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Field Studies for Ecological Risk Assessment

Ecological risk assessments of Superfund sites evaluate the actual or potential effects of site contaminants on plants and animals and assess the need for remediation, including considering remedial alternatives and evaluating ecological effects of remediation. Such ecological risk assessments make use of a variety of desktop, laboratory, and field approaches, which may include chemical analyses of media, toxicity testing, literature searches, evaluation of the condition of organisms, and ecological field studies. As the name implies, **ecological field studies** are investigations that take place in the actual area under scrutiny, focusing on the site's habitats and **biota**[1] (resident organisms) and comparing them with unimpacted conditions. The ecological risk assessment of a Superfund site nearly always requires some type of field study. At a minimum, some field study is necessary in order to identify organisms and **habitats**[2] that may be at risk. By themselves, hazard indices based on literature values rarely prove adequate for characterizing ecological effects.

Rather than studying individual organisms, field studies generally focus on populations or communities. **Populations** are groups of organisms belonging to the same species and inhabiting a contiguous area. **Communities** consist of populations of different species living together. For example, a forest community consists of the plants, animals, and microorganisms found in a forest. A community also can be a more restricted group of organisms. Within the forest, the soil community consists of only those organisms living in, or in close association with, the soil. Less frequently, a field study evaluates an **ecosystem**, which consists of both the organisms and the nonliving components of a specific, limited area.[3] In the case of a forest, the ecosystem includes the soils, rocks, streams, and springs as well as the resident organisms that make up the forest community.

Which sites warrant a detailed field study? At many sites the existing information indicates a significant likelihood of present or future adverse impact but is insufficient to support remedial decision making. At such sites, field studies can identify actually or potentially exposed organisms, exposure routes, ecological effects, and also the potential of the site to support biota. In the initial phase of an ecological risk assessment, a field study can take the form of a site's habitats and many of its species and also note any obvious adverse ecological effects. If a site warrants further study, a more intensive effort during the analysis phase can help to provide evidence of a link between a site's contaminants and an adverse effect. As Table 1 shows, field studies can contribute information at different stages of the ecological risk assessment and can assist with each of the assessment's components:

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[1] The first time that a technical term appears, it is bolded and either defined in the text or in a footnote.

[2] A habitat is the place that a species naturally inhabits.

[3] Although ecologists often use this term to include much larger resources, this definition gives the word dimensions usable at a Superfund site.

ECO Update is a Bulletin series on ecological risk assessment of Superfund sites. These Bulletins serve as supplements to *Risk Assessment Guidance for Superfund, Volume II: Environmental Evaluation Manual* (EPA/540-1-89/001). The information presented is intended to provide technical information to EPA and other government employees. It does not constitute rulemaking by the Agency, and may not be relied on to create a substantive or procedural right enforceable by any other person. The Government may take action that is at variance with these Bulletins problem formulation, analysis, and risk characterization.[4]

The specific role of field study in an ecological risk assessment varies with the site. Site managers[5] should consult with the Biological Technical Assistance Group (BTAG) in their Region to determine the best approach to each site.[6] This consultation should occur at the earliest possible stage of site investigation. The BTAG may suggest other methods—in addition to, or instead of, techniques discussed in this document—that are especially appropriate for a particular site.

This Bulletin provides site managers with an overview of field study options. Four main sections follow this Introduction. The first considers the organisms, which are the major focus of most field studies, and the second describes the remaining elements in the design of a field study. The third section presents a catalogue of common field study methods, while the fourth section summarizes the contributions that field study can make to an ecological risk assessment.

This Bulletin is intended only as quick reference for site managers, not as a comprehensive review of field methods or of the ecological attributes evaluated using these methods. Those who want to examine the subject in greater depth should consult the list of references at the end of the Bulletin and also the list of additional resources available from the federal government.

The Organisms in a Field Study

Although a large number of species can inhabit a site, an ecological risk assessment of a Superfund site concerns itself only with those that are actually or potentially adversely affected by site contamination or that can serve as surrogates for such species. Such organisms are among a site's **ecological components**. Ecological components are populations, communities, habitats, or ecosystems actually or potentially affected by site contamination. A field survey conducted as part of a reconnaissance visit can help to identify the potentially affected organisms at a site. Some factors to consider in making this identification include which media (e.g., soil, surface water, ground water) have become contaminated, the site's contaminants, their environmental concentrations, and their **bioavailability**.[7]

Table 1. Field Study Contributions to Ecological Risk Assessments

Task	Site Reconnaissance Visit	Intensive Field Study
Problem Formulation	Identify ecological components potentially exposed to contaminants.	Identify specific ecological components and the exposure pathways for populations and communities.
Analysis	Identify ecological components likely to be exposed to contaminants. Identify readily apparent effects.	Describe populations and community attributes with respect to exposure. Quantify exposure of specific ecological components. Quantify effects on specific ecological components
Risk Characteri-zation	Develop hypothesis of relationship between exposure and effects.	Characterize and document links between exposure and effects. Quantify relation-ship between exposure and effects.

