



# Tri-Service Procedural Guidelines For Ecological Risk Assessments

**TRI-SERVICE PROCEDURAL GUIDELINES FOR ECOLOGICAL  
RISK ASSESSMENTS**

**MAY, 1996**

## FOREWORD

In an effort to improve efficiency within DOD, the Air Force Center for Environmental Excellence (AFCEE), the Army Environmental Center (AEC), and the Naval Facilities Engineering Service Center (NFESC) have combined to coordinate projects of mutual interest. One such project has been the development of procedural guidelines for ecological risk assessment. The product of this effort will maximize the transfer of programmatic and technical information in ecological risk assessment to the Tri-Service Centers.

The purpose of this report is to provide guidance for conducting ERAs for use by risk assessors at Navy, Air Force, and Army installations. Each of the three services has a support center which is available to provide guidance and programmatic services. The three members are: U.S. Army Environmental Center (AEC), Naval Facilities Engineering Service Center, and the Air Force Center for Environmental Excellence. Using this approach will provide Tri-Service Centers with cost-effective, tiered procedures with which to direct and coordinate the scientific and technical efforts of contractors involved in ecological risk assessment.

The Procedural Guidelines for Ecological Risk Assessments is representative of the DOD trend toward partnership, and the goal to use increasingly scarce DOD dollars as efficiently as possible. With this vision in mind, the tri-services have joined efforts to produce this procedural guidance document that will benefit each of the services equally.

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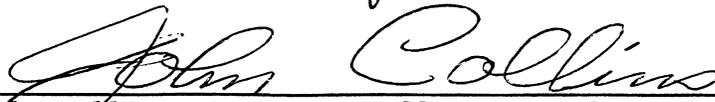
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## **PREFACE**

The work described in this report was authorized under MIPR No. 2372 from the U.S. Army Environmental Center (AEC) and work order No. 56015408-05-0000 from the U.S. Army Edgewood Research, Development and Engineering Center.

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### **Acknowledgments**

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VOLUME 1

Table of Contents

1.	INTRODUCTION . . . . .	1
1.1	Report Objectives . . . . .	2
1.2	History of Risk Analysis . . . . .	3
1.2.1	Application of Risk to Environmental Issues . . . . .	3
1.2.2	Initial Activities of Federal Agencies . . . . .	3
1.2.3	National Academy of Science (NAS) Framework . . . . .	4
1.2.4	Cancer and Non-Cancer Guidelines . . . . .	4
1.2.5	Council, Committee, and Society Actions . . . . .	5
1.2.6	Ecological Risk Assessment . . . . .	5
1.3	Comprehensive Environmental Response Compensation and Liability Act (CERCLA) and Ecological Risk Assessment . . . . .	6
1.4	Risk Assessment Framework . . . . .	8
1.5	A Tiered Approach to Ecological Risk Assessment . . . . .	10
2.	PROBLEM FORMULATION . . . . .	17
2.1	General Overview . . . . .	17
2.2	Discussion Between Risk Assessor and Risk Manager . . . . .	19
2.3	Stressor Characteristics . . . . .	19
2.4	Identifying the Ecosystem Potentially at Risk . . . . .	22
2.5	Ecological Effects . . . . .	23
2.6	Endpoint Selection . . . . .	24
2.7	The Conceptual Model . . . . .	27
2.8	Evaluation of Problem Formulation . . . . .	31
3.	ANALYSIS PHASE . . . . .	32
3.1	Exposure Characterization . . . . .	34
3.1.1	Stressor Characterization . . . . .	34
3.1.2	Ecosystem Characterization . . . . .	40
3.1.3	Exposure Analysis . . . . .	41
3.1.4	Exposure Profile . . . . .	46
3.2	Characterization of Ecological Effects . . . . .	47
3.2.1	General Overview . . . . .	47
3.2.2	Method of Characterizing Ecological Effects . . . . .	53
3.2.3	Linking Exposure and Stressor-Response Profiles . . . . .	68
3.2.4	Examples of Linking Biotic Responses and Exposure . . . . .	70
3.2.5	Example of Ecological Risk Assessment at a U.S. Air Force Site. . . . .	75
3.2.6	Example of Ecological Risk Assessment at a U.S. Navy Site . . . . .	76
4.	RISK CHARACTERIZATION . . . . .	78

4.1	General Overview . . . . .	78
4.2	Decision Points . . . . .	80
4.3	Risk Estimation . . . . .	82
4.3.1	Hazard Quotient . . . . .	82
4.3.2	Probabilistic Risk Estimates . . . . .	85
4.4	Simulation and Exposure Modeling . . . . .	90
4.5	Uncertainty Analysis . . . . .	92
4.5.1	Conceptual Model Formulation . . . . .	94
4.5.2	Incomplete Information and Data . . . . .	95
4.5.3	Natural Variability . . . . .	95
4.5.4	Procedural and Design Error . . . . .	95
4.6	Risk Description: Ecological Risk Summary and Interpretation of the Significance . . . . .	96
4.6.1	Ecological Risk Summary . . . . .	96
4.6.2	Weight of Evidence . . . . .	97
4.7	Risk Management . . . . .	100
5.	REFERENCES . . . . .	102

and a decision made concerning the potential for risk to occur in the RC phase, after which a decision will be made whether to proceed to testing at higher tiers. The assessment should proceed if the probability of risk is apparent, but complete characterization of risk cannot be determined due to significant data gaps. The assessment should not proceed if no risk is apparent, or if the risk is so great that action (e.g., remediation, containment, etc.) is warranted immediately. Proceeding to higher tiers in these situations would be a waste of time and money. Tiers are defined on the basis of progressive increases in the level of concern or in levels of manpower and monetary inputs in each successive tier.

Tier 1 (Figure 4) involves primarily a literature study, but adds RI results, historical site information, existing field data, literature and output from fate and effects models, and previous field surveys on the biota (including endangered and threatened species). These studies can be conducted by personnel from the installation, the USFWS, or other governmental agencies. Measurement endpoints rely on available data with underlying conservative assumptions and infer protection for assessment endpoints. These data and results may be used to develop preliminary hazard indices (risk quotients). The purpose of higher tiers (Figure 5) is to address data gaps and reduce uncertainty in the risk characterization and lessen the need for the use of conservative assumptions. This does not necessarily mean that laboratory studies are conducted in Tier 2 and field studies in Tier 3. In many cases, a laboratory study in Tier 3 will answer data gaps in the ERA with more precision than would field studies.

Tier 2 should address site-specific issues, limiting reliance on literature-cited values. This may include more models, laboratory tests, or limited field studies to address data gaps in exposure or ecological effects, and use more sophisticated analyses to develop more rigorous hazard indices to prioritize various locations at the site for potential risk. Measurement endpoints should be more complex, relying on specific laboratory or field studies that address data gaps identified in Tier 1, to better relate to assessment endpoints.

Tier 3 involves increased complexity, combining site-specific field observations with laboratory and field data to refine exposure and ecological effects characterization. Studies may include population- and ecosystem-level complexity and involve substantially longer-term investigations. The uncertainty associated with measurement endpoints is reduced, resulting in stronger data and greater confidence. At this point, the risk characterizations rely on distribution of exposure and effects results to facilitate understanding and interpretation of hazard indices at the site.

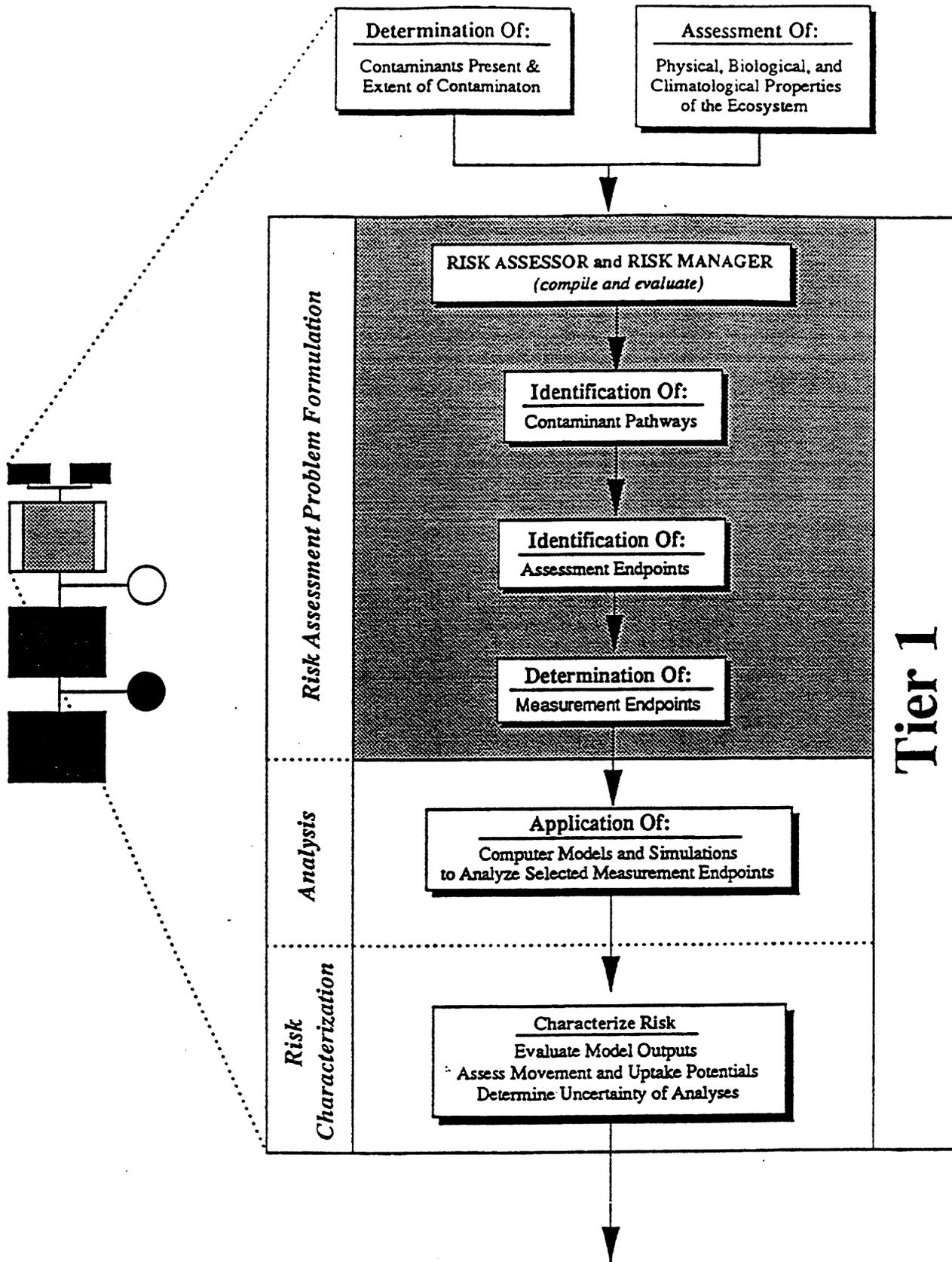


Figure 4. Tier 1 analysis.

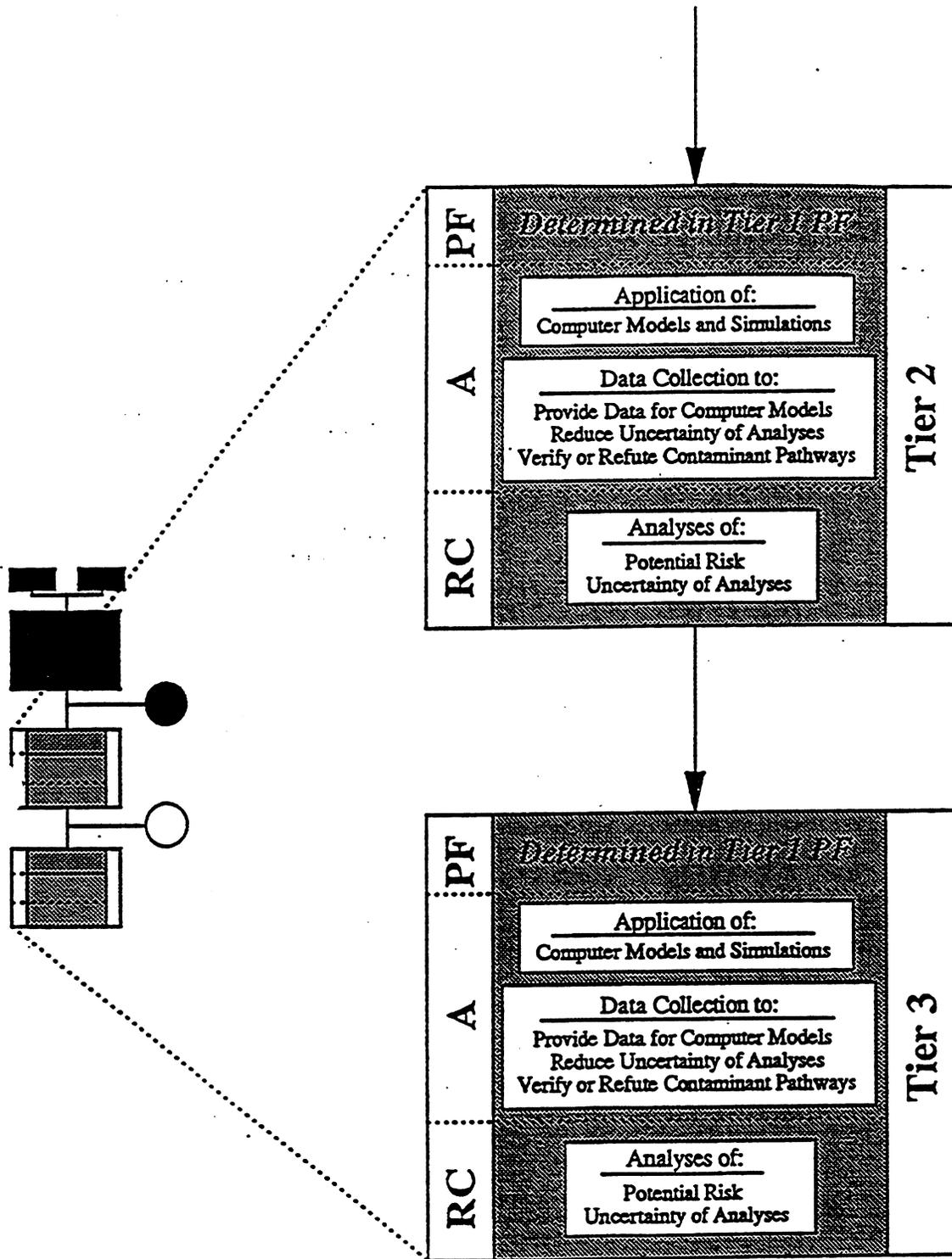


Figure 5. Tier 2 and Tier 3 analyses.

Although each tier is, in essence, an evaluation by itself, it is important that if testing proceeds to higher levels, there exists continuity in the risk assessment among tiers. Continuity is provided by establishing assessment endpoints. The measurement endpoints employed will change if the ERA progresses to higher tiers; however, the focus on assessment endpoints remains intact. For example, for an investigation of dieldrin residues in soils on a population of coyotes, one measurement endpoint in Tier 1 might be "dieldrin concentration in soil and in resident field mice". In Tier 2, measurement endpoints might be "analysis of coyote feeding habits on resident field mice and dieldrin concentrations in coyote tissue". In Tier 3, the procedure might involve a detailed analysis of coyote home range, time spent feeding, reproductive behavior, etc. In each tier, the measurement endpoints differ while the assessment endpoint remains the same. Further, if the assessment were stopped at Tier 1, estimates of risk would have to be conservative (e.g., broad "safety factors"). As the ERA process gathers more data on actual exposure and effects, the conservative assumptions may be relaxed.

## PROBLEM FORMULATION

### 2.1 General Overview

In the Problem Formulation phase (Figure 6), policy and regulatory discussions with the risk manager establish the goals and focus of the risk assessment. The views and values of the various stakeholders concerned with the management of the site are discussed, coordinated and prioritized. In this phase, the major factors to be considered are identified for the particular assessment, and working hypotheses are developed.

The process begins by characterizing exposure and ecological effects, including evaluating the stressor characteristics, the ecosystem potentially at risk, and the ecological effects expected or observed. Assessment and measurement endpoints are then identified. A conceptual model is constructed from this information that describes how a given stressor might affect the ecological components in the environment. The model also describes the relationships among assessment and measurement endpoints, the data required, and the methodologies that will be used to analyze the data. The conceptual model serves as input to the analysis phase of the assessment<sup>4</sup>.

Problem Formulation (PF) should clearly define the goals of the assessment (i.e., what are we trying to protect) and develop a type that is appropriate for achieving those goals within the constraints of available resources and the overall uncertainties of the analyses. To accomplish this, the problem formulation should ensure that the assessment focuses on the stressors, ecological components, and endpoints that are most appropriate for determining whether a cause and effect relationship exists and for making ultimate management decisions. Reviewers of risk assessment case studies<sup>16</sup> observed that establishing cause and effect is especially critical when resources are limited by fiscal constraints. Strengths and weaknesses of the case studies seemed to originate, in large part, from decisions made during the preliminary planning stages.

Steps 1-4 presented in the EPA draft report on an ecological risk assessment process for Superfund sites (Figure 2), are addressed in the PF phase of EPA (Figure 6). After stressor characteristics, ecological effects, and ecosystem parameters have been initially reviewed (after step 2 in the EPA Superfund draft report) a scientific/management decision point (SMDP) is reached to decide whether the data warrants further study. After each of the two remaining parts of the PF phase, endpoint selection and development of the conceptual model, the EPA Superfund report<sup>3</sup> calls for SMDPs to formally agree to the results from these two key planning parts of PF. The use of SMDPs stresses good communication among all parties involved and

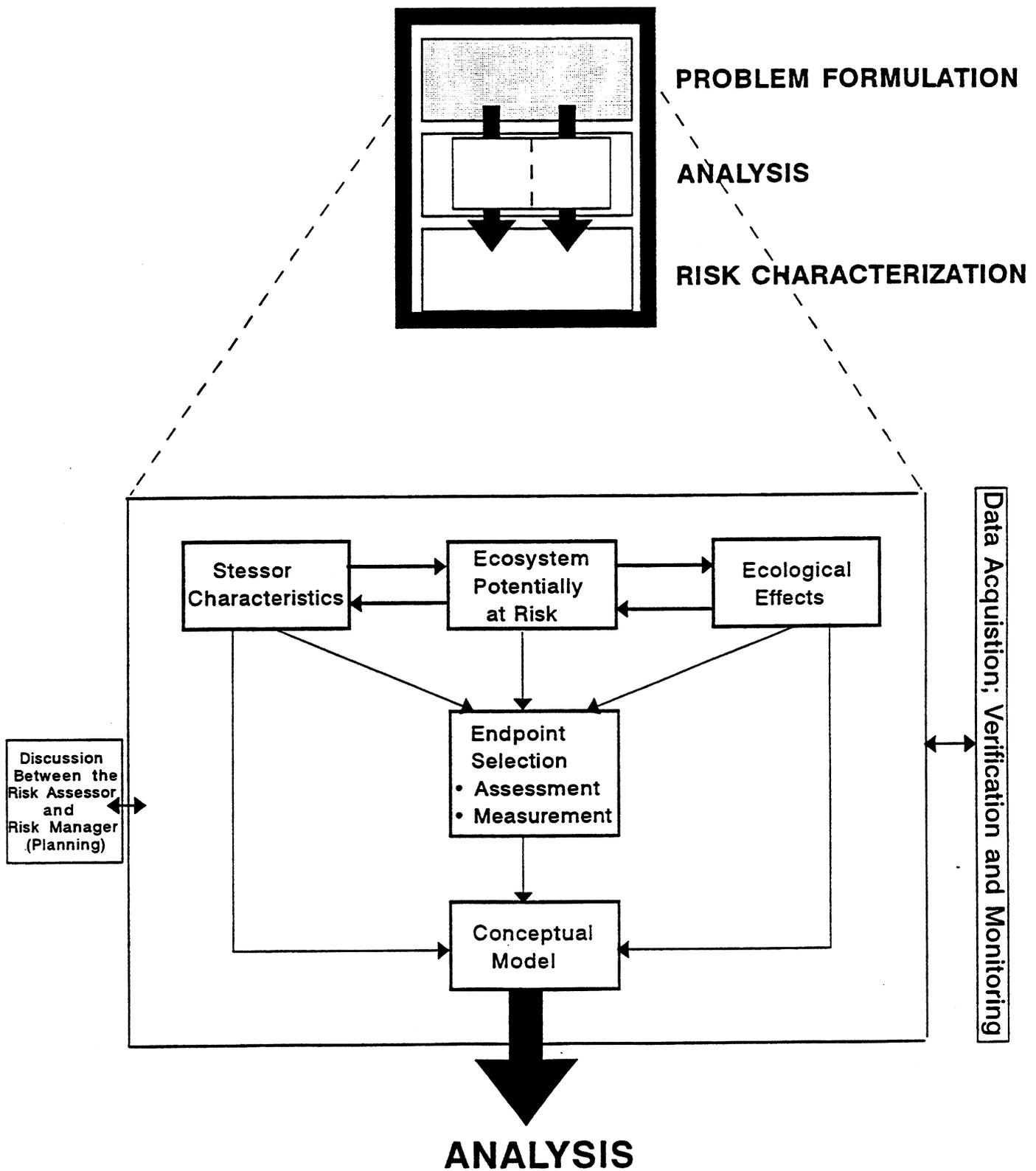


Figure 6. Problem Formulation Phase

keeps the risk assessment process focused and efficient.

## **2.2 Discussion Between Risk Assessor and Risk Manager**

Establishing a two-way dialogue between the risk assessors and risk managers during the problem formulation phase is essential to achieving societal, regulatory, and scientific goals. Risk managers can ensure that the risk assessment will provide answers for questions related to protection of societal values, selection of remediation technologies, policy concerns and cost, whereas, the ecological risk assessor ensures that the assessment addresses important scientific concerns. Both perspectives are necessary to efficiently utilize resources to produce scientifically sound risk assessments that are relevant to management decisions and public concerns<sup>4</sup>. Establishment of SMDPs, as described above, is a good method to ensure that all policy and scientific issues are addressed.

The National Crop Loss Assessment Network (NCLAN) case study<sup>16</sup> was a good example of an assessment where the ultimate management issue was clear from the onset; the stressor, ecological components, and endpoints were clearly defined; and the design of the study was structured around a clear set of hypotheses amenable to scientific inquiry. This level of clarity was achieved, in part, through frequent meetings and interactions among researchers and others involved with the risk assessment/risk management process. The author and reviewers of the case study stressed the importance of this type of communication for clarifying issues and goals.

## **2.3 Stressor Characteristics**

Stressors are chemical, physical or biological influences causing negative impact on the populations or ecosystems at risk. Chemical stressors include not only the contaminants of concern (COCs), but inorganic and organic chemicals inherent in the environment as well. Secondary stressors may arise as a result of primary COCs, such as increased concentrations of chlorofluorocarbons causing stratospheric ozone depletion which, in turn, results in increased exposure to ultraviolet radiation. Physical stressors are generally the abiotic environmental conditions under which the biota find themselves. These include such factors as seasonal and diurnal variance in atmospheric temperature, soil characteristics (soil type, parent material, climate, pH, organic matter content, management practices, etc.), the hydrologic regime (seasonal flooding, tidal influences, etc.) and habitat alterations (logging, construction, urbanization, etc.). Biological stressors also exist and are often important in determining survivorship of populations. Examples of biological stressors include competitor and predator species, introduced pests, such as the gypsy moth and various fungal

pathogens of tree species, or cholera epidemics in bird species. Changes in the physical/chemical environment may lead to subtle changes in competitive abilities of a species or may lead to changes in abilities to avoid predators, infestations, or disease epidemics. Therefore, biological stressors may assume larger roles in determining the maintenance of a population if the habitat has been altered chemically or physically. Stressors may also result from management practices such as harvesting of fishery or forest resources, or cultivation techniques during crop production.

Any stressor cannot be judged as such without reference to the species or community under stress. One cannot isolate the stressors from the species response, as they are interrelated. The degree to which stressors influence the survivorship of species depends on the magnitude of the stress (the intensity), the duration of the stress (how long the species is exposed, relative to its own life history characteristics), the frequency (how often a stress of a particular intensity occurs), the timing (when the stress occurs, relative to critical life history stages of the species). A complex of stress factors influence species responses; hence, creating a map of direct or indirect influences of contaminant stressors onto the "mosaic" pattern of normal stressors involves considerable thought.

The task of the RA in the PF phase is to analyze a suite of previously compiled chemical, physical and biological data. Literature data bases contain a variety of environmental toxicology data for chemicals. A partial listing of such data bases is given in Table 1. Defense Technical Information Center (DTIC), DoD research laboratories and DoD scientists may also be able to guide the RA to relevant toxicity data.

With this information, the RA then evaluates site-specific stressor characteristics in the PF phase of the Tier 1 analysis. During Tier 1, the RA identifies which chemical, physical, and biological stressors are present based on available information and estimates the nature, extent and potential interaction of these stressors. This information may be obtained from databases listed above but also from information previously collected from the site, such as record searches or Installation Assessments, reports on chemical storage, use and distribution, or from DTIC. Information on chemical properties of the contaminants should be examined in the context of biological, chemical, and physical characteristics of the ecosystem.

The manner in which contaminants interact with the physical and biological ecosystem components are predictable, within certain constraints. Interactions among site-specific soil and biotic characteristics influence contaminant distribution, fate and, importantly, allow the RA to estimate the likelihood of the contaminants remaining *in-situ* rather than moving off-site or

through the ecosystem. For example, fairly simple models (SESOIL, EXAMS; see Volume 2) may be called upon in Tier I to estimate the distribution of contaminants downstream or in soils on the site. The input data (e.g., soil moisture, pH, particle size, percent organic matter) for these types of models, if not measured directly, are available from detailed county soil surveys (Soil Conservation Service), USGS topographic maps, or state resource agencies. When more detailed and site-specific information is available, more sophisticated models may be used (CMLS, LEACHM; see Volume 2).

Table 1. Listing of databases available for information on contaminant fate and effect.

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**1. Chemical Information System (CIS)**

AQUIRE - Aquatic Information Retrieval  
CERCLIS - CERCLA Information System  
CHRIS - Chemical Hazard Response Information System  
ENVIROFATE - Environmental Fate  
ISHOW - Information System for Hazardous Organics  
in Water

OHMTADS - Oil and Haz. Materials/Tech. Assist. Data  
System  
PHYTOTOX - Toxic Effects on Plants

**2. National Library of Medicine's Database Selection Menu**

HSDB - Hazardous Substances Data Bank  
IRIS - Integrated Risk Information System  
EMICBACK - Environmental  
EMIC - Environmental  
ETICBACK - Environmental

**3. Dialog Databases**

Oceanic Abstracts  
Enviroline  
Pollution Abstracts  
Aquatic Sciences and Fisheries Abstracts  
Environmental Bibliography

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Bioavailability of chemical constituents should also be considered at this point. For example, is the chemical hydrophilic or hydrophobic?; is it available in the soil water and subject to surface runoff and leaching, or is it tightly bound to soil particles and organic matter?; and how do site specific soil characteristics affect the contaminants' bioavailability?

At the end of Tier 1 PF, the risk assessor should have a good understanding of the stressor characteristics for the particular site under study. Data gaps should be addressed in Tier 2 if the assessment proceeds that far.

#### **2.4 Identifying the Ecosystem Potentially at Risk**

Identifying the ecosystem potentially at risk from a stressor depends in part on how the risk assessment was initiated. Once a stressor is identified, information on the spatial and temporal distribution patterns of the stressor can be helpful in identifying ecosystems potentially at risk. Similarly, if the risk assessment is initiated by observing effects, these effects can directly indicate ecosystems or ecological components of the system that may be considered in the assessment.

Ecosystem properties should be analyzed during PF. These properties include ecosystem structure (including types and abundances of different species and their trophic level relationships), ecosystem function (i.e., ecosystem energy source, pathways of energy utilization, and nutrient processing), bioavailability, and aspects of the abiotic component (see Section 2.3 above). In addition, types and chronology of historical disturbance should be determined to help predict ecological responses to stressors.

At this point, it is important to emphasize that not all aspects of ecosystem structure and function need to be analyzed in every risk assessment. The extent to which ecosystem properties are analyzed depends upon the nature of the stressors and ecosystem components, bioavailability, and the resources available. Analyses should concentrate on those ecosystem components that are determined to be at greatest risk. Knowing the stressor characteristics can help to narrow the focus of the investigation on the components of the ecosystem that are potentially most susceptible.

Once stressor characteristics and the ecosystem potentially at risk have been identified, potential pathways for contaminant(s) through the ecosystem must be identified. Contaminant pathways may be simple and straightforward or complex and highly branched. Pathways are generally defined by naturally occurring physical, chemical, and biological components of the ecosystem. As an example, consider the evapotranspiration potential,

precipitation, soil type, slope, local vegetation, and ground squirrels (*Citellus* sp.) foraging on the vegetation in a given ecosystem. In this example, the movement of an organic contaminant might be a function of the seasonal food source sought by the rodent species. In other seasons, the ground squirrels are absent or dormant; hence, they would not be subject to exposure by the same pathway.

The origin of each contaminant pathway is typically from soil or water, at the site of contamination and the end of each pathway is a component of the ecosystem where adverse effects may occur (such as threatened or endangered species, a resident small mammal population, or fish species in a downstream lake or reservoir). Several assessment endpoints (see Section 2.6 below) may exist at the end of a contaminant pathway because pathways will seldom be unidirectional or linear. Chemical pathways generally branch and proceed in multiple directions; for example, a contaminant may have the potential for moving from a contaminated site into an aquatic system, with no potential impacts (branches) en route to a pond. However, once the contaminant enters the pond, potential contaminant pathways may include uptake of the contaminant by aquatic vegetation, by aquatic organisms (e.g., mollusks, gastropods, aquatic insects), uptake by fish, or amphibians, or transport back to the terrestrial environment via birds or mammals that feed on aquatic organisms.

The number of contaminant pathways are determined by the characteristics of the contaminant and the complexity of the ecosystem. Contaminant pathways must be identified on each Army Superfund site; however, similarities in pathways will likely exist among many sites resulting from similar ecosystems. Greater definition (closer focus) of specific contaminant pathways will be a function of Tier 2 and Tier 3 chemical analyses. Ultimately, however, if a pathway is incomplete or does not exist at a particular site, no cause and effect relationship exists and there is no associated risk.

## **2.5 Ecological Effects**

Ecological effects in Tier 1 of the PF phase should be derived from studies in the literature that are applicable to the stressors and ecological components of concern in the assessment, and from reports of previous studies (e.g., RI/FS) conducted at the site. Published data may come from a variety of sources including field observations (e.g., fish kills, changes in aquatic community structure), laboratory tests (e.g., single species or microcosm bioassays), and chemical structure-activity relationships. Home range, feeding area, and migratory patterns of the biota of concern at the site should be determined from USFWS, site specific sources (i.e., state fish and wildlife agencies, military installation records, etc.) or the open

literature. These data, together with spatial and temporal patterns of the COC within the site can help characterize the extent of ecological effects. Analysis of this information can help focus the assessment on specific stressors and on ecological components relevant to the site.

Caution must be taken so that the ecological effects data are properly utilized in Problem Formulation. For example, applicability of laboratory-based tests may be affected by extrapolations to various field conditions, whereas the interpretation of field observations may be influenced by site-specific factors such as natural variability or the presence of stressors other than the COCs. Ecological effects data obtained in PF can then be used to identify data gaps and to characterize ecological effects in the Analysis Phase of the assessment.

## 2.6 Endpoint Selection

Ecologically based endpoints are selected after the societal, regulatory, and biological goals have been established following review of stressor characteristics, the ecosystem potentially at risk, and the potential ecological effects. It is important that the RA and RM collaborate and agree on the endpoints selected before proceeding to the Analysis phase. An endpoint is defined as a characteristic of an ecological component (e.g., increased mortality in fish) that may be affected by exposure to the stressor<sup>17</sup>. Two types of endpoints, assessment and measurement, are used in the ERA to determine risk to the ecosystem.

An assessment endpoint is defined as:

An explicit expression of the environmental value to be protected.<sup>4</sup>

For best use, assessment endpoints should have biological as well as societal value so that scientific information can be linked to the risk management process (e.g., policy goals). For an ERA to produce sound, acceptable results, there are five criteria necessary for choosing assessment endpoints<sup>7,4</sup>:

- 1) policy goals and societal relevance;
- 2) ecological relevance;
- 3) unambiguous operational definition;
- 4) accessibility to prediction and measurement; and
- 5) susceptibility to the hazardous agent.

When choosing assessment endpoints, two general questions must be answered: (1) what valued components of the environment are considered to be at risk; and (2) how should effects be defined? Some assessment endpoints are mandated legally or politically; however, the RA should also determine what endpoints should be

Table 3. Examples of assessment endpoints. Possible indicators of effects on those endpoints, and possible endpoints for measurements of those indicators.<sup>7</sup>

azard/Policy Goal	Assessment Endpoints	Indicators of Effects	Measurement Endpoints
Herbicide used for weed control in southern lakes/No acceptable loss of fisheries	Probability of >10% reduction in game fish production	Laboratory toxicity to fish	Fathead minnow LC <sub>50</sub> Larval bass concentration/mortality function
		Laboratory toxicity to food-chain organisms	<i>Daphnia Magna</i> LC <sub>50</sub> <i>Selenastrum capricornutum</i> EC <sub>10</sub>
		Field toxicity to fish	Percent mortality of caged bass
		Populations in treated lakes	Catch per unit effort Size/age ratios by age class
Agriculture insecticide associated with bird kills/No acceptable reductions in avian populations function	Proportion of raptors killed within the region of use	Laboratory toxicity to prey	Rat LD <sub>50</sub> Japanese quail dietary LC <sub>50</sub>
		Laboratory toxicity to raptors	Sparrow hawk dietary concentration/response Japanese quail dietary LC <sub>50</sub>
		Avian field toxicity	Number of prey carcasses per hectare Number of dead moribund raptors per hectare
	Increase in rates of decline of declining bird populations within the region of use	Avian laboratory toxicity	Japanese quail dietary LC <sub>50</sub> , Starling dietary LC <sub>50</sub>
		Avian field toxicity	Number of bird carcasses per hectare by species
		Trends in populations of declining birds	Rates of decline in areas of use as proportions of reference areas

At this stage of the RA, the conceptual model should be used to predict the impact of the chemicals on individuals, populations and communities. The exposure scenario for chemical stressors usually involves consideration of sources (e.g., explosives burning ground), environmental transport (e.g., rate of movement through soil column), partitioning of the chemical among various environmental media (e.g., soil particles vs. organic matter), chemical/biological transformation or speciation processes (e.g., photolysis, biodegradation), and identification of potential routes of exposure (e.g., ingestion, plant root absorption, etc.). Exposure scenarios for non-chemical stressors such as soil compaction, or habitat alteration describe the ecological components exposed and the general temporal and spatial patterns of their co-occurrence with the stressor. For example, the exposure scenario may describe the extent and distributional pattern of compacted and disturbed soil in a field used for military training with tracked vehicles, the soil microflora, vegetation and wildlife occupying or using this field, and a comparison of the size and distribution of these populations with those in adjacent undisturbed fields<sup>19</sup>.

The hypotheses formulated must first be "weeded out" for those considered most likely to contribute to risk. Then the risk assessor should further narrow down the choices to focus only on those hypotheses that can be addressed with available resources. These hypotheses are then evaluated in the Analysis phase. It is important that any hypotheses not originally used in the Analysis phase be re-visited when uncertainty is addressed in the Risk Characterization (RC) phase. Uncertainty considerations of model predictions in the RC phase may require that previous hypotheses explaining the assessment endpoint be reviewed. Professional judgement is needed to select the most appropriate risk hypotheses; further, it is needed to document the rationale underlying the selection process<sup>4</sup>.

