL, SEDIMENT, AND SURFACE-WATER SAMPLING AT TWO HAZARDOUS WASTE SITES

Two hazardous waste sites with different histories were sampled. Site 1 (Friedman, New Jersey) was initially ranked high as a potentially hazardous site because of eyewitness reports that it had received drums and free-flowing liquids during the late 1950s and early 1960s. A subsequent site survey and detailed chemical study suggested that there would be few environmental or human health problems at this site. Excavations conducted during the survey revealed that the site had actually been used as a dumpsite for household waste and building debris. However, analysis of site sediments indicated the presence of very low levels of a few priority pollutants. Because the site is near residential wells, an extensive bioassay study was designed and carried out to assess toxicity.

Site 2 (Combe, New Jersey) is an abandoned commercial landfill situated in a partially wooded rural residential area. The site is bordered on one side by a hardwood wetland containing the headwaters of a brook that is the source of the water for a fishery in a nearby state park. In addition, water from the brook is the source for some residential ponds. The site contained an old landfill, closed in 1972, that contains waste from the 1940s. A newer landfill was closed in 1981. There were no records available to provide information about the types or volumes of chemical or industrial wastes deposited at the site. Because some offsite surface-water samples contained >100 ppb of dichloromethane, carbon tetrachloride, trans-1,2-dichloroethene, nonane, and 1,1-dichloroethane, and because similar concentrations had been reported from two onsite seeps, this site was presumed to be contaminated.

8.3.1 Assemble Information Relevant to the Problem

Site 1 (Friedman, New Jersey, Figure 8.16) includes 0.68 ha located near the intersection of two county highways. It is adjacent to an unnamed stream that is tributary to a nearby river. Several residences and two trailer parks are located within 402 m of the site, and all the residents obtain water from private wells. In 1983 field investigations were begun on the presence of hazardous substances and their areal extent and migration because of public concern resulting from rumors that hazardous substances had been dumped into the site. The site exists as an open vacant lot bordered to the east by scrub vegetation running into a pine and hardwood grove. This grove is nearly flat with a <1% slope toward the northeast.

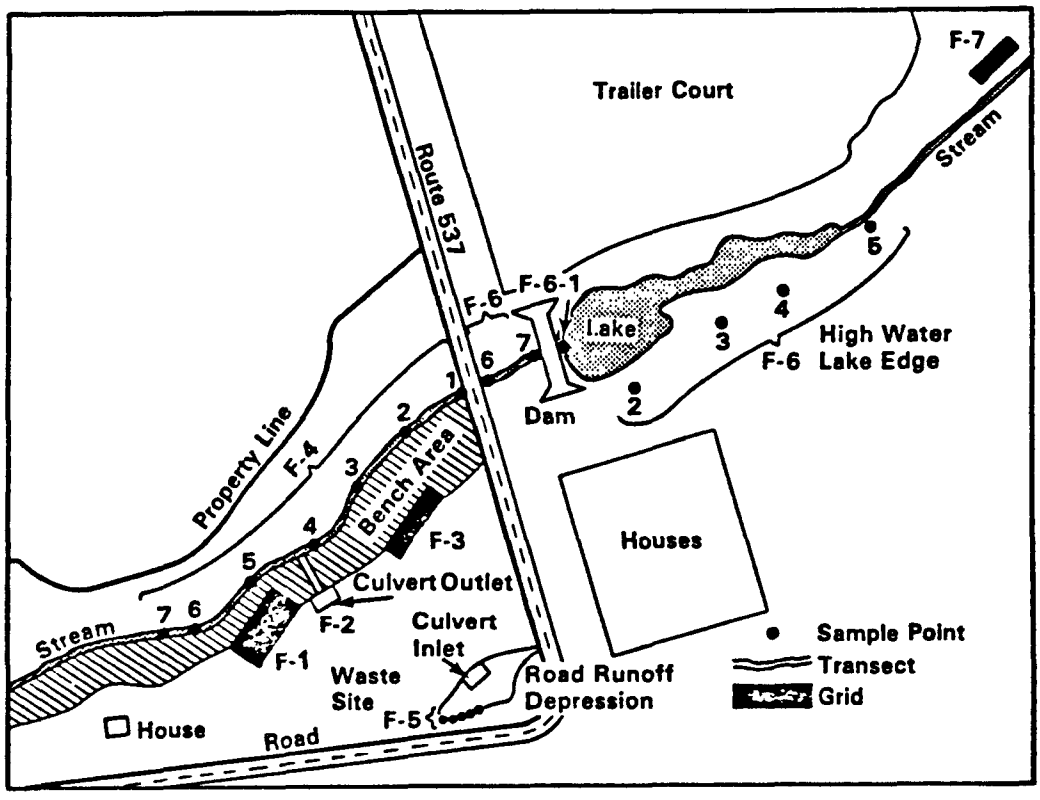


FIGURE 8.16. Seven Sampling Locations at or near Hazardous Waste Site 1. For clarity, individual sampling points are shown for locations 4-6 (•).

A patch of dense, woody vegetation approximately 30.5 by 61.0 m is located in the southwest corner of the property at the junction of the two county roads. This dense vegetation covers a surface depression that is approximately 2 m lower than the surrounding ground surface (location F-5, Figure 8.16). The depression receives drainage from the west side of one road and the south side of the other. Drainage water leaves the depression via a 0.9-m-diameter corrugated metal culvert pipe that runs north-northeast under the dump site at a slope of 2.3%. This culvert runs through the site for 83.9 m to its discharge at a marshland, where it flows 30.5 m through a small channel to the stream.

At the northeast and eastern portions of the site the slope of the land increases (approximately 3.1 m of relief), forming a wooded escarpment with slopes varying between 10% and 40%. This escarpment slopes into a marshland formed by the tributary. The total relief through the site is about 6.2 m, with an average slope of 3.3%.

Based on chemical concentrations from six shallow wells and eight local domestic wells, the shallow ground water and deep aquifer were judged to be either not contaminated or to contain inconsequential levels of chemicals. Similarly, samples of site soils and actual waste (obtained from trenching) revealed no pollution problems. However, stream sediment analysis indicated that several priority pollutant organics and inorganics were present at very low levels in sediments in and around the site. A comparison of the 1983 sediment concentrations with previous sampling results indicated that a group of polynuclear aromatic hydrocarbons, most likely a result of previous road maintenance operations, had been trapped in the surface drainage sediments. The comparison also showed that the areas of highest concentration appeared to be moving downstream. Lead, a common pollutant associated with roadway runoff, was also found in the stream sediments. Overall, there did not appear to be any significant contributions from organic or inorganic priority pollutants in the ground water, surface water, or stream sediments that could be attributed to site waste.