General Considerations in Selecting Organisms for Study

Most sites have a large number of species, making it necessary for the investigator[8] to focus on a limited number of these for detailed study. A variety of site-

[4] In preparing this Bulletin every effort was made to use terminology found in the *Framework for Ecological Risk Assessment* (U.S. EPA. 1992. Office of Research and Development, Risk Assessment Forum. EPA/630/R-92/001. Washington, D.C.). The three phases listed in the text are equivalent to the four components of an ecological risk assessment described in "Ecological Assessment of Superfund Sites: An Overview" (*ECO Update* Vol. 1, No. 2). The *Framework's* analysis phase corresponds to the "Overview's" exposure assessment and the ecological effects assessment phases.

[5] Site managers include both remedial project managers and on-scene coordinators.

[6] These groups are sometimes known by different names, depending on the Region. Readers should check with the appropriate Superfund manager for the name of the BTAG coordinator or other sources of technical assistance in their Region. A more complete description of BTAG structure and function is available in "The Role of BTAGs in Ecological Assessment" (*ECO Update* Vol. 1, No. 1).

[7] Bioavailability is the occurrence of a contaminant in a form that organisms can take up.

[8] The term "investigator" refers to the individual charged with responsibility for designing and/or carrying out any part of an ecological risk assessment. Investigators can include government scientists, contractors, or university scientists. However, the site manager (remedial project manager or on-scene coordinator) retains ultimate responsibility for the quality of the ecological risk assessment.

specific factors—including the size of the site and the types of habitats that have become contaminated—contribute to making this selection. In their role as protectors of natural resources, trustees also can influence the selection of organisms for study.

Species-specific factors also enter into the selection. These include:

- *Intensity of exposure*. Species vary in the intensity of their contact with contaminated media. For example, earthworms and other invertebrates inhabiting a contaminated medium receive longer and more intense exposures than wider-ranging invertebrates such as butterflies. Some animals have limited mobility early in their life cycle—as eggs, larvae, or nestlings, for example—so have greater exposure than older animals.

- *Relative sensitivity to contaminants.* Evaluation of a highly sensitive species can bring about *de facto* consideration and protection of other species inhabiting the site. However, at a site with a complex mixture of contaminants, the investigator may be unable to identify one sensitive species that is most appropriate for study.

- *Ecological function, along with significance of a species' contribution to this function*. An investigator may select a species for study based on its ecological function. For example, at a site with contaminated surface water, the investigator may choose to study algae as the aquatic community's **primary producers**.[9] The investigator may specifically focus on those algal species that make a large contribution to this function.

- *Time spent on-site.* To qualify for further study, a species should inhabit the site during either a considerable portion or a critical stage of its life cycle.

- *Ease of difficulty of conducting field studies with the organisms*. A field study that requires capturing birds is resource-intensive, and unlikely to occur at a typical Superfund assessment. Where fish-eating birds are at risk because bioaccumulating substances have contaminated the surface water or aquatic organisms, the investigator might choose instead to focus primarily on the fish that the birds eat or to study a mammal or reptile with feeding habits similar to birds.

- *Appropriateness of surrogate species*. In the case of a site with an endangered or threatened species, the investigator may elect to study a surrogate species with similar exposure. Surrogate species offer the advantage of sampling and analysis options that cannot be employed with threatened or endangered species. However, in selecting a surrogate species, the investigator should identify one that resembles the site species in behavior, feeding, and physiological response to the contaminants of concern. The best choice for a surrogate is not necessarily the one most closely related to the site species.

- *Other recognized values*. At some sites the investigator may want to consider a species because of other values associated with it, such as economic or recreational value.

Based on the above considerations, the investigator generally selects no more than a few species as subjects of the field study. However, an investigator can choose to study a community. For example, at a lake with contaminated water and sediment the investigator can study the benthic community that lives in association with the lake's bottom. When selecting a community as an ecological component, the investigator needs to ascertain that the study includes populations representing multiple **trophic levels**.[10]

Although a large number of species can inhabit a site, an ecological risk assessment of a Superfund site concerns itself only with those that are actually or potentially adversely affected by site contamination or that can serve as surrogates for such species.

At some Superfund sites, investigators have selected a wetland habitat as the ecological component for further study. Such choice can prove more protective of the environment since the investigator can document a variety of adverse effects on the habitat rather than having to demonstrate significant impact to only selected species. Studying a habitat becomes especially important when one or more of the remedies under consideration could adversely affect the habitat. However, designating a habitat as the ecological component can prove costly, depending upon how the ecological risk assessment delineates the habitat.

[9] A primary producer is an organism, such as an alga or a terrestrial plant, that converts the energy from sunlight to chemical energy.

[10] A trophic level is a stage in the flow of food from one population to another. For example, as primary producers, plants occupy the first trophic level, and grazing organisms occupy the second trophic level.

To extend these general guidelines for selecting ecological components, the following sections consider different kinds of organisms that inhabit either aquatic or terrestrial environments and detail how each can increase an investigator's knowledge of the ecological conditions at the site.

Aquatic Organisms

At a site where contaminated surface water is a medium of concern, field studies can focus on periphyton, plankton, benthic macroinvertebrates, or fish. **Periphytons** are microscopic algae that grow on sediment, stems and leaves of rooted water vegetation, and other surfaces that project above the bottom of a body of water. Studying periphyton provides information about primary producers in an aquatic environment. These organisms also include many species useful in assessing the cause, extent, and magnitude of contaminant problems.