A detailed work plan should then be written describing objectives, data requirements (including assessment and measurement endpoints), experimental design, procedures and methods, quality assurance objectives, and a time schedule to estimate duration and completion dates of various phases of the assessment. Work plans will vary according to the specific needs of each assessment but should be formulated and agreed upon by all parties involved. The work plan should be included in the remedial investigation. In formulating a work plan, it is critical to address how data gaps will be handled and to explicitly state the data quality objectives<sup>4</sup>. The conceptual model describes the approach that will be used for the Analysis phase and the types of data and analytical tools that will be needed.

## 2.8 Evaluation of Problem Formulation

At the conclusion of PF, it is important for the risk assessors and risk managers to determine the attributes and focus of the rest of the assessment and to decide if indeed the assessment should continue. The EPA<sup>3</sup> has compiled a list of scientific/management decision points (Figure 2) that include factors that should be agreed upon before proceeding further with the risk assessment such as:

- (1) Deciding whether or not the risk assessment should proceed further based on available information;
- (2) Selecting assessment endpoints, testable hypotheses, and measurement endpoints;
- (3) Agreement upon the exposure pathways;
- (4) Selection of specific investigation methodology;
- (5) Selection of data reduction and interpretation methods.

Agreement by all involved parties on the decisions and methodologies shown above will help to keep the risk assessment focused and save time and money.

### 3. ANALYSIS PHASE

During the Analysis phase (Figure 8), the working hypotheses developed during the PF phase link exposure assessment to ecological effects. This phase acknowledges that the abiotic and biotic characteristics of the ecosystem of concern will impact the ecological effects and the exposure profile. The various steps in this phase lead to the development of a stressor-response profile and an exposure profile. These profiles are used as the basis for risk characterization.

The most effective tool available to the ecological risk assessor is a site visit. During this visit the ecosystem is qualitatively assessed to determine potential receptors present at the site, determination of routes of exposure, and other stressors present (e.g., dredging activity, prop wash, lack of riparian habitat on the banks of a stream, etc.). Signs of direct effects may be noted during the site visit such as stressed vegetation around a seep.

On the basis of this site visit as well as existing data for the site, the risk assessor has to determine what additional data are necessary. Ecological risk assessment is commonly performed using a "weight of evidence" approach. An excellent description of this approach applied to a terrestrial ecosystem can be found in Menzie et al.<sup>20</sup>. They utilized predictive modeling based on measured surface water, sediment and soil concentrations of COCs, laboratory toxicity tests, field toxicity tests, and other field methods to assess potential ecological impacts.

It is important to realize that many potential hazardous waste site assessments have been designed by engineers without consultation with risk assessors. What often results is a large amount of data, none of which is of value to the risk assessor. For example, many metal water quality criteria are dependent upon site-specific water hardness, but water hardness is often not analyzed, or even thought of as important for analysis by the workplan author. Another important data quality often overlooked is the required detection limits necessary to perform risk assessment. The CLP procedure does analyze for polycyclic aromatic hydrocarbons (PAH), however CLP reporting limits are much above concentrations at which one may expect potential ecological impacts. Listed below are parameters commonly overlooked and chemicals which alternative analytical methods which provide lower detection limits may be appropriate:

#### \* Parameters Commonly Overlooked

Hardness in surface water,  
Total organic carbon in sediment and soil,  
Lipid content in biological samples

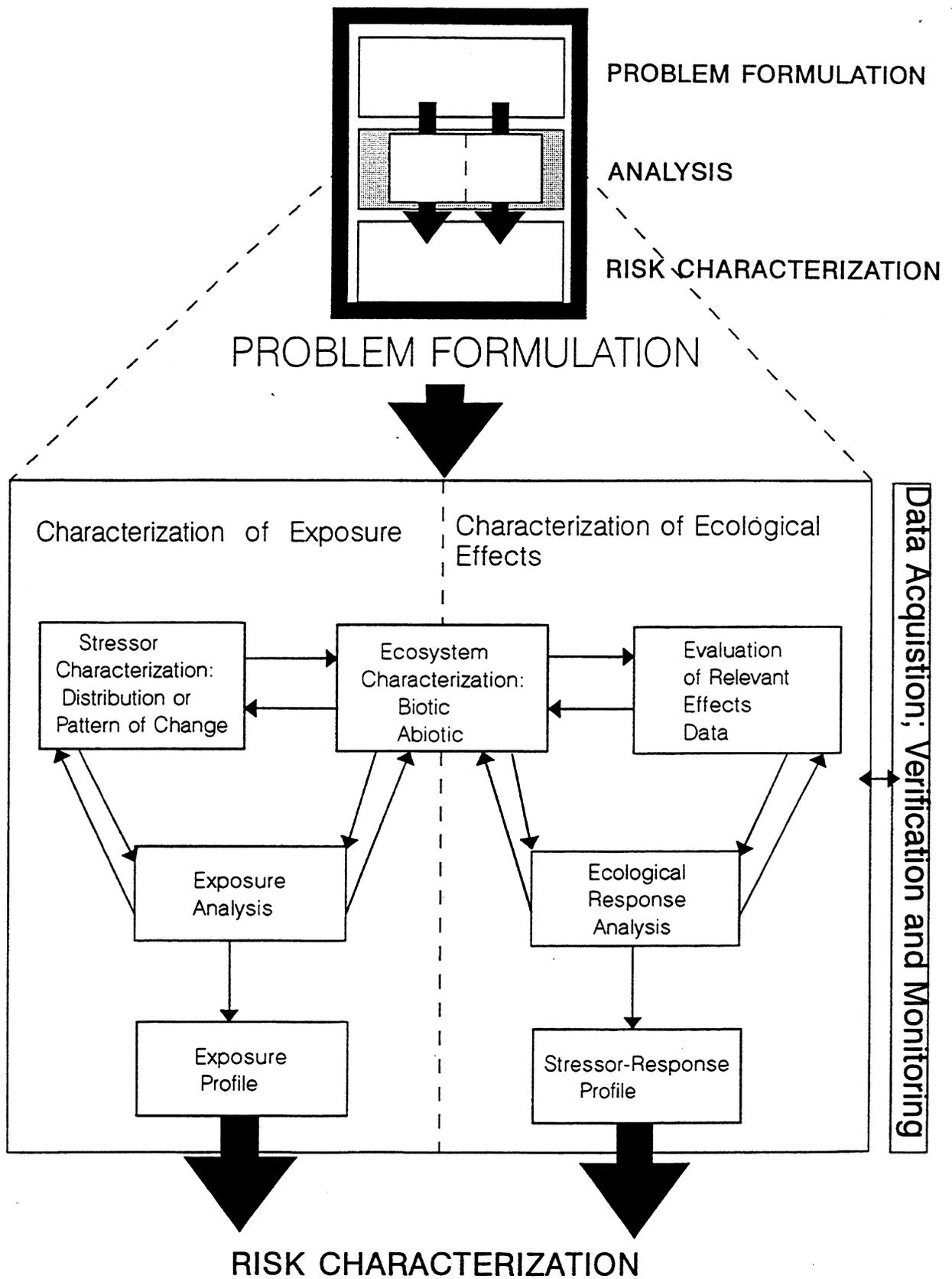


Figure 8. Analysis Phase

## \* Chemical Types Commonly Measured at High Reporting Limits

Polycyclic Aromatic Hydrocarbons (PAH),  
Pesticides, PCBs, and some metals.

To correct this situation it is necessary to involve the risk assessment personnel early in the workplan stage. Their role should be to assure that all necessary parameters are being measured at appropriate reporting limits. Alternative analytical chemistry methods are available which allow reporting much lower detection limits than those reported using CLP standards. The risk assessment personnel should work with the analytical laboratory to determine appropriate analytical methodology. In addition, the sampling plan should be assessed to assure that proper numbers and types of samples are being taken. Biota samples will commonly be completely unsampled, and because the waste engineers tend to focus on "hot spots", by definition a biased sampling procedure, exposure will often be overestimated.

### 3.1 Exposure Characterization

#### 3.1.1 Stressor Characterization

Characterization of exposure begins with determining what stressors are present at the site. Ecological risk assessment is complicated by the necessity of determining multiple stressors, often including stressors such as habitat and human actions like dredging a stream or water body.

This step determines the stressor's distribution over space and time at the study area. The primary stressor is evaluated as well as any secondary effects which have occurred due to impacts from the initial stress to the system. Background or preliminary information on the chemical-of-concern is important for the stressor characterization because such information points towards expected stressor-responses. For example, lipid-soluble organochlorine pesticides bioaccumulate fairly readily in aquatic ecosystems. Organic chemicals with low  $K_{ow}$  do not accumulate readily and direct toxicity, rather than tissue uptake, is the primary concern for exposure.

Characterization of exposure begins with determining where the contaminant is on the site, where, if and how the contaminant moves from the site, and what physical/chemical characteristics lead to its bioaccumulation, degradation, transport, etc. For many chemicals, historical files provide information on quantities produced, used, stored on-site, or sprayed (pesticides, solvent cleaners). Often, chemical characteristics of the contaminant, including rates of degradation (via photolysis, hydrolysis, microbial), adsorption, solubility in water or lipid may be obtained from literature sources, on-line

chemical databases (Table 3), Material Safety Data Sheets (for industrial chemicals), and technical reports. An excellent source for environmental degradation rate is Howard et al.<sup>21</sup>, general fate and transport data can be found in the Lewis Publishers (Chelsea, Michigan) series titled "Handbook of Environmental Fate and Exposure Data for Organic Chemicals". This series, ultimately to have seven volumes, presently consists of Large Production and Priority Pollutants (Volume I), Solvents (Volume II), Pesticides (Volume III), and Solvents 2 (Volume IV). Data provided in these volumes include basic chemical and physical properties (boiling point, melting point, molecular weight, water solubility, octanol-water partition coefficient, vapor pressure, etc.) and a description of basic fate and exposure potential including sources, important transport processes, and reported concentrations in the environment. While there are many computer databases available, the most current and reliable database encountered so far for fate and transport data is produced by the Syracuse Research Corporation, Merrill Lane, Syracuse NY 13210. They maintain several databases including BIOLOG (Biodegradation database) and CHEMFATE. CHEMFATE can be used to search for many properties and characteristics ranging from soil adsorption constants to photolysis degradation rates. The above references refer to fate and transport of organic chemicals. There are several excellent references available regarding fate and transport of metals in the environment<sup>22,23</sup>.

The information required for a Tier 1 exposure characterization would be obtained via the documents described above. Ecological assessments may be "effects-driven" or "stressor-driven." For example, the abundance of a sediment benthic community is often used as a measure of sediment "health". If the benthic community is found to be deficient, it is commonly used as an "effects-driven" assessment. Alternatively, known dump sites, with no apparent ecological effects are an example of a "stressor-driven" assessment. This implies that the initial focus may be on understanding how the measured effects were induced ("effects-driven") or on understanding the behavior of the chemical(s) of concern ("stressor-driven"). In characterizing exposure, the RA identifies measurement endpoints along each contaminant pathway where data collection or computer simulations and models are applied to evaluate contaminant fate and consequent ecological impacts. Data collected for these measurement endpoints help reduce uncertainty by validating or refuting whether predicted contaminant movement is actually occurring. In characterizing exposure, the RA identifies measurement endpoints along each contaminant pathway where data collection or computer simulations and models are applied to evaluate contaminant fate and consequent ecological impacts. Data collected for these measurement endpoints help reduce uncertainty by validating or refuting whether predicted contaminant movement is actually occurring.

The environmental fate and potential transport of contaminants is crucial to effective risk assessment because the bioaccessibility (whether organisms come in contact with toxicants) and bioavailability (whether contact leads to uptake) are controlled by these processes. For pesticides, degradation, volatilization, binding, leaching, and aging determine ultimate exposure concentrations<sup>24</sup>. Metals availability is controlled largely by pH and oxidation-reduction relationships in environmental media<sup>22,25</sup>. The chemistry and distribution of the compounds of interest must be thoroughly understood for effective risk analysis. It is crucial for the risk assessment/risk management team to understand that the bulk concentration of chemical compounds as measured in typical laboratory extraction tests (such as those provided with Contract Laboratory Program quality assurance documentation under CERCLA) do not reflect the biologically active concentrations. In practice, binding and uptake processes depend on complex environmental processes which need to be accounted for in projecting risks.

The environmental fate and transport of mercury in anoxic (oxygen depleted) environments is shown in Figure 9. Mercury has been identified as a chemical of concern in many areas of the country, primarily due to its volatilization and transport within the atmosphere. For example, within the everglades of Florida mercury has been identified as a chemical of concern for many fish, raccoons, and cougars preying on the raccoons. Obviously, there are no point sources of mercury directly in the everglades, pointing to long range transport from outside the boundaries of the everglades. The fate and transport of mercury is complex, and involves bacteria who can methylate the ion and form a highly bioaccumulative methylmercury.

Similar fate and transport figures can be produced for other metals and organic chemicals. Environmental factors will influence chemical fate and transport dependent upon the type of chemical of concern. For example, lipid-soluble (high octanol-water partition coefficient,  $K_{ow}$ ) organochlorine pesticides bioaccumulate readily in aquatic ecosystems. Alternatively, low  $K_{ow}$  chemicals do not readily bioaccumulate and direct toxicity, rather than tissue uptake, is the primary route of exposure.

Models in Tier 1 analyses serve as "screening analysis" to provide initial qualitative assessments of contaminant transport into the environment. They are designed to (1) identify each transport process controlling movement of various contaminants within and among media, (2) estimate the direction and rate of chemical movement from the site and, (3) identify areas to which contaminants have been or may be transported. Fugacity models<sup>26,27</sup>, which calculate where a given chemical will tend to accumulate in the environment, are an example of this level of

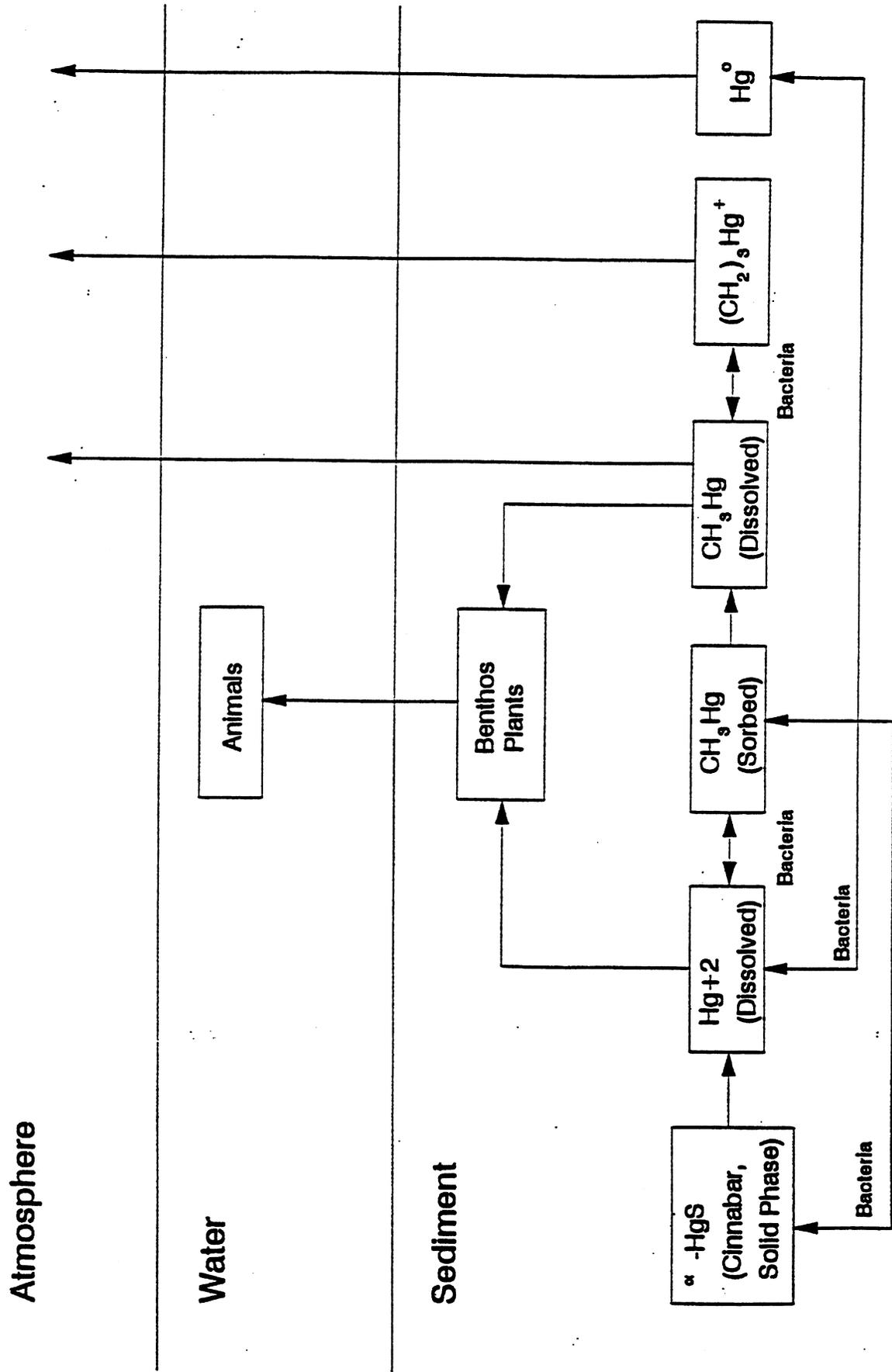


Figure 9 Environmental Fate and Transport of Mercury in Anoxic Aquatic Environments

detail. This level of modeling provides an initial organization and direction for subsequent in-depth analyses of contaminant transport. When a more in-depth analysis of environmental fate is desired, the RA should seek advice on which modeling procedure is most appropriate to the circumstances. In general, the more sophisticated models are data-, time- or resource-intensive. Table 4 is a ranking of relatively simple to complex models<sup>28</sup>. Criteria to consider when selecting in-depth environmental fate models are:

- (1) capability of the model to account for important transport, transformation and transfer mechanisms;
- (2) the fit of the model to site-specific and substance-specific parameters;
- (3) data requirements of the model, in relation to the availability and reliability of site-specific data; and
- (4) the form and content of the model output. That is, does the model output address relevant questions and provide data required for use as input to further analyses.

At the end of a Tier 1 study for the exposure characterization, the RA should have:

- 1) identified the major COCs,
- 2) listed physical and chemical parameters of the COCs,
- 3) collected environmental fate information from the literature,
- 4) compiled site-specific sampling data on COCs,
- 5) identified contaminants that may bioaccumulate,
- 6) identified data gaps.

As the exposure characterization progresses to tiers 2 and 3, contaminant pathways examined in Tier 1 of exposure characterization will continue to be evaluated through such options as data collection of previously unsampled measurements endpoints identified in the Tier 1 PF phase or a more intensive sampling over the same habitats to more closely characterize contaminant distribution. In Tiers 2 and 3 more intensive chemistry sampling may allow sampling of degradation products spread in a more diffuse manner throughout the site. Further data collection reduces the uncertainty of environmental fate and distribution estimates.

Monitoring data are useful for analyzing contaminant transport and fate. However, monitoring data may not allow discrimination of the contributions of contaminant loadings from point versus non-point sources. A combination of monitoring data with modeling techniques is necessary in Tiers 2 and 3 to conduct

Table 4. Progressive Levels of Aquatic Chemical Models

Level	Features	Data Needs	Answers
0	Dilution model, yields initial complete mix concentration	Effluent design flow, critical low flow in receiving water or allowable mixing radius/zone, upstream chemical concentration, effluent load or ambient standard-model solves for missing parameter	Worst case ambient concentration in the water column following mixing; additional calculations using $K_{oc}$ yields information on the expected phase distribution (particulate or dissolved)
1a	Steady-state model, simple one-dimensional (1-D) segmentation, first order loss from the water column	River physiography, chemical concentration versus river mile and/or knowledge of first-order loss rates	More realistic estimate of concentration as a function of distance from the effluent, rough estimate of the chemical retained in the system
1b	Steady-state model, 1-D segmentation, partitioning to solids, net settling links water to sediment	Solids loads, solids versus river mile, solids characteristics, and partitioning coefficient	Chemical distribution in particulate and dissolved phases in the water column
1c	Steady-state model, 1-D segmentation, partitioning, full solids dynamics	Literature and site-specific analysis of resuspension and gross settling rates	Provides chemical levels in the sediment and the water compartments
1d	Steady-state model, 1-D segmentation, partitioning, separation of abiotic and biotic solids	Information on water column abiotic-biotic solids origin and transport rates	More accuracy, better differentiation of biotic component
2a	Time-variable model, 1-D segmentation, partitioning, full solids dynamics	Time variable loads and environmental conditions, better vertical solids transport rates	Response as a function of time and distance from the source(s)
2b	Steady-state model, 2-D segmentation, partitioning, full solids dynamics	Hydraulic transport or routing, more spatially distributed field data	Spatially distributed (2-D) results, better representation of certain systems, a broader range of questions addressable to correspond to locations of specific interest
2c	Time-variable model, 2-D segmentation, partitioning, full solids dynamics	Typically more highly resolved data (time and space)	Temporal and spatially related questions
3	More hydraulic (3-D), sorbent, chemical, or biological complexity	Additional problem-specific site data and potentially supporting research	More complex questions of source, chemical interaction, fate, transport, or effects

analyses of contaminant fate in sites for which Tier 1 results do not allow a sufficiently accurate determination of exposure and risk.

### 3.1.2 Ecosystem Characterization

In ecosystem characterization the abiotic and biotic parameters of the system of concern are evaluated. Their impact on the distribution and bioavailability of the stressors of concern are critical parts of the exposure assessment. Migration and resource use by biota and behavioral effects of the stressors on organisms are also considered.

To fully characterize exposure and develop an exposure profile for the site, the RA must recognize the ecosystem components and functions described as important in the conceptual model formulation.

Included in the ecosystem characterization are physical characteristics of the ecosystem, including topography, geology, and hydrology, climatic patterns of the area such as precipitation, insolation, temperature, humidity, and the flora and fauna of the sites. Understanding these components and their interrelationships, in conjunction with data on the contaminant distribution, allows the RA to evaluate whether the contaminants are confined to specific areas and remain *in situ*, or whether the contaminants have the potential to move through various ecosystem components.

Barnthouse et al.<sup>29</sup> presented modeling approaches to link water quality to reductions in "dose" under various scenarios of ecosystem productivity. One example of a modeling approach that illustrates how ecosystem trophic status modifies the bioavailability of toxicants and decreases the subsequent dose to biota was performed by McCarthy and Bartell<sup>30</sup>. Their model predicts the association of a contaminant with dissolved organic material (DOM) or particulate organic material (POM) significantly lessens the bioavailability of a toxicant and, thus, the potential dose experienced by the organisms. Importantly, this paper shows the necessity of estimating the true bioavailability of a contaminant in the environment.

Seasonal or habitat variances in bioavailability can be modeled (e.g., mapped onto expected environmental chemical concentrations for species of known life history, feeding, and habitat requirements) and are a cost-effective approach to the hazard characterization of complex chemicals. For a given concentration, species may be subject to exposure for a relatively longer period of their life-span if they are smaller or less likely to move beyond the boundaries of the contaminated area (examples are earthworms, burrowing invertebrates, or small

mammals). Further, if a chemical is susceptible to being bound by organics, burrowing (or thigmotactic) benthic invertebrates (or benthos-feeding fish) may be subjected to higher exposures than would otherwise be predicted. Volume 2 includes certain models available for evaluating transport, transformation and fate of contaminants in the environment (e.g., EXAMSII, LPMM). In addition, several models estimate biotic exposure or uptake of contaminants (e.g., FGETS).

If available data indicate little potential for movement, the assessment may move in the direction of evaluating the potential for uptake by flora and fauna in the immediate vicinity of contamination. Questions might focus on whether the material is being bound within the soil by specific soil constituents or within specific soil horizons, or taken up by plants or burrowing invertebrates. These initial lines of inquiry may lead to further questions about the potential for effects on plant distribution and floral composition. Questions stemming from the hypotheses formulated in the PF phase may include: Are soil microorganisms affected to the extent that soils become infertile or soil-plant interactions disrupted? Are processes of nutrient cycling disrupted? Answers may lead to other lines of inquiry, such as the potential for movement of contaminants into animal matrices.

### 3.1.3 Exposure Analysis

Once stressor characteristics and the ecosystem potentially at risk have been identified, potential pathways for contaminant(s) through the ecosystem must be identified. The spacial and temporal distribution of the stressors and the ecological characteristics of the system of concern are combined to evaluate exposure. The concentrations of the stressor are combined with assumptions about contact or uptake by biota to determine co-occurrence with measurement endpoints. However, concentration of a contaminant does not equate to exposure. Bioavailability and the environmental fate of the chemical must also be considered. The environmental fate and potential transport of contaminants is crucial to effective risk assessment, because the bioaccessibility (whether organisms come in contact with toxicants) and bioavailability (whether contact leads to uptake) are controlled by these processes. For pesticides, degradation, volatilization, binding, leaching, and aging determine ultimate exposure concentrations<sup>24</sup>. Metals availability is controlled largely by pH and oxidation-reduction relationships in environmental media<sup>25</sup>. The chemistry and distribution of the compounds of interest must be thoroughly understood for effective risk analysis. It is crucial for the risk assessment/risk management team to understand that the bulk concentration of chemical compounds as measured in typical laboratory extraction tests (such as those provided with Contract Laboratory Program

quality assurance documentation under CERCLA) do not reflect the biologically active concentrations. In practice, binding and uptake processes depend on complex environmental processes which need to be accounted for in projecting risks.

The environmental fate of a contaminant will generate pathways that may be simple and straightforward or complex and highly branched. Pathways are generally defined by naturally occurring physical, chemical, and biological components of the ecosystem. As an example, consider the evapotranspiration potential, precipitation, soil type, slope, local vegetation, and ground squirrels (*Citellus* sp.) foraging on the vegetation in a given ecosystem. In this example, the movement of an organic contaminant might be a function of the seasonal food source sought by the rodent species. In other seasons, the ground squirrels are absent or dormant; hence, they would not be subject to exposure by the same pathway.

The origin of each contaminant pathway is typically from soil or water, at the site of contamination and the end of each pathway is a component of the ecosystem where adverse effects may occur (such as threatened or endangered species, a resident small mammal population, or fish species in a downstream lake or reservoir). Several assessment endpoints may exist at the end of a contaminant pathway because pathways will seldom be unidirectional or linear. Chemical pathways generally branch and proceed in multiple directions; for example, a contaminant may have the potential for moving from a contaminated site into an aquatic system, with no potential impacts (branches) en route to a pond. However, once the contaminant enters the pond, potential contaminant pathways may include uptake of the contaminant by aquatic vegetation, by aquatic organisms (e.g., mollusks, gastropods, aquatic insects), uptake by fish, or amphibians, or transport back to the terrestrial environment via birds or mammals that feed on aquatic organisms.

The number of contaminant pathways are determined by the characteristics of the contaminant and the complexity of the ecosystem. Contaminant pathways must be identified on each Army Superfund site; however, similarities in pathways will likely exist among many sites resulting from similar ecosystems. Greater definition (closer focus) of specific contaminant pathways will be a function of Tier 2 and Tier 3 chemical analyses. Ultimately, however, if a pathway is incomplete or does not exist at a particular site, no cause and effect relationship exists and there is no associated risk.

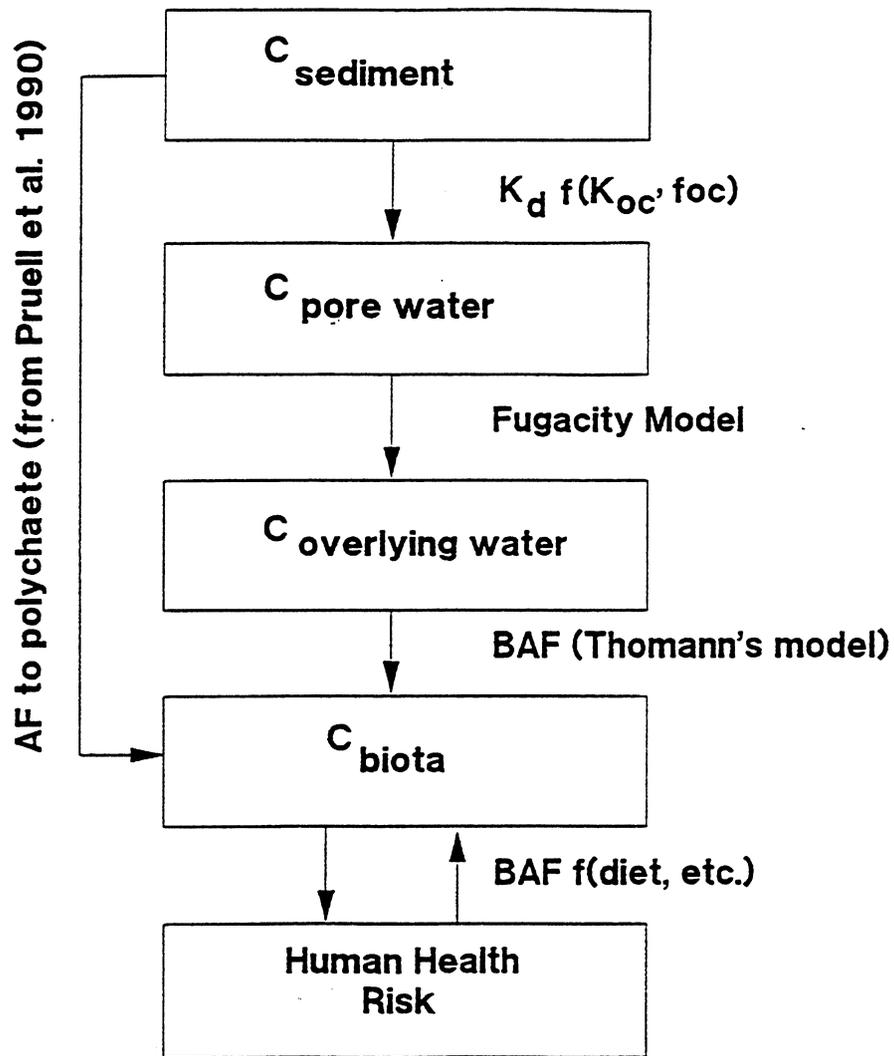
Several models are currently used to assess the fate and distribution of toxic chemicals in ecosystems and link distribution to exposure and effects assessment. Many of these are discussed in detail in Volume 2 of this document. Most exposure models tend to be conservative because they are based on

an assumption of equilibrium, and thus overestimate exposure. Thus model validation is very important when using any predictive model. For example, if one is modeling bioconcentration of chemicals into fish at a site, the results can be compared to measured concentrations of chemicals in fish at the site to validate the model. The text that follows is meant as an introduction of modeling efforts which have been successfully used to assess chemical fate, transport and exposure.

Estimation of contaminant bioaccumulation (the net accumulation of a chemical by an organism as a result of uptake from all routes of exposure) at the site through the food web is very important to address because, in many cases, it provides a link to human health risk assessment. For example, the octanol-water partition coefficient ( $K_{ow}$ ) may be known or estimated for organic chemicals. Typically,  $\log K_{ow}$  values less than 4.3<sup>31</sup> to 5.0<sup>32</sup> do not biomagnify in fish. Garten and Trabalka<sup>33</sup> reviewed terrestrial food-chain data and concluded that only organic chemicals with  $K_{ow}$  values greater than 3.5 significantly bioaccumulate in mammals or birds. Models such as FGETS (Food and Gill Exchange of Toxic Substances) and SARAH (Surface Water Back Calculation Procedure) can be used to predict bioaccumulation potential (see Volume 2).

An example of the use of fate, transport, and exposure models were used to predict risks to humans can be found in a Newark Bay study<sup>34</sup>. Dredged material from Newark Bay containing dioxin was proposed for disposal at a disposal site in the New York Bight. Models were used to predict human exposure via ingestion of fish by humans (Figure 10). Accumulation factors (AF) found in Pruell et al.<sup>35</sup> were used to directly model transfer of dioxin from sediment to benthic organisms associated with that sediment. In order to estimate the exposure of dioxin associated with the dredged material to other aquatic organisms, it was initially partitioned to sediment interstitial water. An equilibrium fugacity model developed by Mackay<sup>26,27</sup> was then used to predict sediment overlying concentrations of 2,3,7,8-TCDD (Dioxin). Thomann<sup>36,37</sup> developed a simple aquatic food chain model using contaminant body burdens of organisms in various trophic levels, thus quantifying bioaccumulation. This same model was expanded to include interaction of aquatic biota with sediment chemicals in Thomann et al.<sup>38</sup>. These models were used to predict concentrations of dioxin in lobster, flounder, and bluefish in a food web. Ultimately the risk to humans ingesting these fish was calculated.

Fordham and Reagan<sup>39</sup> developed a food web model to evaluate potential exposure pathways for a site (Figure 11). Data collection can be complex and many assumptions on exposure and uptake are made. The model estimates acceptable concentrations



**Figure 10 Schematic presentation of the approach used to assess risk of dioxin associated with sediments.**

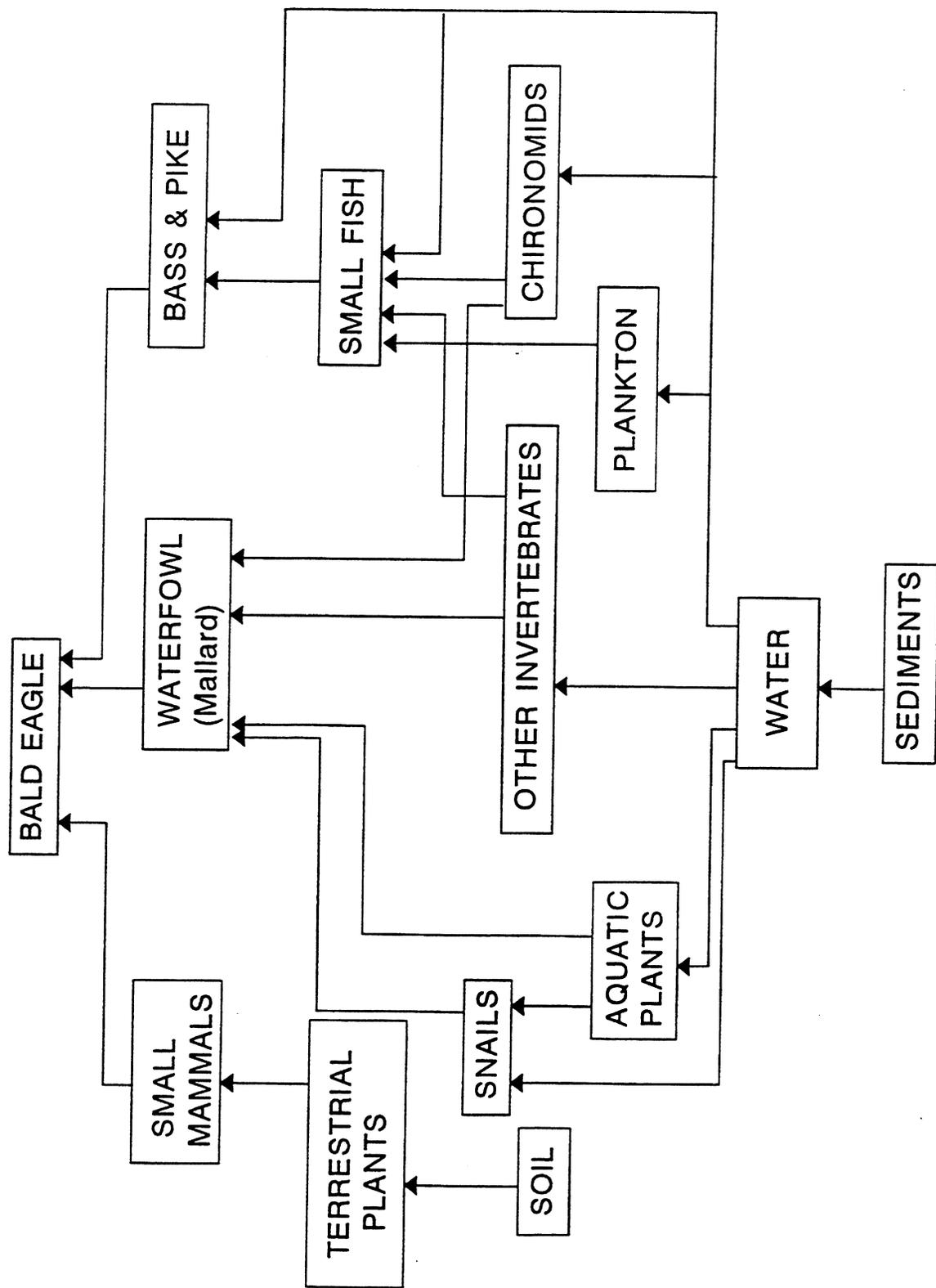


FIGURE 11. Pathways of contaminants from sediment and water to a target organism in a representative aquatic ecosystems at Rocky Mountain Arsenal.<sup>39</sup>

in abiotic media for each exposure pathway. Further, it develops a site-specific food web by entering data from on-site sampling as well as literature sources. Finally, the model addresses bioaccumulation in multiple food chains that terminate in a high trophic level species (e.g., bald eagle). Uncertainty and data gaps need to be stated when using this method. Data from this type of study can be utilized in ecological risk assessments when evaluating risk to populations of biota exposed to site-related contaminants via different pathways.