Site 2 (Combe, New Jersey) is a 40-ha inactive sanitary landfill situated atop a hill in a partially wooded residential area. Portions of the landfill appear to extend above the preexisting ground surface, with elevations ranging from 244 to 268.4 m above mean sea level. The site is

bordered to the east and south by a county road, to the north by private properties on residential streets, and to the west-southwest by a 20-ha tract described as a hardwood wetland (Figure 8.17). This wetland contains the headwaters of a brook that is also a river tributary. Surface runoff from the site drains to both the east and west branches of the brook.

The old landfill consists of two areas totaling approximately 12 ha. These older landfill areas were filled and partially reclaimed in 1972 and may contain refuse deposited during the 1940s. No record exists of the type of wastes disposed in the old landfill. The present extent and configuration of the old landfill areas are not yet confirmed.

The new landfill area is located to the south and west of the older landfill and extends west to the wetland where some landfilling operations may have been conducted. The new landfill was closed and regraded in September 1981. Existing cover material consists of coarse permeable local soils and crushed bedrock. Severe sheet erosion has occurred on the steep slopes at the western and southern edges of the landfill where vegetation was not established. Numerous brownish-black-stained seep areas are present both at the base of the landfill and on the side slopes. The exact extent and configuration of the new landfill are also unknown. In addition to the landfill operation, several open fields near the site may be of importance. Local residents have suggested that a field at the northwest corner may have been used for unauthorized dumping of refuse, chemical wastes, and industrial wastes.

A potential problem at the landfill site is the discharge of contaminants into the local streams from surface runoff, ground-water baseflow, and leachate seeps. All streams mentioned previously eventually discharge into a source of public water supply. It is expected that the wastes contained in eight identified seeps (location C-8, Figure 8.17) have or will penetrate and contaminate site cover soils as well as offsite soils in established flow paths. Eleven known and 24 unknown organics have been found in the residential well samples near the site. Tetrachloroethylene and chloroform were the most common. Both substances have been identified as potential carcinogens by the EPA and were measured at levels that may constitute a risk via water ingestion. Because of these identified priority pollutants and the available information, this site was considered contaminated.

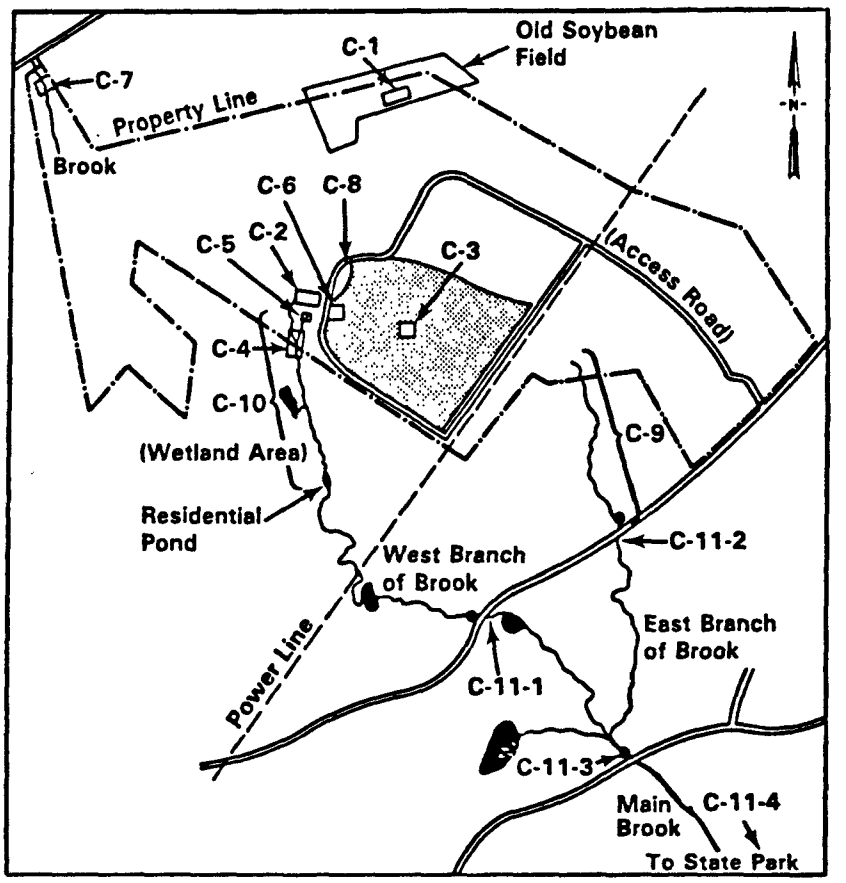


FIGURE 8.17. Sampling Locations at Landfill Site 2

8.3.2 Prepare a Statement of Study Objectives

Based on chemical analyses of soil (including samples from trenches) and surface- and ground-water samples, Site 1 was judged not to be an immediate problem to either the environment or human health. Thus, the working hypothesis for bioassay studies at this site was that the site did not contain dangerous levels of priority pollutants.

There were two objectives in the bioassay program for this site:

1. To validate, insofar as possible, that samples from the same areas that were previously declared to be chemically clean were also biologically inactive
2. To evaluate the toxic potential of the site "bench" (area of streambed periodically flooded) and the onsite stream area using results from composited soil and water samples (see Figure 8.16).

Site 2 had both a prior history and outward signs of chemical contamination. However, the extent of surface and subsurface contamination was unknown because of intermittent ground-water seeps and the possibility of undocumented dumping. For these reasons, bioassay studies at this site were necessary to determine the degree and geographic extent of any offsite or unidentified onsite chemical contamination and to assess the environmental hazard. The objectives of the bioassay program for Site 2 were as follows:

1. To determine the toxicity of surface soils and seep areas in the new landfill and open field area
2. To determine whether samples of sediment and water collected in the east and west branches of the brook and the wetland to the southwest of the landfill were toxic.