Plankton are microscopic organisms that float or swim weakly in the water column. Plankton includes algae, protozoa, and small crustaceans. Planktonic algae are called **phytoplankton**, and the protozoa and crustaceans are referred to as **zooplankton.** Because plankton include primary producers, which supply food for larger animals and also increase the amount of oxygen dissolved in water, these organisms make an important contribution to the aquatic community. Like periphyton, plankton includes species that are sensitive indicators of ecological injury resulting from contamination or enrichment of water bodies.

As defined by EPA, **benthic macroinvertebrates** are invertebrate animals that live in or near the bottom of a body of water and that will not pass through a U.S. Standard No. 30 sieve, which has 0.595 mm openings. Such organisms occur in gravel, sediments, on submerged logs and debris, on pilings, and even on filamentous algae. Freshwater benthic macroinvertebrates include insects, worms, freshwater clams, snails, and crustaceans. The benthic macroinvertebrate communities of marine and estuarine environments include worms, clams, mussels, scallops, oysters, snails, crustaceans, sea anemones, sponges, starfish, sea urchins, sand dollars, and sea cucumbers. When water becomes contaminated, some of the contaminants migrate to the sediment and accumulate there. Field studies of benthic macroinvertebrates can indicate the degree to which sediment contamination can adversely affect biota. In addition, the composition and diversity of benthic macroinvertebrate communities can indicate the overall well-being of the aquatic ecosystem.

Because fish occupy a range of trophic levels, they serve as useful indicators of community-level effects. The relative ease of identifying most juvenile and adult forms makes fish particularly convenient subjects for field study. In addition, field study methods for fish are relatively simple and inexpensive. In selecting a species for sampling, the investigator will want to consider its characteristic home range. For a species that spends little time on-site, a field study may not be able to establish whether any adverse effects result from exposure to site-associated contaminants.

Semi-aquatic and Terrestrial Animals

Semi-aquatic and terrestrial animals—including insects, other invertebrates, and vertebrates—can all provide useful information about ecological effects associated with the site. Soil fauna, the organisms most intimately associated with this medium, include many species that perform important functions in terrestrial ecosystems. For example, earthworms aerate the soil. Other soil-dwellers—such as some small insects, soil mites, and certain nematodes (a kind of worm), break down organic wastes and dead organisms—releasing the elements and compounds they contain and making these available to living organisms. Both soil aeration and organic decomposition support the growth of terrestrial plants. Consequently, plants can suffer impact if soil fauna are affected.

Insects' small size and their large numbers make them convenient subjects of study. Further, a site generally has a large number of species. Because these species occupy a variety of microhabitats and also differ in their behaviors, the investigator can measure a range of effects. For example, because insects include species at different trophic levels, a field study can assess the potential for **biomagnification**[11] of a site's contaminants.

Field studies focusing on such vertebrates as amphibians, reptiles, and mammals can contribute to a site's ecological assessment. Depending on their trophic level, these vertebrates may ingest contaminants as a result of consuming contaminated plants, other terrestrial animals, or fish. Burrowing animals, such as voles, can show greater ecological effects from contaminated soil than animals that have less intimate contact with the soil. Where investigators at Superfund sites decide to study terrestrial vertebrates, they generally choose small species, which are likely to range over a smaller area than larger species. As a result, the smaller species tend to spend more of their time on the site, making it easier to estimate exposure.

Field studies of birds present certain difficulties at a Superfund site. These organisms can range far off-site, making it difficult for a field study to establish whether an adverse ecological effect results from exposure to site-associated contaminants. In addition, bird studies can prove especially resource-intensive. However, at a large

[11] Biomagnification is the increasing of concentration of a bioaccumulating contaminant as it passes up a food chain or a food web. A food chain is a series of organisms that sequentially feed on one another. For example, mice eat seeds and are in turn eaten by owls. A food web, which is a group of interrelated food chains, takes into account a species' participation in multiple food chains. For example, birds, insects, and other mammals also eat seeds, and cats, as well as owls, prey on mice.

site or a site with a complicated contaminant picture, the investigator and the BTAG may decide that avian field studies are worth the effort. For example, many sites have large populations of waterfowl that can potentially suffer adverse effects from site contaminants.

Terrestrial Vegetation

When a site has contaminants associated with soil, field study can focus on terrestrial vegetation. In particular, investigators may want to conduct field studies of vegetation at Superfund sites where plants show signs of stress, such as stunted growth or yellowing, or where pollution-tolerant species are abundant. Since plants are the primary producers in terrestrial environments, an ecological impact to vegetation can affect other terrestrial biota.

Elements in the Design of a Field Study

As with any study, an ecological field study has several elements. In addition to selection of the organisms, the elements of a field study encompass the study's objectives, a reference site, endpoints, methods, level of effort, sample design, quality assurance/quality control standards, and the statistical analysis of the data. When the investigator carefully crafts each of these elements, the resulting study should achieve its objectives and should further the overall ecological risk assessment of the Superfund site. Under-developing any of these elements can weaken the study's results and adversely affect the results of the overall assessment.

Objectives

To ensure that the study will have clear direction, the investigator needs to establish study objectives that address ecological concerns for that site. The objectives should ensure that the field study supports the over-all objectives of an ecological risk assessment for a Superfund site. These objectives are (1) to determine whether site contamination poses a current or potential threat of adverse ecological effects; (2) if a threat does exist, to decide whether remediation is required; and (3) if remediation is required, to set cleanup levels.