#### 3.1.4 Exposure Profile

The exposure profile presents the concentration of the stressor and its distribution over the area of study. Exposure over time can also be addressed so that the units match those presented in the stressor-response profile. The exposure profile evaluates pathways and determines exposure or dose to measurement endpoints. The extent to which ecosystem properties are analyzed depends upon the nature of the stressors and ecosystem components, bioavailability, and the resources available. Analyses should concentrate on those ecosystem components that are determined to be at greatest risk. Knowing the stressor characteristics can help to narrow the focus of the investigation on the components of the ecosystem that are potentially most susceptible.

The exposure profile for chemical stressors usually involves consideration of sources (e.g., explosives burning ground), environmental transport (e.g., rate of movement through soil column), partitioning of the chemical among various environmental media (e.g., soil particles vs. organic matter), chemical/biological transformation or speciation processes (e.g., photolysis, biodegradation), and identification of potential routes of exposure (e.g., ingestion, plant root absorption, etc.). Exposure profiles for non-chemical stressors such as soil compaction, or habitat alteration describe the ecological components exposed and the general temporal and spatial patterns of their co-occurrence with the stressor. Shaw and Diersing<sup>19</sup> described the extent and distributional pattern of compacted and disturbed soil in a field used for military training with tracked vehicles, the soil microflora, vegetation and wildlife occupying or using this training field. They compared the size and distribution of these populations with those in adjacent undisturbed fields.

Statistical techniques commonly used in the exposure profile are geostatistical techniques (kriging) to determine loci of contaminant residues in soil or water and multivariate techniques (cluster analyses, canonical correlation, principal components). Perland<sup>40</sup> presented an effective integration of chemical fate and transport information into an exposure profile of an ecological

risk assessment. In this case, groundwater was contaminated with benzene and barium in the vicinity of valuable wetlands habitat. Surface water exposure concentrations were projected based on measured groundwater data and information regarding local precipitation, soil chemistry, contaminant binding, pH, Eh, and volatilization and dilution. It was concluded in the risk characterization that potential ecological risks were not associated with groundwater contamination and site remediation proceeded as dictated by non-ecological issues.

### **3.2 Characterization of Ecological Effects**

#### **3.2.1 General Overview**

The determination of ecological effects at a site is a critical component of the ERA because data generated in this section may drive the decision making for the rest of the assessment. Assessment endpoints guide what data or measurement endpoints are required to assess impacts. To quantify ecological effects, data can range from sublethal or behavioral effects, to lethal effects, to population shifts, to community changes, habitat loss, ecosystem structural and/or functional changes, to biomagnification of chemicals through a food web (Volume 2). Subcellular biomarkers may be useful for identifying subtle effects. Data on threatened or endangered species offer special consideration because individuals, as well as populations, must be protected<sup>41</sup>. Evaluating ecological effects at a particular site is made more difficult because site-specific toxicity data or specific data on a species of concern are often lacking. Ecological surveys and Geographical Information Systems (GIS) are used to support a qualitative determination of ecological health, diversity, and habitat distribution and they can help to fill such data gaps.

Potential cause and effect relationships between a contaminant and the ecological measurement endpoint must be established. Hill's criteria<sup>4</sup> provide a listing of the primary questions that should be addressed (Table 5). The major criteria such as strength (a high magnitude of effect associated with exposure to the stressor), consistency (the association is repeatedly observed under different circumstances) and specificity (the effect is diagnostic of a stressor) need to be recognized and considered. We caution against establishing a cause - effect relationship based on simple observations (i.e., the contaminant is present in a forest soil and the forest is in decline, therefore the decline is caused by the contaminant). Many factors such as drought, insect infestation, disease, nutrient stress, management practices, etc. may be contributing to the decline.

Table 5. Hill's Criteria for evaluating causal associations<sup>4</sup>.

- 
1. **Strength:** A high magnitude of effect is associated with exposure to the stressor.
  2. **Consistency:** The association is repeatedly observed under different circumstances.
  3. **Specificity:** The effect is diagnostic of the stressor.
  4. **Temporality:** The stressor precedes the effect in time.
  5. **Presence of biological gradient:** A positive correlation between the stressor and the response.
  6. **A plausible mechanism of action.**
  7. **Coherence:** The hypothesis does not conflict with knowledge of natural history and biology.
  8. **Experimental evidence.**
  9. **Analogy:** Similar stressors cause similar responses.

note: Not all of these criteria must be satisfied, but each incrementally reinforces the argument for causality. Negative evidence does not rule out a causal association but may indicate incomplete knowledge of the relationship.

At most DoD hazardous waste sites, the initial environmental effects may have occurred years ago. Cause and effect evidence of contaminant toxicity may be difficult to determine because of adaptation of the community or system. Therefore, it is important to determine as much of the natural history and biology of the site as possible and to determine whether a continuing exposure pathway exists and whether it poses a threat to the currently-existing ecosystem. The ecological system in which the contaminants or stressors are present influence the impact they have on the biota. For instance, it is well-documented that physical and chemical changes in aquatic systems affect the toxicity and distribution of chemicals. An example is the inverse correlation between toxicity of heavy metals and increasing water hardness<sup>42</sup> and pH<sup>43</sup>. Terrestrial systems can act in a similar fashion with various soil parameters such as CEC or organic matter content, enhancing the ability of a soil to adsorb chemicals<sup>44</sup>.

Thus physical, chemical, and biological components of the ecosystem need to be considered for their impact on the bioavailability and exposure of the contaminants at the site. Furthermore, if the contamination or stress occurred years ago, the ecosystem may have had time to recover to another state. The adapted state of the system needs to be evaluated to judge habitat change, and to determine whether the changes have reduced the "value" or productivity of the site. System resilience is also important in assessing the impact of the contaminant on the biota. Resilience, defined as the capacity of the system to return to a "pre-disturbed" state, has to be defined in terms of the important effects endpoints. For example, it may be the time it takes for a bird or small mammal population to re-establish itself (years to decades) or a soil invertebrate fauna to re-establish (months to years). Resilience is most often measured in lower trophic level animals or plants, simply because of the ability of the assessor to measure their ability to recover.

Selecting appropriate reference sites is difficult but very important to accurately evaluate the ecological effects in a risk assessment. The reference habitat should be similar in all aspects but for the contamination. For example, a terrestrial location with contaminated soil should have as a reference site one that has a similar soil type with similar vegetation and wildlife habitat. It may be useful to study soil survey maps obtained from the Soil Conservation Service, consult with the National Wildlife Federation about wildlife habitats, seek categories of "reference watershed" from the EPA EMAP program, or to link gradients of chemical contamination to observed effects or measured body burdens. Lacking such data, information from regional or state parks, undisturbed areas on the site (and known to not have been subject to previous contamination) may serve for use under Tier 1.

Various data on cause and effect of the contaminant(s) at the site then need to be formatted into a contaminant/response profile. Each measurement endpoint should, in theory, have its own profile. The profile may include NOEL's and LOEL's, LC<sub>50</sub>'s, LD<sub>50</sub>'s, EC<sub>50</sub>'s or other quantitative measures, as well as the percentile of the population community or system affected versus exposure dose. In practice, these data can be hard to find and difficult to generate.

An example method of profiling toxicity and exposure assessment is provided by Toxicity Reference Values (TRV) (Figure 12). The TRV method uses available toxicity data on a specific COC to generate an estimated No Observed Adverse Effects Level (NOAEL) for a species of concern at the site with safety factors or uncertainty values included in the process. Laboratory-generated TRVs for a given time period (i.e., the lowest observed effect concentration, LOEC, for a 10-day exposure) may be linked to a specific exposure duration for the population in the field. Although there are sets of limiting assumptions required for the use of TRVs, they can provide an estimate of expected toxicity for given exposure periods.

Multi-contaminated sites offer unique problems. Often, many receptors are exposed to multiple stressors simultaneously. Ecological risk is much more difficult to discern at these sites. Individual as well as synergistic effects of the stressors must be estimated to accurately determine risk. Chemical mixtures influence toxicity in two ways. First, chemical mixtures can cause a toxic effect that is qualitatively or quantitatively different from any of the individual stressors acting alone. Second, the effects of one chemical may influence the kinetics of uptake, metabolism, and excretion of other chemicals. Examples include coating of fish gills by thick mucus when exposed to excessive aqueous concentrations of zinc and damage to nephridia that may be caused by cadmium-metallothioneine complexes. The metabolic kinetics of a chemical may also be affected by other chemicals that induce or inhibit enzymes, or that simply reduce the physiological capacities of an organism<sup>7</sup>.

Direct effects of stressors on variables such as mortality or growth need to be evaluated at higher levels of organization (population, community, or system) than the organismal (individual or species) level alone. These variables will typically be harder to measure, but usually will provide more pertinent information on the ecological effects caused by the stressors. A population shift, in and of itself however, does not imply a negative impact on the community. The relevance of

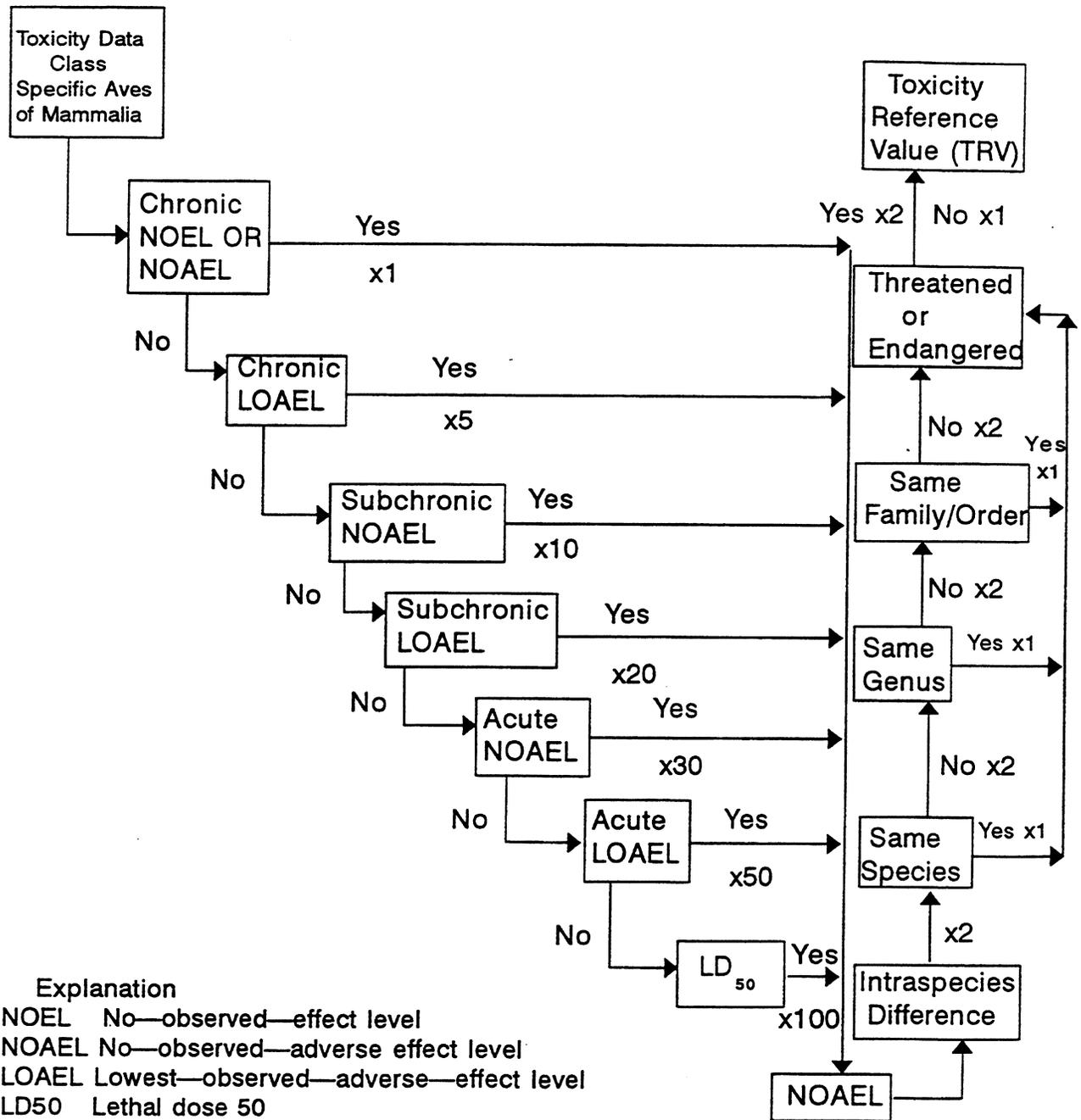


Figure 12. Methodology to derive toxicity reference values (TRV's) from class—specific toxicity data.

effects at the population level to the stressors of concern must then be determined.

Indirect effects must also be considered and include impacts on habitat, effects on biota in the food web, changes in reproductive capacity, etc. The interaction of all indirect effects to each other and to direct effects should be obtained in order to accurately characterize risk. The simplest assumption is that indirect effects are additive, but more complex interactions are possible. The best understood of the nonadditive effects are thresholds<sup>7</sup>. For instance, populations of a certain species will not be supported once habitat area drops below a certain size; anoxia occurs once the organic input into a water body rises above a certain level, and extinction occurs when mortality rates rise above a certain level in a population. Identification and quantification of such thresholds is a critical component of cumulative effects assessment. Synergistic and antagonistic relationships are more difficult to delineate. Mixtures of chemicals may have more or less than additive effects. In the case of the California condor (*Gymnogyps californianus*), habitat degradation and toxic exposures had a joint effect (extinction in the wild) that was greater than would have been expected from simply adding the losses that either would have caused acting alone<sup>7</sup>.

In ecological effects analyses, information collected on measurement endpoints must relate to appropriate assessment endpoints. Extrapolations may include those between species, between responses, from laboratory to field, or from field to field. For example, the responses of organisms (earthworms, plants, small mammals) exposed to soils in the laboratory could be extrapolated to similar populations in the field<sup>45</sup>. An example of a field-to-field extrapolation is provided by La Point et al.<sup>46</sup>, in which the diversity of soil invertebrates in ten heavy-metal contaminated sites were compared. The more heavily contaminated sites had fewer insects, leading to the determination that management practices were influencing insect distribution. Assessment endpoints may also be predicted by analysis of indirect effects such as relating removal of long-leaf pine to reduced populations of the red-cockaded woodpecker, or by analysis of higher organizational levels, e.g., relating reduced individual fecundity to reduced population size. These extrapolations require professional judgment. The thought process must be clearly and carefully described to avoid confusion. Conservative assumptions are often used during Tiers 1 and 2. If and when the risk assessment proceeds beyond Tier 2, the data and information gathered to this point reduces uncertainty and fills data gaps to enable the risk assessor to use less conservative assumptions in Tier 3. The assumptions should be clearly stated so a reviewer or risk manager is aware of them. These assumptions should be restated in the Risk

Characterization phase so that reviewers are, once again, aware of the thought process.

### 3.2.2 Method of Characterizing Ecological Effects

#### \* Tier 1

Methods used in Tier 1 should focus on available information, estimation methods, and literature searches. Available information includes past site reports, surveys or assessments, on-site record searches and Installation Assessments. Much of this information would be gathered under the RI. Wildlife and habitat information may be available from the installation, National Biological Survey, U.S. Fish and Wildlife Service, the State Natural Resources Dept, or other local resources (Table 6). Regional Biological and Technical Assistance Group (BTAG) of U.S. EPA (Table 7) and the U.S. Army BTAG (Table 8) should be able to provide further sources of contacts, information and technical assistance.

Critical focus needs to be placed on threatened or endangered species at the installation. A threatened or endangered species may dominate the concerns of ecological effects and drive the decision on risk characterization. The reason for this is because individuals of threatened or endangered species must be protected as assessment endpoints instead of general populations, communities or ecological systems.

At the end of a Tier 1 study for ecological effects of contaminants at the site, the risk assessor should have:

- (1) the available toxicity data on the chemicals of concern (COC);
- (2) any available ecological information and information on biological incidents e.g., fish kills, dead birds;
- (3) identified threatened or endangered species at the site and estimated their homerange or migrational pattern;
- (4) identified any contaminants that may bioaccumulate;
- (5) identified habitat areas of concern and areas known to be adversely affected by contaminants; and
- (6) identified data gaps.

This information is summarized in a contaminant/response profile for the COC. At this stage and level of effort, the degree of uncertainty may be high and data gaps will occur, but the risk

**Table 6. Sources of Site Information**

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U.S. EPA Environmental Research Laboratories

U.S. Department of Agriculture  
(e.g., Southern Forest Experiment Station, New Orleans, LA)

U.S. Soil Conservation Service  
(e.g., County soil surveys, Natural resources inventories)

U.S. Fish and Wildlife Service

National Oceanic and Atmospheric Administration

State Parks and Wildlife Departments

Agricultural Experiment Stations (within University systems)

Sierra Club (e.g., Naturalist's guides)

**Table 7. U.S. EPA Regional BTAG Coordinators/Contacts**

**EPA HEADQUARTERS**

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(908) 321-6724 FAX

Steve Ells  
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John Miller  
(703) 603-9076

EPA/OWPE (5502G)mail  
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(703) 603-9124 FAX

Jeffrey Langholz  
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**REGION 4**

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(404) 347-0076 FAX  
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**REGION 5**

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**Table 7. U.S. EPA Regional BTAG Coordinators/Contacts (Cont'd.)**

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**REGION 7**

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(206) 553-0119 FAX  
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email: NWOSU.JULIUS@EPAMAIL.  
EPA.GOV

assessor must use professional judgement to summarize this information with appropriate uncertainty included.

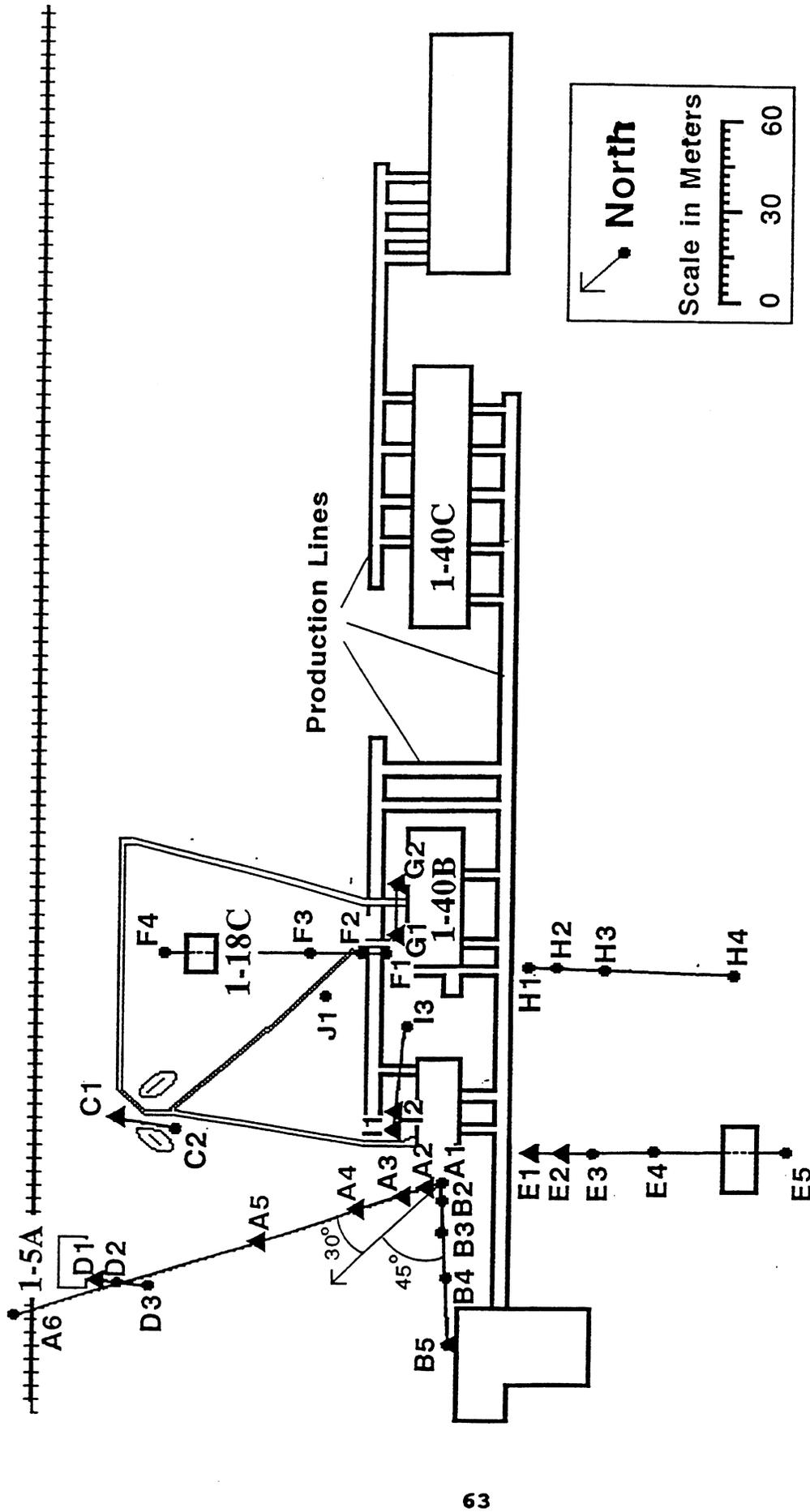
\* Tier 2

The purpose of Tier 2 is to build on information gathered in Tier 1 by addressing data gaps to reduce uncertainty. Ecological effects data need to focus on the main COCs and reduce uncertainty when addressing their impacts on threatened or endangered species, habitat, or important populations. Measurement endpoints used in Tier 1 may become more complex or sophisticated in Tier 2. An example would be the use of literature toxicity data in Tier 1 versus specific laboratory toxicity studies in Tier 2.

Pathways where COCs could biomagnify in the food web to affect threatened or endangered species are addressed in this tier. Simple estimation methods of contaminant biomagnification for Tier 1 need to be upgraded in Tier 2 to reduce uncertainty or to fill data gaps. An example of a simplified approach to measuring biomagnification is a food-chain laboratory microcosm<sup>47</sup>, in which lower trophic level organisms are exposed to contaminated water or sediments and subsequently fed to top predators to develop estimates of biomagnification. Estimation methods based on  $K_{ow}$  values and other physical and chemical parameters of the COCs should provide a technically sound estimate of the ability of the COCs to biomagnify. If the COC has been estimated by models or by use of  $K_{ow}$  values to biomagnify in the food web, then field or laboratory tissue studies will provide confirmation of model estimates.

Laboratory toxicity studies using site specific soil or sediment may also serve to reduce uncertainty and data gaps identified in Tier 1. Soil or sediment tests for sites contaminated with multiple COCs provide useful specific data on toxicity of mixtures of COCs. The results from laboratory toxicity studies, used as Tier 2 measurement endpoints, should provide information to better define areas at the site where the soil, water or sediment are toxic or nontoxic. An example of how toxicity testing can help delineate between toxic and non-toxic areas at a site was a study of soils conducted at Joliet Army Ammunition Plant, Joliet, IL.

In the Joliet study, six sites were identified by a remedial investigation as potentially having high concentrations of explosives and heavy metals<sup>48</sup>. Soil sampling was performed along transects through areas suspected of having high contamination at each site. Subsequent toxicity testing and chemical analyses identified the two most toxic sites, defined the shape and extent of the toxic areas within each site (Figures 13 and 14) and



## Area L7 Group 1 Transects and sampling locations

Figure 13. Joliet Army Ammunition Plant Group 1 load, assemble, and pack area showing transects and locations with non-toxic ● or toxic ▲ response to at least one toxicity test.<sup>45</sup>

# Area L2 Explosive Burning Ground

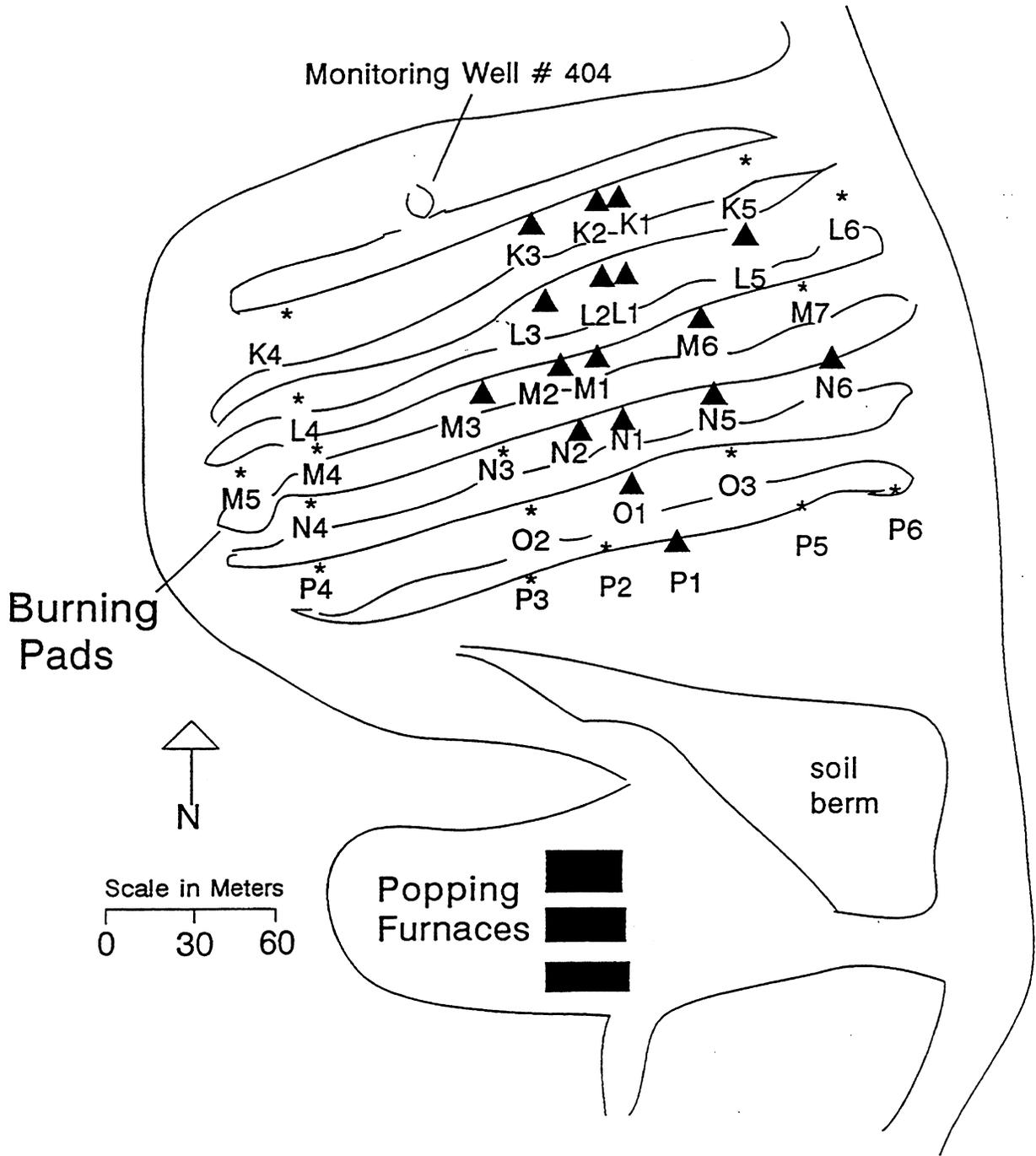


Figure 14. Joliet Army Ammunition Plant Area L2 explosive burning ground showing transects and soil sampling locations with non-toxic • or toxic \* response to at least one toxicity test.<sup>45</sup>

against concentration values of explosives. TNT was determined to have the greatest  $R^2$  (coefficient of determination) value of the eight compounds detected. Lowest observed effects concentrations (LOEC) of TNT were then extrapolated from these data.

In the preceding study, relatively inexpensive, short term ( $\leq 14$ -day) toxicity tests provided information to risk managers that will save time and money in the long run. For instance, remediation can be concentrated on the two sites that pose the greatest ecological risk. Within sites, risk managers can use these results, together with results from studies of other components of the ecosystem, to decide on the extent and type of remediation. Managers may also use these results to decide if further, more extensive testing is necessary in areas where soil concentrations are on the borderline of causing toxic effects. Furthermore, this study incorporated a series of bioassays to investigate effects at different levels of biological organization. This approach is more effective than using bioassays at the same organizational level because response to a stressor may vary among organisms at different levels of organization.

It is important to note that ANOVA results, LOEC's, and  $R^2$  values in this study are site-specific and highly dependent upon soil characteristics and concentrations of other soil contaminants. As cited previously, toxicity of many chemicals, and soil explosives in particular are highly dependant on pH, organic matter, CEC and other characteristics of the site soil. Therefore, soil characteristics should be considered before extrapolating toxicity data between sites and between studies.

The Joliet case study is example of the use of toxicity testing established gradients of concentrations of explosives in site soils<sup>45</sup>. Plant (two species), earthworm, and Microtox<sup>™</sup> bioassays were used to assess soil toxicity. Highly toxic, moderately toxic, or not significantly toxic soils were determined based on statistical significance compared to control soils. These categories were used to define the shape of toxic areas at each site. Soil samples with significant toxicity, according to at least one test, and representative samples displaying no toxicity, were analyzed for explosives at each site. The explosives, trinitrotoluene (TNT), cyclotrimethylenetrinitramine (RDX), and their degradation products were identified via HPLC analyses. End points of toxicity tests were then regressed to assess risk in Tier 2 of an ERA. An extensive compilation of ecological effects methods is presented in Volume 2. The reader is referred to Volume 2 for measurement endpoints to support specific goals of the ERA.

Field studies conducted in Tier 2 will be focused to address data gaps identified in Tier 1 and the overall assessment endpoints for the ERA. Ecological assessment may be necessary if the installation or other agencies do not have the information on biota present at the site. GIS can be used to identify habitat and land use patterns at the installation. Biotic surveys can determine species diversity, predominant populations, and identify population shifts.

Results from the Tier 2 ecological effects studies will further support cause and effect relationships between the COCs and the biota, community or ecological system. Uncertainty will have been reduced and most data gaps addressed. Various measurement endpoints will be "mapped" onto site locations to generate contaminant response profiles of species tested at the sites. These will, as in Tier 1, be related to the assessment endpoint(s) identified in the initial phase of the ERA.

\* Tier 3

Tier 3 should involve larger levels of effort reflecting increased levels of concern to reduce ERA uncertainty and address ecological effects data gaps in the ERA. Investigations in Tier 3 are not meant to deal with the highly toxic or hazardous areas within a site. The highly toxic sites could, and should, be identified in Tier 1 as areas where significant ecological effects occur and significant risk is probable. In Tier 1 or 2, these areas would be recommended for remediation. In Tier 3, there is no need to analyze the specific toxicity of contaminants or conduct more in-depth ecological studies on the highly toxic sites, if it is clear from Tiers 1 and 2 that they will be remediated. Tier 3 should focus on the "gray" areas, where it is still uncertain if significant ecological effects occur. By the end of the Tier 2 investigation, sites should have been identified that are clearly affected by COCs, as well as sites where no effects occur following COC exposure. Further laboratory and field toxicity tests may be required to establish NOEL concentrations. These refined measurement endpoints are designed to reduce uncertainty and address data gaps not covered in Tiers 1 and 2. In Tier 3, collecting field data to determine tissue concentration in wildlife should be conducted to confirm the presence and extent of bioaccumulation, bioconcentration, and/or biomagnification that was suspected from results of Tier 2 studies. Additionally, if chronic physiological effects are suspected, they should be performed in Tier 3, particularly if evidence for such effects is obtained in previous tiers. However, these types of studies are often time consuming and expensive. Work should proceed only if all parties agree that the studies are essential to adequately complete the risk assessment and enough funds and resources are available to do quality experimentation.

A study by McBee et al.<sup>49</sup> is a good example of focused field research appropriate for a Tier 3 study to examine subtle, chronic ecological effects. In this study, the existence of environmental mutagenesis was determined by examining standard metaphase chromosome preparations from resident small mammals (*Peromyscus leucopus*, *Sigmodon hispidus*) trapped over a two-year period at a site polluted with petrochemical waste products, heavy metals, and PCBs. Significant differences in levels of chromosomal aberrations were found between animals collected at the contaminated site and those captured at two uncontaminated sites, even though acute toxicity was not apparent. Levels of chromosomal aberrations were not significantly different between the control sites. Potential longer-term, chronic effects suggested by the cytogenetic analyses, however, clearly indicated responses relevant to site assessments evaluating adverse ecological effects, and reinforced the importance of reference sites when correlative analyses are considered in the assessment of biological effects in the field.

Food web sampling is more complex but offers more complete information on contaminant pathways through the food web. Fordham and Reagan<sup>39</sup> (Figure 11) developed a food web model to evaluate potential exposure pathways for a site. The model estimates acceptable concentrations in abiotic media for each exposure pathway. Further, it develops a site-specific food web by entering data from on-site sampling as well as literature sources. Finally, the model addresses bioaccumulation in multiple food chains that terminate in a high trophic level species (e.g., bald eagle). Data from this type of study can be utilized in ecological risk assessments when evaluating risk to populations of biota exposed to site-related contaminants via different pathways.

When conducting any field study, various problems must be anticipated. The data collected will be more variable than laboratory studies. Analytical detection limits for tissue, soil, or water need to be known before data are collected. Detection limits in tissue need to be low enough so a no effect level can be related back to soil or water concentrations. Estimates from Tier 2 should be used to provide a guide for setting detection limits in Tier 3.

