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Selecting and Using Reference Information in Superfund Ecological Risk Assessments

Ecological risk assessments at Superfund sites estimate the adverse effects of chemical contaminants on the plant and animal life inhabiting the area associated with the site. This process depends on the collection of data from the impacted areas of the Superfund site; however, these data alone often cannot show whether adverse ecological effects have occurred or might occur as a result of site contamination. To evaluate actual impacts or likely ecological risks more completely, site data normally are compared to reasonable expectations for the site. Such expectations commonly are referred to as **reference information**. This Bulletin reviews approaches for defining and using reference information.

What Is Reference Information?

Reference information, as used in this Bulletin, is data of a comparative nature needed by investigators[1] to evaluate the environmental condition of a Superfund site. Simply put, reference data are baseline values or characteristics. If the results of site investigations suggest that environmental (physical, chemical, or biological) conditions are degraded in comparison to those defined by reference data, adverse impacts to the site's ecological resources are indicated.

Reference information generally falls into one or more of the following categories:

- *Relevant Existing Data*. Data specific to the site-associated area collected before contamination occurred, or data from elsewhere that can be applied to site conditions.
- *Models*. Indices and similar mathematical simulations of relationships among habitats, populations, and communities.
- *Reference Samples*. New data collected from the least impacted (or unimpacted) area of the Superfund site, or from a nearby site that is ecologically similar to the Superfund site and is not affected by the Superfund site contaminants.

Information from each of these categories has certain benefits and appropriate uses. Commonly, investigators use more than one source of reference information in completing Superfund ecological risk assessments. Knowledge of exact pre-contamination conditions or finding a reference site that

[1] The term "investigator" refers to the individual charged with responsibility for designing and/or implementing any part of an ecological risk assessment or other environmental assessment at a Superfund Site. Investigators can include scientists from the government, contractor organizations, or universities. The site manager (remedial project manager or on-scene coordinator), however, retains ultimate responsibility for the quality of any assessment.

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exactly reproduces pre-contamination status simply is not possible; some approximation, estimation, projection, or prediction will be necessary in almost all cases to define appropriate reference conditions. Also, the kind and extent of reference information can vary considerably depending on the characteristics of the site, its contaminants and potentially exposed resources, and the remediation decisions that must be made. For these reasons, site managers are encouraged to consult with the regional BTAG[2] to help identify reference information needs and apply reference data.

Existing Relevant Data

This category refers to data that can be found in government reports, library archives, scientific periodicals, etc., and can be applied to site conditions. In other words, investigators obtain "existing data" by conducting an extensive literature search—a basic step in any scientific investigation.

Applicable reference information may be found in the published scientific literature. Especially useful is information on the types and extent of ecological resources in the general area of the Superfund site, and on the condition of those resources through time. Data bases maintained by state and federal natural resource agencies often contain information on the distribution, abundance, and condition of natural resources. The U.S. Fish and Wildlife Service (USFWS), for example, has conducted an annual breeding bird survey for decades. Other examples include EPA's STORET data base on water quality conditions, the Agency's BIOS data base on aquatic biota, and the U.S. Geological Survey's WATSTOR data base on stream flow.

Private conservation organizations also can be useful sources of existing data, particularly concerning the types, extent, and condition of habitats and species in a region. The most common sources include local affiliates of the Nature Conservancy and state affiliates of the National Audubon Society and the National Wildlife Federation. Such groups often employ professionally trained staff and include scientists who can provide useful information and observations about the region.

Sometimes data are available that were collected on site prior to contamination. Possible sources include regulatory documents, such as Environmental Impact Statements or NPDES permit files, or (as noted earlier) the files of federal and state natural resource agencies. College and university science departments often maintain archives that might include information on the pre-disturbance conditions of the site-associated area.

A word of caution is in order, however, because historical site data often are not available and, when they are available, they generally lack the level of detail necessary to meet the needs of a Superfund ecological investigation. Environmental Impact Statements, for example, often list every possible species that might have inhabited or passed through the area. This can include extremely rare visitors; species whose current ranges have changed and are now too distant to inhabit the site again or to be affected by present site conditions; or, sometimes, species that are now locally or regionally extinct. Also, if enough time has passed, historical data may become irrelevant because land use in the area has changed significantly since the data were collected, such that fewer or different species are supported by the available habitats.