Investigators will find that study objectives help to indicate the appropriate level of effort. In an initial field study to identify ecological components, for instance, an investigator might find that a qualitative survey method would achieve the study's objective. The later study of adverse effects to a population might require a more resource-intensive approach, such as semi-quantitative or quantitative sampling to estimate population sizes or capture of organisms and transport to a laboratory for biochemical analysis.

Although the specific objectives for a field study will vary both with the site and its stage in the ecological risk assessment process, investigators should keep in mind that a field study performed as part of a Superfund site's ecological risk assessment is not a research project. Generally, a snapshot of site characteristics can provide the needed information.

In addition to stating the purpose of a field study, the objectives also should indicate whether the field study is occurring in conjunction with another type of study, such as a toxicity assessment. In such a case, the two studies have a shared goal: to determine whether adverse ecological effects correlate with toxicity. To meet this goal, the studies' objectives should emphasize the need for integrating sampling plans and coordinating the collection of data. When sampling for coordinated studies occurs at the same time and location, and with similar data quality objectives and levels of precision, the investigator can more convincingly compare results.

Reference Site

A reference site is a location that closely resembles the Superfund site in terrain, hydrologic regime, soil types, vegetation, and wildlife. A well-chosen reference site provides background conditions, allowing the investigator to draw conclusions about the ecological effects of contaminants on the Superfund site. The more closely the reference site resembles the Superfund site, the more valid will be the conclusions based on comparisons of the two. In some cases, no single reference site adequately approximates the Superfund site. In such a case, the investigator may need to identify multiple reference sites.

The investigator should try to locate a reference site as close as possible to the Superfund site so that it will accurately reflect the conditions prevailing at the Superfund site. Yet the reference site should lie at a great enough distance from the Suprerfund site to be outside its sphere of influence and relatively contaminant-free. For example, an upstream location often can provide appropriate reference site conditions for a site with contaminated surface water. A woodland site used as a reference site needs to lie at a great enough distance from the Superfund site that ranges of organisms will not include both sites. Failure to choose appropriate reference sites can result in inaccurate conclusions. For example, if the surface water at the Superfund site consists largely of soft-bottomed pools, then an area having fast-running streams with gravel bottoms will not provide an appropriate comparison. Differences in species composition and other features at the two sites will, at least in part, reflect their very different aquatic habitats rather than contamination at the Superfund site.

In the absence of suitable reference sites, an investigator may need to turn to historical information about the site and/or a large database in order to make a comparison between site conditions and conditions in an uncontaminated area. If this approach becomes necessary, the investigator needs to choose a data source that reflects the site's geologic, hydrologic, and ecological traits as closely as possible. In making this selection, the investigator can obtain advice and suggestions from the BTAG.

A field study performed as part of a Superfund site's ecological risk assessment is not a research project.

Endpoints

The identification of **endpoints**, which are ecological characteristics that may be adversely affected by site contaminants, is essential to a successful ecological risk assessment as a whole and also to each of the studies that make up this assessment. Ecological risk assessors have found it useful to recognize two levels as endpoints, assessment and measurement endpoints. An **assessment endpoint** is an explicit expression of the environmental characteristic that is to be protected (24, 29).[12] At a Superfund site an assessment endpoint is an endpoint that may drive remedial decision-making. Determining potential contaminants of concern and potential ecological components and developing a conceptual model of a site's contaminant situation generally indicates which ecological traits are assessment endpoints at a particular site.

A **measurement endpoint** is an ecological trait that is closely related to an assessment endpoint and that is, in addition, readily measurable. A measurement endpoint, then, is a response that can approximate or represent an assessment endpoint which is not amenable to direct measurement. A site's ecological components and assessment endpoints drive the selection of the measurement endpoint(s). (See Figure 1.) For example, at a site where lead is a contaminant of concern and which has resident species sensitive to lead, the investigator may identify lead poisoning as an assessment endpoint and one or more lead-sensitive species as ecological components. The investigator's choices of measurement endpoints are then limited to accepted ways of measuring the presence of lead in organisms or its effects on them.

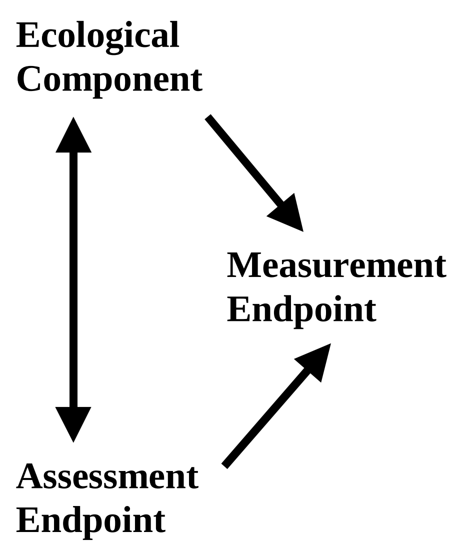
Table 2 summarizes measurement endpoints for field studies. Although a field study can focus on one population, an entire community, or even two or three communities, as both Table 2 and the following discussion indicate, measurement endpoints for field studies are much more than simply "head counts."

[12] Numbers in parentheses refer to references listed at the end of this Bulletin.