Co-locating tissue samples with soil or water samples at the site of collection may be necessary to accurately assess the toxicity of the COCs. The spatial relationship of data points collected during a field survey will be important for relating tissue concentration to exposure<sup>15</sup>. Maps have been used extensively to study and display spatial patterns. Many cartographic and GIS techniques are available for displaying spatially varying quantitative data. For example, if the variable of interest (e.g., distribution of TNT) is spatially continuous, it can be

conceptualized as a surface in three dimensions. The surface can be displayed as contour lines, isopleths, or as perspective plots. Alternatively, if the variable is discontinuous, the magnitude of an observation at a point can be represented by a symbol size or color. Synopses of these methods with references are found in Volume 2 of this publication.

Additional data needed to assess wildlife impacts include: home range, feeding area, and migratory patterns of the biota of concern at the site. This information can be provided by USFWS, site specific sources (i.e., state fish and wildlife department, military installation records, etc.) or the open literature. Identification of critical habitat to species of concern should be conducted. These data, together with spatial and temporal patterns of the COCs within the site help characterize the extent of ecological effects. Contaminant effects on local habitats, if extensive enough, can be related to cumulative impact on the watershed in which the site or sites are contained. These data may be used later to mitigate impacts through the additional critical habitat areas to the site. Mitigation options need to be viewed in light of minimizing further damage or risk to the resource. For example, if a habitat has been shown to be critical for a top avian predator (e.g., old-growth tree snags for osprey), it would not be suitable to suggest grading and incinerating of the vegetation from the site, unless similar habitat were set aside elsewhere as a mitigation option. Additional laboratory studies may focus on establishing no effect levels for the COCs. These studies should include tissue analyses so toxicity responses can be related to COC concentrations in tissues. These data are valuable for determining no effect levels of COCs in soils, water, or sediment. Other Tier 3 studies may be driven by regulatory or local concerns that may arise only after previous studies have been performed.

### 3.2.3 Linking Exposure and Stressor-Response Profiles

During the final stages of the Analysis phase, ecological effects and exposure are characterized concurrently. Data on fate and effects are objectively evaluated for their utility in ascribing cause and effect of the stressor. The degree to which organisms are adversely affected beyond those due to "normal" physical or biological stressors must be quantified. To this end, collected data are often subject to statistical methods to describe the inherent mean tendency and distribution of the population parameters (behavior, growth, reproduction, mortality, etc.). Among the statistical techniques commonly applied to such situations are geostatistical techniques (kriging) to determine loci of contaminant residues in soil or water, multivariate techniques (cluster analyses, canonical correlation, principal components) and univariate approaches to measure the organismal or population responses (e.g., differences in mean body burden of

chemical in an exposed set of organisms; differences in reproductive success of exposed small mammals).

The paths by which contaminants move from the point of origin through the biota and ecosystem may be simple and straightforward or complex and highly branched. Contaminant pathways will generally be defined by naturally-occurring physical, chemical, and biological components of the ecosystem (e.g., soil, vegetation growing on those soils, and microtine rodents foraging on the vegetation). The necessity of moving to Tier 2 or 3 under an ERA will largely depend on the complexity of the pathways, as determined by stressor-response and ecological analysis portion of the Analysis Phase.

On typical CERCLA sites, most contaminant pathways branch and proceed in multiple directions; for example, contaminants may have the potential for moving from the point of contamination into an aquatic system, with no potential impacts (branches) en route. An example of such a scenario is provided by groundwater movement of soluble nitrogenous compounds or pesticides, emerging via seepage into a stream or pond. Once the contaminant enters the water body, potential contaminant pathways may include uptake of the contaminant by aquatic vegetation, aquatic organisms (e.g., mollusks, gastropods, aquatic insects), fish or amphibians, or transport of the contaminant to birds or mammals feeding on aquatic organisms. Within each tier, contaminant pathways must be identified on each Superfund site. However, similarities in pathways will likely exist among many sites because of similarities of habitats and organisms in similar ecosystems (e.g., grasslands, deciduous forest, bottomland hardwood, etc.). It should be re-emphasized that the number of contaminant pathways are determined by the characteristics of the contaminant and the complexity of the ecosystem. Under situations of high complexity and/or diversity, when the magnitude, frequency or duration of the stressor varies in unpredictable ways, the estimates of ecological response(s) and exposure scenarios may require effort and cost beyond Tier 1.

A summary of the Analysis phase is provided by a stressor-response profile. In developing such, the RA identifies measurement endpoints along each contaminant pathway where data collection or computer simulations and models are applied to evaluate contaminant fate or assess potential impacts. This can be conducted early in Tiers 1 or 2. Data collected for these measurement endpoints will help validate or refute whether predicted movement or effects on assessment endpoints are actually occurring. As testing progresses to higher tier levels, these same contaminant pathways will continue to be evaluated through such options as data collection at previously unsampled measurements endpoints identified in the Tier 1 PF phase, or by more intensive data collection at previously sampled measurement endpoints to reduce the uncertainty of analyses. The Tier 1 or

Tier 2 identification of contaminant pathways (and modeling efforts) thus unify the investigative efforts of the ecological risk assessment through all levels of the tier structure.

Under Tier 1, the RA analyzes a suite of previously-compiled data and evaluates site-specific characteristics collected in the PF phase of the analysis (Figure 3). The RA might consider which contaminants were present and estimate the extent of contamination. Under Tier 1, information on chemical/physical properties of the contaminants would be examined in the context of tabulated or otherwise compiled physical, biological, chemical and climatological characteristics of the ecosystem. How the contaminants interact with the physical and biological components of the ecosystem will be predictable, within certain constraints. In any case, using reports, maps and some preliminary sample collections would allow the RA to estimate the likelihood of the contaminants remaining *in situ* or moving off-site or through the ecosystem. In the final components of the Analysis phase, information should have been collected to link contaminant exposure to biotic response of critical species and/or habitats. The linkage is made by measuring the response in toxicity, biomagnification, reduction in population density, or other critical measurement endpoints to exposure. Hence, model development is critical at this juncture to understand how the COCs are accumulated by the biota and what a given tissue concentration means to the organisms<sup>18</sup>. In Tier 1, the models used may be as in Thomann<sup>36</sup> or relating concentrations in the soil to toxicity and species presence (as in Apparent Effects Threshold<sup>50</sup>). Under Tiers 2 and 3, model predictions are approximated empirically using on-site or laboratory exposures of naive organisms to measure uptake and consequent effects.

It should be stressed that in Tier 1 analyses, highly conservative risk measures should be developed from the assessments. As more information is collated under Tier 2 and 3 investigations, the need for "application factors" of safety should diminish. This means that, as effects are measured directly or via the use of surrogate organisms exposed on-site, the need for wide confidence limits around the estimates of effect lessens. The measures of risk become more direct.

#### 3.2.4 Examples of Linking Biotic Responses and Exposure

Three examples linking exposure to biotic response are provided to describe situations in which exposure is related to biotic effects. The first involves birds subject to agricultural pesticides used on crops in the midwest. The second is an example of mammals located on a terrestrial grassland site. The third example describes assessment in an aquatic system.

- \* Avian example integrating exposure and stressor-response profile.

Birds are often used in evaluating wildlife exposure to, and trophic transport of, environmental contaminants<sup>11</sup>. Birds have a high metabolic rate and, therefore, consume large amounts of food relative to their body weight. This may lead to elevated bioaccumulation or biomagnification, even with contaminants less persistent in the environment. The avian respiratory system, characterized by lungs with air sacs, is highly efficient, moving large amounts of air through the lungs. This physiological characteristic may yield avian species highly susceptible to exposure and accumulation of particulate or vaporized air-borne contaminants. Additionally, many species of birds prey heavily on larval or adult insects during the breeding season<sup>8</sup>. Most of these insects spend all or a portion of their life cycle on or in soil or thatch where they are highly likely to come in contact with environmental contaminants. Other bird species prey upon flying adult insects as they emerge from aquatic and benthic larval forms, and are thus exposed to contaminants in water and sediments. Birds are often numerous in natural and disturbed habitats and can provide an adequate sample size to satisfy quantitative analyses.

Procedures for sampling exposure and response to contaminants in birds can be designed for each level of effort and costs relative to Tier 1, 2, or 3 studies as defined in this document.

A Tier 1 effort may involve avian censusing techniques to determine relative frequency and abundance of bird species on the study area. Habitat use and activity data collected during the census, graphically displayed, will quickly identify which species are most likely to be exposed to the contaminant(s) of concern. Once susceptible species are identified, efforts to assess exposure may be concentrated on these species. In certain situations, susceptible species may have been extirpated from the site.

At the Tier 2 level, the RA can attempt to answer more complex questions. In such cases, reference sites are necessary to determine reference (e.g., "control") estimates of contaminant uptake. Contaminant levels may be determined by collecting individuals and conducting residue analyses. A limited sampling of food items of targeted species may provide insight into the nature of exposure route. If the species included for study are cavity nesting birds, nest boxes can be erected on the study site to increase that species' presence and activity level on the site and increase access for sampling<sup>51</sup>. In some cases, one species may naturally nest in abundance on the study site, providing adequate sampling opportunity.

Sampling across several environmental matrices, such as soil, water, invertebrates, and adult and nestling birds can quantify contaminant availability to the species under investigation at different trophic levels. Monitoring contaminant intake in nestling birds quantifies exposure; then, measuring endpoints such as enzyme response (i.e., cholinesterase in the case of organophosphorus or carbamate insecticides), immune system response, growth and survival, quantifies effects at the measured exposure levels.

Tier 3 levels of funding and personnel would allow thorough assessment of exposure and effects along several food chains, each of which having a different bird species as the top predator. Exposure duration may play a significant role in the degree of effects observed in higher trophic levels. This is particularly true for the more environmentally persistent contaminants. Therefore, selecting a food chain with a long-lived, resident, predacious bird (e.g., bald eagle; Figure 9) at the top would likely provide an assessment of the worst case exposure scenario.

Certain birds of prey such as barn owls (*Tyto sp.*), screech owls (*Otus asio*) and barred owls (*Strix varia*) utilize nest boxes, thus providing easy access to nestlings. By selecting several top predators, each representing a different food chain, adequate data can be gathered to predict risk to a broader array of species. European starlings (*Sturnus vulgaris*), tree swallows (*Iridoprocne bicolor*), and barn owls, for example, represent diverse food chains that would provide exposure and effects data applicable to numerous other species.

In a Tier 3 study, long term monitoring of adult birds using tarsus banding or radio telemetry provides valuable data on survival and demographics relative to exposure and accumulation of environmental contaminants. In some cases, multiple captures and non-lethal sampling of blood or fecal urates over extended periods of time provide temporal patterns of exposure. For example, repeated blood samples from an individual bird provides insight into exposure to certain heavy metals or to exposure to anticholinergic compounds. The more information determinable in diverse food chains about routes of exposure, bioaccumulation, biomagnification, and organism response to exposure, the more accurately the RA can predict risk for various avian species.

\* Example of integrating exposure and stressor-response profile for small mammals on a hazardous waste site.

Initially, maps of the site provide estimates of "hot spots," on which small mammal distributions are mapped. Species lists of mammals and birds were collected from local resource managers. In situations in which small mammals are known to be abundant on a site, the collection and study of small mammals provides an

excellent "model" with which to relate exposure characterization and ecological effects. For example, deer mice (*Peromyscus* sp.) or cotton rats (*Sigmodon hispidus*) are often widely distributed over terrestrial sites, are easily live-captured, and respond to contaminants<sup>49,52,53</sup>. Small mammals have relatively small home ranges, ensuring that they are exposed to on-site contaminants. Depending on the local species, rodents, shrews (Insectivora) and mustelids (e.g., badgers) represent different trophic levels, feeding on a variety of food sources, from grasses and seeds to meat. Hence, using such local populations, observed individual or population responses can be readily attributed to contaminants at a particular site.

Under Tier 1, the estimates of effect would stem from, initially, estimates of contaminant concentration in the soil and developing a quotient of soil concentration to body burden. In addition, published information on effects of given concentrations for other small mammals (e.g., laboratory mice) would provide estimates of expected effects for given body burdens. However, variance in the diversity and concentration of contaminants at hazardous waste sites and in "reference" sites may make it desirable to empirically determine exposure using individual mammals with a known, uncontaminated history. This procedure moves to efforts and cost related to Tiers 2 and 3.

Under Tiers 2 or 3, the use of clean, "sentinel" animals introduced onto the site(s) allow quantification of contaminant accumulation and any consequent biological effects. The use of such organisms also experimentally controls for differences in intra-specific variability. Finally, linking the use of biomarkers to population dynamics in introduced organisms allows a conservative estimate of how successful remediation efforts are to minimize biological effects subsequent to site clean-up. If sufficient justification for exposure is determined and justification for closely assessing exposure the organisms experience "removed" some distance from the highly hazardous areas.

Tier 3 calls for measuring endpoints of controlled-exposure small mammals. Such endpoints include monitoring metabolic enzyme activity, such as hepatic microsomal ethoxyresorufin-O-deethylase<sup>54</sup>, immunological endpoints<sup>53</sup> and reproduction<sup>55</sup>. Such biomarkers of exposure may be linked to population presence and abundance, the final measure of continued population survival at a site.

### \* Impacts of multiple contaminants

An example of a study that sought to determine the ecological effects and potential risk of multiple contaminants to multiple receptors was the Commencement Bay study<sup>56</sup>. This study investigated the extent of sediment contamination and adverse biological effects in a heavily industrialized area at the southern end of the main basin of Puget Sound. The tide flats area comprises seven waterways and associated shoreline with water depths less than 60 feet. Chemicals of concern included eight metals and 18 organic compounds. Exposure was evaluated by measuring concentrations of chemicals in sediments. A model was used to predict natural recovery. Effects were evaluated by determining benthic abundance, occurrence of liver abnormalities in fish, and various measures of sediment toxicity.

Risks to the fish and invertebrates in Commencement Bay were characterized by comparing conditions at contaminated sites to benchmark or reference locations, applying apparent effects threshold (AET) values for chemical concentrations in sediments. An AET was defined as the concentration in sediments above which statistically significant biological effects (relative to reference sediments) would always be expected. This study included several notable examples for a successful ERA: 1) multiple chemical measurements and biological endpoints were used; 2) the combination of field-collected sediment bioassays and AET's helped to differentiate between effects associated with different contaminants; and 3) by expressing all chemical and biological measures as elevations relative to a reference site, comparisons among these measures and demonstrations of concordance were straightforward.

The Commencement Bay ERA has certain limitations, including: 1) the ecological assessment was neither predictive nor probabilistic, although not originally conceived as a risk assessment; 2) the empirical significance of some endpoints was not explained, particularly with respect to individual site characteristics; 3) the definition of AET as the highest concentration at which no effect is observed (rather than the lowest concentration at which any effect is observed) is the least protective of possible definitions for effects thresholds. This method assumes a consistently increasing biological response at increasing concentrations of chemical. Unmeasured chemicals, physical conditions, species interactions, and other community-level processes may alter the dose-response relationship.

The Commencement Bay study was a multi-year, multimillion dollar effort to explain the ecological effects of many stressors on biota within an ecosystem. Other case studies offer smaller-scale, less expensive, but equally effective methods to examine individual and synergistic effects caused by multiple stressors<sup>16</sup>.

### 3.2.5 Example of Ecological Risk Assessment at a U.S. Air Force Site

Massachusetts Military Reserve (MMR) / Otis Air National Guard Base on Cape Cod, MA, has several groundwater plumes contaminated with organic and inorganic contaminants from leachate caused by spills of fuels and solvents and from a landfill.<sup>57</sup> Preliminary Ecological Risk Assessments were performed on seven plume areas to assess the potential for groundwater contaminants to impact surface water and sediment receptors. These ERAs were based on data gathered from monitoring wells during remedial investigations (RIs). ERAs were also performed on two ponds, Ashumet Pond and Johns' Pond, that would potentially receive plume contaminants in the future.

Monitoring wells were placed at strategic locations within each plume and sampled several times during the remedial investigations. However, most wells were concentrated near the original source of contamination or near the leading edge of each plume. Also, organic compounds were sampled more frequently than were inorganic compounds. COCs were identified based on Ambient Water Quality Criteria (AWQC) for aquatic and marine organisms. Ecosystems potentially at risk were surface water bodies and sediments in ponds, rivers and estuaries. Hazard Quotients were calculated based on the AWQCs. No adjustment was made to the risk estimations to account for percentage of loading of contaminants relative to the flux of total water entering surface water bodies. Nor were adjustments made to account for degradation of contaminants over time or solubility of inorganics relative to water chemistry. Therefore, risk estimates were extremely conservative.

The Risk identified some heavy metals as COCs in most of the sites. Organics such as trichloroethylene (TCE), parachloroethylene (PCE), ethylene dibromide (EDB), xylenes and benzene were of concern in a few areas in some plumes. In most cases, the organic compounds were well below reference levels for toxicity to ecological receptors.

A Plume Containment Plan was formulated to remediate the plumes. This plan involves pumping and treating groundwater to remove contaminants followed by re-injection into the ground. A team of experts was assembled to assess the ecological risk of the plume contaminants based on the original RIs and Preliminary Risk Assessments as well as data obtained from more recent sampling. Review of the previous RIs and Ecological Risk Assessments by the team of experts led to adjustment of the Hazard Quotients based on the latest reference values for ecological receptors. For example, a food chain model designed to predict uptake of manganese and copper in Osprey were originally calculated using reference values determined for monkey and swine, respectively.

monkey and swine, respectively. Hazard Quotients were re-calculated using new reference values that were determined for Osprey. Risk was also re-evaluated using contaminant concentrations found in wells sampled subsequent to the RIs. In addition, some studies performed in the RIs were found to be of poor quality and, hence, not suitable for use in risk assessments.

Reviewers constructed weight-of-evidence tables to evaluate measurement endpoints, data quality objectives, strength of relationship between measurement and assessment endpoints, study design, potential for risk, magnitude of risk, and uncertainties associated with the risk based on the RIs. Risk was then re-evaluated using contaminant concentrations taken from the latest sampling, new Hazard Quotients, and estimates of contaminant flux into ground water bodies over time. Data gaps were identified based on the uncertainty associated with each plume and water body. Recommendations for future studies were then made based on these data gaps. The TRET recommended that several of the Tier One studies, such as surveys of fish papillomas and levels of heavy metals in fish and mussels be done again since statistical design of the original studies was inadequate to accurately assess risk.

Conclusions drawn by the TRET, which exposed weaknesses in the original Risk Assessments, underscored the significance of knowing the quality of data generated and the importance of using up-to-date data before characterizing risk and making recommendations for remediation or future studies. The lesson learned in this case study is that risk assessors should not extract numbers from previous studies to estimate risk without first assessing the quality of those numbers.

### 3.2.6 Example of Ecological Risk Assessment at a U.S. Navy Site

Phase I of an Estuarine Ecological Risk Assessment was performed for Portsmouth Naval Shipyard, Kittery, Maine<sup>58</sup>. This cooperative effort was performed by the Navy, USEPA, and University scientists. The approach followed the USEPA Region 1 guidance and the USEPA Framework to assess ecological risk from past disposal practices of the Portsmouth Naval Shipyard on the Great Bay Estuary.

A network of stations was established in depositional areas where the greatest likelihood of contamination would occur to determine the temporal distribution of contaminants and to assess ecological effects. Other sites were established near the shipyard to provide information on the extent of contamination from the shipyard, identify other sources of contamination in the estuary, and establish background reference levels of contaminants of concern (COC). This network was established for

the problem formulation phase which included: identification of the stressor characteristics, the ecosystems potentially at risk, ecological effects, selection of assessment and measurement endpoints, and formulation of initial first-tier and second-tier conceptual models.

A number of studies were performed to address the above-mentioned parameters including texture of sediments, sediment toxicity, characterization of water-column conditions, water toxicity, fecal-borne microbial contamination, hydrodynamics, eelgrass analysis, fucoid analysis, flounder and lobster analysis, mussel analysis, infaunal invertebrate analysis, analysis of field samples of marine sediments, tissues, water, and analysis of organic chemical markers to distinguish shipyard contamination from contamination caused by other sources in the estuary.

Results of field and laboratory investigations indicated limited toxicological impact and absence of severe environmental contamination. Elevated levels of Hg, Pb, Cr, and Ni were found in mussel tissue. The authors suggested that chronic exposure to these levels could possibly cause long-term impacts. However, contamination likely originated from many sources, some of which may not be from the shipyard. Data gaps were identified, including initial assessment of the health of the salt grass communities, and additional information about the trophic transfer of contaminants. These studies were recommended for Phase II investigations. The Conceptual Model was revisited and modified based on the results of sampling and toxicity testing.

This study represents a logical, step-wise procedural example of a Tier I Problem Formulation. Statistically sound studies were based on well thought out estimates of the nature of potential contaminants and their spatial and temporal patterns. Contaminant loading into the estuary from sources other than the shipyard were considered. Although all sources of contamination were not found in Phase I of the program, the initial studies determined COCs, characterized exposure and effects and identified data gaps to be considered in Phase II of the program.

## 4. RISK CHARACTERIZATION

### 4.1 General Overview

Risk characterization is the critical process in an ERA. In the risk characterization, information on exposure, exposure-effects relationships, and defined or presumed target populations (whether from direct sampling efforts or from estimates derived from reports and literature) is integrated to attribute the likelihood, severity, and characteristics of adverse effects to environmental stressors present at the site (Figure 15). It is these parameters which determine the ecological significance of risk, and therefore the appropriate level of risk management response.

It is important to understand that "risk" is an integrative concept, not a single, directly measurable value. Risk is estimated by calculation from information on exposure and contaminant fate. However, risk assessment findings and conclusions may be verified and confirmed by measurement. Direct measures of impact and effect may be important in developing the weight of evidence which supports the attribution of risk to different sources of stress.

The framework document<sup>4</sup>, outlined in Section 1, emphasizes the possible interaction of alternate sources of stress and the necessity to identify contaminant-related effects in this context. Draft guidance<sup>3</sup> provides a conceptual foundation for implementing this evaluation. The various components of a weight-of-evidence evaluation should be developed in advance of conducting the analyses, and the relative importance of each should be determined *a priori*. This procedure helps prevent biased conclusions by employing previously agreed-upon input information in deriving risk estimates. In many cases it will be up to the risk manager to understand the administrative record for project plan approvals and act accordingly, because experience has shown that when preconceived notions of risk are not supported by site-specific evidence, risk assessors may come to disagreement or indeed attempt to stretch the assessment process by undertaking further, unplanned and possibly unnecessary studies.

Risk calculations must always be related to assessment endpoint(s) via measurement endpoints. It is this relationship that supports the utility of risk assessment for risk management. It is crucial that assessment and measurement endpoints be understood in the context of the range of ecological stressors present at a site, and that the ERA be conducted to effectively attribute effects (if any) to site-related contaminants. Ecological risk assessment is one of a number of sources of information that must be considered in evaluating the possible

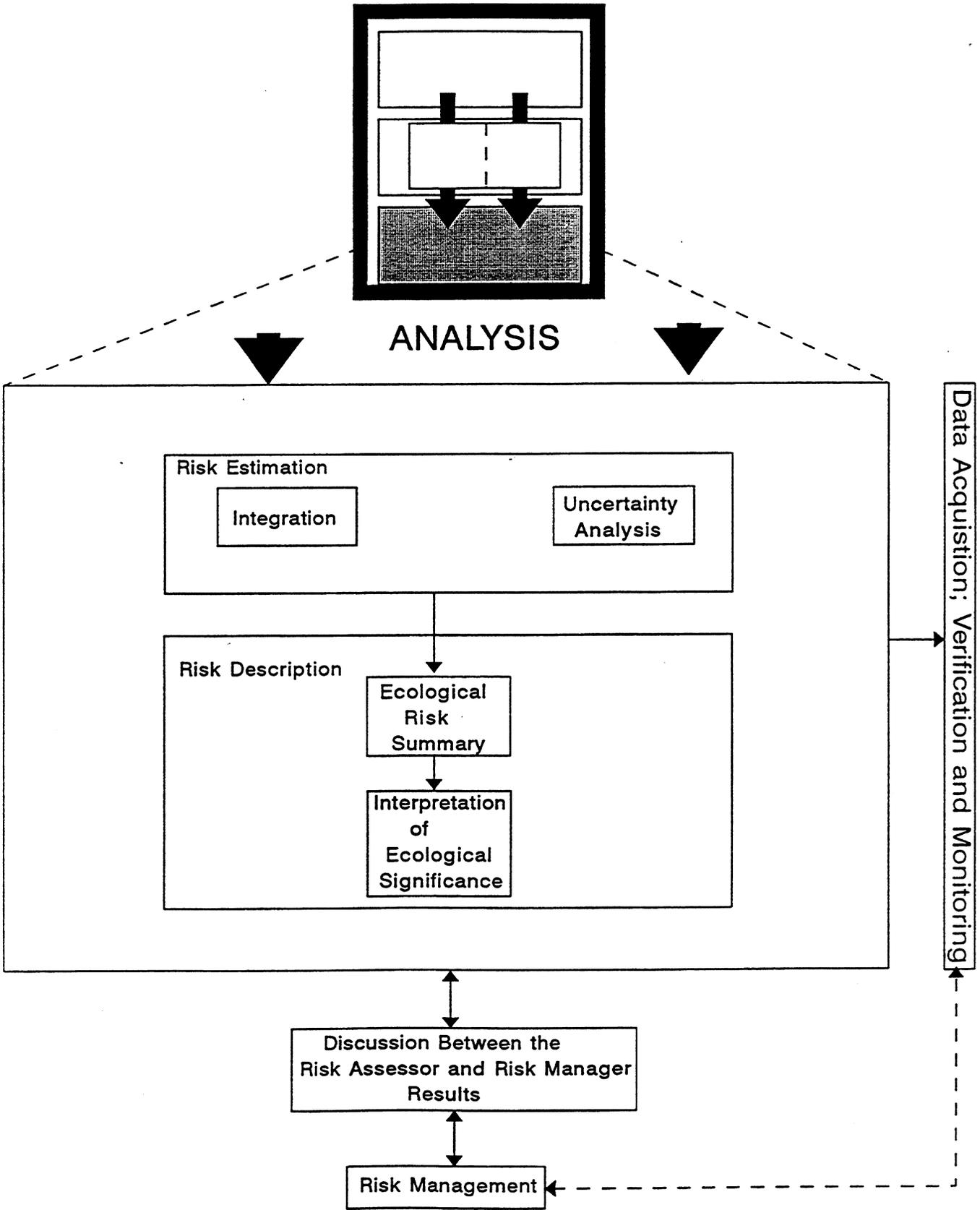


Figure 15. Risk Characterization Phase

remediation of a contaminated site. For ecological assessment to play a proper role in this process, ecological risk characterization must be as accurate and scientifically sound as possible<sup>59</sup> in keeping with the objectives of the assessment. These objectives are identified during the problem formulation phase. Risk assessment objectives in the tiered approach are related to specific decision points which can be useful in determining possible need for further data gathering, evaluation effort, or management actions. Decision points are fundamental to successful implementation of a tiered ERA<sup>3</sup>.

#### 4.2 Decision Points

The tiered approach to ecological assessment provides an effective framework for risk estimation. The key to successful implementation of the phased approach at the risk characterization stage is the *a priori* provision of decision points for the risk assessment. Review Draft Guidance<sup>3</sup> identifies a series of administrative decision points relating to the review and approval of certain documents. In practice, the risk assessment/risk management team needs to identify technical decision points at which the possible requirements for further investigation, uncertainty evaluation, or risk management consideration are characterized. It is important that such decision points be built in to project planning, to avoid the truncation of the process by time and effort constraints which fail to account realistically for the needs of the assessment process.

Action-oriented decision points will vary with site conditions and assessment objectives, and thus cannot be detailed generically. However, certain categories of decision points can be identified based on habitats present and the overall role of risk assessment in the site management and weight-of-evidence evaluation processes. This section provides brief examples of decision points appropriate for different habitats and levels of assessment. For any particular site, the risk assessment/risk management team should develop in advance detailed decision points on which to base technical progress.

Terrestrial Habitats. Tier 1 investigations in terrestrial habitats will identify areas of heavily contaminated soils. Tier 2 and 3 investigations will focus on the margins of the heavily contaminated zones, and quantify risks associated with contaminant transport and chronic exposure. Presence of elevated concentrations of organic toxicants or metals relative to "reference" conditions is a primary decision threshold determining the need for further investigation. The need for quantitative assessment beyond Tier 1 can be ascertained by simple, point estimate of exposure vs. known effects concentrations. In general, simple point estimates of risk are

most valuable as indicators of need for further evaluation, and not for defining risk management. Decisions to move to Tier 3 level of investigation should be based on the nature of contamination (bioaccumulative organic compounds, for example) and the complexity of site conditions. For example, presence of endangered or threatened species in areas of elevated contamination suggest the need for advanced analyses.

Risk management decisions in terrestrial habitats should incorporate realistic estimates of exposure based on bioaccessibility and bioavailability of toxicants (Section 3.1). Hypothetical risks based on highly conservative assumptions should not, in general, define active remediation.

Three categories of biota are often the focus for decision making in terrestrial habitats. Vegetation is often not demonstrably impacted (except by herbicide discharge) unless contaminant concentrations are very high. However, vegetation can be a key exposure route through uptake to the consumer food web. Soil fauna, because of local nature of exposure and intimate contact with the primary medium, may provide excellent decision points, and some promising techniques for assessing contaminant effects on soil fauna communities are being developed<sup>60</sup>. Vertebrate organisms are often exposed primarily through the food web. Probabilistic risk estimates based on all exposure routes (see discussion of Conceptual Models in Section 2) provide the decision making thresholds for these receptors.

Aquatic Habitats. Tier 1 investigations in aquatic habitats may focus on point estimates of exposure compared with effects levels such as the available EPA Ambient Water Quality Criteria. Such comparisons should not be made simplistically, however. The published criteria for some metals are weighted relative to water hardness, and this should be accounted for in making decisions on this basis. In addition, the criteria may be modified on a site specific basis to account for resident species (with a recalculation based on supporting toxicity data) or based on site specific toxicological testing. The latter should be considered Tier 2 and 3 studies, respectively, with the decision to undertake such investigations dependent on the level of risk inferred from simple point estimates.

Beyond criteria comparisons, aquatic food web models and probabilistic exposure estimates should be applied when Tier 2 and 3 studies are warranted by potential contaminant-related effects. Effects may be verified by community structure measurements of water column and benthic biota, and perhaps direct toxicity testing. These techniques have the disadvantage, however, of integrating all sources of impact and exposure. They should only be employed when the potential site risks are

sufficient to support the level of technical effort necessary to apportion impacts.

Monitoring and Assessment Validation. In all habitats and under all risk management scenarios, post-assessment monitoring or assessment validation data collection may be important. In general, monitoring is useful in situations where residual contamination will be present after the remedial alternative is implemented. The decision to undertake post-cleanup monitoring is best based on: 1) the relative uncertainty of the risk assessment (more uncertain assessments, especially those based on single point estimates, may need a greater investment in monitoring); and 2) projected exposure reductions associated with the remediation. Properly designed monitoring programs serve simultaneously to assure the efficacy of the cleanup and to validate the risk assessment and its application, i.e., determine the accuracy of the original estimate of risk<sup>61</sup>.

The most elaborate and expensive monitoring and validation programs will be used where Tier 1 and 2 assessments have been employed to support cleanup decisions. Tier 3 assessments will generally include intensive field investigations to validate risk assessment parameters. The low uncertainty associated with this greater investigation effort may be reflected in reduced monitoring requirements.

#### 4.3 Risk Estimation

The fundamental tools of risk estimation are the simple hazard quotient and probabilistic risk estimates. Each has its uses, and each supports certain decision points for a particular site.

##### 4.3.1 Hazard Quotient

The simple hazard quotient is a tool primarily useful in the Tier 1 and some Tier 2 levels of investigation. Simple hazard quotients are point estimates relating presumed exposure concentrations to known or extrapolated effects levels of toxicants. Conceptually, the hazard quotient is represented as:

$$HQ = \frac{EEC}{TEC}$$

where EEC is the expected exposure point concentration and TEC is the appropriate toxicological endpoint concentration. As a basis for risk assessment, separate hazard quotients are calculated for each contaminant/receptor pair. It may be possible to derive hazard indices by combining hazard quotients for different compounds for a single receptor taxon. Such indices are generally constructed by simple addition, and the result is very poorly supported by existing toxicological data. Assessment uncertainty is greatly increased by combining hazard quotients. Where necessary, such combinations should only be made of compounds likely to have similar modes of action. For example, some organochlorine pesticides which each act to suppress brain enzyme activity, or some metals which each act to damage kidney cells might be combined for risk assessment. It would be inappropriate and ineffective to construct a hazard index which combined hazard quotients of, for example, trichloroethane, PCB Arochlor 1248, and arsenic. Each of these compounds has a different mode of action, and their effect in combination is not additive or even directly related, particularly at the chronic dose level usually observed in relation to hazardous sites.

Uncertainties surrounding point estimates arise from extrapolation of the available toxicity data bases and inference regarding exposures. Because the hazard quotient is a point estimate only, the estimate itself must account for uncertainty in application to the field situation. As illustrated in Figure 12, the process of extrapolating toxicity data for point estimates sometimes incorporates divisors which compensate for possible uncertainties but which could lead to inflated and unrealistic hazard estimates. Similarly, inflated exposure assumptions could be employed to compensate for presumed uncertainty. Despite these drawbacks, the quotient method is a useful and appropriate tool for Tier 1 and certain Tier 2 investigations. The risk assessor must, however, be vigilant in deriving realistic, site-specific quotients rather than simply applying generic, overly conservative values<sup>62</sup>.

LD<sub>50</sub> estimates, ambient water quality criteria, and reproductive effects thresholds are examples of single number effect and exposure profiles. The LD<sub>50</sub> is that level of exposure dose that is lethal to 50% of the population exposed. The ratio, or quotient, of the exposure value to the effect value provides the relative estimate of risk. Under any tier, the quotient method may be employed to estimate the possibility of an adverse effect from single sources<sup>63</sup>. In general, ratios of EEC to TEC greater than 1.0 are considered to indicate a potential risk. Because the quotient method yields only a point estimate, effects probabilities cannot be easily specified. To account for this, safety factors are sometimes considered in interpreting findings. For example, Menzie et al.<sup>20</sup> interpreted HQs between 1 and 10 as having "some small potential" for adverse effects, HQs between 10

and 100 as having "significant potential", and HQs greater than 100 as indicating "expected" adverse effects. However, it is important to note that no statistical analysis supports this interpretation, and indeed none is possible within the context of a single site investigation.

For more quantitative assessment, lower ( $F_L$ ) and upper ( $F_U$ ) safety factor(s) may be included in the basic HQ equation so that if the ratio is less than the lower-bound factor ( $EEC/TEC < F_L$ ), the release is considered potentially "safe". If the quotients exceed some upper-bound factor(s) ( $EEC/TEC > F_U$ ), exposure concentrations are considered "unsafe". Quotients between  $F_L$  and  $F_U$  indicate uncertainty about safety and imply the need for further assessment. In many cases, such boundary limits cannot be specified, and a single factor ( $F$ ) is used (i.e., if  $EEC/TEC < F$ , the release is considered safe; otherwise, it is not). The quotient is deterministic, in that it establishes a number without an associated variance.