Models

Mathematical models have been developed to simulate the relationships among populations, communities, and habitats. Depending upon the characteristics of the Superfund site, models can be used to derive reference values for comparison with a Superfund site, to select reference locations, or as a tool to compare reference and Superfund site conditions.

Before using a model, however, it is important to understand that not all models are appropriate or practical for all sites or site personnel. Input data criteria, for example, can be so specific that using a particular model could require collection activities that were not factored into work plans, or could require data that cannot be collected at the site under investigation. When considering whether to use models, availability of qualified personnel may be a concern because specific training often is needed to ensure that appropriate input data are collected, both to run the model and to interpret and apply the results.

Three examples of mathematical models currently in use are the Index of Biotic Integrity, Habitat Evaluation Procedures, and the Geographic Aquatic and Wildlife Survey. These are discussed in the paragraphs below. It is important for readers to recognize that these are *only* examples; many other models exist, and the applicability of any model to any particular site is a matter for careful consideration by qualified professionals.

The Index of Biotic Integrity (IBI) is an aggregate of fish community measures that distinguishes disturbed from undisturbed midwestern steams.[3] Streams are scored with respect to twelve metrics based on their similarity to nominally undisturbed streams of similar size in the same region. For example, species richness is scored relative to the maximum species richness of streams of the same order in the region. These scores are then combined into a total score which is converted to one of six integrity classes (excellent, good, etc.). Since its original development, other scientists have adapted IBI to a variety of diverse geographic regions including streams in New England, Colorado, and northern California, and estuaries in Louisiana and the Chesapeake Bay.[4]

For Superfund ecological assessments, investigators can use IBI scores to evaluate current site conditions and to compare the Superfund site to the reference site(s). However, it is important to enlist the aid of the BTAG when analyzing data from indices such as IBI. For instance, in discussing the problems associated with indices, Suter[5] described how IBI combines dissimilar measures which can lead to index ambiguity (i.e., you cannot tell why the

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[2] 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 in their region. For a description of BTAG structure and function, readers are directed to "The Role of BTAGs in Ecological Assessment" (*ECO Update* Vol. 1, No. 1).

[3] Karr, J.R., Fausch, K.D., Angermeier, P.L., Yant, P.R. and I.J. Schlosser. 1986. Assessing biological integrity in running waters; a method and its rationale. Illinois Natural History Survey Special Pub. 5. Champaign, IL.

[4] Karr, J.R. 1991. Biological integrity: A long-neglected aspect of water resource management. Ecological Applications 1(1):66-84.

[5] Suter, G.W., II. 1991. Ecological Risk Assessment. Lewis Publishers, Chelsea, MI.

index is high or low) and a phenomenon known as eclipsing (i.e., a serious negative effect on one measure may be hidden by measures that are not affected or are positively affected).[6]

Habitat Evaluation Procedures (HEP)[7] provide a habitat approach to impact assessment and evaluation. The USFWS originally developed HEP to analyze the habitat impacts of planned land and water development projects. However, HEP also can be used to determine the amount of habitat change resulting from contamination by hazardous substances such as those occurring at Superfund sites. In this type of application, comparisons of the Superfund site are made against reference (control) areas representing the assumed baseline condition. In HEP analyses, the end result is to derive and compare Habitat Units, which represent both the quality and quantity of habitat for chosen wildlife indicator species.

Like any other approach used for impact or risk assessment, HEP has limited application and requires good professional judgment.[8] A habitat approach is restricted to those situations in which measurable and predictable habitat changes are important variables, but there are no assurances that wildlife populations will exist at the levels predicted by habitat analyses. Also, the methodology is applicable only for the wildlife species evaluated and does not necessarily relate to other wildlife species associated with other ecosystem components. Even if habitat is optimal, wildlife populations may not exist. Nevertheless, users of HEP can make scientifically sound, qualitative statements about potential beneficial or adverse impacts to other important flora and fauna in the study area, as long as such statements include caveats that they are qualitative inferences and not reliable facts.