Biomass, the total weight of individuals, can be a measurement endpoint for both populations and communities. An investigation may measure biomass directly by weighing collections of some organisms or, for larger organisms, by weighing individuals and summing their weights. Alternatively, he or she may use an indirect method, such as applying length-to-weight regressions to data detailing the number and length of individuals in a population, an especially common approach for fishes.

Figure 1. The Relationship Between Ecological Components, Assessment Endpoints, and Measurement Endpoints

In designing a field study, the ecological components and assessment endpoints generally drive the selection of measurement endpoints.



Productivity, the rate of increase, is another measurement endpoint that can apply to both populations and communities. For plants, the rate of increase in biomass indicates productivity. For many animals, investigators take the rate of increase in numbers as the measure of productivity. Because productivity is a rate, measurements or estimates must occur at least twice during growing season. However, investigators can infer productivity by conducting seed or egg counts or by studying the age structure of a population. At Superfund sites, investigators can infer relative productivity by comparing data from the Superfund site and the reference site(s).

A common measurement endpoint for terrestrial plant population is **cover**, which is the percentage of ground area that lies beneath the **canopy** (uppermost branches) of a tree or shrub species. In evaluating a stand of trees, an investigator can instead measure **basal area**, which is the sum of the cross-sectional area of the trees' trunks.

Some measurement endpoints relate specifically to community parameters. **Species richness** is the number of species in a community. **Species density** refers to the number of individuals of a given species per unit area, while **relative abundance** is the number of individuals in a particular species compared to the total number of individuals. **Dominance**, in the sense of commonness at a site, describes a species that occurs in high abundance, as indicated largely by species density. **Diversity** relates the abundance of individuals in one taxon (level of classification) to the total abundance of individuals in all other taxa. **Evenness** measures how evenly distributed individuals are among the community's taxa**.** **Guild structure,** which refers to the different types of feeding groups in a community, also can be used to evaluate community structure.[13] A number of similarity and different **indices**[14] can compare community structure at Superfund and reference sites.

Another community-level measurement endpoint concerns **indicator species**, which are species whose presence, absence, or population density helps to indicate whether the environment is contaminated. Some species are associated with thriving communities, so either absence or a reduced population can indicate an ecologically disturbed environment. For example, the larval stage of insects in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), referred to collectively as the "EPTs," show sensitivity to metals and other inorganic contaminants. Reduced populations of EPTs, then, can indicate toxic levels of metals or other inorganics in a stream. Conversely, some species occur in association with a disturbed habitat, where they dominate or kill native species weakened by exposure to contaminants. For example, such plant species as *Phragmites* and cattails (*Typha*) characteristically grow abundantly in disturbed wetlands. Consequently, dense growth of *Phragmites* or cattails indicates that a wetland may have suffered ecological stress.

Methods

Field studies gather information about the site by observing organisms, noting signs of an animal species' presence, or collecting organisms for further study. With respect to methods, the work plan for a site should include detailed instructions for sampling organisms and collecting the relevant data. This data may include such physical measurements as the temperature at a sampling site. Proper methodology ensures that the data collected can be analyzed and results interpreted.

Observation indicates whether a species occurs at the site. For vegetation and for animals with limited mobility, the observer also can note the condition of the organisms. Investigators need to keep in mind that for more mobile animals, observation indicates only whether the species occurs at the site, not what percentage of time it spends there. Observing organisms can be as straightforward as walking the site or can involve the use of specialized equipment, as in remote sensing of terrestrial vegetation. Remote sensing by such means as infrared or multispectral photography can prove an effective way of detecting stressed vegetation at a large site.

The signs of an animal's presence include **scat** (feces); burrows, nests, or dens; cast-off larval cases or cocoons; tracks; and carcasses. Characteristic sounds, such as bird song, also can reveal an animal's presence and sometimes provide limited information about relative abundance. Like direct observation of resident organisms, observation of animal sign indicates only whether a species occurs at the site, not the percentage of time it spends there.

Table 2. Measurement Endpoints in Field Studies

Type	Measurement Endpoint
Measurement Endpoints for Populations	Biomass Productivity -Cover (terrestrial vegetation) -Basal area (terrestrial vegetation)
Measurement Endpoints for Communities	Biomass -Productivity and Respiration (aquatic communities) -Species richness -Species density -Relative abundance -Dominance -Diversity -Evenness -Similarity/difference between Superfund site and reference site -Similarity/difference in guild structure between Superfund site and reference site. -Presence, absence, or population density of indicator species.

[13] Guilds, also called **functional feeding groups,** are groups of animals occupying the same trophic level and feeding either in the same way or in the same location. For example, among terrestrial plant-eaters there are five guilds: stem-eaters, root-eaters, leaf-eaters, bud-eaters, and nectar-sippers.

[14] An index is a single number that incorporates information from a class of data.

Methods of collecting samples for further study vary with the kind of organism being studied. A field worker can catch a fish in a net, capture a mouse in a trap, or sieve organisms from a soil or sediment sample. Depending on the species and objectives, once the investigator has collected the organisms, he or she may make direct observations and then release them. Alternatively, the investigator may retain the organisms for further study, such as analyzing tissues for their contaminant content or examining them microscopically for indications of contaminant-related abnormalities.

From among the wide variety of available field methods, those used at a Superfund site should provide data at a reasonable cost and within a reasonable timeframe for that site. They should be readily reproducible, reliable, and relevant to the site. The BTAG can assist investigators in selecting methods appropriate to a site. In choosing a method, investigators also need to be aware that they should obtain permission from federal and state fish and wildlife agencies before collecting vertebrates. Some states also require permits for collecting certain other organisms.