A practical example of a Tier 1 application of the Quotient Method is an evaluation of DDT residues at a Superfund site. Because DDT is known to accumulate in earthworms, and because American robins feed almost exclusively on earthworms in the spring, the robin would be a good population on which to base a bird safety assessment using the Quotient Method. Assume we determined from the literature the DDT 6-month  $LC_{50}$  for robins is 5 ppm and a conservative upper allowable exposure level for the site ( $F_U$ ) was established at 50% of the  $LC_{50}$ . If the mean residue level in earthworms on site was 3.7 ppm, the quotient equation would be  $EEC (3.7 \text{ ppm}) / TEC (5 \text{ ppm}) = 0.74 > F_U (0.5)$ . Therefore, the site contamination level is greater than the acceptable safety criteria. In this case, the decision is made to remediate the site and no further study on the site is required. If, however, there are not adequate data in the literature regarding the TEC, there is tremendous uncertainty about what level of exposure may be considered safe, or there are numerous species for which risk estimation is needed, the Quotient Method may still be applicable but would be elevated to a Tier 2 or 3 effort.

One example of the use of Quotient method in Tier 2 of a RA was conducted by Charters, et al.<sup>64</sup> at a PCB and lead contaminated wetland. They evaluated three pathways of exposure and established measurement and assessment endpoints for each. The measurement endpoints were toxicity values or body contaminant burdens; assessment endpoints were population maintenance (continuance of viable populations). Exposure estimates incorporated field and laboratory measurements and information derived from available scientific literature. In keeping with the objectives of a Tier 2 level of effort, risk estimates were focused on sensitive receptors and suggested the need for further

action (quantitative Tier 3 site evaluation and remedial actions).

Another effective application of the simple quotient method in a Tier 2 assessment is described in Boucher<sup>65</sup>. In this case, protective criteria for representative receptor organisms were derived based on extrapolated toxic hazards and site-specific exposure levels. Exposure concentrations were verified with field data, and point estimates were incorporated in a weight-of-evidence evaluation of cleanup alternatives. Some of the uncertainties inherent in the point estimate approach were accounted for by the use of site-specific measurement data on concentrations in environmental media and biotic tissues. Others were accounted for by employing realistic, technically sound estimates for toxicity and exposure parameters.

#### **4.3.2 Probabilistic Risk Estimates**

Probabilistic risk estimates provide a technically sound basis for evaluating possible contaminant hazards in the "gray zone" beyond heavily contaminated areas and for cases where remedial activities would be costly and highly destructive. Probabilistic approaches allow much more precise quantitation of risks and the nature and location of contaminants driving risks. In general, probabilistic estimates are most useful in Tier 2 and 3 investigations, where the level of site complexity and decision making importance warrant more accurate and precise risk evaluation.

Probabilistic approaches require more investment of resources in the assessment, but provide a substantial return on this investment by more clearly and effectively guiding risk management engineering. Probabilistic risk estimates are based on ranges of input values manipulated mathematically to yield an ecologically realistic picture of potential site related exposure and exposure related effects. Statistical distributions of input data are derived from available scientific information, and risk quantitation is calculated for various combinations of these distributions. Risk quantitation by this approach avoids the highly conservative uncertainty divisors which are often applied to assure the protective nature of risk estimates based on single point estimates. Probabilistic assessment also offers the risk manager objective specification of the level of protection provided by cleanup scenarios which may require understanding of the trade offs inherent in environmental destruction associated with active remediation vs. the benefit of contaminant removal or exposure reduction.

A detailed description of a comprehensive approach to probabilistic risk estimation is provided in Bartell et al.<sup>1</sup>. The fundamental components of a probabilistic assessment are:

- identify contaminants of primary concern;
- develop statistical distributions of concentration-dependent effects of contaminants on representative receptor organisms;
- develop statistical distributions of site-specific exposure of receptor organisms to contaminants;
- combine effects and exposure distributions to yield probabilistic estimates of effect.

Because the distributions account for data-driven uncertainties, elaborate and conservative uncertainty factors are not applied. The distributional nature of the estimates allows the risk assessor to provide the risk manager with clear statements of risk probability. Thus, for example, should risk management objectives include "protecting 95% of species present in a body of water from adverse effects of cadmium", the distributions of exposure and toxicological effect allow the risk assessor to determine, in light of site specific bioaccessibility and bioavailability, realistic and protective concentration objectives.

Analysis of distributions of exposure and effects, rather than using single values, makes probabilistic risk estimates possible. Risk is quantified by an expression of the overlap between the two distributions, with greater overlap indicating greater risk (Figure 16). Figure 16 presents a simplistic view of the overlap between exposure and effect, relating to risk. In reality, exposure varies temporally and spatially. The heuristic model presented in Figure 16 can be expanded in other dimensions (time and space), with an integration of the multi-dimensional curves, to arrive at a more realistic estimate of the risk. We are unaware of such an approach being taken to date. One method which has been applied to multidimensional risk evaluation is fuzzy modeling<sup>66</sup>. Such an approach could be used to fully incorporate spatial and temporal considerations in risk quantitation.

An example of this method, Analysis of Extrapolation Error (AEE), is described in Suter<sup>7</sup>. The AEE approach uses the variability in and relationship between responses of particular species to a range of contaminants to predict effects of unstudied contaminant-receptor pairs. For example, the distribution of effects of varying concentrations of various contaminants may be known for fish species A and B, while the contaminant of interest may only be known for species A. Relative sensitivity to other contaminants predict, with quantifiable uncertainty, the response of species B to the untested contaminant of interest. When data are available to support AEE, the approach has substantial value.

# Exposure-Response Risk Model

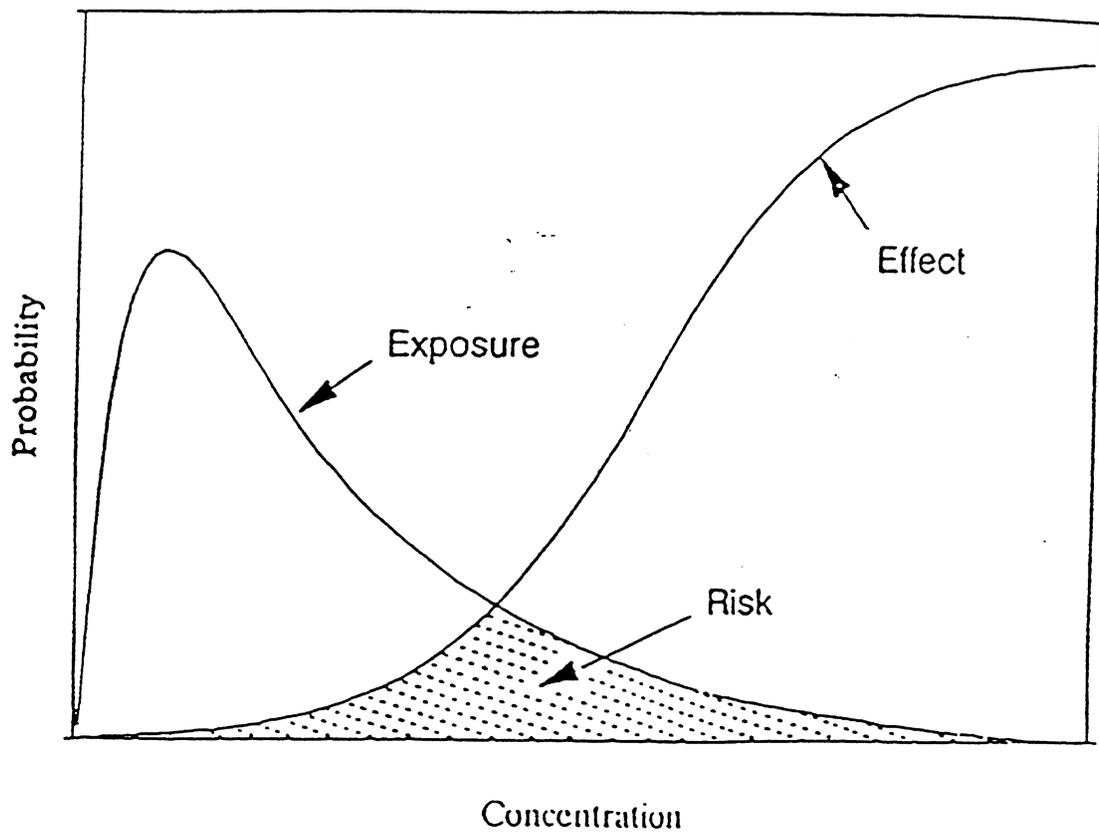


Figure 16. Exposure-Response Risk Model.

As Suter<sup>7</sup> states:

The main advantage of the AEE method is that it clearly distinguishes, quantifies, and displays both the extrapolations that must be made from the toxicity data and relate it to the assessment endpoints and the uncertainties associated with the process of extrapolation. In contrast, the quotient method with factors treats uncertainties and correlations as equivalent and does not systematically account for either one.

However, AEE only addresses the response component of risk. The exposure component must often be measured or modeled directly in Tier 2 and 3 assessments, accounting as necessary for contaminant bioaccessibility and bioavailability (Section 3).

Probabilistic approaches to risk assessment have been applied for investigations at hazardous waste sites. For example, Cardwell et al.<sup>67</sup> employed effects and exposure distributions to estimate risk probabilities associated with metals contamination of river ecosystems (Figure 17). In this approach it is relatively simple to visualize the proportion of species in the community potentially at risk of chronic or acute contaminant effects. In this case, test species (measurement endpoints) were assumed to represent the balanced, indigenous community in the rivers (assessment endpoints). Because the presentation is essentially a cumulative probability density function (CPDF) of the toxicity data obtained, it is critically important that the assumption of representativeness is realized to the greatest extent possible. If the species and endpoints used in the presentation are not representative of the community potentially at risk, the CPDFs generated will not accurately reflect potential risks in the environment. For example, if the CPDF is constructed from data for *Daphnia* and *Hyalella*, two invertebrate species, but is used as a reference for fish, the results may be far too uncertain to use. The concept of balance is also critically important when using this form of presentation. If the data upon which the CPDF is based are not balanced with respect to numbers and types of test species and endpoints (e.g., 20 *Daphnia* values and only 2 for fathead minnow values), the resulting CPDF will be biased toward the one test species and again, comparisons will be very uncertain. If CPDFs are constructed from data which accurately represent the composition and balance of the community potentially at risk, the technique presented by Cardwell et al.<sup>67</sup> can contribute a valuable additional layer to the presentation of uncertainty.

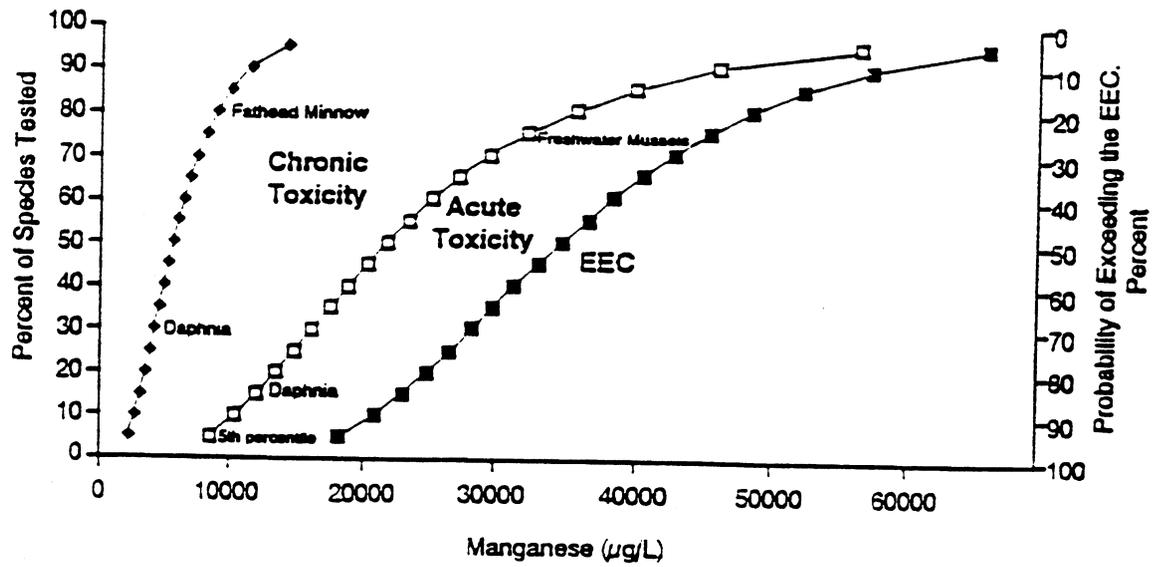


Figure 17. Comparison of Expected Environmental Concentrations of dissolved manganese with concentrations causing acute and chronic toxicity (from Cardwell et al. 1993).

#### 4.4 Simulation and Exposure Modeling

Simulation and exposure modeling may be useful in any investigation tier. For Tier 1, simple exposure models incorporating estimated bioaccumulation factors and initial engineering investigation data on the nature and extent of contamination in environmental media can be used to "screen" sites or areas for further investigation. For Tiers 2 and 3, modeling, usually with integral probabilistic components, is often crucial to the overall weight of evidence evaluation.

It is desirable in risk characterization to obtain probabilistic estimates of risk for a species or group of species. Simulation models can provide such estimates by integrating exposure and stressor-response profiles. These profiles may include information on the frequency, timing, and duration of the exposure in addition to the variables which characterize the stressor-response.

There are two basic types of simulation models used in ecological risk assessments: 1) single-species population models and 2) multi-species models. Single species population models are used to predict direct effects on single populations, using measurement endpoints at the individual organism level. Multi-species models include various components of the ecosystem, such as food-web relationships (i.e., predator-prey, competition), plant succession, etc. Multi-species models evaluate both direct and indirect effects. An example of an indirect effect predicted through modeling is the potential for a change in avian behavior that would tip the balance of interspecific competition for nest sites or behavior that reduces some aspect of parental care. The influence such responses may have on population status may be either very obvious or subtle and only substantiated by empirical results or complex models. When the population response is less complex, such as reduced fledgling success in a bird species, it may be advantageous to use simpler, single-species population models to predict the probability of a given response level. When selecting a model, it is important to thoroughly consider the appropriateness of the model for the particular application.

Information needed to develop an estimation of risk may come from field studies, existing literature, or a combination of the two. In some cases risk estimation need not require a full-scale field study conducted over several years or seasons. As stated in the examples under the Analysis phase, above, the risk characterization may proceed using key sentinel species, with known life-history requirements (feeding, reproduction, habitat). Use of such surrogate species, which may be free-ranging wild individuals or individuals introduced to the site, may be far less costly than full-scale field surveys. When naturally occurring individuals or introduced individuals are exposed on the site for a defined time, the body burdens,

biochemical responses, and/or alterations in behavior may be correlated to distributions of the contaminants. Such an assessment would provide the variety of measures (measurement endpoints) and allow estimates of variance within each set. In this manner, site-specific probabilities could be associated with each of the expected adverse effects.

The Rocky Mountain Arsenal Environmental Risk Assessment is used as a case history example in "A Review of Ecological Assessment Case Studies from a Risk Assessment Perspective"<sup>16</sup>. This case study presents an example of a food chain-based model developed to predict effects on animal species on the site. The model is developed and tested using data from Tier 3 level field studies of exposure and effects in sentinel species.

For probabilistic estimates of risk, there are a wide variety of available models useful in any of the tiers (Volume 2, Appendix A). Several models focus on how the environment modifies the contaminant bioavailability (e.g., FGETS model, Volume 2). Modeling approaches presently exist to link water quality to reductions in "dose" under various scenarios of ecosystem productivity<sup>29</sup>. One example of a modeling approach that illustrates how ecosystem trophic status modifies the bioavailability of toxicants and decreases the subsequent dose to biota was performed by McCarthy and Bartell<sup>68</sup>. Their model predicts the association of a contaminant with dissolved organic material (DOM) or particulate organic material (POM), which significantly lessens the bioavailability of a toxicant, and thus, the potential dose experienced by the organisms. Importantly, this paper shows the necessity of estimating the true bioavailability of a contaminant in the environment.

Model projections which include seasonal or habitat variances in bioavailability (e.g., mapped onto expected environmental chemical concentrations for species of known life history, feeding, and habitat requirements) are a cost-effective approach to the hazard characterization of complex chemicals. For a given concentration, species may be subject to exposure for a relatively longer period of their life-span if they are smaller or less likely to move beyond the boundaries of the contaminated area (examples are earthworms, burrowing invertebrates, or small mammals). Further, if a chemical is susceptible to being bound by organics, burrowing (or thigmotactic) benthic invertebrates (or benthos-feeding fish) may be subjected to higher exposures than would otherwise be predicted. Volume 2 includes certain models available for evaluating transport, transformation and fate of contaminants in the environment (e.g., EXAMSII, LPMM). In addition, several models estimate biotic exposure or uptake of contaminants (e.g., FGETS).

Environmental and ecological monitoring data may be evaluated

using a Geographical Information System (GIS) as part of a Tier 3 effort to gain a higher level of understanding of potential contaminant-associated problems and approaches to effective risk management. Coupling modeling and GIS is particularly effective when geographic distributions of contaminants and the integration of these contaminants and wildlife activities on the study site are important parts of the risk analysis and characterization. For example, animal home-range analyses can be incorporated to GIS software and home-range use can be correlated with geographic distributions of contaminants to estimate potential for exposure. From this information, risk management alternatives can be evaluated on a "what if" basis by having remediation engineers identify contaminant parcels most amenable to control. The risk assessment benefit of such projected risk management efforts can then be evaluated directly through the GIS. Such an approach is being explored for remediation at Rocky Mountain Arsenal<sup>69</sup>. In this case, the site-wide risk reduction associated with local "hotspot" removal is clearly demonstrated by linking exposure models to GIS for immediate evaluation of the benefits of various remediation scenarios. This is illustrated in Figure 18 which contains "risk surfaces" for burrowing owls exposed to dieldrin via diet at Rocky Mountain Arsenal. The upper surface is prior to remediation and clearly shows the dieldrin "hot spot" (HQ=434). The bottom surface is a post-remediation projection with no HQ greater than 1.0.

Using GIS in the risk assessment process is also a highly effective way to produce graphics and visual aids to demonstrate and explain (to military and regulatory personnel, and to the public) the critical environmental relationships that influence ecological risk.

#### **4.5 Uncertainty Analysis**

Risk estimation infers a degree of uncertainty. The estimation is derived from comparison of organism exposure to organism response to the stressor(s) under investigation. The stressor-response profiles used in this process may involve a single value response such as an LD<sub>50</sub>, or a suite of responses such as immune system function responses combined with contaminant blood levels. The degree of uncertainty around the estimate is related to the precision of the stressor-response profiles used. When the response evaluated is death, or death of 50% of the population (LD<sub>50</sub>), the uncertainty of an adverse effect will be greater than if the response level of concern is a measured level of sublethal immune system response. The more conservative response variables are more likely to err on the safety side of the equation, and result in lower uncertainty of the negative effects under consideration. Within each tier, there will be assumptions and uncertainties involved in characterizing the ecological risk. By the very nature of the lower effort and cost at the lower tiers,

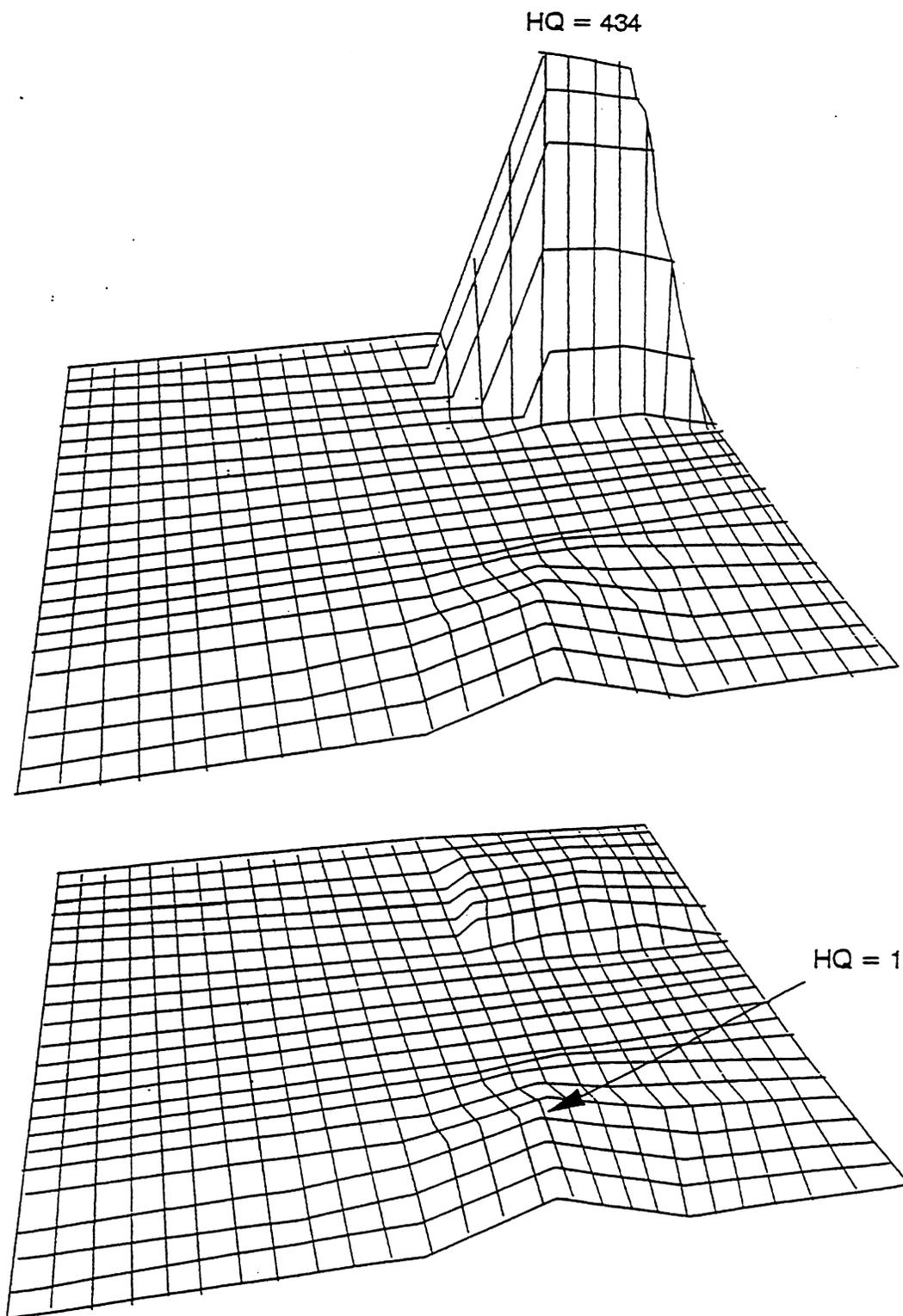


Figure 18. GIS-based "risk surfaces" for dieldrin at Rocky Mountain Arsenal.

risk characterization will have larger uncertainties. The benefit of more focused effort in the higher tiers becomes primarily one of incorporating more site-specific information, thus reducing the need for simplifying assumptions, and therefore reducing the level of associated uncertainty.

Uncertainty analysis is thus an important part of the Risk Characterization phase and occurs as a function of questions and variances from all phases of an ERA. The objective of uncertainty analysis is to identify and quantify, to the highest degree possible, the cumulative uncertainty surrounding the estimates of risk. Products of the uncertainty analysis are an evaluation of the effects of uncertainties on the overall assessment and on the risk management process. For example, if risk assessment uncertainty is high, and conservative assumptions were used to suggest a major cleanup effort, additional investigation to reduce uncertainty might be warranted. However, if conservative or realistic risk estimates yield an objective, credible risk management program, the level of uncertainty is clearly appropriate to the assessment goals.

Sources and effects of uncertainty overlap throughout the risk assessment. The reader can find in-depth discussions of the subject in the references listed in the Risk Assessment Framework<sup>70,71,72</sup>. Some major sources of uncertainty include:

- 1) formulation of the conceptual model: are the correct working hypotheses established?
- 2) incomplete information and data: if the correct data are not collected, little can be said of the exposure or response.
- 3) natural variability: variance in spatial, temporal distributions of the COC, biotic and abiotic stressors, and population at risk.
- 4) procedural or design error: unless data quality assurance plan is formulated, it is likely that errors and greater uncertainty will increase from incorrect or inappropriate analyses.

#### **4.5.1 Conceptual Model Formulation**

Flaws in the conceptual model may be the most pervasive source of uncertainty, and the most difficult to identify, quantify or reduce. The conceptual model, which is the product of the problem formulation phase, provides the basis for the analysis phase and the development of the exposure and stressor-response profiles. If incorrect assumptions are made during the

conceptual model development regarding the potential effects of a stressor, the influence of environmental variables, the interaction of wildlife species with the stressor, or the sensitivity of organisms to the stressor, the final risk assessment will be flawed. Once the conceptual model is correctly developed during the course of the ERA, care should be taken not to incorporate factors that erroneously increase uncertainty, lead to incorrect conclusions, or limit management decisions. Awareness and avoidance of factors that unduly increase uncertainty are critical at all phases of the assessment.

#### **4.5.2 Incomplete Information and Data**

The risk assessor will invariably encounter situations where information or data are incomplete. In some cases the assessment may be halted until further information is obtained or further study completed to fill in data gaps. However, there will be cases when the resources, technology, or fundamental ecological knowledge needed to close such gaps are not available. In these cases, the risk assessor must rely on professional judgement and cautious use of assumptions. When judgement and assumptions are inserted into the assessment, they must be clearly identified as such throughout the various phases of the assessment, and thoroughly explained and evaluated during the Risk Characterization phase.

#### **4.5.3 Natural Variability**

Natural variability (stochasticity) is an ever-present condition that influences the distribution, availability and influence of stressors in the environment. It equally biases our perception and interpretation of these factors. Variability inherent in the physical environment (moisture, nutrients, organic material, temperature, etc.) causes variability in biological components of the environment (animal health, size, sensitivity, exposure level, etc.). Although the uncertainty caused by variability may be complex, it can be acknowledged and described, but not reduced<sup>72</sup>. When sufficient databases exist, stochasticity can be quantitatively estimated and analyzed via such methods as Monte Carlo simulation and statistical uncertainty analyses<sup>73,74</sup>.

#### **4.5.4 Procedural and Design Error**

Errors in measurement and sampling can be reduced through adherence to a good quality control program or Good Laboratory Practices Guidelines. Raw data review and data entry verification procedures are invaluable in reducing the introduction of human errors. Errors in study design are best avoided by assuring a strong peer review of protocols. Errors and uncertainty in the development of simulation models can be

addressed through sensitivity analysis and field verification or model validation.

#### **4.6 Risk Description: Ecological Risk Summary and Interpretation of the Significance.**

The EPA Framework<sup>4</sup> describes two elements of ecological risk description: 1) a summary of the risk estimation results to describe the confidence level in the risk estimates; and 2) interpretation of ecological significance, identified in the Framework Document as the magnitude of the risks relative to the assessment endpoints. This approach has been carried into the Review Draft Superfund Guidance<sup>3</sup> as a weight-of-evidence foundation for ecological risk assessment. A weight-of-evidence approach incorporates the judgement of how variable are estimates of contaminant distribution, exposure and biotic uptake potential, and the probability of adverse effects of residual contamination and possible remedial activities.

##### **4.6.1 Ecological Risk Summary**

The ecological risk summary succinctly reports results of the risk estimation phase and discusses the uncertainty of previous phases of the assessment. This involves an overview of measured endpoints (or estimates) of exposure and response at the individual or population level, bioaccumulation potential, integration of single or distributional exposure and stressor-response profiles, and/or model predictions. This overview must also include a discussion of the uncertainty inherent in each phase of the assessment. Whenever possible, the conclusion of the risk estimation should be expressed as a quantitative expression (there is a 30% probability of 25% mortality in American robins). Another example consists of a study on the effects of molybdenum mine tailings on marine fish and invertebrates<sup>16</sup>. The scientists calculated the risk to aquatic organisms by developing a probability of exceeding a water quality criterion level for copper (over a 55 year period) and - conservatively- assuming 100% mortality if organisms were exposed to concentrations higher than the criterion. Hence, the probability of greater-than-criterion levels for copper in water and sediments becomes the probability of effect. The conservatism of this approach could be made less, with greater accuracy, if more data were collected from the field or laboratory exposures were developed using native organisms. However, the example does provide a case where the effects are cast in probabilistic terms.

However, ecological risk assessments completed to date usually express the risk estimation in qualitative format with terms such as "high likelihood", "moderate", "low likelihood" of a given negative impact (e.g., avian mortality). Uncertainty also will

be expressed in quantitative or qualitative terms. In the discussion of uncertainty, it is important to include evaluation of the relative contributions of the uncertainties from different aspects of the assessment to the final estimate of risk.

#### 4.6.2 Weight of Evidence and Ecological Significance

Weight of evidence for projecting risks and impacts is a conceptual approach which dictates that all sources of information be considered in making risk management decisions. Because the weight of evidence links the risk assessment to the risk management process, it is imperative that the risk assessor provide clear characterization of uncertainty in each component of the weight of evidence and the meaning of each component for ecological impacts.

The evaluation of the ecological significance of risk is a process at the very edge of the capability of ecological science. Biological populations are very dynamic and population measures and models are relatively simple compared to the underlying ecological complexity. Yet it is at the population level that ecological significance must be evaluated (except for endangered or threatened species, which are often evaluated at the individual level). Suter<sup>7</sup> provides an example of an approach to quantifying population level effects of toxicological risks. Yet this exercise cannot be validated, and is only tested by additional modeling<sup>75</sup>.

An instructive example of the difficulty of projecting the significance of risk estimates is provided in Barnthouse et al.<sup>76</sup>. While this paper discusses the impacts and importance of power plant withdrawals on finfish communities, the principles developed apply to contaminated site assessments. Barnthouse et al. evaluated more than ten year's worth of effort to extrapolate the effects of cooling water withdrawal on fish populations in the Hudson River. Such withdrawals are inevitably associated with the loss of individuals. At issue was the relative ecological importance of such losses. In practice, despite highly certain estimates of the loss rates, estimates of importance at the population and ecosystem levels were so uncertain as to be useless.

Whenever possible, the assessment should clearly distinguish between impacts to individuals, or even portions of populations, and those impacts that affect whole populations. For example, Hinckley and Porter<sup>77</sup> demonstrated at a midwestern NPL site some individual impacts to white-footed mice from lead. However, impacts to the population as a whole were minimal. In contrast, although many fewer red-tailed hawks were impacted, a much greater proportion of their population was involved.

Thus, the current state of ecological science is not conducive to elucidating "ecological significance" of estimated risks. The most productive approach for most sites, in keeping with the conclusions of Barnthouse et al.<sup>76</sup>, is to document for risk managers the potential impacts of contamination and remediation and make site specific decisions on risk reduction. It may be appropriate at large, complex sites to undertake attempts to quantify ecological significance as a component of Tier 3 evaluation, but such efforts should be tempered by sound risk management judgement.

Weight of evidence in an ERA is supported by the quality and sufficiency of data. Quality assurance programs are paramount in any ERA and provide confidence in precision, reproducibility, etc. Sufficiency of the data is addressed relative to the effort involved. Tier 1 information provides primarily corroborative information, such as lists of known chemicals (and, hence, toxicity and physico-chemical characteristics), suspected distribution on the site, and limited data on direct measures of exposure and effects. Models applied at this tier may require several default assumptions for parameters.

In Tiers 2 and 3, the information on exposure and ecological effects provide a higher degree of correlation between the stressor and consequent effects. For example, a better resolution of contaminant effects of metals in a wetland on waterfowl may be obtained for migratory avian species when the timing and distribution of the migratory species is matched to times when their food base (burrowing insects) lead to exposure to the contaminant. To discern how much of the exposure stems from on-site, relative to exposure elsewhere takes time and effort not available under Tier 1. Ultimately, to reduce uncertainty in analyses, one must understand the situation in greater detail. Hence, it may be necessary to conduct follow-on studies to corroborate initial judgement calls.

When a population responds to a contaminant, its response may range from biochemical or physiological responses at the cellular level to behavioral changes or (ultimately) death and the reduction of population numbers. The significance of the responses need to be addressed in an ERA relative to the ecological context. Organismal responses (physiological, behavioral) may be transient enough, relative to the exposure duration or life history characteristics of the species, that they have little or no influence on the assessment endpoint. However, it may be that such "lower level" responses provide sufficient questions as to sub-lethal effects that another problem formulation may be called for. As an example, such a situation might exist within a site with multiple contaminant point sources, such as certain hazardous waste sites with a history of uncontrolled dumping of multiple, complex wastes. The

tiered assessment may focus on chemicals known to have been dumped at the site; however, some animals may be exposed to an unknown or unrecognized source. Biomarkers of exposure (cf., cytochrome P450 induction, porphyrin profiles; Volume 2, Appendix B) would indicate that exposure has occurred and that the potential for adverse effects on the population may warrant further investigation of the nature and extent of risk.

The interpretation of ecological effects also needs to take into account the spatial and temporal nature of the stressor and population exposed. Risk stemming from a wide area of diffuse contamination will be more difficult to summarize than areas with defined "hot spots" of contamination. Further, if the area and duration of exposure are long enough relative to the generation time of the species, then one may expect sublethal toxicity to be expressed. For certain species, a small area of contamination may lead to local population extermination if the stress is high. This might occur if a species requires a very specific habitat (e.g., wood ducks in wetlands). Should the habitat be altered even a little, the effect on the species could be catastrophic.

In addition to local, catastrophic effects, stressor responses identified throughout the risk assessment process may have ecological significance of a broader, more diffuse nature. For example, it may be determined that the response of nestling birds to a contaminant consumed in their food is 25% mortality. However, a follow-on evaluation of nestling fledgling rates and post-fledgling survival indicates there is an increase in overall fledgling and survival. For these results, the explanation is that nestling survival is density-dependent and the loss of an average of one nestling per nest resulted in more parental attention and more food for the remaining nestlings. Thus remaining nestlings were of greater body weight at fledgling and this equated to greater overall post-fledgling survival compared to non-dosed nestlings. In a case such as this, we may conclude that while there was a significant effect to individuals, the effect on population was positive, not negative. Therefore, there was little or no ecological significance.

The interpretation of ecological significance places risk estimates in the context of the types and extent of anticipated effects. Interpretation of these factors relies heavily on professional judgement. The significance of effects may be evaluated in context of several variables:

- 1) the nature and magnitude of effects,
- 2) the spatial and temporal patterns of effects,
- 3) the duration of effects, and
- 4) the potential for the system or species to recover from the effects.

All the above factors help to place expected risks into broader

ecological perspectives. Interpretation of significance may take into consideration other ecological components not specifically addressed in the risk assessment. For example, the risk assessment may have addressed reduction in a population of breeding voles (a species of small mouse-like mammals) thought to be due to a stressor. The reduction in vole numbers may not be discernable following the reproductive season, when autumn vole populations are no different on the impact site than on reference sites. The significance of the toxic effect to the vole population may prove to be small. However, as part of the interpretation of the significance of the spring decline in adult voles, the risk manager may make the connection with a separate report that northern harrier production in the area has declined and question whether this is related to the decreased availability of voles, the harrier's staple diet.

A final strength of the tiered approach to risk assessment is related to resolving the question "how does one go about measuring when clean is clean enough?" The tiered approach provides some guidance: for example, if surrogate organisms are used as part of a Tier 3 evaluation of exposure (i.e., nest boxes), this assessment process could be left intact, or repeated, as an on-site biomonitoring assessment following mitigation. If mitigation truly reduced bioavailability, the exposure in the surrogate species should measurably decline. If biochemical markers of exposure indicate no exposure, then the contaminant (even if at detectable levels in soil) is not being taken up by the organisms. Hence, a measure of the success of clean up efforts becomes available.

The summary decisions and projections of risk within the Risk Description phase concludes the risk assessment process and provides the basis for communication between the risk assessor and the risk manager, ultimately responsible for making the appropriate regulatory decisions.