The Geographic Aquatic and Wildlife Survey (GAWS) was developed by the USDA Forest Service to inventory and monitor steep, fast-flowing Rocky Mountain streams in Idaho. GAWS can be adapted to other regions, as long as this is accomplished by a fisheries biologist who is trained to use the model and is knowledgeable about the ecology of the region in which it is to be used.

At the Clear Creek, Colorado, Superfund site, investigators were uncertain as to whether decreased trout populations were attributable to metals toxicity, habitat destruction, or both. In an attempt to determine whether physical habitat parameters were the limiting factor, GAWS was used to compare the physical characteristics of Clear Creek basin against ideal (i.e., reference) conditions for trout. The GAWS investigation demonstrated that the carrying capacity of the physical habitat of Clear Creek could support more biomass and numbers of fish than were found in field surveys; therefore, investigators concluded that a lack of physical habitat did not cause the observed decrease in Clear Creek fish populations.

[6] Ott, W.R. 1978. Environmental Indices—Theory and Practice. Ann Arbor Science Publishers, Ann Arbor, MI.

[7] U.S. Fish and Wildlife Service. 1987. Type B Technical Information Document: Guidance on use of Habitat Evaluation Procedures and Suitability Index Models for CERCLA Application. National Technical Information Service Publication No. PB88-100151.

[8] Brody, M.S., Valette, Y. and M.E. Troyer. 1993. Modeling Future Losses of Bottomland Forest Wetlands and Changes in Wildlife Habitat Within a Louisiana Basin. In A Review of Ecological Assessment Case Studies from a Risk Assessment Perspective. EPA/630/R-92/005.

Reference Samples

Most often, sampling for new data is the best approach to establishing reference conditions. Depending upon the circumstances, reference samples are collected on-site (or the site-associated area) or from a separate location, known as a reference site.

Ideally, on-site reference samples are collected from the unaffected portions of contaminated habitats. This requires enough unaffected habitat to allow investigators to obtain an adequate sample size for comparison with contaminated samples. In general, only the larger or more complex sites allow for the collection of this type of reference sample. More commonly, on-site samples are taken along a gradient from lowest to highest contaminant concentration. The area of lowest impact or lowest measured concentration becomes the "reference" target for the site. This type of "reference" information can become necessary when all habitats are affected to some extent by site contaminants and a suitable reference location cannot be found.

Preferably, reference samples are collected from a reference site. Reference sites should match the Superfund site in all aspects except contamination: the former should be upstream, upwind, or higher in the drainage system but otherwise located as close as possible to the latter. A general guideline is to select reference locations that reflect the overall environmental conditions that can reasonably be expected in the site area given current uses other than those associated with the contamination under investigation.

Characteristics to Consider When Selecting Reference Locations

It is not possible to find the ideal reference location—that is, one which exactly mirrors the physical, climatic, chemical, and biological aspects of the Superfund site. Natural environmental characteristics vary widely, even among the same kinds of habitats. Therefore, the most one can hope for is a high degree of similarity between reference locations and the impacted area.

The following paragraphs present some of the characteristics one might consider during the selection of reference locations.

Physical Characteristics

Water. Among the many variables to consider when characterizing aquatic habitats are the current flow rates and patterns, water depth, temperature regimes, depth and intensity of light penetration, dissolved oxygen concentrations and dynamics, acidity, hardness, alkalinity, and nutrient concentrations. In mountainous areas, elevation can be an important consideration due to its strong correlation to temperature, climate, and related environmental characteristics. For estuaries, salinity gradients and tidal cycles are important considerations.

Upstream areas often serve as useful reference locations because they are part of the same aquatic system and can have physical, ecological, and historical characteristics similar to those for the Superfund site. This is not universally true, however. The area upstream might be wider or narrower, shallower or deeper, faster- or slower-moving, warmer or colder, or have a coarser or finer sediment than the contaminated stream segment.