At sites in the nation's temperate areas, investigators need to coordinate site studies with seasons. Floristics surveys and photosynthetic measurements must be conducted during the growing season. Surveying a migratory population, such as most bird species, will require coordination with season. In addition, natality studies should occur during the warm months, when most animal species produce young.

The final section of this Bulletin provides additional information about techniques available for field studies of different types of populations and communities.

Level of Effort

A site's characteristics, its contaminant picture, and a proposed field study's objectives together indicate the level of effort appropriate to carrying out the study. For example, an objective to identify potentially affected animal species at a small site with few habitats will require a lower level of effort than one that specific to the evaluation of community structure at a large site with several habitats. The number and nature of ecological components are endpoints specified by the objectives also may affect a study's level of effort. As the number of ecological components and assessment and measurement endpoints increases, the level of effort generally will increase. Additionally, some organisms are more difficult to observe or sample than others, and some measurements are more difficult to make. For example, collecting insects usually entails less effort than collecting fish.

In conducting field studies, the level of effort varies with the sampling method chosen: qualitative, semi-quantitative, or quantitative (17). Qualitative sampling which has as its goal to observe or sample as many taxa as possible in the available time, requires the least effort. Qualitative sampling attempts to sample all habitats, using several collection methods at each sample station. Such an approach can prove useful for a site reconnaissance visit, with the objective of identifying potentially exposed ecological components and readily apparent effects.

Rapid Bioassessment Protocol II is an example of a semi-quantitative, or intermediate-level, sampling method for benthic macroinvertebrates inhabiting flowing waters (21). This approach offers a time-saving and cost-effective means of obtaining information about benthic macroinvertebrates. In this approach, the field team uses a net to collect organisms from two approximately one-square-meter areas, one in a fast-flowing part of the stream and the other in a slow-flowing area. The organisms are then enumerated and classified only to family level, which requires less time than classification to genus or species. A sub-sample of 100 organisms is then classified according to guild. An additional sample is collected from an area with coarse particulate organic matter, such as a leafpack or an area near the shore. The organisms in this sample are classified simply as shredders or non-shredders.

Quantitative sampling uses methods that sample a unit area or volume of habitat. Generally, these methods are applied to randomly selected sampling units. As with semi-quantitative sampling, the organisms collected are counted and classified.

In deciding on level of effort, investigators should be aware that limited sampling efforts could provide enough data for an adequate ecological risk assessment of a Superfund site. It is true that differences in life cycle characteristics and diurnal effects do prevent limited sampling from providing a comprehensive estimate of all species. However, if field studies at the site sample enough biota, these kinds of variations will have minimal effect on the overall assessment.

Sampling Plan

A sampling plan for field study indicates the number of sampling points, the number of replicates for each sampling point, the method for determining sampling locations, holding times for samples, and any sample preparation required for laboratory analysis. In making these decisions for an ecological field study, the investigator needs to consider the study objectives, the level of effort, the site's soil, the ecological component(s), the measurement endpoints, the method, the statistical method of analyzing data, and the available resources. For example, the approach to statistical analysis will affect sampling size. If the field study is one of a group of coordinated studies, then the investigator also needs to consider whether a particular sampling method can apply to all the studies in the group.

In general, sampling locations can be selected either non-randomly or randomly. Qualitative and semi-quantitative surveys make use of non-random sampling, taking into account the habitat and mobility of the organisms and the location of contaminant "hot spots." For quantitative sampling, investigators generally use random sampling methods.

When the investigator has decided on the number of sampling locations and the method of selecting them, he or she must also decide how many replicate samples to collect per site. Both the study objectives and the data quality objectives (discussed below) influence this decision. While natural variability makes replicate sampling desirable, for some field studies the sampling population cannot specify a fixed number of replicates. For example, field biologists have no control over trapping success.

Quality Assurance/Quality Control (QA/QC) Standards

Quality assurance and quality control standards are an essential element in the study plan. Included among the QA/QC considerations are the **data quality objectives (DQOs)**. These are statements that define the level of uncertainty that the investigator is willing to accept in environmental data used to support a remedial decision. DQOs address the purpose and use of the data, the resource constraints on data collection, and any calculations based on the data. In particular, DQOs help investigators to decide how many samples and replicates to collect in order to limit uncertainty to an acceptable level.

DQOs also guide decisions about the level of detail necessary for the study. For example, in field studies involving certain groups of organisms, such as insects, DQOs establish the level to which the investigation should take the identification of organisms. Identification to the level of family or genus requires less expertise and time than identification to the level of species. However, the DQOs may require identification to the species level to obtain detailed enough information about the site's ecological condition.

In addition to defining acceptable uncertainty, QA/QC standards address other concerns:

- *Reference sites*. As discussed earlier, the investigator should achieve a careful match between the Superfund site and one or more reference sites.
- *Accurate identification of organisms*. The investigator must identify organisms accurately. A common means of ensuring the accuracy of identification involves having the classification of a subset of organisms verified by independent experts.
- *Adherence to sampling plan*. Field biologists must adhere closely to the sampling plan in order to collect valid data. Consequently, the study's design will need to incorporate methods for checking how precisely personnel have followed the sampling plan. For example, QA/QC standards may require field biologists to maintain field notebooks and submit copies of these. Chain of custody documents provide another means of tracking small collection, transfer, and analysis.
- *Contractor*. The contractor selected must have personnel with the expertise needed to perform the particular type of field study and interpret the data. In addition, the contractor must have the necessary equipment and personnel skilled in its use and maintenance.