#### **4.7 Risk Management**

Environmental cleanup actions have technical and social foundations. At many sites, various stakeholders and stakeholder groups have divergent interests and concerns. Remedial activities are truly effective when stakeholder interests are satisfied. For example, the site assessment team might agree that low, but elevated, concentrations of a particular contaminant could remain in place without adverse effects. Owners of adjacent properties, concerned about real estate values, might be more concerned about *de minimis* residual contamination. Or the risk management team might determine that destructive remediation of a wetland is warranted by contaminant levels, while local recreational boaters might desire simple monitoring.

Clearly, there is a trade off in risk management, between destructive remediation (all currently available technologies destroy the habitat in place) and residual contamination. While it is desirable to make decisions on a "risk averse" basis, it is not always clear what is "riskier": site remediation or site contamination. Risk assessment uncertainty (described below) plays a crucial role in this decision threshold, because the risk of remedy associated with site cleanup is highly certain, and must be balanced against the weight of evidence for contaminant-related risks.

The trade off between risks due to existing contaminants and those due to remediation was illustrated at a midwestern site by Hinckley and Porter<sup>77</sup>. These authors demonstrated that removal of lead from a wetland entailed its destruction, while only providing minimal reduction in hazard quotients for mice and raptors.

Once the decision has been made to undertake site cleanup, the nature and extent of remedial activities must be determined. With the exception of highly contaminated "hotspots", these definitions are best supported by Tier 2 and 3 evaluations with decision criteria developed in advance.

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**Tri-Service Procedural Guidelines for Ecological Risk Assessments**

**VOLUME 2**

**Research and Biomonitoring Methods for the Characterization of  
Ecological Effects**

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CONTENTS

INTRODUCTION . . . . . 1

APPENDIX A - MODELS OF USE IN ECOLOGICAL RISK ASSESSMENT

AERIS . . . . . A-1  
Aquatic Food Chain Models . . . . . A-2  
CREAMS . . . . . A-3  
Enpart . . . . . A-5  
EXAMSII . . . . . A-6  
FGETS . . . . . A-8  
GEOTOX . . . . . A-10  
GLEAMS . . . . . A-12  
MINTEQAII . . . . . A-13  
Persistence . . . . . A-14  
PIRAHNA . . . . . A-15  
QWASI . . . . . A-16  
SIMPLESAL . . . . . A-17  
SMCM . . . . . A-18  
Toxscreen . . . . . A-19  
WQAM . . . . . A-20  
SLSA . . . . . A-21  
MICHRIV . . . . . A-22  
SARAH . . . . . A-23  
SESOIL . . . . . A-24  
LPMM . . . . . A-25  
RAPS . . . . . A-26  
RWSTM . . . . . A-27  
CFEST . . . . . A-28  
SWIFT AND SWIFT II . . . . . A-29  
CTAP . . . . . A-30  
HPSF . . . . . A-31  
TODAM . . . . . A-32  
CHNTRN . . . . . A-33  
FETRA . . . . . A-34  
WASP4/DYNHYD5 . . . . . A-35  
Sediment Chronology Models . . . . . A-36  
PRZM . . . . . A-37  
SERATRA . . . . . A-38  
CMLS . . . . . A-39  
LEACHM . . . . . A-41

**APPENDIX B - TECHNICAL TEST METHODS FOR USE IN ECOLOGICAL  
RISK ASSESSMENT**

**Biochemical**

Cytochrome P450 Induction . . . . .	B-1
Cholinesterase Inhibition . . . . .	B-3
Porphyrin Profiles . . . . .	B-5
Delta-Aminolevulinic Acid Dehydratase . . . . .	B-7
Metabolic Products . . . . .	B-9
Metallothionein Induction . . . . .	B-11
Stress Protein Induction . . . . .	B-13

**Genetic**

Clastogenicity Tests . . . . .	B-15
Ames Test . . . . .	B-17
Detection of DNA Adducts . . . . .	B-19
Secondary Modification of DNA . . . . .	B-21

**Histopathological**

Trace Metals in Tissues . . . . .	B-23
Skeletal Abnormalities . . . . .	B-25
Hepatic Histopathology . . . . .	B-27

**Immunological**

Macrophage Phagocytosis . . . . .	B-29
Lymphocyte Blastogenesis . . . . .	B-31

**Microbial**

Microbial Toxicity Tests . . . . .	B-33
Microbial Ecological Effects Tests . . . . .	B-35
Microtox™ . . . . .	B-37
Soil-core Microcosm . . . . .	B-39
Soil Microbial Activity . . . . .	B-42
Soil Lipid Chemistry . . . . .	B-45
Nitrogen Cycling in Soil . . . . .	B-47
Uptake and Utilization of Organic Compounds by Microbes . . . . .	B-49
Soil Respiration . . . . .	B-51

**Vegetative**

Seed Germination and Root Elongation . . . . .	B-53
Early Seedling Survival and Vegetative Vigor . . . . .	B-55
Rooted Aquatic and Wetland Plants . . . . .	B-57
Plant Uptake Bioassay . . . . .	B-59
TOXSCREEN . . . . .	B-61
Vascular Plant Life-cycle Tests . . . . .	B-63
Plant Tissue Culture Tests . . . . .	B-65
Plant Community Structure Mesocosm . . . . .	B-67

Phytotoxicity Testing with Ambient Air Exposure Systems	B-69
Chlorophyll Fluorescence Bioassay . . . . .	B-71

Aquatic

Aquatic Organism Toxicity Tests . . . . .	B-73
Sediment Toxicity Tests . . . . .	B-76
Amphibian Test Methods . . . . .	B-78
Static Microalgae Toxicity Test . . . . .	B-80
Early Life Stage Fish Toxicity Test . . . . .	B-82
Conducting Three Brood, Renewal Toxicity Tests with <u>Ceriodaphnia dubia</u> . . . . .	B-85

Invertebrate

Earthworm Survival and Sublethal Effects . . . . .	B-87
Free-Living Nematode Survival and Sublethal Effects. . .	B-90
Terrestrial Arthropods (Insects) . . . . .	B-92
Terrestrial Arthropods (non-insect) and Isopods . . . .	B-94
Invertebrate Immunotoxicity . . . . .	B-97
Mollusks (Terrestrial and Wetland) . . . . .	B-99

Mammalian

Use of Small Mammals to Assess Exposure and Effects . .	B-101
---	-------

Avian

Use of Avian Species to Assess Exposure and Effects . .	B-103
Avian Eggshell Thinning . . . . .	B-105

Biotic Surveys

Fish Survey . . . . .	B-107
Plankton Survey . . . . .	B-109
Periphyton Survey . . . . .	B-111
Analysis of Benthic Macroinvertebrate Populations . . .	B-113
Presence/Absence of Indicator Species . . . . .	B-115
Remote Sensing of Vegetation . . . . .	B-117
Digital Imaging Analysis of Vegetation . . . . .	B-119
Plant Ecological Surveys . . . . .	B-121
Soil Fauna Microcosm . . . . .	B-123
Bacterial Biomass in Soils . . . . .	B-125
Fungal Biomass in Soils . . . . .	B-127
Protozoan Numbers and Diversity . . . . .	B-129

**APPENDIX C - TECHNICAL TEST METHODS UNDER DEVELOPMENT  
FOR ECOLOGICAL RISK ASSESSMENT**

Adenosine Triphosphatase Activity . . . . .	C-2
Adenylate Energy Charge . . . . .	C-3
Plant Enzyme Activity . . . . .	C-4
Scope for Growth . . . . .	C-5
Protein Synthesis . . . . .	C-6

Oncogene Activation . . . . .	C-7
Genetic Mutation Rates . . . . .	C-8

## INTRODUCTION

Volume 2 of this report contains summaries of research and biomonitoring methods useful in characterizing ecological effects at hazardous waste sites. The information presented in these summaries is intended to present the Risk Assessor with an overview of test methods and to provide references about the suitability of a particular test for a particular application.

There are two caveats before using any tests in this volume:

1) The test summaries should not be used as the sole source of information when deciding which tests to use to characterize ecological effects. However, with professional judgement and using teams of experts, the RA may decide on a suite of tests, given the personnel and cost limitations within the Tiered approach.

(2) It is important for the RA to realize that there may be considerable variation in certain of the described test methods, as several summaries describe closely related tests. Summaries of this sort describe tests in which different analytical techniques are used to assess the same or similar test endpoints. Further, several of the tests are not standardized in the sense of having EPA or ASTM approval; hence, professional expertise is required before deciding on which to use.

There may be considerable variation from the stated times required to perform a particular test. During the development of the summaries, the amount of training or time required to perform a particular test was often difficult to categorize. For example, many biochemical tests require very little time to run individual samples; however, it is seldom the case when characterizing ecological effects that the RA perform analyses on single samples. As is the case with field testing, assessment of many samples may be arduous and time consuming.

The Technical Summaries are organized in a manner intended to make the information easy to assimilate. Categories for each summary (e.g., description, references) are self explanatory; however, a few need further explanation. The category "Logistical Considerations" is subdivided into two subfields, "Sample Collection" and "Sample Analysis." Categorical entries of "Minimal," "Moderate," and "Extensive" have been used instead of definitive values for these subfields. We have defined the categories as follows:

### Training

Extensive - six months experience or greater performing the analysis or sample collection.

Moderate - Less than six months experience but more than a high school education.

Minimal - High school education.

Time

Intensive - three months or more to assess a single sample.

Moderate - one week to three months.

Minimal - Less than one week.

## APPENDIX A

### MODELS OF USE IN ECOLOGICAL RISK ASSESSMENT

**Model Name:** AERIS

**Model Type:** Fate, Multimedia

---

#### **Description:**

This is a risk assessment model that estimates environmental concentrations and subsequently, human exposure in the vicinity of contaminated land sites. It is intended for use at sites where redevelopment is under consideration. The model runs within a user-friendly expert system programming environment. An "intelligent" preprocessor interrogates the user about the redevelopment scenario to be assessed, assisting where necessary, or supplying default values.

It estimates pseudo steady-state concentrations of contaminant in compartments such as air and groundwater based on the concentration of the contaminant in the soil. These predicted environmental concentrations are used to estimate the exposure incurred by a site user. It is intended to provide a consistent approach to establishing soil guidelines and identifying cleanup objectives.

---

#### **Key References:**

Senes Consultants. 1989. Contaminated Soil Cleanup in Canada, vol. 5, Development of the AERIS model, Final Report prepared for the Decommissioning Steering Committee.

Senes Consultants. 1989. Contaminated Soil Cleanup in Canada, vol. 6, User's guide for the AERIS model, Prepared for the Decommissioning Steering Committee.

---

#### **Logistical Considerations:**

**Equipment:** IBM-PC compatible computer

---

#### **Critique/Comments:**

Use of the model requires many input parameters. The model employs a user friendly interactive computer program to examine on-site human health risks of relatively old contamination. It is inappropriate for recent spills. AERIS allows the user to calculate risks from a site or to develop cleanup levels. It lacks transport features and is not useful for predicting the fate of complex mixtures. Unfortunately, the model is currently unavailable for public use.

**Model Name:** Aquatic Food Chain Models  
**Model Type:** Exposure, Bioaccumulation, Aquatic, Toxicant Uptake, Food Chain

---

**Description:**

The models were developed for calculating the concentration of organic chemicals in a simple generic aquatic food chain. Chemical uptake efficiency from water, excretion rate and chemical assimilation efficiency are variable as a function of the octanol water partition coefficient,  $K_{ow}$ . The models indicate the significance of the growth rate and variable efficiency of uptake in the calculation of a bioconcentration factor BCF under field conditions.

The models extend a previously developed steady state bioconcentration model of the distribution of chemicals as a function of trophic level in the ecosystem.

---

**Key References:**

Clark, J.R., F.A.P.C. Gobas and D. Mackay. 1990. Model of organic chemical uptake and clearance by fish from food and water. Environ. Sci. Technol. 24:1203-1213.

Landrum, P.E., H. Lee II and M.J. Lydy. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ. Toxicol. Chem. 11:1709-1725.

Thomann, R.V. 1988. Deterministic and statistical models of chemical fate in aquatic systems. In: Ecotoxicology: Problems and Approaches. Springer Advanced Texts in Life Sciences. Springer, New York. Chapter 10, pp. 245-277.

Thomann, R.V. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol. 23:699-707.

Thomann, R.V. and J.P. Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18:65-71.

---

**Logistical Considerations:**

**Equipment:** IBM-PC compatible computer

---

**Critique/Comments:** The model equations have been used successfully to calculate the concentration of organic chemicals in a diversity of aquatic food chains.

**Model Name:** CREAMS (Chemicals, Runoff and Erosion from Agricultural Management Systems)

**Model Type:** Fate, Nutrient runoff, Inorganic Chemical

---

**Description:**

The CREAMS model is structured into three components: hydrology, erosion/sedimentation, and chemistry. The model is useful in simulating stormloads and sediment-associated and dissolved chemicals in the runoff, sediment and percolate fractions. For example, it estimates soluble and sediment attached nitrogen (N) and phosphorus (P) in runoff. A nitrogen submodel also considers plant uptake, denitrification, mineralization of organic nitrogen and leaching of nitrate. Fertilizer nitrogen and phosphorus can be added to the surface or incorporated in the profile in single or multiple applications during the year.

---

**Key References:**

Heatwole, C.D., K.L. Campbell and A.B. Bottcher. 1988. Modified CREAMS Nutrient Model for Coastal Plain Watersheds. Trans. Amer. Soc. Ag. Engineers 31:154-160.

Knisel, W.G. 1980. CREAMS: A field scale model for chemical runoff, and erosion from agricultural management systems. U.S.D.A. Conservation Research Report Number 26.

Knisel, W.G., G.R. Foster and R.A. Leonard. 1983. CREAMS: A system for evaluating management practices. In: Schaller, F.W. and G.W. Bailey (eds.) Agricultural Management and Water Quality Iowa State University Press, Ames, pp. 178-199.

---

**Logistical Considerations:**

**Equipment:** Unavailable

---

**Critique/Comments:** A major utility of CREAMS has been the evaluation of alternate management practices for control or minimization of runoff of sediment and chemicals. Several alternate practices might be proposed for a given site. Each could be evaluated with CREAMS, and the responsible party could select a practice to minimize chemical movement offsite. CREAMS has been tested in a number of research watersheds in several land resource areas. Results show that the model can be applied successfully by estimating parameter values from information in Conservation Research No. 26 (Knisel, 1980). Use of observed data for a site may be useful in improving model accuracy for the site for Tier II or III testing, but is unnecessary for Tier I tests. Testing has shown that the model adequately represents changes in

management practices and that relative differences between practices are valid.

**Model Name:** Enpart (Environmental Partitioning Model)  
**Model Type:** Fate, Exposure, Multimedia

---

**Description:**

Enpart was developed by the U.S. EPA as a first-level screening tool for new and existing organic chemicals of possible concern. It is a fugacity based model which estimates the steady-state equilibrium or dynamic partitioning of organic chemicals among environmental compartments. It identifies dominant pathways and data gaps, and estimates the chemicals persistence and bioconcentration potential.

The data required by the model include the properties of the chemical and some environmental parameters such as soil and sediment density, suspended sediment and biota concentrations. The output is in the form of concentration ratios between compartments rather than absolute concentrations.

---

**Key References:**

- OECD (Organization for Economic Cooperation and Development). 1989. Compendium of Environmental Exposure Assessment Methods for Chemicals. Environment Monographs, No. 27, OECD, Paris.
- Mackay, D. and S. Paterson. 1993. Mathematical models of transport and fate. In: G. Suter (ed.), Ecological Risk Assessment, Lewis Publishers, Chelsea, Michigan. pp. 129-152.
- 

**Logistical Considerations:**

**Equipment:** Unavailable

---

**Critique/Comments:** It is an easy-to-use approximate method intended to indicate chemicals which may require further testing (Mackay and Paterson, 1993).

**Model Name:** EXAMSII

**Model Type:** Fate, Transport, Exposure, Aquatic

---

**Description:**

This is an interactive mass balance model developed at the U.S. EPA Research Laboratory in Athens, GA, which predicts the fate of organic contaminants in stratified surface waters as a result of continuous or intermittent releases. It is widely used by the EPA and other environmental agencies in the U.S.

The water body is subdivided into zones, or segments, the mass balance of each segment being described by a differential equation. The resulting set of equations, which describes the mass balance of the entire system, incorporates comprehensive transport and transformational processes. It allows for loadings by point or nonpoint sources, dry fallout or aerial drift, atmospheric wash-out and groundwater seepage to selected segments.

The user has the choice of three operating models determined by the complexity of the problem under study. The modes range from a steady-state solution for a continuous release of a contaminant to the dynamic solution of a time varying source. Input data requirements are generally intensive.

The latest version of EXAMS is applied in formulating aquatic ecosystem models and rapidly evaluating the fate, transport and exposure concentrations of synthetic organic chemicals-- pesticides, industrial materials and leachates from disposal sites. EXAMS contains an interactive Database Management System (DBMS) designed for storage and management of project databases. User interaction is enhanced with an on Command Line Interface (CLI), context sensitive help menus, an on-line data dictionary and CLI user's guide and plotting capabilities for review of output data. EXAMS has 20 output tables which both document datasets and provide integrated results for aid in ecological risk assessments.

---

**Key References:**

Burns, L.A., D.M. Cline and R.R. Lassiter. 1982. Exposure Analysis modeling system (EXAMS): User manual and system documentation. EPA/600/3-82/023, U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

---

**Logistical Considerations:**

**Equipment:** Vax

---

**Critique/Comments:** The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** FGETS (Food and Gill Exchange of Toxic Substances)  
**Model Type:** Exposure, Bioaccumulation

---

**Description:**

This is a fortran simulation that predicts temporal dynamics of a fish's whole body concentration ( $\mu\text{g}$  chemical/ grams live weight fish) of non-ionic, non-metabolized, organic chemicals that are accumulated from water and food. The model is based on a set of diffusion and forced convection partial differential equations, coupled to a process-based fish growth formulation. The theoretical basis and equation development are presented in Barber, et al. (1991). The model also calculates the time to reach a lethal activity in fish assuming that the chemical has a narcotic mode of action.

The model considers both biological attributes of the fish and physico-chemical properties of the chemical that determine diffusive exchange across gill membranes and intestinal mucosa. Important biological characteristics used by the model include the fish's gill morphometry, body weight, and fractional aqueous, lipid and structural organic composition. Physico-chemical properties of importance include the chemical's aqueous diffusivity, molar volume, and n-octanol/water partition coefficient ( $K_{ow}$ ), which is used as a surrogate to quantify chemical partitioning into the fish's lipid and structural organic fractions.  $K_{ow}$  is used in calculating the fish's bioconcentration factor, molecular volume is used to estimate aqueous diffusivity, and melting point is used in conjunction with  $K_{ow}$  to calculate the chemical's activity within the fish.

The model is parameterized for a particular fish species by means of a morphological and physiological database that delineates the fish's gill morphometry, feeding and metabolic demands, and body composition. The database currently holds data for five fish families: salmonidae, centrarchidae, cyprinidae, percidae and ictaluridae.

Three simulation modes in FGETS v.3 are (a) laboratory, (2) food chain and (3) food web. The first mode is for description of bioconcentration or bioaccumulation under controlled laboratory conditions. The latter two modes are for modelling these processes in field conditions.

---

**Key References:**

Barber, M.C., L.A. Suárez and R.R. Lassiter. 1991. Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Great Lakes salmonids. Canadian Journal of Fisheries and Aquatic Sciences 48:318-337.

---

**Logistical Considerations:**

**Equipment:** IBM-PC compatible computer

---

**Critique/Comments:** A maximum of 10 chemicals may be simulated simultaneously by the VAX version; the PC version has a limit of 4 chemicals. A maximum number of species is 5 for the VAX version; the PC version has a limit of 3 species. The maximum number of observations per species is 50 for the VAX version; the PC version has a limit of 20 observations per species. The maximum number of age classes per species is 15 for both VAX and PC versions. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** GEOTOX  
**Model Type:** Fate, Multimedia

---

**Description:**

A multimedia compartmental model developed under contract from the U.S. government (DOE and Army) which calculates chemical partitioning, degrading reactions, and diffusive and nondiffusive interphase transport. The concentrations estimated for environmental compartments are combined with human inhalation and ingestion rates and absorption factors to calculate exposure. It consists of eight compartments: air (gas), air (particles), biomass, upper soil, lower soil, groundwater, surface water and sediments. These compartmental media are assumed to be composed of subphases of gas, liquid and solid. Environmental dimensions and characteristics can be adjusted to represent other regions. Chemical partitioning between compartments, interphase transport, reaction and advective losses are described by first-order rate constants. The model can be applied to constant or time-varying chemical sources.

The soil is treated as three layers: upper soil layer, lower soil layer and groundwater zone. The soil layers are described by depth, bulk density, porosity, water content, and fraction organic carbon parameters. The groundwater zone consists of solids with fluid filled pore space. Processes of adsorption, ion exchange, precipitation, colloidal infiltration and irreversible mineralization in the groundwater are incorporated by means of sorption partitioning constants and expressions. The water phase consists of water, biota and suspended solids in equilibrium.

Concentration of a chemical in land biomass is calculated as the product of the soil concentration and the plant/soil partition coefficient. This is an equilibrium type expression incorporating vegetation production or growth rate. Animal biomass is not considered in the mass balance but is treated as an exposure vector.

The model output is in the form of environmental concentrations, intake by various exposure pathways and total intake. A measure of relative health risk can be calculated for a number of chemicals.

---

**Key References:**

- McKone, T.E. and D.W. Layton. 1986. Screening the potential risks of toxic substances using a multimedia compartment model: Estimation of human exposure. *Regul. Toxicol. Pharmacol.* 6:359-380.

---

**Logistical Considerations:**

**Equipment:** Unavailable

**Data Requirements:**

- physical-chemical properties
- degradation rate constants
- emission data
- environmental characteristics  
for example, fraction of land surface covered by water  
and average depth

---

**Critique/Comments:**

**Model Name:** GLEAMS

**Model Type:** Fate, Groundwater Nutrient Loading

---

**Description:**

This is a modified version of the CREAMS model that takes into account vertical pesticide movement in the root zone. The model retains the same runoff component structure found in CREAMS, but links the hydrology, erosion and pesticide submodels into one program for better efficiency in computer operation. Input files for the model are simpler and the maximum simulation time is increased to 50 years.

---

**Key References:**

Leonard, R.A., W.G. Knisel and D.A. Still. 1987. GLEAMS: Groundwater loading effects of agricultural management systems. Trans. ASAE 30:1403-1418.

Knisel, W.G. and R.A. Leonard. 1986. Impact of irrigation on groundwater quality in humid areas. Water Forum 86. pp. 1508-1515. In: World Water Issues in Evolution. Proc. of ASAE Spec. Conf., Long Beach, CA. 4-6 Aug. Am. Soc. Civil Engineering, Boise, ID.

---

**Logistical Considerations:**

**Equipment:** Unavailable

---

**Critique/Comments:** The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** MINTEQAII  
**Model Type:** Fate

---

**Description:**

This is an equilibrium metal speciation model applicable to metallic contaminants in surface and groundwaters. It is different in purpose than the mass balance models for organic contaminants. It calculates the equilibrium aqueous speciation, adsorption, gas phase partitioning, solid phase saturation states and precipitation-dissolution of 11 metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium and zinc). It contains an extensive thermodynamic base and is designed to make minimal demands on the user.

A degree of expertise regarding kinetic limitations at particular sites is required for proper application of the model. The output is a description of the major metal species in the system.

---

**Key References:**

Brown, D.S. and J.D. Allison. 1987. MINTEQAII Equilibrium metal speciation model: A user's manual. U.S. EPA, Athens, GA.

---

**Logistical Considerations:**

**Equipment:** IBM compatible PC

---

**Critique/Comments:** The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** Persistence  
**Model Type:** Fate, Aquatic

---

**Description:**

This model was developed for the National Research Council of Canada as a screening method to estimate the fate of organic chemicals, especially pesticides, that are released into the aquatic environment. There are four compartments in the model: water, catch-all (including suspended solids, invertebrates and other aquatic life, excluding fish), sediment and fish. The model can calculate both steady-state or time-dependent solutions. Default environments for the model are a Standard Pond and a Standard Lake simulating a small eutrophic pond and a deep, oligotrophic lake. Removal pathways include photodegradation, volatilization and hydrolysis in water; biodegradation in fish; and microbial degradation in suspended solids and sediments.

Output for the steady-state model is in a tabular form only, whereas solutions for the dynamic model can take the form of tables or concentration-time curves for various compartments. The overall persistence of the system is also calculated.

---

**Key References:**

Asher, S.C., K.M. Lloyd, D. Mackay, S. Paterson and J.R. Roberts. 1985. A critical examination of environmental monitoring-modeling the fate of chlorobenzenes using the persistence and fugacity models. Rep. No. NRCC 23990. National Research Council of Canada.

Roberts, J.R., M.S. Mitchell, M.J. Boddington and J.M. Ridgeway. 1981. A screen for the relative persistence of lipophilic organic chemicals in aquatic ecosystem- An analysis of the role of a simple computer model in screening, Part I. National Research Council of Canada. Rep. No. NRCC 18570, Ottawa, Canada.

---

**Logistical Considerations:**

**Equipment:** Mainframe

---

**Critique/Comments:** Requires measures or estimates of photodegradation, volatilization and hydrolysis in water; biodegradation in fish.

**Model Name:** PIRAHNA (Pesticide and Industrial Chemical Risk Analysis and Hazard Assessment, version 2.0)  
**Model Type:** Fate, Bioaccumulation, Databases

---

**Description:**

PIRAHNA is an ecological risk tool designed for analysts who have environmental safety responsibilities for synthetic chemicals. It is a vehicle for transmittal of results of the U.S. EPA's Office of Research and Development (ORD) Ecological Risk Assessment ("EcoRisk") Research program to the user community-- EPA and State regulatory scientists, and industrial chemical safety specialists. The documentation for PIRAHNA is being released in annual updates over the period from 1990 to 1995. The final version will encompass analytical capabilities ranging from the estimation of chemical properties from chemical structure through risks attending chemical releases to whole ecosystems.

---

**Key References:**

Burns, L.A., B.W. Allen, Jr., M.C. Barber, S.L. Bird, J.M. Cheplick, D.R. Hartel, C.A. Kittner, F.L. Mayer, L.A. Suarez and S.E. Wooten. 1991. PIRAHNA, version 2.0.

---

**Logistical Considerations:**

**Equipment:** IBM compatible PC

---

**Critique/Comments:** PIRAHNA is composed of three models described individually in this chapter (PRZM, EXAMSII, FGETS). PIRAHNA also includes agricultural crop census and ichthyofaunal geographic range databases.

**Model Name:** QWASI  
**Model Type:** Fate, Multimedia, Fugacity

---

**Description:**

The model was developed by Mackay et al. (1983) to treat the fate of a chemical discharge to a water-air-sediment system using Z and D values, volumes, areas, flows and the input parameters to give a steady-state algebraic solution. Algebraic and numerical time-dependent solutions can be written by the user. The program is not "user-friendly". The conditions simulated in the program are similar to those described by Mackay (1989) for the fate of PCBs in Lake Ontario.

---

**Key References:**

Mackay, D. 1989. Modeling the long term behaviour of an organic contaminant in a large lake: Application to PCBs in Lake Ontario. J. Great Lakes Res. 15:283-297.

Mackay, D., S. Joy and S. Paterson. 1983. A quantitative water air sediment interaction (QWASI) model for describing the fate of chemicals in lakes. Chemosphere 14:335-374.

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**Logistical Considerations:**

**Equipment:** IBM compatible PC

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**Critique/Comments:** The user must specify conditions by editing the appropriate lines of code. Some users find it more convenient to write the equations into a spreadsheet, such as Lotus 1-2-3 or Quattro Pro, from which the results can be plotted directly.

**Model Name:** SIMPLESAL

**Model Type:** Fate, Exposure, Multimedia, Fugacity

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**Description:**

This is a spreadsheet-based model which can be used to estimate steady-state or time-dependent concentrations of organic compounds as well as heavy metals. It determines dominant environmental pathways and processes for contaminants, and was designed for use in the Netherlands as a screening tool to predict results of various scenarios for emission control of new and existing chemicals such as benzene, cadmium, lindane and copper. It considers air, water, suspended solids, aquatic biota, sediment and soil compartments.

The model incorporates processes of advective flows, diffusive and non-diffusive transfer, bioconcentration in aquatic biota, leaching from soil to groundwater, and biotic and abiotic transformation.

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**Key References:**

OECD (Organization for Economic Cooperation and Development). 1989. Compendium of Environmental Exposure Assessment Methods for Chemicals. Environment Monographs, No. 27, OECD, Paris.

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**Logistical Considerations:**

**Equipment:** Mainframe

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**Critique/Comments:** Data required to run the model include dimensions, properties of and emissions into environmental media; air and water residence times; parameters for intercompartment transfer in association with particulates; physico-chemical properties of water solubility, vapor pressure and octanol-water partition coefficient and degradation rate constants.

**Model Name:** Spatial Multimedia Compartmental Model (SMCM)  
**Model Type:** Fate, Multimedia

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**Description:**

The model was developed by the National Center for Intermedia Transport at UCLA. It describes the fate of chemicals in a conventional air-water-soil-sediment system under steady-state or dynamic conditions. It allows for concentration variation with depth in the soil or sediment.

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**Key References:**

Cohen, Y. 1989. The Spatial Multimedia Compartments Model (SMCM), User's Manual Version 3.0, NCITR, UCLA, CA

Cohen, Y., Tsai, W., S.L. Chetty and G.L. Mayer. 1990. Dynamic partitioning of organic chemicals in regional environments: A multimedia screening-level modeling approach, Environ. Sci. Technol. 24:1549-1558.

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**Logistical Considerations:**

**Training:** Software can be run with virtually no background in transport phenomena.

**Equipment:** IBM compatible PC

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**Critique/Comments:** The model is user friendly with help menus and the capacity of presenting data output in tabular or graphical form.

**Model Name:** Toxscreen  
**Model Type:** Fate, Multimedia

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**Description:**

Toxscreen is a time-dependent multimedia model, developed by the U.S. EPA to assess the potential for environmental transport and accumulation of chemicals released into the air, surface water or soil. It is modular in concept and incorporates intermedia transfer processes. It is a screening tool to assess the potential for human exposure to chemicals.

Atmospheric dispersion is incorporated into the model using a Gaussian plume dispersion model. Contaminant migration in soil following direct application and transport between other media is estimated by means of the SESOIL model. Pollutant concentrations in water bodies are estimated over a period of time using a method similar to the EXAMS model.

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**Key References:**

Hetrick, D.M. and L.M. McDowell-Boyer. 1983. User's manual for TOXSCREEN: A multimedia screening level program for assessing the potential fate of chemicals released into the environment. ORNL/TM-8570. Oak Ridge National Laboratory, Oak Ridge, TN.

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**Logistical Considerations:**

**Equipment:** Mainframe

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**Critique/Comments:** Data requirements are extensive, including information on the characteristics of the environment treated and emission sources; chemical and degradation parameters; and climatological, meteorological and hydrological parameters. Data files on climatic and soil conditions for some regions are included with the model. The output is in the form of an estimation of the fate of a chemical over a period of time.

**Model Name:** WQAM (Water Quality Assessment Methodology)  
**Model Type:** Surface Water Fate

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**Description:**

This is a steady state, one dimensional model; requiring only desktop calculations. It provides canonical information. The methods are useful for modeling lakes, rivers and estuaries.

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**Key References:**

Mills, W.B., J.D. Dean, D.B. Porcella et al. 1982. Water quality assessment: a screening procedure for toxic and conventional pollutants: parts 1, 2 & 3. Athens, GA: USEPA. Environmental Research Laboratory. Office of Research and Development. EPA 600/6-82/004 a,b,c.

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**Logistical Considerations:**

**Equipment:** Hand calculator.

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**Critique/Comments:** Recommended if time, costs or information are restrictive.

**Model Name:** SLSA (Simplified Lake/Stream Analysis)  
**Model Type:** Surface Water Fate

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**Description:**

This is a steady state, one dimensional model. It may be solved by desk top calculation or via a simple computer program in languages such as Fortran, Pascal or BASIC or through calculations in a spreadsheet. It is suited to simplified lake or river systems.

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**Key References:**

Hydroqual, Inc. 1982. Application guide for CMA-Hydroqual chemical fate models. Prepared for: Chemical Manufacturer's Association, Washington, DC. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington,DC: US EPA contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** Hand calculator or IBM compatible PC

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**Critique/Comments:** Well documented and suggested for use prior to use of a more sophisticated model.

**Model Name:** MICHRIV (Michigan River Model)  
**Model Type:** Surface Water Fate

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**Description:**

This is a steady state, one dimensional model. It is a computer program written in Fortran. It is similar to SLSA, but is capable of handling more than one reach. It is intended for modeling metals fate and is suitable for rivers and streams.

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**Key References:**

Delos, C.G., W.L. Richardson, J.V. DePinto et al. 1984. Technical guidance manual for performing wasteload allocations, book II: streams and rivers. USEPA. Office of Water Regulations and Standards. Water Quality Analysis Branch. Washington, DC. (Draft Final).

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**Logistical Considerations:**

**Equipment:** IBM compatible PC

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**Critique/Comments:** Well documented and suggested for use prior to use of a more sophisticated model. It is easy to set up and use and requires minimal computer programming.

**Model Name:** SARAH (Surface Water Back Calculation Procedure)  
**Model Type:** Contaminant Fate in Surface Waters.

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**Description:**

This is a steady state, one dimensional analytical solution coded in Fortran. It is designed for simulation of contaminated leachate plume feeding the down gradient surface waterbody (stream or river). The solution is generated from a Monte Carlo simulated generic environment. It considers degradation, volatilization, dilution and sorption, and bioaccumulation in fish.

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**Key References:**

Jan. 14, 1986 Federal Register, Hazardous Waste Management System, Land Disposal Restrictions, Proposed Rule

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**Logistical Considerations:**

**Equipment:** Mainframe.

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**Critique/Comments:** The model requires minimal data input. Data on degradation, volatilization, dilution and sorption, and bioaccumulation in fish may be estimated by model default parameters.

**Model Name:** SESOIL (Seasonal Soil Compartment Model)  
**Model Type:** Unsaturated Zone and Groundwater Fate

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**Description:**

This is an integrated screening level soil compartment model designed to simultaneously model water transport, sediment transport and pollutant fate. It was developed for the EPA offices of Water Quality, and Toxic Substances (OTS). The model was originally coded in fortran but has since been encoded in PCGEMS (Graphical exposure modeling for the PC). It is a complete information management tool developed for EPA-OTS and designed to help users perform exposure assessments. PCGEMS has subsequently been transformed into a system called RISKPRO, which is an upgraded PCGEMS system.

The model may be used to simulate chemical releases to soil from sources such as landfill disposal, accidental leaks, agricultural applications, leaking underground storage tanks, or deposition from the atmosphere. Potential applications include long-term leaching from waste disposal sites, pesticide and sediment transport on watersheds, studies of hydrologic cycles on watersheds and water balances of soil compartments. The effect of site management or design strategies on pollutant distributions and concentrations in the environment may also be simulated.

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**Key References:**

Bonazountas, M. and J. Wagner. 1981. SESOIL, a seasonal soil compartment model. Cambridge, MA: A.D. Little, Inc. for USEPA. Contract No. 68-01-6271.

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**Logistical Considerations:**

**Programming Language:** Fortran

**Equipment:** PC-GEMS system (or RISKPRO) on a IBM compatible PC, VAX 11/780, IBM 370

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**Critique/Comments:** Versatile, easy to use.