8.3.3 Define the Evaluation Criteria and Reliability Requirements for the Results

Because samples from both sites were to be composited for screening bioassays, the number of samples selected for compositing was calculated using the method outlined in Section 5.2:

$$n \leq \frac{MAL}{MDL}$$

where

n = the number of component samples to mix into a single composite

MAL = maximum acceptable limit. We selected 50% reduction in the Selenastrum yield (relative to control) at an 80% elutriate concentration, which is the highest sample strength that can be bioassayed with algae. Such a limit would be liberal compared to the arbitrary <20% EC50 standard in Porcella (1983).

MDL = minimum detectable limit. Previous results led us to believe that control samples might cause up to 5% reduction in Selenastrum at an 80% elutriate concentration.

Thus, $n \leq 50 / 5 \leq 10$. According to these calculations, when 10 components are mixed and eluted with water (4:1 volume:weight) and the resulting 80% concentration material causes a reduction in Selenastrum numbers >50%, 1 or more of the 10 components is over the MAL, and each would have caused a reduction >50% if tested alone. It is possible that only one sample might be over the MAL (50%) if it contained very high levels of toxicant. However, testing individual components at full strength may not identify this high concentration since percent reduction in Selenastrum numbers (compared to control) cannot exceed 100%. Thus, EC50 determinations on each component may be needed. If the elutriate causes a reduction of 5% or less, then none of the 10 components would show a 50% reduction when tested alone. However, the above calculations depend on the negative linearity of the dose (units of toxicant extracted from the composite) versus percent reduction function. Intermediate results (i.e., between 5% and 50%) would require a statistical test such as given in Skalski and Thomas (1984).

Clearly, there are problems and research questions to resolve relative to compositing samples from chemical waste sites. In order to use the statistical test in Skalski and Thomas (1984), background parameters (e.g., based on the distribution of control effects) must be available. Since the statistical test could not be used at either site, a three-stage laboratory analysis strategy was used. Thus, intermediate and sometimes high reductions in Selenastrum or Daphnia caused by composite elutriates triggered a final-stage EC50 determination on all components. Often, a second-stage screening using root elongation and Microtox was conducted. Moreover, when spatial

patterns in final stage component EC50 values are found, kriging techniques can be used to prepare a map (see Section 8.1.4 for an example).

8.3.4 Determine What Is to Be Sampled in the Field

To meet the objectives at Site 1, the following media and locations were sampled (see Figure 8.16):

1. Soil and/or sediments in the wetland between the landfill and the stream
2. Sediments and water in the stream
3. Sediments and water at the juncture of the two county roads
4. Sediments and water upstream of the site.

Samples of the following media were collected from Site 2 (Figure 8.17):

1. Soil and/or water at all eight identified seep areas (C-8)
2. Soil/sediments/water in the wetlands southwest of the site
3. Sediment and water samples in the east and west branches of the brook
4. Neutral (control) soil and water samples collected north of the site (C-7).

8.3.5 Choose Test Organisms for the Bioassays

The Selenastrum and Daphnia bioassays have been shown to respond to more than 90% of the hazardous chemical wastes tested (Miller et al. 1985). Therefore, to reduce costs, these bioassays were selected as a first-stage screen to test the composited samples from Sites 1 and 2. EC50 determinations were not conducted in this stage. Instead, each composite was tested using algae [three replicates each of two elutriate concentrations (one at 80%, rather than 100%, because nutrients must be added to the growth media, and a second concentration of 25%)]. Only one replicate of each of two elutriate concentrations (100% and 25%) was tested using the Daphnia bioassay. When results from the 80% concentration exceeded 50% reduction, components were individually bioassayed as a second stage. Definitive EC50s for Daphnia or algae were generally conducted as a final stage. Depending on the second-stage results for the Microtox or root elongation bioassays, as well as first-stage screening results, definitive EC50s for earthworms and plants were conducted. The lower first-stage screening elutriate

concentrations (25%) were included to evaluate multicomponent causes of toxicity and/or to judge the existence of very toxic single components.

8.3.6 Define the Data Analysis Techniques

For Site 1 studies, objective 1 (see Section 8.3.2) simply required a finding of no biological toxicity. For composite samples where results were below 5% reduction in Selenastrum numbers, components were judged to be biologically unaffected (see Section 8.3.3). Elutriate samples that inhibited algae growth by more than 50% were reassayed to define their EC50 values (see Section 8.3.5). Samples with reductions between 50% and 75% were usually rescreened with Microtox and root elongation bioassays.

To meet objective 2 at Site 1, two grids were established in the "bench" region (see Section 8.3.7 and locations F-1 and F-3; also see Figure 8.16). If the composite components proved to be toxic, kriging could be used to assess the areal extent of toxicity. Both objectives for Site 2 (see Section 8.3.2) could have been met using the methods for Site 1, objective 1. However, as a precaution, several grid designs were used even though questions about spatial distribution were not included in the objectives. Rectangular grids ranged from 1.5 to 25 m on the longest side, depending on the area targeted for sampling [e.g., a 3-m^2 pond at the top of the landfill or a 100- x 28-m area in the soybean field (Figure 8.17)]. These "extra" samples allowed the compositing strategy to be used to assess areal toxicity potential, and if toxicity was found, components were available to assess either small- or large-scale patterns.

8.3.7 Design the Field Sampling and Laboratory Studies

Because there was very little chemical concentration data available for either site, a varied field sampling protocol was developed. At each of the sites an array of sampling schemes was used to collect soil/sediment and surface-water samples. Rectangular grids were established at various locations to systematically sample selected regions. In other instances (i.e., along streams and apparent waste flows), samples were collected along line transects or by opportunistic sampling of suspected contaminated areas. Sample locations were mapped and identified using transit, compass, and meter tapes. Soil and sediment samples were collected to a depth of 15 cm using a metal hand spade. Water samples were obtained by submerging 1-L polyethylene

bottles below the surface of water sources. Samples collected under these protocols were evaluated using screening bioassays of composited samples (see Section 8.3.3). A second, and sometimes third, laboratory analysis stage was used to evaluate individual composite components when toxicity was found in first-stage screening tests.

An example of a 25-point (component) rectangular grid from location F-3 at Site 1 (see Figure 8.16) is shown in Figure 8.18. A similar grid was used at location F-1, while a 12-point grid was used at F-7. In Figure 8.19, an example of a transect sampling approach at Site 1 is shown (location F-4, Figure 8.16). Finally, in Figure 8.20, an example of opportunistic sampling at location C-11 of Site 2 is shown (Figure 8.17). All other locations at Sites 1 and 2 were sampled using variants of the methods shown in Figures 8.18 through 8.20 or in Section 8.1.