Statistical Analysis of Field Data

In performing statistical analyses of field data from Superfund sites, two issues require special consideration: lack of randomness and use of indices.

Lack of Randomness. Neither the Superfund site nor the reference site is selected randomly. As a result of this lack of randomness, the investigator must use one of the following approaches in statistically analyzing differences between the Superfund site and the reference site:

- The investigator selects sampling stations randomly at both the Superfund site and the reference site(s), and then tests the hypothesis that observed differences between these stations result from conditions at the stations in the Superfund site.
- The investigator tests the hypothesis that the reference site(s) and the Superfund site differ. If such a difference exists, the investigator then employs nonstatistical methods to evaluate whether contamination at the Superfund site causes this difference.

Use of Indices. A field study can generate a volume of data too large to be analyzed efficiently. In such a case, reducing classes of data to a single number, called an index, simplifies the analysis. Some of the community traits discussed earlier, including evenness and diversity, are examples of indices calculated from taxonomic data. Indices also include biotic indices, which examine the environmental tolerances or requirements of particular species or groups of species.

While indices can make field data more manageable, investigators need to appreciate that indices have properties that can preclude standard statistical comparison of results among sampling locations (9). If an ecological risk assessment makes use of indices, the discussion of uncertainty needs to address the limitations of the indices and acknowledge the assumptions that they make.

Field Methods

The following list includes a brief description, by type of organism, of field methods useful in ecological risk assessments at Superfund sites. Some methods focus on ways to collect organisms, while others concern ways to examine them.

These methods represent only a selection of those available. In designing a field study, the investigator should consult the BTAG, which may suggest approaches not described here. Please note also that this catalogue includes only methods used in studying biota. For methodology relating to the study of physical and chemical characteristics of a site, investigators should consult the following EPA documents:

- *Sampler's Guide to the Contract Laboratory Program.* EPA/540/P-90/006, December 1990.
- *Compendium of ERT Surface Water and Sediment Sampling Procedures.* EPA/540/P-91/005, January 1991.
- *Compendium of ERT Soil Sampling and Surface Geophysics Procedures.* EPA/540/P-91/006, January 1991.
- *Compendium of ERT Groundwater Sampling Procedures.* EPA/540/P-91/007, January 1991.
- *Compendium of ERT Waste Sampling Procedures.* EPA/540/P-91/008, January 1991.

Periphyton

Scraping, coring, or suction. Field studies of periphyton can involve collecting these organisms from their natural environment by means of devices that scrape, core, or use suction (18,30).

Artificial substrate. Materials such as granite, tile, plastic, and glass can serve as an artificial substrate on which periphyton communities can develop (18, 30).

Data Analysis. Investigators can study the taxonomic composition, biomass, species richness, and relative abundance of periphyton communities from either natural habitats or artificial substrates. In addition, analysis of data can yield information about diversity, evenness, and similarity (18, 30).

Plankton

Trapping, pumping, netting, and using closing samplers. Samples can be collected from natural substrates by means of traps, pumps, nets, and closing samplers such as tubes and bottles (3, 18, 30).

Data Analysis. After identifying the organisms in the sample investigators can determine species richness, relative abundance and diversity (18).

Benthic Macroinvertebrates

Dredging and digging. Dredging and digging provide qualitative samples from the natural environment (17, 18).

Stream netting, coring, or sampling with a grab. Stream netting, coring and sampling with a grab collect quantitative samples from the natural environment. Stream netting involves using specialized nets to collect samples. The Surber and the Hesser are stream nets commonly used to sample macroinvertebrates in or on substrate. Some types of stream nets collect macroinvertebrates drifting in the water column (a normal occurrence with benthic macroinvertebrates inhabiting flowing water). A grab is a sampling device with jaws that penetrate and extract an area of the substrate (1, 4, 12, 17, 18, 21).

Sweep netting. Sweep nets collect qualitative samples associated with aquatic vegetation (17, 18).

Sampling with other devices. More quantitative methods of sampling benthic macroinvertebrates associated with aquatic vegetation involve using either the Wilding stovepipe or the Maca the Minto, or the McCauley samplers (17, 18, 21).

Artificial substrate. Communities of benthic macro-invertebrates can develop on artificial substrates introduced in the site's water (17, 18, 21).

Data Analysis. Once the sample has been collected, the investigator can identify the species present and measure biomass. Further analysis of data can disclose such parameters as species richness, species density, diversity, and relative abundance (17, 18, 21).

Fish

Seining. Seines are effective sampling devices for shallow water such as stream, nearshore areas of lakes, and shallow marine and estuarine locations. The most commonly used seines consists of a specialized net attached to long vertical poles (4, 21, 30).

Trawling. In deeper waters that have no obstructions, investigators use tapered conical fishing net called a trawl. A boat pulls the trawl through the water at a specified depth (18, 21, 30).

Passive Netting. For passive netting, the field biologist attaches a net to the bottom of a river or lake. Fish that swim into the net become entangled or unable to escape. Passive nets include gear trammel, and hoop nets (20, 18, 21, 30).