**Model Name:** LPMM (Leachate Plume Migration Model)  
**Model Type:** Unsaturated Zone and Groundwater Fate

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**Description:**

This is a continuous source model that simulates dispersion from the a source. Degradation mechanisms are taken into account by the model. It is a simplistic model useful as a screening tool, but not for level III work.

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**Key References:**

Kent, D.C., W.A. Pettyjohn, F. Witz and T.A. Prickett. 1982. Prediction of leachate plume migration and mixing in groundwater. Solid and Hazardous Water Research and Development Annual Symposium proceedings. Columbus, OH: National Water Well Association. As reviewed in: Versar, Inc. 1983. Theoretical evaluation of sites located in the zone of saturation. Draft final report. Chicago, IL: USEPA. Contract No. 68-01-6438.

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**Logistical Considerations:**

**Programming Language:** must be written by user.

**Equipment:** Handheld calculator or computer.

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**Critique/Comments:** The model has been field-verified and is easy to use.

**Model Name:** RAPS (Remedial Action Priority System)  
**Model Type:** Fate, Remediation

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**Description:**

This system was developed by the U.S. Department of Energy to set priorities for investigation and possible cleanup of chemical and radioactive waste disposal sites. It is intended to be used in a comparative rather than predictive manner. The methodology considers four major pathways of contaminant migration: groundwater, surface water, overland, and atmospheric. Estimated concentrations in the air, soil, sediments, and water media are used to assess exposure to neighboring populations.

Data used to run RAPS includes on site and pollutant characteristics to simulate migration and fate from source to receptor by various pathways.

The estimated environmental concentrations form the basis of subsequent human exposure calculation and determination of the Hazard Potential Index (HPI). The modular development makes it useful for the inclusion of additional components.

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**Key References:**

Whelan, G., D.L. Strenge, J.G. Droppo, B.L. Steelman and J.W. Buck. 1987. The remedial action priority system (RAPS): Mathematical Formulations, PNL-6200. Pacific Northwest Laboratory, Richland, WA.

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**Logistical Considerations:**

**Equipment:** Mainframe

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**Critique/Comments:** The model methods are not truly multimedia, because it is based on use of independent modules which do not interact spatially or temporally; transfer of a pollutant is unidirectional. This modular framework permits updating of, or inclusion of, addition components with improvement of technology. The user supplies appropriate routes of chemical from waste site to neighboring populations through various media including air, groundwater, soil and vegetation.

**Model Name:** RWSTM (Random Walk Solute Transport Model; aka TRANS)  
**Model Type:** Unsaturated Zone and Groundwater Fate

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**Description:**

This is a one-dimensional or two-dimensional model accounting for time-variant release rates. The model accomodates well injected releases, incorporates dispersion and retardation and accounts for well pumping. It is capable of providing estimates of nonconservative pollutant concentrations at user selected points.

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**Key References:**

Prickett, T.A., T.G. Naymik and C.G. Lonquist. 1981. A "random-walk" solute transport model for selected groundwater quality evaluations. Champaign, IL: Illinois Department of Energy and Natural Resources. ISWS/BUL-65/81. As reviewed in: Versar Inc. 1983. Theoretical Evaluation of sites located in the zone of saturation. Draft Final Report. Chicago, IL: USEPA. Contract No. 68-01-6438.

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**Logistical Considerations:**

**Equipment:** Mainframe

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**Critique/Comments:** Model use requires mathematical programming and hydrogeological knowledge on the part of the user.

**Model Name:** CFEST (Coupled Fluid, Energy and Solute Transport)  
combined with UNSAT-ID  
**Model Type:** Unsaturated Zone and Groundwater Fate

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**Description:**

This is a three-dimensional model combination accommodating heterogeneous, anisotropic, multilayered soil configurations. The model combination can be utilized for saline and freshwater aquifers. Dispersive and advection transport mechanisms are simulated by the models; sorption and degradation mechanisms are not. It can be used in unsaturated and saturated zones for simulation of time-variant releases and flow rates.

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**Key References:**

Gupta, S.K., C.R. Cole, C.T. Kincaid and A.M. Monti. 1987.  
Coupled fluid, energy and solute transport (CFEST) model:  
formulation and user's manual. Columbus, Ohio: Office of  
Nuclear Waste Isolation, Battelle Memorial Institute.

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**Logistical Considerations:**

**Equipment:** Mainframe

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**Critique/Comments:** The model combination has been applied to arsenic and organic wastes.

**Model Name:** SWIFT and SWIFT II (Sandia Waste Isolation Flow and Transport Model)

**Model Type:** Unsaturated Zone and Groundwater Fate

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**Description:**

This is a three-dimensional model accommodating heterogeneous, anisotropic, multilayered soil configurations. The model combination can be utilized for saline and freshwater aquifers. Dispersive and advection transport mechanisms are simulated by the models. Sorption and degradation mechanisms are also taken into account. It is appropriate for use in waste-injection, waste-isolation simulation.

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**Key References:**

Finley, N.C. and M. Reeves. 1968. SWIFT self-teaching curriculum. Washington, DC: Nuclear Regulatory Commission. NUREG/CR-1968, SAND 81-0410. As reviewed in: Lo T.Y.R., B.H. Scott and R.R. Benjamin. 1983. Remedial action assessment models for hazardous waste sites. Review draft. Athens, GA: USEPA. Contract No. 68-03-3116.

Reeves, M. and R.M. Cranwell. 1981. User's manual for the Sandia Waste-Isolation Flow Transport Model (SWIFT). Washington, DC: Nuclear Regulatory Commission. NUREG/CR-2324, SAND 81-2516. As reviewed in: Lo T.Y.R., B.H. Scott and R.R. Benjamin. 1983. Remedial action assessment models for hazardous waste sites. Review draft. Athens, GA: USEPA. Contract No. 68-03-3116.

Software: National Energy Software Center  
Argonne National Laboratories  
Argonne, IL 60439

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**Logistical Considerations:**

**Programming Language:** Fortran

**Equipment:** Has been used on CDC systems, including CDC 7600

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**Critique/Comments:** The model has been field-verified. It comes with a user's guide written in a self-teaching format.

**Model Name:** CTAP (Chemical Transport and Analysis Program)  
**Model Type:** Surface Water Fate

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**Description:**

This is a steady state, three dimensional compartmental model. It is a computer program written in Fortran IV and suitable for numerous personal computers. It is similar to SLSA except more sophisticated. Each component of the model is equivalent to one SLSA lake. It is intended for modeling fate in streams, stratified rivers, lakes, estuaries and coastal embayments.

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**Key References:**

Hydroqual, Inc. 1982. Application guide for CMA-Hydroqual chemical fate models. Prepared for: Chemical Manufacturer's Association, Washington, DC. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: US EPA contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** Suitable for IBM 360/370, UNIVAC 108, CDC 6600 mainframe computers and IBM compatible PC

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**Critique/Comments:** Well documented and suggested for use following use of a less sophisticated model. The model requires extensive data input.

**Model Name:** HPSF (Hydrological Simulation Program-FORTRAN)  
**Model Type:** Surface Water Fate

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**Description:**

This is a time varying, one dimensional model. It is a computer program written in Fortran and suitable for numerous personal computers. It is designed for year-round simulation of organic pollutant fate in non-tidal rivers, streams and mixed lakes according to a second order decay mechanism.

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**Key References:**

Johanson, R.C., G.C. Imhoff and H.H. Davis. 1984. Hydrocomp Inc. User's manual for hydrological simulation program - Fortran (HSPF). Athens, GA: Office of Research and Development, USEPA. EPA-600/9-80-015. As reviewed in: Versar, Inc. 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** IBM compatible PC. Can be used on mainframes.

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**Critique/Comments:** The model requires extensive data input. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** TODAM (Transient One-Dimensional Degradation and Migration Model)

**Model Type:** Surface Water Fate

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**Description:**

This is a time varying, one dimensional model. It is designed for simulation of second order decay processes in river and estuarine systems. The model requires use of an exterior hydrodynamic model to provide channel and flow velocities.

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**Key References:**

Onishi, Y., G. Whelan and R.L. Skaggs. 1982. Development of multimedia radionuclide exposure assessment methodology for low-level waste management. Athens, GA: Office of Research and Development, USEPA. As reviewed in: Versar Inc. 1983. Methology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** VAX or PDP 11/70

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**Critique/Comments:** The model requires extensive data input. It is a complex FORTRAN computer program written in the preprocessor language, FLECS, or in Fortran IV.

**Model Name:** CHNTRN (Channel Transport Model)  
**Model Type:** Surface Water Fate

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**Description:**

This is a time varying, one dimensional model. It is a complex FORTRAN IV computer program. It is designed for simulation of second order decay processes of organic pollutants in rivers, lakes, estuaries and coastal waters. The model can be coupled to a hydrodynamic model, CHNHYD, to estimate flow dynamics where such data are not available.

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**Key References:**

Yeh, G.T. 1982. CHNTRN: a chemical transport model for simulating sediment and chemical distribution in a stream/river network. Washington, DC: Office of Pesticides and Toxic Substances, USEPA. Contract No. W-7405-eng-26. As reviewed in: Versar 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** IBM 3933 and others.

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**Critique/Comments:** The model requires extensive data input and has an extensive set up time.

**Model Name:** FETRA (Finite Element Transport Model)  
**Model Type:** Surface Water Fate

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**Description:**

This is a time varying, two dimensional (longitude and latitude) model. It is designed for simulation of second order decay processes of organic pollutants in rivers, estuaries, coastal systems and completely mixed lakes. The model can be coupled to a hydrodynamic model, EXPLORE, to estimate flow dynamics where such data are not available.

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**Key References:**

Onishi, Y. 1981. Sediment-contaminant transport model. Journal of the Hydraulics Division, ASCE. 107(HY9):1089-1107. Proc. Paper 16505. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** IBM, VAX or CDC-7600 computers.

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**Critique/Comments:** It is written in FORTRAN IV computer programming language. The model requires extensive data input and has extensive setup and execution time requirements.

**Model Name:** WASP4 (Estuary and Stream Quality Model)  
**Model Type:** Surface Water Fate

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**Description:**

This is a time varying, three dimensional model. It is designed for simulation of second order decay kinetics of organic pollutants in rivers, lakes and estuaries.

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**Key References:** Unavailable

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**Logistical Considerations:**

**Equipment:** IBM 370 or PDP-11/70

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**Critique/Comments:** The user must provide hydrodynamic flows between model compartments. The model requires extensive data input and has extensive setup and execution time requirements. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** Sediment Chronology Models  
**Model Type:** Fate, Aquatic

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**Description:**

Bottom sediments serve as sinks for many metallic and hydrophobic contaminants. These models typically assume the possibility of estimating the historic condition of a lake by examining the variation of contaminant concentration with depth of burial. Considerable effort has been devoted to deducing the likely fate of buried chemicals when subject to diagenetic processes. These efforts usually take the form of multilayer models in which the year by year transport and transformation rates are estimated in slices of buried sediment. Examples of studies of this type are exemplified in Hites and Eisenreich (1987).

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**Key References:**

Hites, R.A. and S.J. Eisenreich (eds.). 1987. Sources and Fates of Aquatic Pollutants. Advances in Chemistry Series 216, ACS, Washington, D.C.

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**Logistical Considerations:**

**Equipment:** Personal computer, micro-computer

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**Critique/Comments:** Horizontal movement of contaminants should be considered when applying this type of model.

**Model Name:** PRZM (Pesticide Root Zone Model)  
**Model Type:** Unsaturated Zone and Groundwater Fate, Vertical  
Runoff

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**Description:**

PRZM is a dynamic compartmental model for use in simulating chemical movement in the unsaturated soil systems within and below the plant root zone. It is capable of simulating movement of potentially toxic chemicals, particularly pesticides, that are applied to soil or to plant foliage as pulse loads, predicting peak events and estimating time-varying mass emissions or concentration profiles.

There are three major components to the model: (a) water movement, (b) soil erosion and (c) chemical transport and transformation.

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**Key References:**

Carsel, R.F., C.N. Smith, L.A. Mulkey, J.D. Dean and P. Jowise. 1984. User's manual for the pesticide root zone model (PRZM) Release 1. USEPA EPA-600/3-84-109. U.S. Gov't Printing Office.

Williams, J.R. 1975. Sediment yield predictions with universal equation using runoff energy factor. In: Present and prospective technology for predicting sediment yields and sources. USDA-ARS, U.S. Gov't Printing Office. pp. 244-252.

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**Logistical Considerations:**

**Training:** PIC is designed to be easily assessible to the novice user.

**Time:** Basic understanding of the system can be gained within a day.

**Equipment:** Can be run utilizing a PC equipped with a math coprocessor.

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**Critique/Comments:** The current state of model utilization is through the PRZM Input Collator (PIC), which provides an interface between PRZM and a series of databases to allow efficient generation and modification of PRZM input data sets. PIC also contains utilities to allow the user to explore the data bases and screen geographically based information. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

**Model Name:** SERATRA (Sediment-Contaminant Transport)  
**Model Type:** Surface Water Fate

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**Description:**

This is a time varying, two dimensional (longitudinal and vertical) model that accounts for complex sediment transport mechanisms. It is designed for simulation of second order decay processes of organic pollutants in rivers and lakes.

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**Key References:**

For documentation: ORD Publications. Center for Environmental Research, Information, USEPA, Cincinnati, OH 45268 (513)-546-7562.

Onishi, Y. and S.E. Wise. 1982. Mathematical model, SERATRA, for sediment-contaminant transport in rivers and its application to pesticide transport in Four Mile and Wolf creeks in Iowa. Athens, GA: Office of Research and Development, USEPA. EPA-600-3/82-045. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Onishi, Y. and S.E. Wise. 1982. User's manual for instream sediment-contaminant transport model SERATRA. Athens, GA: Office of Research and Development, USEPA. EPA-600/3-82-055. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

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**Logistical Considerations:**

**Equipment:** Vax, mainframe

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**Critique/Comments:** It is written in FORTRAN preprocessor language FLECS< in batch mode. It has been field tested and is available for use. The model requires extensive data input and has extensive setup and execution time requirements. It is estimated to require a person 750 hours to prepare the model to run, assuming all data are readily available.

**Model Name:** CMLS (Chemical Movement in Layered Soils) Ver. 4.2  
**Model Type:** Unsaturated/Saturated Flow, Chemical Fate in Soils

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**Description:**

CMLS is an interactive microcomputer model. It was written to serve as a management tool and decision aid for the application of organic chemicals to soils. CMLS is an integrated soil compartment model designed to simultaneously model water and chemical transport, evapotranspirative effects, and the fate of non-polar organic chemicals. The model estimates the location of the peak concentration of the chemicals as they move through a soil in response to downward movement of water, and the relative amount of each chemical still remaining in the soil at any time. The model can handle soils with up to 20 different layers or horizons, so soil properties need not be assumed uniform by depth. Different partition coefficients and degradation half-lives for the chemicals of interest can be designated for each layer within the soil, to account for differing chemical/soil property interactions. Chemical movement and degradation can be simulated for up to 15 years. Results may be displayed in graphical as well as tabular form. The user may also request output designating the amount of time required for selected chemicals to move to user-specified depths within the soil profile.

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**Key References:**

Nofziger, D.L. and A.G. Hornsby. 1987. Chemical Movement in Layered Soils: User's Manual. Circular 780, Computer Series, Software in Soil Science, Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.

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**Logistical Considerations:**

**Training:** Software can be run on the basis of the information provided in the user's manual, however selecting appropriate input for the model requires knowledge of soil science and/or transport phenomena.

**Equipment:** Minimal requirements for CMLS Ver. 4.2 are an IBM compatible XT microcomputer with 512K bytes

of random access memory (RAM), two disk drives, and enhanced graphics array (EGA). Operating system must be DOS, version 2.0 or later. A printer and math coprocessor are beneficial.

**Data Requirements:**

For each soil layer/horizon

- depth
- percent organic carbon (%OC)
- soil bulk density
- water content at -0.01 -1.5 MPa, and saturation

For each chemical

- partition coefficient
- degradation half-life

Max. rooting depth of plants at soil surface

Initial depth of chemicals

Daily effective precipitation and evapotranspiration records

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**Critique/Comments:** Well documented, interactive, flexible and user-friendly. Model may be used for simple or more sophisticated modeling. CMLS is especially useful for modeling situations where diverse soil horizons or soil properties exist.

**Model Name:** LEACHM (Leaching Estimation And CHemistry Model)  
**Model Type:** Unsaturated Zone, Chemical Fate in Soils

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**Description:**

LEACHM is a process-based model that simulates water and solute movement in soils, chemical transformations and fate, plant uptake, and chemical reactions in the vadose zone. LEACHM is a general acronym that refers to three linked simulation models that describe the chemistry, transport, sorption, degradation, and volatilization of chemical compounds in the plant root zone and through the soil profile, with the three linked models sharing the same numerical solution scheme to simulate water and chemical transport. Chemical fate is determined in response to a variety of environmental parameters including soil characteristics; precipitation, evaporation, and transpiration of water; uptake of solutes and plant growth; and heat flow (temperature profiles). LEACHM includes the flexibility of simulating layered or non-homogeneous soil profiles. The three linked models consist of LEACHMP, LEACHMS, and LEACHMN. LEACHP simulates the chemistry, transport, and degradation of organic compounds, pesticides (P), in soils. LEACHMS is formulated to describe transient movement of inorganic salt solutes (S) (including Ca, Mg, Na, K, SO<sub>4</sub>, Cl, CO<sub>3</sub>, and HCO<sub>3</sub>) and corresponding soil chemical reactions. LEACHMN is organized to describe the transport and transformation of nitrogen (N) in soils.

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**Key References:**

- Wagenet, R.J. and J.L. Hutson. 1987. LEACHM: Leaching Estimation And CHemistry Model - A process-based model of water and solute movement, transformations, plant uptake and chemical reactions in the unsaturated zone. CONTINUUM, Vol. 2, Water Resources Institute. Center for Environmental Research, Cornell University, Ithaca, NY.
- Hutson, J.L. and R.J. Wagenet. 1988. Leaching Estimation And CHemistry Model: LEACHM - A user's guide. Department of Agronomy, Cornell University, Ithaca, NY.
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**Logistical Considerations:**

**Programming Language:** FORTRAN

**Training:** LEACHM is a sophisticated and relatively complex model. Before running the software, the user must read the references cited above and have a working knowledge of soil science and transport phenomena.

**Equipment:** Microsoft FORTRAN77 compiler (Ver. 3.2), on an IBM compatible PC operating under MS-DOS. A printer and math coprocessor are recommended.

**Data Requirements:**

Starting and last day nos.

Number of soil segments (layers/horizons)

Number of depth nodes (sub-segments)

Largest time interval for modeling

Max. theta (water) change/time step

Min. time interval/day

No. of water applications

No. of pesticide (organic chemical) applications

No. of fertilizer applications

Initial depth and concentrations

Initial theta (volumetric water content)

Initial temperature

Initial matric potential

Air entry value (AEV)

Selectivity coefficients for ion exchange

Pan evaporation

Water application and composition

- time
- amount
- rate

If plant cover is specified

- planting time (day)
- seedling emergence (day)
- plant maturity (day)
- mature root profile (day)
- harvest (day)
- roots (constant, or growing)
- relative root distribution (fraction)
- crop cover at maturity (fraction)
- min. and max. root zone water potential

- root flow resistance
- max. actual transpiration/potential transpiration

For each soil layer/horizon

- depth
- percent organic carbon (%OC)
- soil bulk density
- hydraulic conductivity value
- bottom boundary condition

For each chemical

- partition or distribution coefficient
- molecular diffusion coefficient
- empirical diffusion constant values (a and b)
- dispersivity
- saturated vapor density
- water solubility
- Campbell's exponent for retentivity
- initial concentration
- transformation/degradation products
- initial concentrations trans./degrad. prods.
- degradation half-life
- transformation constant

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**Critique/Comments:** Well documented, complex but flexible. Assistance running the model is available from co-author John L. Hutson (telephone: (607) 255-7631), Cornell University. Model is recommended for more sophisticated quantitative modeling of chemical fate. LEACHM is especially useful for modeling situations where diverse soil horizons or soil properties exist and there is substantial supporting soil/environmental data.

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**A-44**

## APPENDIX B

### TECHNICAL TEST METHODS FOR USE IN ECOLOGICAL RISK ASSESSMENT

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**Technique Name:** Cytochrome P450 Induction

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**Technique Type:** Enzyme Induction

**Matrix Type:** Biological Tissue

**Ecosystem Level:** Individual

**Test Location:** Laboratory

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#### Description:

This technique can be used to analyze the induction of enzymes such as cytochrome P-450IIB (phenobarbital type) and P-450IA (3-methylcholanthrene type) in the liver and kidney. These enzymes are involved in the oxidative metabolism of such compounds as fatty acids, steroids, prostaglandins, leukotrienes, biogenic amines, pheromones, and plant metabolites. Further, cytochrome P-450s metabolize innumerable drugs, chemical carcinogens, mutagens, and other environmental contaminants. In one assay used to detect the induction of cytochrome P450s, liver or kidney samples are taken from environmentally exposed individuals and are processed to create microsomes, which contain the membrane-bound cytochrome P-450s. The amount of cytochrome P-450s present in the sample can be assessed by measuring fluorescence after exposing microsomes to ethoxyresorufin-O-deethylase (EROD) or pentoxyresorufin-O-deethylase activity (PROD), two enzymes which fluoresce in the presence of specific forms of cytochrome P-450s.

---

#### Logistical Considerations:

##### Sample Collection:

**Training:** MODERATE

**Time:** MODERATE

**Equipment:** Liquid nitrogen, liquid nitrogen storage container - Samples must be stored at -80°C immediately after collection until microsomes can be prepared.

##### Sample Analysis:

**Training:** MODERATE.

**Time:** MINIMAL to run an individual sample, MODERATE to run larger numbers of samples

**Equipment:** Centrifuge, ultracentrifuge, fluorometer, spectrophotometer, 96-well plate reader for fluorimeter, 96-well plate reader for spectrophotometer, -80° C freezer

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**Critique/Comments:**

After extraction, organs from exposed individuals must be frozen at temperatures less than -80° C immediately after the individual is removed from the contaminated site.

Results of this test must be interpreted cautiously because factors other than exposure to anthropogenic materials can induce cytochrome P-450s. These factors include naturally occurring plant toxins.

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**Key References:**

- Hofius, J.L. 1992. Characterization and induction of hepatic and renal detoxification enzymes in nestling European starlings (*Sturnus vulgaris*). Master of Science Thesis unpublished. Clemson University, Clemson, SC.
- Nebert, D.W. and F.J. Gonzalez. 1987. P450 genes: structure, evolution, and regulation. *Ann. Rev. Biochem.* 56:945-993.
- Payne, J.F., L.L. Fancey, A.D. Rahimutula and E.L. Porter. 1987. Induction of hepatic mixed function oxidases in the Herring gull (*Larus argentatus*) by Prudhoe Bay crude oil and its fractions. *Comp. Biochem. Physiol.* 94(C):461-463.
- Rattner, B.A., D.J. Hoffman and C.M. Marn. 1989. Use of mixed-function oxygenases to monitor contaminant exposure in wildlife. *Environ. Toxic. Chem.* 8:1093-1102.
- Simmons, G.J. and M.J. McKee. 1992. Alkoxyresorufin metabolism in white-footed mice at relevant environmental concentrations of Aroclor 1254. *Fund. Appl. Toxicol.* 19:001-006.
- Walters, P., S. Kahn, P.J. O'Brien, J.F. Payne and A.D. Rahimutula. 1987. Effectiveness of a Prudhoe Bay crude oil and its aliphatic, aromatic, and heterocyclic fractions in inducing mortality and aryl hydrocarbon hydroxylase in chick embryo in *ovo*. *Arch. Toxicol.* 60:454-459.
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**Technique Name:** Cholinesterase Inhibition

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**Technique Type:** Enzyme Inhibition  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Field, Laboratory

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**Description:**

In unstressed cells, cholinesterases are responsible for the degradation of neurotransmitters. Inhibition of these enzymes causes a variety of neurotoxic responses in invertebrates, birds, and fish. Various assays have been developed to measure cholinesterase activity, and normal activity levels have been established for several species of birds and mammals. The role of organophosphate and carbamate pesticides in the inhibition of acetylcholinesterase has been extensively researched. Other contaminants, including mercury, have been shown to cause inhibition.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** MODERATE

**Time:** MODERATE

**Equipment:** Samples need to be placed on wet ice immediately after collection to prevent enzyme reactivation. Samples stored for periods > 8h should be stored at temperatures < -20°C.

**Sample Analysis:**

**Training:** MODERATE

**Time:** MINIMAL

**Equipment:** A spectrophotometer is required for sample analysis.

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**Critique/Comments:**

Data reported in the literature suggests there may be variation in baseline ChE values within a population, and variation among seasons. Reactivation by 2-pyridine aldoxime methiodide (2-PAM) seems to occur infrequently, but is a strong indicator of exposure when it is found. Collecting the samples is relatively simple with training personnel and analysis costs are relatively inexpensive. Cholinesterase analyses of blood plasma can be repeatedly collected from the same individual over time.

---

**Key References:**

- Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7:88-95.
- Fairbrother, A., B.T. Marden, J.K. Bennett and M.J. Hooper. 1991. Methods used in determination of cholinesterase activity, In P. Mineau, ed., *Cholinesterase-inhibiting Insecticides*. Elsevier Science Publishers, Amsterdam, pp.35-71.
- Grue, C.E., W.J. Fleming, D.G. Busby and E.F. Hill. 1983. Assessing hazards of organophosphate pesticides to wildlife. *Trans. N. Am. Wildl. Nat. Res. Conf.* 48:200-220.
- Grue, C.E., G.V.N. Powell and N.L. Gladson. Brain cholinesterase (ChE) activity in nestling starlings: implications for monitoring exposure of nestling songbirds to ChE inhibitors. *Bull. Environ. Contam. Toxicol.* 26:544-547.
- Hill, E.F. and W.J. Fleming. 1982. Anticholinesterase poisoning of birds: field monitoring and diagnosis of acute poisoning. *Environ. Toxicol. Chem.* 1:27-38.
- Jett, D.A. 1986. Cholinesterase inhibition in meadow voles (*Microtus pennsylvanicus*) following field applications of Orthene. *Environ. Toxicol. Chem.* 5:255-259.
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**Technique Name:** Porphyrin Profiles

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**Technique Type:** Enzyme Inhibition  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

This technique assesses levels of intermediates in the heme synthesis pathway in tissues, blood, and excreta of mammals, birds, and aquatic organisms. Research to date has indicated this methodology provides both qualitative and quantitative biomarkers of exposure to toxicants such as polyhalogenated hydrocarbons and heavy metals. Levels may be measured in liver, kidney, and fecal-urate excreta. Measurements are made of 8-, 7-, 6-, 5-, 4-, and 2-carboxyl porphyrin concentrations.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** MODERATE

**Time:** MODERATE

**Equipment:** Liquid nitrogen, liquid nitrogen storage container - Samples must be stored at -80°C immediately after collection.

**Sample Analysis:**

**Training:** MODERATE

**Time:** MINIMAL for individual samples.

**Equipment:** Spectrofluorometer and HPLC are both needed for analysis.

---

**Critique/Comments:**

Sample storage temperature is critical since porphyrin intermediates rapidly degrade after sacrifice of the specimen.

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## Key References:

- Akins, J.M., M.J. Hooper, H.D. Miller and J.S. Woods. 1993. Porphyrin profiles in the nestling European starling (*Sturnus vulgaris*): a potential biomarker of field contaminant exposure. *Journal of Toxicology and Environmental Health* 40:47-59.
- Bowers, M.A., L.D. Aicher, H.A. Davis and J.S. Woods. 1992. Quantitative determination of porphyrin in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. *J. Lab. Clinic. Med.* 120:272-281.
- Fox, G.A., S.W. Kennedy, R.J. Norstrom and D.C. Wingfield. 1988. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. *Environ. Toxicol. Chem.* 7:831-839.
- Kennedy, S.W. and G.A. Fox. 1990. Highly carboxylated porphyrin as a biomarker of polyhalogenated aromatic hydrocarbon exposure in wildlife: confirmation of the presence in Great Lake herring gull chicks in the early 1970's and important methodological details. *Chemosphere* 21:407-415.
- Woods, J.S., M.A. Bowers and H.A. Davis. 1991. Urinary porphyrin profiles as biomarkers of trace metal exposure and toxicity: studies on urinary porphyrin excretion patterns in rats during prolonged exposure to methyl mercury. *Toxicol. Appl. Pharmacol.* 110:464-476.
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**Technique Name:** Delta-Aminolevulinic Acid Dehydratase

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**Technique Type:** Enzyme Inhibition  
**Matrix Type:** Terrestrial and Aquatic Organisms  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Delta-aminolevulinic acid dehydratase (ALAD) is an enzyme that catalyzes the formation of porphobilinogen, a precursor of heme. Often, ALAD is inhibited before other signs of toxicity become apparent. Field studies have revealed ALAD inhibition in fish, birds, and mammals exposed to various forms of lead. ALAD inhibition is best used as an indicator of exposure but not necessarily toxicity. ALAD inhibition can be measured in blood and/or liver samples.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** Minimal  
**Time:** Minimal-MODERATE; Depends on the species.  
**Equipment:** Dry ice is required to preserve field-collected samples.

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MODERATE  
**Equipment:** A spectrophotometer is required.

---

**Critique/Comments:**

Assays of ALAD have the advantage of being relatively simple, inexpensive, accurate, and precise.

---

**Key References:**

- Haux, C., A. Larsson, G. Lithner and M.L. Sjobeck. 1986. A field study of physiological effects on fish in lead-contaminated lakes. *Environ. Toxicol. Chem.* 5:283-288.
- Hodson, P.V., B.R. Blunt and D.M. Whittle. 1984. Monitoring lead exposure of fish, In V.W. Cairns, P.V. Hodson and J.O. Nriagu, eds., *Contaminant Effects on Fisheries*. John Wiley & Sons, Toronto. pp 87-97.
- Jakim, E. 1973. Influence of lead and other metals on fish  $\delta$ -aminolevulinate dehydratase activity. *J. Fish. Res. Bd. Canad.* 30: 560-562.
- Johansson-Sjobeck, M.-L. and A. Larsson. 1979. Effects of inorganic lead on delta aminolovelinic acid dehydratase activity and haematological variables in the rainbow trout (*Salmo gairdneri*). *Arch. Environ. Contam. Toxicol.* 8: 419-431.
- Scheuhammer, A.M. 1987. Erythrocyte  $\alpha$ -aminolevulinic acid dehydratase in birds. I. The effects of lead and other metals in vitro. *Toxicology* 45:155-163.
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**Technique Name:** Metabolic Products

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**Technique Type:** Biochemical  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Many xenobiotic chemicals are converted to metabolic products in the organism soon after exposure. Detection of metabolites provides evidence of exposure to the xenobiotic chemical. Metabolites that can currently be used in environmental monitoring include metabolites of chlorinated hydrocarbons and PAHs, which can be detected in tissues, and metabolites of chlorinated phenols, resin acid metabolites, and PAHs, which can be detected in bile.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** MODERATE

**Time:** MODERATE

**Equipment:** Equipment depends on the metabolic product of interest. Liquid nitrogen and appropriate holding containers are needed for samples that may contain short-lived compounds while wet ice may be suitable for more stable compounds.

**Sample Analysis:**

**Training:** EXTENSIVE

**Time:** MODERATE

**Equipment:** Varies with the analyte of interest. Spectrophotometry, fluorimetry, GC, HPLC, or some combination of these analytical techniques are very commonly applied methods for detection of toxicants in biological samples.

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**Critique/Comments:**

This method provides direct evidence of exposure to compounds of interest.

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**Key References:**

- Krahn, M.M., M.S. Myers, D.G. Burrows and D.C. Malins. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. *Xenobiotica* 14:633-646.
- Malins, D.C., B.B. McCain, D.W. Brown, S.-L. Chan, M.S. Myers, J.T. Landahl, P.G. Prohaska, A.J. Friedman, L.D. Rhodes, D.G. Burrows, W.D. Gronlund and H.O. Hodgins. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. *Environ. Sci. Technol.* 18:705-713.
- Melancon, M. J., R. Alscher, W. Benson, G. Kruzynski, R. F. Lee, H. C. Sikka and R. B. Spies. 1992. Metabolic products as biomarkers, In R. J. Huggett, R. A. Kimerle, P. M. Mehrle, Jr. and H. L. Bergman, eds., *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*. Lewis Publishers, Boca Raton, FL, pp. 87-123.
- Oikari, A. and T. Kunnamo-Ojala. 1987. Tracing of xenobiotic contamination of water with the aid of fish bile metabolites: a field study with caged rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol.* 9:327-341.
- Thakker, D.R., H. Yagi, W. Levin, A.W. Wood, A. H. Conney and D.M. Jerina. 1985. In M. W. Anders, ed., *Bioactivation of Foreign Compounds*. Academic Press, Orlando, FL, pp. 177-242.
- Varansi, U., W.L. Reichert and J.E. Stein. 1989. <sup>32</sup>P-postlabeling analysis of DNA adducts in liver of wild English sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectes americanus*). *Cancer Res.* 49:1171-1177.
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**Technique Name:** Metallothionein Induction

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**Technique Type:** Enzyme Induction  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Metallothioneins are low molecular weight, metal-binding proteins and oligonucleotides that are induced by exposure to a wide variety of heavy metals such as cadmium, copper, zinc, mercury, cobalt, nickel, bismuth, and silver. Metallothioneins can be used to assess exposure to heavy metals.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** Minimal  
**Time:** MINIMAL-MODERATE; Depends on the species.  
**Equipment:** Field collected samples must be frozen in liquid nitrogen.

**Sample Analysis:**

**Training:** EXTENSIVE  
**Time:** MODERATE  
**Equipment:** A centrifuge capable of 100,000 g is required.

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**Critique/Comments:**

Considerable effort is required for calibration, using proteins of known molecular weights, of the described technique.

Data from studies using metallothioneins as biomarkers must be interpreted cautiously since many of these proteins can be induced by environmental stresses other than exposure to a toxicant.

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## Key References:

- Benson, W.H., K.N. Baer and C.F. Watson. 1990. Metallothionein as a biomarker of environmental metal contamination: species-dependent effects, In J.F. McCarthy, L.R. Shugart, eds., Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, Fl. pp 255-287.
- Cope, W.G., J.G. Weiner, and G.J. Atchison. 1994. Hepatic cadmium, metal-binding proteins and bioaccumulation in bluegills exposed to aqueous cadmium. Environ
- Hamilton, S.J. and P.M. Mehrle. 1986. Metallothionein in fish: review of its importance in assessing stress from metal contaminants. Trans Am. Fish. Soc. 115:596-609.
- Langston, W.J. and M. Zhou. 1986. Evaluation of the significance of metal binding proteins in the gastropod *Littorina littorea*. Mar. Biol. 92:505-515.
- Roch, M. and J.A. McCarter. 1984. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River System. Can J. Fish Aquat. Sci. 39:1596-1601.
- Roesijadi, G. 1981. The significance of low molecular weight, metallothionein-like proteins in marine invertebrates: current status. Mar. Environ. Res. 4:167-179.
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**Technique Name:** Stress Protein Induction

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**Technique Type:** Enzyme Induction  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Certain classes of low molecular weight proteins are induced in response to a variety of environmental stressors including hyperthermia, sulfide-reactive agents, heavy metals, ethanol, glucose deprivation, viral infection, and anoxia. It has been suggested that these inducible proteins function to renature other proteins that have been denatured by exposure to an insulting agent. The presence of stress proteins can serve as an indication of exposure to an environmental stressor.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL-MODERATE; Depends on the species.