The compositing strategy used for the samples from the locations in Figures 8.18 through 8.20 is shown in Table 8.4. Approximately 250 g of soil or sediment yielded 1000 ml of elutriate for the bioassays (e.g., about 4:1 volume:weight). This quantity of material was more than adequate for conducting three replicate Selanastrum tests at 80% and 25% dilutions each (six tests) and one replicate Daphnia test at two dilutions, 100% and 25% (two tests).

The basis for including up to 10 components in a single sample composite was explained in Section 8.3.3. At location F-3 (Figure 8.18), samples from two of the three bench locations with standing water (F-3-17 and F-3-23) were pooled and assayed to assess toxicity. The streamside soil samples (F-3-16 through F-3-25) and samples from farther away (F-3-1 to F-3-10) were composited to examine the bench for toxicity (Figure 8.18). Location F-4 (Figure 8.19) provided the opportunity to assess toxicity in the stream water and sediments from where the stream entered Site 1 (F-4-1) to beyond the site boundary (F-4-7). To facilitate an upstream/downstream onsite comparison, four water and sediment samples from two upstream (F-4-2 and F-4-3) and two downstream stations (F-4-5 and F-4-6) were composited into samples F-4-C1 and F-4-C2, respectively (Table 8.5). All water and sediment samples collected at individual sites at location C-11 (Figure 8.20) were bioassayed to determine if either branch of the brook or the unbranched brook itself in or out of the state park contained toxic compounds.

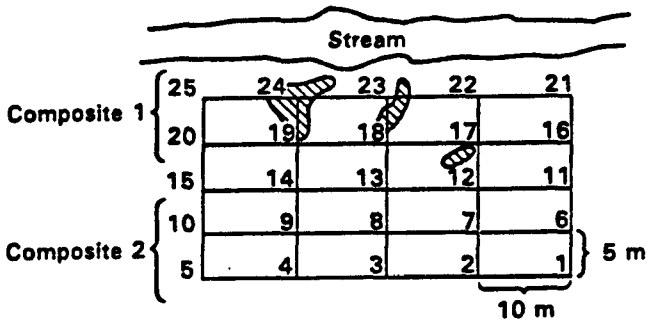


FIGURE 8.18. Grid Sampling at Location F-3 of Site 1

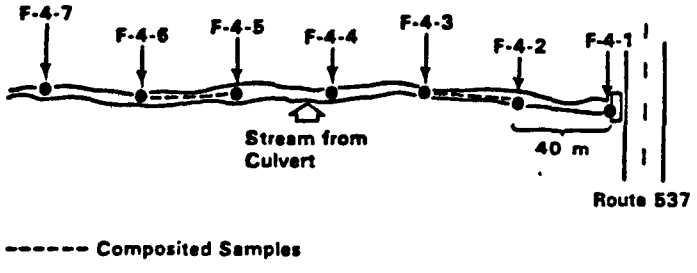


FIGURE 8.19. Transect Sampling at Location F-4 of Site 1

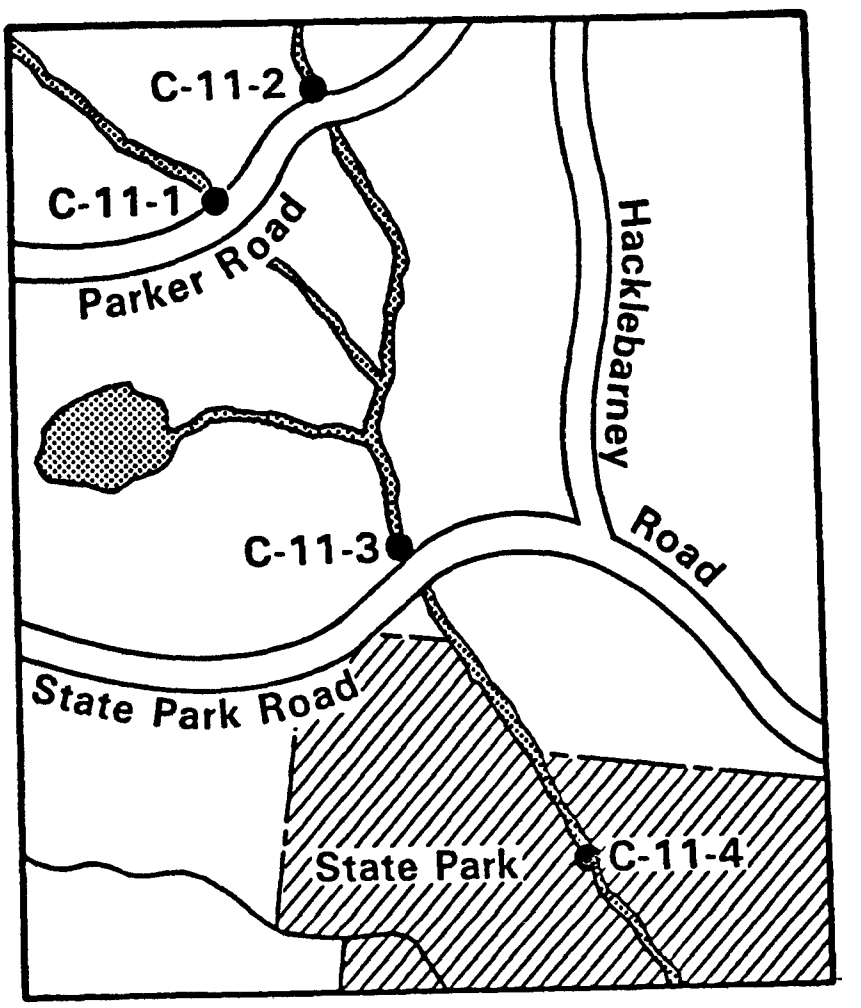


FIGURE 8.20. Opportunistic Sampling at Location C-11 of Site 2

TABLE 8.4. Description of Composite Components Used in Screening Bioassays of Samples Taken from the Locations Shown in Figures 8.18 to 8.20