Electrofishing. This technique which applies an electrical charge to a small area in a body of water momentarily immobilizing fish. Electrofishing is effective for sampling fish in streams, rivers, and lakes (18, 21, 30).

Chemical collection. This specialized technique involves exposing the animals to fish toxicants (21). Investigators should familiarize themselves with state regulations regarding the use of these substances. While use of such chemicals is a standard procedure, this method is not preferred because of its negative effects.

Fish tissue collection. Methods for collecting fish tissue are described in References 25, 27, and 28.

Data analysis. Once the investigator has identified the fish, he or she can determine such measurement endpoints as relative abundance and species richness (18, 21, 30).

Terrestrial Vegetation

The methods described for terrestrial vegetation work equally well for upland and wetland areas. This is true even though in wetlands, by definition, the prevailing vegetation is typically adapted to saturated soil conditions.

Remote sensing. Remote sensing, which uses either satellite imagery or aerial photography, is useful when contamination of a site has resulted in restricted access or when initial site reconnaissance requires surveys of large areas. The technique provides information about general landscape patterns, gross features of the vegetation, and photosynthetic rates. Infrared and multispectral remote sensing also can be used to identify and map areas of stressed vegetation (13). Usually, some limited ground-level survey (ground-truthing) is required to verify identification of species and condition.

Quadrats and transects. Quadrats and transects are often used in vegetation survey and sampling methods to provide a more quantitative approach to collecting data. **Quadrats** are closed sampling units or plots. **Transects** consist of belts, strips, or lines used as a sampling unit. Both methods define precise, isolated areas for sampling, recording, mapping, or studying organisms within a larger area. Both methods allow investigators to estimate characteristics such as cover, species frequency, and density (2, 10).

Point method. The point method estimates cover using sampling points (2, 10).

Distance methods. Distance methods provide a means of estimating coverage and species density in forests, which would require large quadrants to sample trees adequately. There are several different versions of distance methods but in general the methods are based on measuring distances between random points and the sampled plants, or between individual plants (2, 10).

Soil Fauna

Coring. Field biologists collect samples by coring devices (23).

Driving organisms from soil sample. Heat, moisture, or chemical stimuli drive the organisms from the soil into collection chambers (23).

Sieving. Sieving can be used to retrieve the fauna from the soil. Dry sieving separates soil fauna from fallen leaves and friable soil. Wet sieving, also called soil washing, is used to extract organisms from fine mud, sediments, and leaf litter (23).

Density separation. Floatation, centrifugation, and sedimentation separate organisms from soil on the basis of density (23).

Data analysis. After the investigator has identified the organisms, he or she can determine parameters that characterize the community.

Terrestrial and Flying Insects

Trapping. Traps can be used to collect insects of particular species, groups of species, insects at specific life stages, or insects with specific behaviors. Trapping methods include the use of attractant chemicals, light, hosts, host substitutes, and insect sounds. Traps include emergence, pitfall, and sticky traps, to name a few (23). The type of trap used affects both the range of species collected and the types of data collected. When collecting several kinds of insects, the investigator can determine such measurement endpoints as species diversity (23).

Sign. Frass (feces), nests, cast-off larval cases or cocoons, and auditory signals indicate the presence of particular insects (23).

Amphibians, Reptiles, and Mammals

Auditory and visual study. Visual studies can determine the presence of species on a site. In addition, sounds can indicate the presence of certain amphibians and mammals (19, 22).

Sign. Tracks, nests, burrows, dens, scat, or carcasses indicate which species occur on a site (19, 22).

Trapping. Traps and nets can provide more quantitative means of sampling, and depending on the breadth of the study, allow an investigator to determine population and/or community parameters relative to the reference site. Trapping methods include both live traps and kill traps. Depending on the study objectives, the investigator either makes observations on live-trapped animals and leases them or retains the animals for further study (5, 6, 7, 19).

Tissue collection. Methods for collecting tissues are described in Reference 26.

Birds

Auditory and visual studies. Ornithologists identify the species at a site by walking specified areas or distances (e.g., along a transect) and record birds sighted or identified through their songs (5).

Nest success. The evaluation of nest success on the basis of measures such as clutch size and number of fledglings is practical only for very large Superfund sites (7).

Trapping. Not practical for most Superfund sites, a variety of traps and nets can be used to capture birds (6).

Field Studies: Their Contribution

As the previous sections of this Bulletin indicate, field studies can contribute to all phases of the ecological risk assessment of a Superfund site and in a variety of ways. Specifically, a well designed field study can allow investigators to:

- Identify and describe the habitats and species (including those of special concern) actually or potentially exposed to waste site contaminants.
- Indicate detrimental ecological effects that may have occurred on or near the site.
- Provide information adding to the weight of evidence linking adverse effects to the site's contaminants.
- Provide samples for **biomarker** studies, such as bioaccumulation studies, biochemical analyses, and histopathological studies. [15]
- Aid in identifying remedial alternatives that are protective of natural resources.
- Assist in monitoring remediation effectiveness.

Investigators should consult with their Region's BTAG to determine whether and when to conduct field studies and to select the studies most appropriate to their sites.

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[15] A biomarker is a physiological, biochemical, or histological response that is measured in individual organisms and that indicates either exposure or sub-lethal stress.

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