Sediment. Trying to find a reference location with sediment characteristics that approximate those found at the Superfund site can be difficult, because sediment composition is highly variable

Some Characteristics to Consider When Selecting Reference Locations

Physical	Water	Temperature, chemistry, depth, flow
	Sediment	Total organic carbon; total acid-volatile sulfides; percent sand, silt, clay, water
	Soil	Particle size distribution, organic matter content, hydrologic regime, chemistry
	Habitat Structure	Stream/lake bottom structure, stream-/lake-side cover, vertical stratification, horizontal variation, percent cover
Climatic	Regional	Latitude, proximity to mountains and large water bodies
	Local	Topography (valleys, hilltops), altitude, aspect (north- or south-facing slopes), solar radiation
Biological	Community Characteristics	Species abundance, species richness, diversity, trophic structure, history of species introductions

One or Several Reference Sites?

The Rocky Mountain Arsenal (RMA), located north of Denver, CO, is a large (27 mi.^{2}) Superfund site composed of many habitats. To meet specific sampling needs, investigators selected several smaller reference areas representing specific habitats at RMA. The Wellington Wildlife Refuge, for example, more than 50 miles north of RMA, was selected for collecting mallard ducks, mallard eggs, cottontail rabbits, mule deer, and prairie dogs for chemical analyses. And investigators selected land five miles away as a reference site for soils because it was composed of the same soil type as that found at RMA.

The Popile Wood Treating site, on the other hand, is small in comparison to RMA, comprising 40 acres bordering Bayou de Loutre in Arkansas. However, like RMA, Popile affects several different habitat types including upland forested areas, wetlands, and surface water, requiring investigators to identify separate reference locations for the affected habitats. For example, because downstream freshwater communities were affected by site contaminants, investigators found a suitable reference site 300 feet upstream where they collected sediment and water samples for toxicity testing.

within and between aquatic environments. Widely ranging organic carbon content, particles size distribution, clay content, clay type, redox characteristics, cation exchange characteristics, and pH are observed under both natural and anthropogenically altered conditions.[9]

It is important, however, to consider all of these characteristics when selecting reference locations. For example, several investigators have demonstrated that the sorption of neutral organic chemicals to sediments is directly related to the sediment's organic carbon content,[10,11] and metals tend to be more available in oxidized sediments than reduced ones.[9] Therefore, when neutral organic chemical contamination is the issue, determining whether a difference exists between reference and site samples will require a high degree of similarity in organic carbon content, as a minimum. The same situation would exist for metals contamination and the extent to which reference and site sediments are oxidized.

Soil. Each combination of parent material, biota, topography, and climate produces a unique soil. Nevertheless, scientists recognize certain general types of soils, based on similarities in texture, moisture content, and chemistry. Soil texture describes the proportion of different particle sizes (sand, silt, and clay). Texture also is important for determining the ability of a soil to retain moisture. For example, clays and clay loams retain more moisture than sandy soils.

When selecting reference locations, investigators should try to match soil characteristics—such as particle size distribution, organic matter content, hydrologic regime, and pH—as closely as possible. For example, organic matter content, pH, and texture (particularly the type and proportion of clay) determine a soil's cation exchange capacity, or its ability to adsorb positively charged ions such as H^+, Mg^{+2}, or Cd^{+2}. These cations may include pollutants as well as nutrients.

Habitat Structure. The physical structure of the environment is another critical determinant of the composition of populations inhabiting an area. For example, lake and river bottom characteristics (i.e., organic sediment, silt, clay, sand, gravel, boulders, etc.)

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[9] Cockerham, L.G. and B.S. Shane. 1994. Basic Environmental Toxicology. CRC Press, Ann Arbor, MI.

[10] Chiou, C.T., Peters, L.J. and V.H. Freed. 1979. A physical concept of soil-water equilibria for nontoxic organic compounds. Science 206:831-832.

[11] Means, J.C., Hassett, J.J., Wood, S.G., and W.L. Banwart. 1979. Sorption properties of energy-related pollutants and sediments. In Polynuclear Aromatic Hydrocarbons. P.W. Jones and P. Leber, Eds. Ann Arbor Science Publ., Inc., Ann Arbor, MI.