Location	Media	Samples to be Composited	Sample Size from Each Container	Total Sample Size
F-3	Water	F-3-17 A & B	250 ml	500 ml
		F-3-23 A & B	250 ml	500 ml
		F-3-24 A & B	250 ml	500 ml
F-3	Soil	F-3-1 to F-3-10	100 g	1000 g
		F-3-16 to F-3-25	100 g	1000 g
F-4	Water	F-4-2 A & B and F-4-3 A & B	125 ml	500 ml
		F-4-5 A & B and F-4-6 A & B	125 ml	500 ml
F-4	Sediment	F-4-2 A & B and F-4-3 A & B	100 g	400 g
		F-4-5 A & B and F-4-6 A & B	100 g	400 g
C-11	Water	C-11-1 A & B	250 ml	500 ml
		C-11-3 A & B	250 ml	500 ml
		C-11-4 A & B	250 ml	500 ml
C-11	Sediment	C-11-1	250 g	250 g
		C-11-2	250 g	250 g
		C-11-3	250 g	250 g
		C-11-4	250 g	250 g

TABLE 8.5. Bioassay Results from Three Stages of Sample Analysis at Site 1. Results from other samples that did not exhibit toxicity are not presented

Site 1 Location	Sample Number[a]	Media	First-Stage Screen: Algae 80%[b]	First-Stage Screen: Algae 25%	First-Stage Screen: Daphnia 100%	First-Stage Screen: Daphnia 25%	Second-Stage Screen: RE Reduction[c] (%)	Final-Stage Screen: Algae EC50	Final-Stage Screen: Daphnia EC50	Final-Stage Screen: RE EC50	Final-Stage Screen: NEU[d]	Final-Stage Screen: Earthworm Loss[e] (%)
F-1	F-1-C1	Soil	-33[f]	-25	NE[g]	NE	NE	NA[h]	NA	NA	NE	NA
F-3	F-3-C1	Soil	-91	-76	-71	NE	NE	20[i]	94	NA	38	-20
F-7	F-7-C1	Soil	NE	NE	NE	NE	-39	NA	NA	NA	NE	--[j]
	F-7-1		NE	NE	NE	NE	-69	NA	NA	28	35	--[j]
	F-7-10		NE	NE	-100	NE	NE	NA	89	NA	74	--[k]
	F-7-11		NE	NE	-100	NE	NE	NA	89	NA	63	NE
F-2	F-2-C1	Sediment	-67	-48	NE	NE	NE	22	NA	NA	27	--[j]
F-4	F-4-C1	Sediment	-97	-60	NE	NE	NE	33	NA	NA	NE	--[k]
	F-4-C2		-94	-67	NE	NE	NE	36	NA	NA	NE	--[k]
F-5	F-5-C1	Sediment	-100	-69	-100	-14	NE	20	31	NA	NE	--[k]

(a) A "C" in the suffix indicates a composite.
(b) Percent dilution of elutriate.
(c) RE = Root Elongation.
(d) NEU = Neubauer Test.
(e) In 75% to 80% soil.
(f) Negative values are mortalities in 100% soil or soil elutriate.
(g) NE = No Effect.
(h) NA = Not Applicable.
(i) Positive values are EC50s.
(j) Analysis not yet completed.
(k) Insufficient sample.

The transect sampling technique was to run transects down the length of the stream starting at the west side of Route 537. Samples of sediment and water were taken at 40-m intervals along the course of the stream. At each sample location, one 2-kg sediment sample and two 1-L water samples were collected. All samples (sediment and water) were similarly coded and numbered F-4-1 to F-4-7.

8.3.8 Analyze and Evaluate the Results

The bioassay results from all locations (in addition to the examples illustrated in Figures 8.18 and 8.19) at Site 1 that exhibited toxicity are listed in Table 8.5. Composited sediments from locations F-2 (not illustrated in detail; however, the composite contained four components), F-4 (shown in Figure 8.19), and F-5 (not illustrated in detail, but it contained two components) were toxic to algae in the first-stage screen and very toxic to algae (low EC50 values) in the final screen. Further, the sediment composite from location F-5 was also toxic to Daphnia, while the F-2 composite was toxic to lettuce seeds; however, water samples from these locations were not toxic. These toxic composite sediment samples were from an area between the culvert outlet and the stream (F-2); instream, upstream from the culvert entrance (F-4-C1); instream, below the culvert entrance (F-4-C2); and in the depression where the two roads meet (F-5). Figure 8.16 shows the general locations. Previous chemical analyses of site sediments (Section 8.3.1) indicated that polynuclear aromatic hydrocarbons had been trapped in site surface sediments. This information, in concert with the fact that no toxicity was found in sediments upstream (location F-6) or in any of the water samples from the site, tends to support the existence of a non-site-related toxicant, bound in sediments. The toxicity to lettuce seeds of sediment composite F-2-C1 (Table 8.5) may indicate some other toxicant is present in the bench area.

A 25-point rectangular sampling grid was set up on the east side of the site between the landfill bank and the stream. Grid point F-3-5 was located at well W-5. Two 1-L water samples (A and B) were collected on each of three open-water patches in the grid near points F-3-17, F-3-23, and F-3-24. One 2-kg soil sample was collected at each grid node (F-3-1 to 25 on Figure 8.18).

Opportunistic sediment and water samples were collected at the road intersections with the east and west branches of the brook (samples C-11-1 and C-11-2, respectively). Sample C-11-3 was obtained beyond the confluence of the two branches near State Park Road, and sample C-11-4 was collected in the park. Each sample consisted of two 2-kg sediment and four 1-L water samples (except C-11-2, where no standing water existed).

The 10 components contained within the soil composites that were taken closest to the stream (see Figure 8.18 and Table 8.5) were slightly toxic (F-1-C1) to very toxic (F-3-C1) for algae and Daphnia in the first-stage screen. Further analysis (final screen, Table 8.5) of sample F-3-C1 indicated that this upstream onsite location contained soils that were toxic to algae and lettuce seeds and slightly toxic to earthworms. Moreover, both composites were located closest to the stream where deposition of road runoff would be likely during periods of high water. As with the instream water samples, standing water from location F-3 was not toxic. However, this sample also provides additional evidence for an insoluble phytotoxic component in F-3 soils (sample F-3-C1) closest to the stream. Thus, there may be a site area of localized phytotoxicity, but most biotoxicity appears to have resulted from road maintenance.

The Neubauer phytoassay results from individual soil samples taken far upstream from the site (principally samples F-7-1, F-7-10, and F-7-11) provide evidence (EC50s range from 35% to 74% soil) that either a naturally occurring phytotoxic component may exist in regional soils, or the component is only bound in a few soils or sediments over the entire region of the stream. Thus, it appears that those areas of the site that were previously shown to be chemically "clean" are also biologically inactive (objective 1, Section 8.3.2). Results from onsite and offsite stream sediment and water samples implicate road runoff as a likely source of the insoluble toxicants detected. One onsite phytotoxic soil and one sediment composite sample were found. Because there were three single phytotoxic soil samples far upstream, it appears that site phytotoxicity may not be caused by site-related chemicals.

The results from locations C-8 to C-10 at Site 2 (location C-11 is shown in Figure 8.20) that showed evidence of toxicity relevant to the study objectives are presented in Table 8.6. The location of each sampling site is shown in Figure 8.17.

For convenience, the bioassay results are discussed for the east branch (location C-9 and sample C-11-2), west branch (location C-10 and sample C-11-1), and joint brook water and sediment samples, followed by the seep (C-8) soil and water samples. A description of samples obtained from locations C-8 to C-10 (for C-11; see Figure 8.20) is given in Table 8.7.

There was no evidence of toxicity caused by water or sediments from the east branch of the brook (including sample C-11-2; see Figure 8.17) since only one sample from location C-9 was toxic (the root elongation bioassay for sample C-9-5 showed some toxicity). However, several water and sediment samples from the west branch were very toxic to algae in samples just below, in, and just above the small residential pond (samples C-10-1 to C-10-3 for water and sample C-10-9 for sediment). Evidence that the toxicity had migrated downstream is provided by the sediment bioassay results from sample C-11-1 where algae, Daphnia, and plant seed toxicity were fairly severe. In addition, residential pond sediment caused earthworm toxicity (C-10-9; 100%) and one of two sediment samples taken below the confluence of the branches (C-11-4; Figure 8.17) was also toxic to earthworms. Both mainstem brook samples (including C-11-4 in the park) indicate that the phytotoxic component in the residential pond (see C-10-9; 6% EC50 for the Neubauer test) may also have migrated. Finally, upstream water samples C-10-5 and C-10-7 exhibited limited toxicity in the algal screen as well in the root elongation assay, but samples taken further upstream (C-10-8 and C-10-10) were not toxic. Thus, it appears that the toxic components may be emanating from the waste site somewhere below location C-4 (Figure 8.17) and that the eastern area of the site and the west branch of the brook either have been and/or are being influenced by chemicals leaking from the waste site. It is probable that these chemicals are being exported beyond the site boundaries (objective 2, Section 8.2.3).

Bioassay results from soils in the six seep areas of location C-8 (Figure 8.17) show that soil from one seep (location C-8-3) caused major biotoxicity to algae, Daphnia, lettuce seeds, and earthworms in the final-

TABLE 8.6. Bioassay Results from Three Stages of Sample Analysis at Site 2. Results from other samples that did not exhibit toxicity are not presented

Site 2 Location	Sample Number[a]	Media	First-Stage Screen: Algae 80%[b]	First-Stage Screen: Algae 25%	First-Stage Screen: Daphnia 100%	First-Stage Screen: Daphnia 25%	Second-Stage Screen: RE Loss (%)	Second-Stage Screen: Microtox X 30	Final-Stage Screen: Algae EC50	Final-Stage Screen: Daphnia EC50	Final-Stage Screen: RE EC50	Final-Stage Screen: Neubauer Loss (%)[c]	Final-Stage Screen: Earthworm Loss (%)[d]
C-8	C-8-4	Water	-99	-25	-100	-25		-23	4	7	14		
	C-8-1	Soil	-4	--[e]									
	C-8-2	Soil											
			+159				-23					66	NE[f]
	C-8-3	Soil	-66	-56					45	46		25	-100
	C-8-4	Soil	+177				-33					31	NE
	C-8-5	Soil	+187				-32					73	NE
	C-8-6	Soil	+243				-34					-43	--[g]
C-9	C-9-5	Water					-37						
C-10	C-10-1	Water	-93	+20					48				
	C-10-2	Water	-95	-9				-28	54				
	C-10-3	Water	-98	-64	-57	NE			17	29	83		
	C-10-5	Water	-39				-32						
	C-10-7	Water					-21						
	C-10-9	Water	-62										
	C-10-9	Sediment	-100	-58			-45		34			6	-100
C-11	C-11-1	Sediment	-92	-71	-100	NE			14	69		-47	NE
	C-11-4	Sediment										-32	-20

(a) A "C" in the sample number suffix indicates a composite.
(b) Percent dilution of elutriate.
(c) Numbers with a minus sign are mortalities in 100% soil. Positive values are EC50s. The screening result was at least -70 in 100% soil.
(d) In 70% to 80% soil.
(e) In general, no test was done because of rules stated in text or because positive results indicated no growth suppression [all other blanks indicate (e)].
(f) NE = No effect.
(g) Insufficient sample.

TABLE 8.7. Description of Samples Collected at Additional Site 2 Locations Where Positive Bioassay Results Were Obtained

Location	Description
C-8	C-8-1 Through C-8-6 Six individual seeps (contained in Area C-8, Figure 8.17) on the west side of the landfill were sampled. Sample C-8-6 was the most northern, followed sequentially south to C-8-1. At each sample location, two 2-kg soil samples were collected and designated A and B. At C-8-4, four 1-L water samples were collected. These water samples were collected at the source of the seep, where water and gas were being emitted.
C-9	C-9-1 Through C-9-7 Starting 50 m upstream of the main road on the east branch of Trout Brook, sediment and water samples were collected every 50 m. Sample C-9-1 was closest to the road while C-9-7 was northernmost, with the rest sequentially located. At each sample location, one 2-kg soil sample was collected. When samples were collected, only occasional standing water existed on the branch (no running water). At C-9-3 two 1-L water samples were collected (C-9-3A and B); at C-9-5, four 1-L water samples were collected.
C-10	C-10-1 Through C-10-10 On the west branch of Trout Brook, samples of sediment and water were collected every 50 m starting at the concrete culvert on the south end of the pond on the site (C-10-1) known as Beam's Pond. Sample C-10-9 was a sample in Beam's Pond. All except sample C-10-9 consisted of one 2-kg sediment sample and two 1-L water samples; sample C-10-9 was two 2-kg soil and four 1-L water samples.

stage screen. These toxicities indicate that both soluble and insoluble compounds are present at this seep. No toxicity was evident in soil from the seep at location C-8-1. The positive results for algae in the first-stage screen for soils from other seeps (locations C-8-2 and C-8-4 to 6) triggered second-stage bioassays. An indication of soluble phytotoxic compounds was evident from results of root elongation bioassays, while third-stage bioassay results indicate potent insoluble phytotoxic compound(s) are also present. The water collected from the seep at location C-8-4 was very toxic to algae and Daphnia, and also inhibited root elongation.