Other Pollutant Sources and Reference Sites

At one Superfund site, investigators attempted to use nearby freshwater wetlands as reference sites because they were similar to the Superfund site with regard to hydrology, vegetation, and sediment. The reference wetlands also were up gradient; however, investigators ultimately determined that the selected reference sites did not provide useful baseline comparisons because one site received runoff from the land application of treated domestic waste water and the other reference site had extremely low levels of dissolved oxygen and highly toxic sediment.

determine the benthic communities of invertebrates and fish living there. The larger physical structure of lakes and rivers, including the extent of cover provided by aquatic vegetation, large woody debris, undercut banks, pool-to-riffle ratio, etc., can be critical in determining the fish species present and their population sizes.

An important physical characteristic of terrestrial habitats is vertical stratification, which is essentially the number of layers of vegetation (e.g., grassland, shrubs, saplings, mature trees, etc.) caused by different life forms and ages of plants. Also important is the horizontal variation resulting from the typically clumped distributions of vegetation. These habitat differences influence the nature and distribution of terrestrial animal life.

Climatic Characteristics

Climatic conditions can vary considerably within a relatively restricted geographic area. No matter how uniform a landscape and its climate may appear, it is not uniformly habitable. Close examination commonly reveals subtle differences in solar radiation, moisture, temperature, and other characteristics. For example, the climate beneath a plant community (microclimate) differs significantly from that in an open field—temperature is lower, humidity and soil moisture are greater, and light intensity is reduced under the vegetation canopy. Each organism primarily occupies only those areas meeting its requirements for life. As a result, plant and animal populations generally have patchy distributions across the larger range of area they are reported to inhabit.

Some of the most pronounced microclimatic differences are found between north- and south-facing slopes. South-facing slopes receive much more solar radiation and are typically warm and dry with wide extremes of micro-climatic conditions, while north-facing slopes tend to be more cool and moist with less variable conditions.

Terrestrial habitat types can change dramatically with fairly small increases in altitude. This can become important when considering up-gradient locations as potential reference sites. Mountainous areas tend to be windier, more exposed, and often drier than valleys, which are calmer, more humid, and tend to hold cold air overnight.

Biological Characteristics

The biotic communities at reference sites should be as similar as possible to those believed to have existed at the Superfund site before contamination occurred. Although the numbers and kinds of species settling within an area and their survival rates are rarely repeated in space or time, scientists often recognize generally recurring patterns that are quite similar for related groups. Such related groups usually are categorized on the basis of general appearance and are named after the dominant life form (e.g., deciduous forest, bog, short-grass prairie communities, etc.).

Three biological characteristics commonly considered when selecting appropriate reference sites for addressing questions concerning population- and community-level impact assessment are abundance, species richness, and species diversity.

- ***Abundance*** is an estimate of the number of organisms inhabiting a specific area.
- ***Species richness*** is the number of species inhabiting an area, such as a deciduous forest containing 20 species of woody plants including white oak, sugar maple, American beech, black cherry, flowering dogwood, and shagbark hickory.
- ***Species diversity*** is a measurement that relates the presence/absence of organisms of each taxonomic group in a habitat to the total number of individuals and species within a habitat.[12] It is usually expressed as an index of diversity. In the deciduous forest example above, each of the 20 species will occupy a percentage of the forest community (e.g., white oak occupies 14.1%, flowering dogwood occupies 1.2%). One of several formulas can be used to calculate a diversity index for this community.

Community Characteristics and Reference Site Selection—Two Examples

An understanding of the community characteristics of the reference site is necessary to enable a useful assessment of Superfund site responses. Community responses to metal contamination, for instance, reflect the impact of metals on the species that inhabit the contaminated ecosystem, changes in interactions within and among populations in the affected system, and possible changes in individual species' responses to the abiotic environment.

It also is important to ensure that the ranges of animals under study at reference locations do not overlap the area affected by the Superfund contaminants. For example, an important consideration when selecting a reference area for a site located on a tributary to a major river would be the transitory nature of the fish living in the tributary and their relationship with the river. If there are no barriers to fish movement, it is likely that fish collected in upstream areas also inhabit the Superfund location at various times. When the probability of such an overlap is unavoidable, it is necessary to use reference areas at greater distances from the Superfund site or to shift the focus of the investigation to organisms with more restricted ranges.

[12] Smith, R.L. 1990. Ecology and Field Biology. HarperCollins Publishers, Inc., New York, NY.