On the basis of these results, it appears that the chemicals that are or have been emitted at each seep exhibit varying toxicities that may reflect different waste compositions within the main site.

9.0 NEEDED ENHANCEMENTS OR ADDITIONS TO BIOASSAY TECHNOLOGY FOR USE AT HAZARDOUS WASTE SITES

Various aspects of the bioassay procedures, the field designs, the size and adequacy of the data base from which specific chemical causation for an observed toxic effect can or cannot be correlated, areal mapping of toxicity, and hot spot detection can be improved by additional research, data, or experience. Moreover, any procedure that can improve cost-effectiveness (e.g., staged designs, compositing) may merit additional exploration. In this section, some of the needed enhancements or improvements to bioassay technology are discussed as well as cost-effective improvements in procedures.

9.1 BIOASSAY STUDIES USING ADDITIONAL PURE CHEMICALS, MIXTURES, AND CHEMICALLY CHARACTERIZED WASTE SITE SAMPLES

At times, we have attempted to infer the chemical cause of an observed bioassay result (Section 4.3). However, there are few data currently available upon which to base such inferences. In fact, the data presented in Section 4.3 represent the few bioassay results for pure chemicals and waste site samples of known chemistry. There appear to be few, if any, bioassay results available for mixtures of chemicals in the proportions found in actual waste site samples. Because of this deficiency, not much is known about the synergistic, antagonistic, or possible additive and/or multiplicative effects of combinations of waste site chemicals. In addition, little is known about bioassay results for classes of priority pollutants. The availability of bioassay results for priority pollutants, alone and in a few meaningful combinations, might be useful in interpreting the chemical cause of observed toxicity for some waste site samples. Finally, a complete chemical analysis of a large number of diverse waste site samples would allow a toxicity prediction (based on chemical content) prior to bioassay. These predictions could subsequently be compared to actual bioassay results. In this way, the usefulness of bioassay results could be demonstrated and the proportion of inaccurate predictions based on chemical analysis could be documented.

9.2 ADDITIONAL WASTE SITE STUDIES

In Sections 8.1 to 8.3, bioassay results are used to assess specific objectives at four waste sites. Additional field studies are needed to ascertain which of the statistical methods is most useful, to define the most informative bioassays, and to rank the ease and expense with which questions (objectives) can be addressed. Further, a comparison (based on actual site experience) of bioassay and chemical analysis approaches to site characterization and predicted toxicity is also needed to validate the superiority of bioassay approaches for ecosystem risk assessment.

9.3 DECISION RULES TO RATE WASTE SITE SAMPLE TOXICITY

The only rule proposed to assess the seriousness of a laboratory-derived EC50 (based on a waste site sample) is from Porcella (1983). In his system, an EC50 < 20% elutriate is referred to as high, an EC50 > 20% or < 75% is moderate, and an EC50 > 75% is low. This system appears to be arbitrary, and is not based on any predicted environmental risk or human health considerations.

In terms of harm to the ecosystem, 50% mortality caused by 100% elutriate might be catastrophic. However, laboratory results do not generally translate directly to field observations (e.g., dilution by surface or ground water would result in lower toxicity). Thus, a ranking system like the one proposed by Porcella (1983), but with different and justified action limits, is needed. Ranking the potential site toxicity to resident organisms may need to be based on a weighting scheme that downweights toxicity obtained from elutriates, because resident organisms are exposed to actual site soils and sediments. In contrast, an argument can be made for extra weighting for very toxic elutriates. Thus, it is evident that additional thought, experiments, and experience are needed to judge which values of toxic effects should lead to remedial action.

9.4 SCREENING BIOASSAYS

In Section 8.3, screening bioassays were conducted using 80% and 25% elutriates for algae and 100% and 25% elutriates for Daphnia (and limited

replications of each level). As a first attempt at two-stage laboratory designs, the screening procedure performed well, but there was no quantitative measurement of performance. Thus, a more formal screening procedure and rationale are needed, and results should be compared to a series of samples run by the usual procedure(s). The usual and new methods can be compared based on the cost per unit of information and the precision and accuracy of each method. Such a structure could be developed and tested at future sites or during the studies suggested in Section 9.2.

9.5 DEVELOPMENT AND ENHANCEMENT OF LABORATORY COMPOSITING STRATEGIES AND CONCOMITANT FIELD DESIGNS FOR DETECTING MOVEMENT AND EXTENT OF CONTAMINATION

The scope of a sampling program generally reflects a preconceived notion of the number of samples that may be ultimately analyzed for contaminants. Since the cost of complete priority chemical analysis is very high and some bioassays are moderately expensive, generally few samples are collected from individual sites. These relatively few samples may poorly represent the extent of surface or subsurface contamination. However, by using group testing methods, large numbers of samples (soil, water, or organism) may be composited to minimize analytic or bioassessment costs without loss of information, thereby permitting better characterization of the potential hazards at a given site. The development of sampling methodology should reflect these relationships between limitations in the bioassay analysis of physical samples and the field sampling designs.

For this purpose, statistical techniques are needed to:

1. devise an optimal field sampling design based on several representative site-specific scenarios (priors), and
2. devise an optimal compositing scheme based on both analytic limits for chemistry and bioassays and a maximum acceptable limit for the compound(s) or assay(s) in question.

Because these sampling purposes are related, they should not be addressed separately.

REFERENCES

American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater. 16th ed. American Public Health Association, Washington, D.C.

Barth, D. S., and B. J. Mason. 1984. Soil Sampling Quality Assurance User's Guide. EPA-600/4-84-043, Environmental Research Center, University of Nevada, Las Vegas, Nevada.

Beckman Inc. 1982. Microtox System Operating Manual. Beckman Instruments, Inc., Microbics Operations, Carlsbad, California.

Clark, I. 1979. Practical Geostatistics. Applied Science, London.

DeVera, E. R., B. P. Simmons, R. D. Stevens, and D. L. Storm. 1980. Samplers and Sampling Procedures for Hazardous Waste Streams. EPA-600/2-80-018, California Department of Health Services, Berkeley, California.