Ecologists sometimes use *similarity and difference indices* to characterize the degree to which communities resemble each other. Such indices may be helpful in evaluating alternative locales as reference sites, or in providing justification for selection of a particular reference site. When interpreting diversity indices, however, investigators need to remember that two or more sites (such as the Superfund site and the reference site) could have identical diversity index values but totally different species compositions. It is best, therefore, to enlist the aid of the BTAG when analyzing data such as diversity indices.

Using Reference Information In Ecological Risk Assessments

Ecological risk assessments begin with a problem formulation phase, continue into an analysis phase (i.e., impact assessment), and end with the characterization of ecological risks.[13] Reference information can play important roles throughout the process. It is important to emphasize, however, that the selection and use of reference information depends on solid upfront planning and good investigation design with appropriate QA/QC documentation.

Problem formulation is a planning phase in which investigators identify contaminants of concern, exposure pathways, potential ecological components, and likely endpoints. Although this is the phase where reference sites themselves might be identified and selected, reference information also might be needed to identify ecological components for further study, since contamination may already have eliminated many species from the Superfund site. Reference data can help establish background levels for certain contaminants that can occur naturally or are otherwise widespread in the environment. Clearly, Superfund ecological risk assessments can require preliminary investigation and analysis to locate useful reference locations. Over the long term, the effort will be rewarded in the form of high-quality reference data. Better data can bolster cases for causality and provide greater support for remediation decisions.

During the *analysis* phase, investigators collect data to characterize exposure and ecological effects. The objective is to characterize habitats in a way that will maximize the investigators' ability to identify important factors. For example, at an upland site it might be important to investigate a contaminant pathway from soil to earthworms to birds, making it necessary to understand ecological factors important to both earthworms and avian species.

During *risk characterization*, reference information can support, or is necessary in most cases to build, a "weight of evidence" which shows a causal link between the contamination and observed effects. Reference information is corroborative by definition, and reference samples, along with site samples, are used to develop relationships that may be statistically based to show that the contaminants are most likely related to the observed impacts.

Remember, perfect reference sites do not exist and professional judgment is the key to proper selection and use of reference information. This is the most important reason for gathering as much reference data as possible from the broad categories described in this Bulletin. Close and early consultation with the BTAG will help to ensure the proper collection and use of reference information.

[13] For a description of the phases of an ecological risk assessment, see U.S. EPA Risk Assessment Forum, *Framework for Ecological Risk Assessment* (Washington, DC: 1992), Publication No. EPA/630/R-92/001 [available from the Center for Environmental Research Information at (513) 569-7562].

Using Reference Information During an Ecological Risk Assessment: The Commencement Bay Superfund Site.

The study area, located in a heavily industrialized area at the southern end of the main basin of Puget Sound, includes more than 281 active industrial facilities. Investigators used reference conditions early on to identify contaminants of concern (COCs). Carr Inlet was selected as the reference site because (1) considerable data were available for metals, organics, grain size, organic carbon content, and other conventional variables, and (2) it was relatively uncontaminated, having the lowest detectable levels for most substances of concern in Puget Sound embayments. Eighteen organic compounds and compound groups were identified as COCs because their concentrations in surface sediments exceeded reference site concentrations.

Investigators next calculated Elevation Above Reference (EAR) values for the COCs. The threshold EAR value for each contaminant was the ratio of the maximum contaminant concentration in site sediments to the average for reference sediments. EAR values were determined for five indicators that were used to characterize site sediments and biota: sediment chemistry, sediment toxicity, benthic macroinvertebrates, bioaccumulation, and fish histopathology. Action level guidelines were applied to the EAR values to identify and prioritize problem areas. An area was designated a problem area if it exhibited a significant elevation in three or more of the five indicators. Using this method, problem areas were identified within all areas and segments in the study area.

Finally, investigators calculated Apparent Effects Thresholds (AETs) within problem areas. An AET was the contaminant concentration above which significant effects could always be expected. Contaminants exceeding their AETs were considered problem chemicals. AETs were calculated for all chemicals exceeding reference levels for three biological indicators: amphipod mortality, oyster larvae abnormality, and abundance of benthic macroinvertebrate taxa.