Douglas, J. 1985. "Measuring and Managing Risk." EPRI Journal 6-7-13.

Dunlap, W. J., J. F. McNabb, M. R. Scalf, and R. L. Cosby. 1977. Sampling for Organic Chemicals and Microorganisms in the Subsurface. EPA-600/2-77-176, Robert S. Kerr Environmental Research Laboratory, Ada, Oklahoma.

Eberhardt, L. L., and J. M. Thomas. 1986. Survey of Statistical and Sampling Needs for Environmental Monitoring of Commercial Low-Level Radioactive Waste Disposal Facilities. NUREG/CR4162, U.S. Nuclear Regulatory Commission, Washington, D. C.

Federal Register. 1980. Part V, Environmental Protection Agency, Water Quality Criteria Documents; Availability. Vol. 45, No. 231, Friday, November 28, 1980. p. 79319.

Finney, D. J. 1978. Statistical Methods in Biological Assay. MacMillan, New York. 508 pp.

Ford, P. J., and P. J. Turina. 1985. Characterization of Hazardous Waste Sites--A Methods Manual, Volume I: Site Investigations. EPA/600/4-84-075, Office of Advanced Monitoring Systems Division, Las Vegas, Nevada.

Ford, P. J., P. J. Turina, and D. E. Seely. 1984. Characterization of Hazardous Waste Sites--A Methods Manual, Volume II: Available Sampling Methods. EPA/600/4-84-076, Office of Advanced Monitoring Systems Division, Las Vegas, Nevada.

Gibb, J. P., R. M. Schuller, and R. A. Griffin. 1980. "Monitoring Well Sampling and Preservation Techniques." In Proceedings of the Sixth Annual Research Symposium, ed. D. Schultz. EPA-600/9-80-101, Municipal Environmental Protection Agency, Cincinnati, Ohio.

Gilbert, R. O., and R. R. Kinnison. 1981. "Statistical Methods for Estimating the Mean and Variance from Radionuclide Data Sets Containing Negative, Unreported or Less Than Values." Health Phys. 40:377-390.

Journal, A. G. 1984. "New Ways of Assessing Spatial Distribution of Pollutants." In Environmental Sampling for Hazardous Wastes, eds. G. E. Schweitzer and J. A. Santolucito. American Chemical Society Symposium Series 267, pp. 109-118, American Chemical Society, Washington, D.C.

Journal, A. G., and C. J. Huijbregts. 1978. Mining Geostatistics. Academic Press, New York.

Kenaga, E. E. 1978. "Test Organisms and Methods Useful for Early Assessment of Acute Toxicity of Chemicals." Environ. Sci. Technol. 12:1322-1329.

Mason, B. J. 1983. Preparation of Soil Sampling Protocol: Techniques and Strategies. EPA-600/4-83-020, Environmental Monitoring Systems Laboratory, Las Vegas, Nevada.

Mausbach, J. J., B. R. Brasher, R. D. Yeck, and W. D. Nettleton. 1980. "Variability of Measured Properties in Morphologically Matched Pedons." Soil Sci. Soc. Am. J. 44(2):358-363.

Miller, M. P., J. D. Cooney, and K. M. Duke. 1984. Chemical Analyses and Aquatic Toxicology Tests on Hazardous Waste Site Soil Samples. Report prepared by Battelle-Columbus Laboratories for the U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory.

Miller, W. E., S. A. Peterson, and C. A. Callahan. 1985. "Comparative Toxicology of Laboratory Organisms for Assessing Hazardous Waste Sites." J. Environ. Qual. 14:569-574.

Porcella, D. B. 1983. Protocol for Bioassessment of Hazardous Waste Sites. EPA 600/2-83-054, Corvallis Environmental Research Lab, Corvallis, Oregon.

Roop, R. D., and C. T. Hunsaker. 1985. "Biomonitoring for Toxics Control in NPDES Permitting." J. Water Pollut. Control Fed. 57:271-277.

Schaeffer, D., H. W. Kerster, and K. G. Janarden. 1982. "Monitoring Toxics by Group Testing." Environ. Manage. 6:467-469.

Sisk, S. W. 1981. NEIC Manual for Groundwater/Subsurface Investigations at Hazardous Waste Sites. EPA-330/9-81-002, National Enforcement Investigations Center, Denver, Colorado.

Skalski, J. R., and J. M. Thomas. 1984. Improved Field Sampling Design and Compositing Schemes for Cost-Effective Detection of Migration and Spills at Commercial Low-Level Radioactive or Chemical Waste Sites. PNL-4935, Pacific Northwest Laboratory, Richland, Washington.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa.

Thomas, J. M., and J. E. Cline. 1985. "Modification of the Neubauer Technique to Assess Toxicity of Hazardous Chemicals in Soils." Environ. Toxicol. Chem. 4:201-207.

Thomas, J. M., W. E. Miller, C. A. Callahan, S. A. Peterson, J. F. Cline, J. C. Simpson, J. C. Greene, J. R. Skalski, and M. C. McShane. 1984a. Characterization of Chemical Waste Site Contamination and Its Extent Using Bioassays. PNL-5302, Pacific Northwest Laboratory, Richland, Washington.

Thomas, J. M., J. R. Skalski, L. L. Eberhardt, and M. A. Simmons. 1984b. "Field Sampling for Monitoring, Migration and Defining the Areal Extent of Chemical Contamination." In Management of Uncontrolled Hazardous Waste Site, Hazardous Material Control Research Institute, Silver Spring, Maryland.

Thomas, J. M., J. E. Rogers, J. F. Cline, L. E. Rogers, K. A. Gano, J. C. Simpson, M. C. McShane, and J. R. Skalski. 1984c. Field Evaluation of Hazardous Waste Bioassessment Protocols. Vol. 2. PNL-4614, Pacific Northwest Laboratory, Richland, Washington.

Thomas, J. M., J. R. Skalski, J. F. Cline, M. C. McShane, J. C. Simpson, W. E. Miller, S. A. Peterson, C. A. Callahan, and J. C. Greene. 1986. "Characterization of Chemical Waste Site Contamination and Determination of Its Extent Using Bioassays." Environ. Toxicol. Chem. 5:487-501.

U.S. Environmental Protection Agency (EPA). 1977. Procedures Manual for Groundwater Monitoring at Solid Waste Disposal Facilities. EPA-530/SW-611, U.S. Environmental Protection Agency, Washington, D.C.