**Equipment:** Field collected samples must be preserved in liquid nitrogen until sample analysis.

**Sample Analysis:**

**Training:** EXTENSIVE

**Time:** MODERATE

**Equipment:** An ultracentrifuge is required. Equipment associated with the Western blotting technique is required.

---

**Critique/Comments:**

Although many stressor specific proteins have been induced and characterized in the laboratory, these proteins have not been widely utilized as a biomarker of environmental contaminant exposure.

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**Key References:**

- Moromoto, R., A. Tissieres and C. Georgopoulos, eds. 1990. The Role of the Stress Response in Biology and Disease. Cold Springs Harbor, New York: Cold Spring Harbor Laboratory.
- Sanders, B.M. 1990. Stress Proteins: Potential as multitiered biomarkers, In L. Shugart, and J. McCarthy, eds., Environmental Biomarkers. Lewis Publishers. Inc., Chelsea, MI, pp. 165-191.
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**Technique Name:** Clastogenicity Tests

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**Technique Type:** DNA Modification  
**Matrix Type:** Biological Tissues  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

This technique is used to examine chromosomal aberrations induced by exposure to contaminants. Cells are usually examined in the mitotic phase for alterations, rearrangements, breakage, and translocations. These effects have been correlated with the presence of mutagens and carcinogens.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL-MODERATE; Depends on the species.

**Equipment:** Samples collected in the field must be preserved in liquid nitrogen.

**Sample Analysis:**

**Training:** EXTENSIVE

**Time:** MODERATE

**Equipment:** Flow cytometer is required for accurate analysis.

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**Critique/Comments:**

Cells must be in the process of dividing for this assay.

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**Key References:**

- McBee, K. J.W. Bickham, K.W. Brown and K.C. Donnelly. 1987. Chromosomal aberrations in native small mammals (*Peromyscus leucopus* and *Sigmodon hispidus*) at a petrochemical waste disposal site: I. Standard karyology. Arch. Environ. Contam. Toxicol. 16:681-688.
- McBee, K. and J.W. Bickham. 1989. Mammals as bioindicators of environmental toxicity, In H.H. Genoways, ed., Current Mammalogy. Plenum Press. New York, NY. pp. 37-88.
- Pesch, G.G. and C.E. Pesch. 1980. *Neanthes arenaceodentata* (Polychaeta: Annelida): a proposed cytogenetic model for marine genetic toxicology. Can. J. Fish. Aquat. Mar. Genet. Toxicol. 37:1225-1228.
- Thompson, R.A., G.D. Schroder, and T.H. Connor. 1988. Chromosomal aberrations in the cotton rat, *Sigmodon hispidus*, exposed to hazardous waste. Environ. Molec. Mutagen. 11:359-367.
- Tice, R.R., B.G. Ormiston, R. Boucher, C.A. Luke and D.E. Paquette. 1987. Environmental biomonitoring with feral rodent species, In S.S. Sandhu, D.M. Demanine, M.J. Mass, M.M. Moore and J.L. Mumford, eds., Short-term Bioassays in the Analysis of Complex Environmental Mixtures, Vol. V. Plenum Press. New York, NY.
- U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines: Final Rules. 40CFR, parts 796, 797, and 798.
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**Technique Name:** Ames Test

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**Technique Type:** DNA Modification  
**Matrix Type:** Water or Extracts of Solid-Phase Materials  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

The Ames test can be used to assess the mutagenic potential of contaminants of a water sample. Test strains of histidine-dependent *Salmonella* bacteria are cultured on media containing nutrients, a microsomal preparation, and the potential mutagen. Standard test strains contain mutations that make them more susceptible to mutations than wild-type bacteria. A toxic response is measured by a decrease in the number of revertants, i.e. cells that are able to grow in the absence of histidine. The number of revertants is a measure of the ability of the mutagen to produce a change in DNA.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** A centrifuge may required for extraction of compounds from soil sediment or other matrices.

**Sample Analysis:**

**Training:** MINIMAL  
**Time:** MODERATE  
**Equipment:** Bacterial culturing facilities are required.

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**Critique/Comments:**

Results should be interpreted cautiously with regard to extrapolation to carcinogenicity and to other species.

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**Key References:**

- Ames, B.N., J. McCann and E. Yamaski. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian mutagenicity test. *Mutation Res.* 31:347.
- Maron, D.M. and B.N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. *Mutation Res* 113:173.
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**Technique Name:** Detection of DNA Adducts

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**Technique Type:** DNA Modification  
**Matrix Type:** Biological Tissues  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Exogenous compounds or their metabolites may covalently bind to DNA. Shortly after exposure, organismal exposure to a contaminant can be ascertained by the detection of these adducts. Currently, techniques used to detect and quantify DNA adducts utilize P-postlabeling, HPLC/fluorescence, and immunological techniques. Tests currently under development utilize gas chromatography, gas chromatography/mass spectroscopy, capillary zone electrophoresis-mass spectroscopy, and fluorescence line-narrowing techniques.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL-MODERATE; Depends on the species.  
**Equipment:** Samples collected in the field must be stored in liquid nitrogen.

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MINIMAL  
**Equipment:** Several techniques can be employed to detect DNA adducts. Equipment needed differs with each of these procedures. A rapid and commonly employed technique requires a fluorimeter.

---

**Critique/Comments:**

Techniques currently used to detect DNA adducts are limited in sensitivity or specificity. The use of DNA adducts for use in assessment of exposure has recently begun to be validated in field studies using the P-postlabeling assay. Currently, the P-postlabeling technique is semi-quantitative, laborious, and moderate in cost.

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**Key References:**

- Dunn, B., J. Black and A. Maccubbin. 1990. <sup>32</sup>P-postlabeling analysis of aromatic DNA adducts in fish from polluted areas. *Cancer Res.* 47:6543-6548.
- Halbrook, R.S., R. L. Kirkpatrick, D.R. Bevan and B. P. Dunn. 1992. DNA adducts detected in muskrats by <sup>32</sup>P-postlabeling analysis. *Environ. Toxicol. Chem.* 11:1605-1613.
- Lower, W.R., F.A. Ireland, and B.M. Judy. 1991. <sup>32</sup>P-postlabeling for DNA adduct determination in plants, In J.W. Gorsuch, W.R. Lower, M.A. Lewis, and W. Wang, eds., *Plants for Toxicity Assessment: Second Volume*. American Society for Testing and Materials, Philadelphia, PA. pp 291-307.
- Rahn, R., S. Chang, J.M. Holland and L.R. Shugart. 1982. A fluorometric-HPLC assay for quantitating the binding of benzo[a]pyrene metabolites to DNA. *Biochem. Biophys. Res. Commun.* 109:262-269.
- Randerath, K., M. Reddy and R.C. Gupta. 1981. <sup>32</sup>P-postlabeling analysis for DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 78:6126-6129.
- Santella, R.M., R. Gasparo and L. Hsieh. Quantitation of carcinogen-DNA adducts with monoclonal antibodies. *Prog. Exp. Tumor Res.* 31:63-75.
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**Technique Name:** Secondary Modification of DNA

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**Technique Type:** DNA Modification  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

These tests are designed to detect modifications of DNA such as strand breakage, changes in minor base composition, or an increase in the level of unscheduled DNA synthesis. The alkaline unwinding assay is a sensitive technique used to detect strand breakage. Exogenous compounds can affect minor base composition by altering the activity of enzymes responsible for controlling the amount of methylated deoxyribonucleoside present in DNA. Alteration of these enzymes can result in hypomethylation of DNA, which can be detected by ion-exchange chromatography. Damaged DNA is repaired by unscheduled DNA synthesis. Detection of unscheduled DNA synthesis serves as a general indicator of genotoxic exposure.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL- MODERATE; Depends on the species.  
**Equipment:** Samples collected in the field must be stored in liquid nitrogen.

**Sample Analysis:**

**Training:** EXTENSIVE  
**Time:** MINIMAL  
**Equipment:** Thin-layer chromatography is employed for this assay. A densitometer is required.

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**Critique/Comments:**

Tests used to detect secondary modifications of DNA have not been fully developed for use as general biomarkers for environmental species. However, the strand breakage assay is currently being evaluated for environmental applications and has been tested using several environmental species, including oysters and mussels, desert rodents, and turtles. Strand breakage assay and minor nucleoside content assay measure a loss of DNA integrity but do not identify the chemical responsible.

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**Key References:**

- Shugart, L.R. 1988. An alkaline unwinding assay for the detection of DNA damage in aquatic organisms. *Marine Environ. Res.* 24:321-325.
- Shugart, L.R. 1990. Biological monitoring: testing for genotoxicity, In J.F. McCarthy and L.R. Shugart, eds., *Biological Markers of Environmental Contaminants*. Lewis Publishers, Inc., Boca Raton, FL, pp 205-216.
- Shugart, L.R. 1990. DNA damage as an indicator of pollutant-induced genotoxicity, In W.G. Landis and W.H. van der Schalie, eds., *13th Symposium on Aquatic Toxicology Risk Assessment*. ASTM Publishers, Philadelphia, PA. pp. 205-216.
- Shugart, L.R. 1990. 5-methyl deoxycytidine content of DNA from bluegill sunfish (*Lepomis macrochirus*) exposed to benzo[a]pyrene. *Environ. Toxicol. Chem.* 9:205-208.
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**Technique Name:** Trace Metals in Tissues

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**Technique Type:** Assessment of Accumulation  
**Matrix Type:** Biological Tissues  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Selected endpoints, typically comprised of biochemical or physiological responses, in individual organisms can be measured to provide sensitive indices of exposure or sublethal stress. Although biomarkers currently cannot be used to determine effects at population, community, or ecosystem levels, carefully selected biomarkers can serve as very sensitive monitoring tools to detect exposure, to assess sublethal stress, and to delineate zones of impact. When feasible, determination of tissue residues is recommended for assessment of exposure. The species chosen and the tissues selected for analysis will depend largely on the ecology of the site and information about contaminating metals. Since most metals bioaccumulate, concentrations in tissues can be measured directly by methods such as atomic absorption spectroscopy, inductively-coupled plasma, and neutron activation analysis. Methods differ in cost, sensitivity to various metals, and availability.

---

**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL - MODERATE depending on the species.

**Equipment:** Depends on the species.

## Sample Analysis:

**Training:** MODERATE

**Time:** MINIMAL

**Equipment:** An atomic absorption spectrophotometer is required for analysis of most metals.

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## Critique/Comments:

Standard methods have been developed for the acquisition and analysis of biological samples for many metals of widespread environmental concern.

Analysis of metal concentrations biological tissue can provide direct evidence of exposure if levels are significantly higher in organisms from contaminated sites than in organisms collected from a control. Despite the usefulness of this technique, it is important to realize that detection of metals in tissue gives little indication of possible effects to the exposed organism.

This method has been widely used with bryophytes; lichens; and terrestrial and aquatic plants invertebrates, amphibians, reptiles, birds, and mammals. A large volume of literature exists, which addresses accumulation of most every heavy metal element.

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## Key References:

- Hunter, B.A., and M.S. Johnson. 1982. Food chain relationships of copper and cadmium in contaminated grassland ecosystems. *Oikos* 38:108-117.
- Jenkins, D.W. 1980. Nickel accumulation in aquatic biota, In J.O. Nriagu, ed., *Nickel in the Environment*. John Wiley and Sons, New York. pp. 283-338.
- Johnson, M.S., R.D. Roberts, M. Hutton, M.J. Inskip. 1978. Distribution of lead, zinc, and cadmium in small mammals from polluted environments. *Oikos* 30:153-159.
- Richardson, D.H.S., P.J. Beckett and E. Nieboer. 1980. Nickel in lichens, bryophytes, fungi, and alga, In J.O. Nriagu, ed., *Nickel in the Environment*. John Wiley and Sons, New York. pp. 367-406.
- Timmermans, K.R. 1993. Accumulation and effects of trace metals in freshwater invertebrates, In R. Dallinger and P.S. Rainbow, eds., *Ecotoxicology of Metals in Invertebrates*. Lewis Publishers, Boca Raton, FL. pp 133-148.

**Technique Name:** Skeletal Abnormalities

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**Technique Type:** Physiological -- Gross Indices  
**Matrix Type:** Whole Body Evaluation  
**Ecosystem Level:** Individual  
**Test Location:** Field

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**Description:**

Various chemical contaminants have been reported to cause skeletal and/or vertebral abnormalities. These chemicals include the heavy metals zinc, cadmium, and lead; the organochlorine compounds kepone, toxaphene, mirex, Aroclor 1254, 2,4-DMA, and chlordecone; the organophosphate pesticides parathion and malathion; trifluralin; and crude oil. Skeletal and vertebral abnormalities of environmental species have been used to monitor pollution effects. Several techniques can be used to assess skeletal abnormalities.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** Can be MINIMAL-EXTENSIVE depending on the elusive nature of the species to be examined and sample size requirements.  
**Equipment:** Depends on the species to be collected.

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MINIMAL for individual samples.  
**Equipment:** Typical laboratory equipment is required.

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**Critique/Comments:**

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## Key References:

- Bengtsson, B.E. 1979. Biological variables, especially skeletal deformities in our fish for monitoring marine pollution. *Philos. Trans. R. Soc. London* 286:457-464.
- Bengtsson, A. and B.-E. Bengtsson. 1983. A method to registrate spinal and vertebral anomalies in fourhorn sculpin, *Myoxocephalus quadricornis* L. (Pisces). *Aquilo Ser. Zool.* 22:61-64.
- Bengtsson, B.-E., A. Bengtsson and M. Himberg. 1985. Fish deformities and pollution in some Swedish waters. *Ambio* 14:32-35.
- Goede, R. 1993. Fish health/condition assessment procedures. Part 1. Procedures manual. Utah Division of Wildlife Resources. Fisheries Exp. Sta. Logan, Utah. 31pp.
- Goede, R. 1993. Fish health/condition assessment procedures. Part 2. A color atlas of autopsy classification categories. Utah Division of Wildlife Resources. Fisheries Exp. Sta. Logan, Utah. 3pp with 64 color plates.
- Mayer, F.L., B.-E. Bengtsson, S.J. Hamilton and A. Bengtsson. 1988. Effects of pulp mill and ore smelter effluents on vertebrae of fourhorn sculpin: laboratory and field comparisons, In W.J. Adams, G.A. Chapman and W.G. Landis, eds., *Aquatic Toxicology and Hazard Assessment*, ASTM STP 971. American Society for Testing Materials, Philadelphia. pp 406-419.
- Meyer, F.P. and L.A. Barclay. 1990. Field manual for the investigation of fish kills. U.S. Department of the Interior, Fish and Wildlife Service. Resource Pub. 177. Washington, DC. 120pp.
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**Technique Name:** Hepatic Histopathology

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**Technique Type:** Histopathological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Several symptoms and forms of liver damage can serve as useful biomarkers of toxicant effects. Coagulative cellular necrosis is a currently useful indicator of toxicant exposure. Hyperplasia, which living cells undergo following necrosis, is another biomarker of exposure. Three types of hepatocytomegaly -- hepatocellular hypertrophy, megalocytosis, and hepatocytomegaly arising from swelling of perinuclear endoplasmic reticulum cisternae -- have been observed as responses to environmental contaminants in numerous fish species. Foci of cellular alteration form as an early stage of hepatic neoplasia. Detection of these foci have been used as a biomarker of exposure. The hepatic adenoma is an intermediate stage between cellular alteration and carcinoma. In addition to foci of cellular alteration, adenomas and hepatocellular carcinomas are present biomarkers. Cholangioma, cholangiocarcinoma, and mixed hepato-cholangiocellular carcinoma can all be used as biomarkers.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL to moderate depending on the species to be collected.  
**Equipment:** Standard laboratory equipment.

**Sample Analysis:**

**Training:** EXTENSIVE  
**Time:** MODERATE  
**Equipment:** A microtome is needed to prepare samples prior to microscopic examination.

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## Critique/Comments:

For each of the biomarkers discussed, links between laboratory results and environmental relevance have been demonstrated.

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## Key References:

- Baumann, P.C., J.C. Harshbarger and K.J. Hartmann. 1990. Relationship between liver tumors and age in brown bullhead populations from two Lake Erie tributaries. *Sci Tot. Environ.* 94:71-87.
- Harshbarger, J.C. and J.B. Clark. 1990. Epizootiology of neoplasms in bony fish of North America. *Sci. Total Environ.* 94:1-32.
- Hendricks, J.D., T.R. Meyers and D.W. Shelton. 1984. Histological Progression of hepatic neoplasia in rainbow trout *Salmo gairdneri*. *Natl. Cancer Inst. Monogr.* 65:321-336.
- Hinton, D.E. and D.J. Lauren. 1990. Integrative histopathological approaches for detecting effects of environmental stressors of fishes, In S.M. Adams, ed., *Biological Indicators of Fish Community Stress*, Amer. Fish. Soc. Special Pub.
- Kent, M.L., M.S. Myers, D.E. Hinton, W.D. Eaton and R.A. Elston. 1988. Suspected toxicopathic hepatic necrosis and megalocytosis in pen-reared Atlantic salmon *Salmo salar* in Puget Sound, Washington, U.S.A. *Dis. Aquat. Org.* 49:91-100.
- Meyers, T.R. and J.D. Hendricks. 1985. Histopathology, In G.M. Rand and S.R. Petrocelli, eds., *Fundamentals of Aquatic Toxicology*. Hemisphere Publishing Corp., Washington, D.C., pp. 283-331.
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**Technique Name:** Macrophage Phagocytosis

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**Technique Type:** Immunological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Macrophage phagocytosis activity is assessed by adding blood cells from environmental organisms to tissue culture medium. The culture is incubated for approximately 24 to 48h. Either fluorescent yeast cells or fluorescent latex particles are then added to the cultures. Phagocytosis activity is measured by counting the number of fluorescent particles macrophages in the culture ingest.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL to MODERATE depending on the species to be sampled.  
**Equipment:** Depends on the species.

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MINIMAL  
**Equipment:** Incubator, Cell Harvester, Sterile Hood, Pipettors, Autoclave, Centrifuge, Coulter Counter, Incubator, Fluorescent Microscope.

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**Critique/Comments:**

Use of any immune dysfunction or suppression assays should be viewed as a component of an integrated risk analysis. As the immune system is closely integrated with many organ systems and functions, and is a network capable of rapid cell proliferation and differentiation, it is susceptible to effects from contaminant exposure. Immune dysfunction or suppression is

a good measure of exposure over time and may reflect the results of simultaneous exposures to contaminants. Further, the ability of the immune system to rapidly proliferate memory cells makes it more suitable for chronic or repeated short exposures to contaminants than for assessing single, acute exposures. However, these tests are currently not capable of identifying the specific compounds responsible for inducing the effects, and no single change in an immune function has been shown to be pathognomic for a specific compound or class of chemicals. There appears to be considerable species-related variation which standardization of assays should help to minimize. Testing for immune dysfunction is appropriate for screening (Tier I) testing, but identification of the mechanisms causing the dysfunction will generally require Tier II testing.

Blood samples taken from individuals must be used the same day as collection.

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### Key References:

- Exon, J.H., L.D. Koller, P.A. Talcott, C.A. O'Reilly and G.J. Henningsen. 1986. Immunotoxicity testing: an economical multiple-assay approach. *Fund. Appl. Toxicol.* 7:387-397.
- Luster, M.I., A.E. Munson, P.T. Thomas, M.P. Holsapple, J.D. Fenders, K.L. White, Jr., L.D. Lauer, D.R. Germolec, G.J. Rosenthal and J.H. Dean. 1988. Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. *Fund. Appl. Toxicol.* 10:2-19.
- McBee, K. and J.W. Bickham. 1988. Petrochemical-related DNA damage in wild rodents detected by flow cytometry. *Bull. Environ. Contam. Toxicol.* 40:343-349.
- Weeks, B.A., R.J. Huggett, J.E. Warinner and E.S. Matthews. Macrophage responses of estuarine fish as bioindicators of toxic contamination, In J.F. McCarthy and L.R. Shugart, eds., *Biomarkers of Environmental Contamination*, Boca Raton, FL, pp. 193-201.
- Zeeman, M.G. and W.A. Brindley. 1981. Effects of toxic agents on fish immune systems: a review, In R.P. Sharma, ed., *Immunologic Considerations in Toxicology*, Vol. II. CRC Press, Inc., Boca Raton, FL.
- Zelikoff, J.T., N.A. Enane, D. Gowser, K.S. Squibb and K. Frenkel. 1991. Development of fish peritoneal macrophages as a model for higher vertebrates in immunotoxicological studies. *Fund. Appl. Toxicol.* 16:576-589.
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**Technique Name:** Lymphocyte Blastogenesis

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**Technique Type:** Immunological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Macrophage phagocytosis activity is assessed by adding a sample of whole blood from environmental organisms to tissue culture medium containing mitogen. Cells are radiactively labeled by adding  $^3\text{H}$ -thymidine to the culture. Cells are then harvested and the blastogenic response measured by comparing the mean disintegrations per minute in mitogen-stimulated wells with that of unstimulated wells.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL to MODERATE depending on the species to be sampled.  
**Equipment:** Depends on the species.

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MINIMAL  
**Equipment:** Incubator, Cell Harvester, Sterile Hood, Scintillation Counter, Pipettors, Autoclave.

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**Critique/Comments:**

Blood samples taken from individuals must be used the same day as collection.

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**Key References:**

- Redig, P.T., J.L. Dunnette and V. Sivanandan. 1984. Use of whole blood lymphocyte stimulation test for immunocompetency studies in bald eagles, red-tailed hawks, and great horned owls. *Am. J. Vet. Res.*, 45:2342-2346.
- Rocke, T.E., T.M. Yuill and R.D. Hinsdill. 1984. Oil and related toxicant effects on mallard immune defenses. *Environ. Res.* 33:343-352.
- Sharma, R.P. and R.V. Reddy. 1983. Toxic effects of chemicals on the immune system, In *Immunotoxicology*. Academic Press, New York, N.Y. pp. 555-591.
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**Technique Name:** Microbial Toxicity Tests

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**Technique Type:** Biochemical  
**Matrix Type:** Leachate, surface water, sediments  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Short-term microbial tests are based on inhibition of the activities of bacteria, algae, and fungi. These tests are versatile and cost-effective tools. Microbial toxicity tests include adenosine triphosphate (ATP) assays, enzymatic activity assays, bioluminescence assays, and microbial growth tests. By measuring ATP synthesis, effects of a toxicant can be gauged by comparing density of a treated bacterial colony with that in a control colony after several generations of bacterial cell growth. Enzymatic inhibition by toxicants could be an underlying cause of toxicity to cells. Toxicity tests, therefore, have been developed to assess inhibition of biosynthesis of enzymes, inhibition of enzyme function, and genetic interference, which leads to the loss of proper enzyme functioning. Bioluminescence assays are based on inhibition of the cellular electron transport system within the marine bacterium *Photobacterium phosphoreum*. Microbial growth tests assess population growth or cell motility.

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**Logistical Considerations:****Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** See references.

**Sample Analysis:**

**Training:** MINIMAL  
**Time:** MINIMAL

**Equipment:** Water extraction techniques require a centrifuge capable of revolution speeds greater than 5000 rpm. Solvent extraction techniques require freeze-drying. Equipment needed may be different for other extraction techniques. A colorimeter is needed for most enzymatic activity tests. Many standardized tests require analytical instruments used specifically for microbial toxicity testing.

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**Critique/Comments:**

These tests can readily be used to assess a wide range of toxicants in water, soil, sediments, sewage effluents, and leachates either directly or after concentration and/or extraction of water and organic solvents. Sensitivity of the test organism to a toxicant can vary with the type of test and toxicant. Many microbial toxicity tests have been standardized and are commercially available through various sources.

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**Key References:**

- ASTM. 1994. Annual book of ASTM standards. Water and Environmental Technology. Volume 11:04. Pesticides; resource recovery; hazardous substances and oil spill responses; waste management; biological effects. American Society for Testing and Materials. Philadelphia, PA. 1619 pp.
- American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. 17th ed. American Public Health Association, Washington, DC.
- Bitton, G. and B. Koopman. 1986. Biochemical tests for toxicity screening, In G. Bitton and B.J. Dutka, eds., Toxicity Testing Using Microorganisms, Vol. 1, CRC Press, Boca Raton, FL, pp.27-55.
- Bulich, A.A. 1986. Bioluminescent assays, In G.Bitton and B.J. Dutka, eds., Toxicity Testing Using Microorganisms, Vol.1. CRC Press, Boca Raton, FL, pp 57-74.
- Carter, M.R. (ed.). 1993. Soil Sampling and Methods of Analysis. Lewis Publishers, Inc. Boca Raton, FL. 864 pp.
- Holme-Hansen, O. 1973. Determination of total microbial biomass by measurements of 90 adenosine triphosphate, In L.H. Stevenson and R.R. Lowell, eds., Estuarine Microbial Ecology. University of South Carolina Press, Columbia, SC.
- Xu, H. and B.J. Dutka. 1987. ATP-TOX system: A new rapid sensitive bacterial toxicity screening system based on the determination of ATP. Toxicity Assess. 2:149-166.

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**Technique Name:**Microbial Ecological Effects Tests

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<b>Technique Type:</b>	Biochemical
<b>Matrix Type:</b>	Soil and surface water
<b>Ecosystem Level:</b>	Population/Community
<b>Test Location:</b>	Laboratory

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**Description:**

Processes that contribute to the cycling of carbon, nitrogen, sulfur, and phosphorus are among the most ecologically significant processes that contribute to the well-being of ecosystems. Certain processes such as nitrification and sulfur oxidation are mediated exclusively by specific groups of microorganisms whose activity can be assessed by their rates of metabolic processes. The cycling of the four elements listed above are especially valuable in environmental assessment. Assays for two of these, nitrogen and sulfur, have been developed. Nitrogen-transformation assays are conducted by adding various concentrations of a water sample or an extract from contaminated soil to a nitrifying soil microbial culture. The effects of a toxicant on sulfur transformations are assessed by adding dilutions of contaminated water or soil extracts to a culture that is actively mineralizing sulfur. Mineralization rates are determined by recovery of the  $^{35}\text{SO}_4^{2-}$  isotope of Sulfoquinovose.

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**Logistical Considerations:****Sample Collection:****Training:** MINIMAL**Time:** MINIMAL**Equipment:** MINIMAL**Sample Analysis:****Training:** MODERATE**Time:** MODERATE**Equipment:** Sulfur-transformation assays require the use of scintillation counting equipment.

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**Critique/Comments:**

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**Key References:**

- Klute, A., ed. 1965. *Methods of Soil Analysis. Part 1: Physical and Mineralogical Methods.* Am. Soc. Agronomy, Madison, WI.
- Lees, H. and J.H. Quastel. 1946. Biochemistry of nitrification in soil. I. Kinetics of, and the effect of poisons on, soil nitrification, as studied by a soil perfusion technique. *Biochem. J.* 40:803-814.
- Strickland, T.C. and J.W. Fitzgerald. 1983. Mineralization of sulfur in sulfoquinovose by forest soils. *Soil Biol. Biochem.* 15:347-349.
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**Technique Name: Microtox™**

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**Technique Type:** Luminescence Bioassay  
**Matrix Type:** Water, extract (water or solvent) for aqueous-phase test; soil and sediment for solid-phase test.  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

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Although aqueous-phase testing with Microtox™ has been readily available for many years, solid-phase testing has only recently been commercially available. Both aqueous-phase and soil-phase tests with Microtox™ directly measure biological activity in water (or soil and sediment-derived extracts), and sediment or soil, respectively. Both test systems use luminescent bacteria (*Photobacterium phosphoreum*) to measure the biological effects on culture metabolism) that may be associated with exposure. Altered cellular metabolism may affect the intensity of light output from the organism. When these changes in light output are expressed, estimates of biological effects may be derived from screening or concentration-response curves that yield EC<sub>50</sub>s (concentration of sample associated with a 50% reduction in light intensity) from plotted data. Unlike the aqueous-phase test where the sample (i.e., surface water, groundwater, sediment pore water or soil eluate) is directly tested (samples may be filtered), the solid-phase test requires a pre-testing extraction step. During the extraction, a "micro-eluate" is prepared from a soil sample (ca 0.3 gram), then incubated at 15°C. Following incubation, the soil-diluent slurry is filtered, and the filtrate is subsequently analyzed using the Microtox™ analyzer.

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**Logistical Considerations:**

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**Sample Collection:**

**Training:** MINIMAL.  
**Time:** MINIMAL.  
**Equipment:** A Microtox™ analyzer is required.

**Sample Analysis:**

**Training:** MINIMAL.  
**Time:** MINIMAL.

**Equipment:** A Microtox™ analyzer is required.

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**Critique/Comments:**

The results of several studies of pure compounds and complex chemical mixtures suggests that aqueous-phase testing with Microtox™ generally agrees with standard fish and invertebrate toxicity tests. Solid-phase testing with Microtox™, however, does not have a comparable data base established to compare with standard soil tests (e.g., earthworms survival). Furthermore, the solid-phase test does not take into account bacteria adsorbed to soil particles. Aqueous-phase testing and solid-phase testing with Microtox™ should both be performed in conjunction with other assessment methods, for example, animal or plant tests as previously noted (Warren-Hicks, et al., 1989). As a direct measure of altered soil structure and function, and for interpretation of ecological effects, the solid-phase Microtox™ currently requires the support of adequately defined site-specific reference soils, as well as a comparative data base that relates solid-phase Microtox™ test results with soil "health". These tests are performed with a marine bacterium; therefore, testing with soil eluates should be compared to standard soils toxicity tests to determine relevance to the soil type being tested.

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**Key References:**

- Bulich, A.A. 1986. Bioluminescent assays. Pages 57-74. In: G. Bitton and B.J. Dutka, eds. Toxicity Testing Using Microorganisms, Vol. 1. CRC Press, Boca Raton, FL.
- Curtis, C., A. Lima, S.J. Lorano, and G.D. Veith. 1982. Evaluation of a bacterial bioluminescence bioassay as a method for predicting acute toxicity of organic chemicals to fish. Pages 170-178. In: J.G. Pearson, R.B. Foster, and W.E. Bishop, eds. Aquatic Toxicity and Hazard Assessment, STP 766, American Society for Testing and Materials. Philadelphia, PA.
- Microbics Corporation. 1992. Microtox™ manual. Microbics Corporation, Carlsbad, CA.
- Munkittrick, K.R., E.A. Power, and G.A. Sergy. 1991. The relative sensitivity of Microtox™, daphnid, rainbow trout, and fathead minnow acute lethality tests. Environ. Toxicol. and Water Quality. 6:35-62.
- Warren-Hicks, W., B. Parkhurst, and S. Baker, Jr. (eds). 1989. Ecological assessment of hazardous waste sites. EPA/600/3-89/013. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.

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**Technique Name: Soil-core Microcosm**

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<b>Technique Type:</b>	Soil Microcosm
<b>Matrix Type:</b>	Soil
<b>Ecosystem Level:</b>	Community, Organismal
<b>Test Location:</b>	Field, and Greenhouse or Environmental Chamber

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**Description:**

The soil-core microcosm test potentially measure the adverse effects, or toxicity, of chemicals in either defined or complex chemical mixture exposures. Originally, the 60-cm deep by 17-cm diameter terrestrial soil-core microcosm was designed to yield chemical effects data in soils collected from grassland or agricultural systems, but the method may be adapted for other soil types as necessary. The cylinder containing the intact soil core is collected from a site using stainless steel extraction tubes; laboratory testing is completed on the intact core. Routine physicochemical analyses are completed on the soil, e.g., percent organic material, cation exchange capacity, and nutrient analysis, and in conjunction with field surveys, vegetation and soil biota are characterized. Once in the laboratory, the soil core can be manipulated following a site-specific sampling and analysis plan, but ideally exposure conditions occur in a greenhouse or environmental chamber. The ASTM E1191 (1991) standard guide outlines numerous exposure methods. Potential endpoints measured in a soil-core microcosm study are numerous, but ecological endpoints that are routinely considered include productivity measurements, and measurements of plant health, nutrient loss and chemical fate testing. Recently, the method has been adapted to evaluate fate and effects of hazardous waste chemicals used in military training (Checkai, et. al., 1993).

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**Logistical Considerations:****Sample Collection:**

**Training:** MINIMAL to collect, set-up and maintain soil cores.

**Time:** MODERATE (several weeks to several months).

**Equipment:** A greenhouse or environmental chamber is required.

**Sample Analysis:**

**Training:** MINIMAL to MODERATE. A good understanding of soil chemistry is required.

**Time:** MINIMAL to MODERATE.

**Equipment:** A wet chemistry laboratory and analytical equipment (i.e., HPLC or GC) is required.

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**Critique/Comments:**

The soil-core microcosm test has been standardized through ASTM E1191 (1991), and has been validated within an ecological risk assessment context for various chemical and biological hazards. The test is designed to evaluate the environmental fate, ecological effects, and environmental transport of chemicals, both liquid and solid, and genetically-engineered microbial agents that may be released to terrestrial systems. For chemicals, the methods can be used to evaluate toxicity or adverse effects on growth and reproduction of native vegetation or crops and the uptake and cycling of nutrients in a soil/plant system. Although soil-core microcosm has been used in various hazard and risk assessment settings, no regulatory precedence exists for routinely testing site soils using the soil-core microcosm. Within applied contexts, the method has proven useful to evaluations of complex chemical waste, hazardous wastes, and agricultural chemicals. The soil-core microcosm potentially yields data that will be directly relevant to any soil contamination evaluation, and its limitations are those inherent to microcosms and laboratory tests in general. However, if reference soils are available for concurrent testing, the soil-core microcosm test can yield information that could be significant to an ecological effects assessment for contaminated soils.

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**Key References:**

- ASTM E1191. 1991. Standard guide for conducting a terrestrial soil-core microcosm test. Annual book of ASTM standards. Volume 11.04. Pesticides; Resource Recovery; Hazardous Substances and Oil Spill Responses; Waste Disposal; Biological Effects. American Society for Testing and Materials (ASTM). Philadelphia, PA 19103.
- Checkai, R.T., R.S. Wentsel, C.T. Phillips, and R.L. Yon, 1993. Controlled environment soil-core microcosm unit for investigating fate, migration, and transformation of chemicals in soils. *J. Soil Contam.* 2(3):229-243.
- Van Voris, P., D. Tolle, M.F. Arthur, J. Chesson, and T.C. Zwick. 1984. Development and validation of terrestrial microcosm test system for assessing ecological effects of utility wastes. EPRI Publication N. EA-3672, Final Project Report. Electric Power Research Institute, Palo Alto, CA.
- Van Voris, P., D. Tolle, M.F. Arthur, and J. Chesson. 1985. Terrestrial microcosms: validation, applications, and cost-benefit analysis. *In* Multi-species toxicity testing, Pergamon Press, New York, NY. pp. 117-142.
- Van Voris, P., D. Tolle, and M.F. Arthur. 1985. Experimental terrestrial soil-core

microcosm test protocol. A method for measuring the potential ecological effects, fate, and transport of chemicals in terrestrial ecosystems. 600/3-85/047, PNL-5450. Environmental Research Laboratory, Corvallis, OR.

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**Technique Name: Soil Microbial Activity**

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**Technique Type:** Biochemical Bioassay  
**Matrix Type:** Soil  
**Ecosystem Level:** Biochemical  
**Test Location:** Laboratory

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**Description:**

Activity rates, as determined by enzyme studies, nucleic acid production and incorporation into biomass or nucleic acids, can be used as indices of total soil microbiological activity. Measurement of microbial activity usually involves addition of a substrate for a particular enzyme to utilize. Incubation times should be kept as short as possible to prevent microbial growth and reproduction. Sorption of the substrate or products on the surfaces of soil and clay particles needs to be prevented, limited, or measured. Measurement of substrate disappearance, enzyme presence, or product appearance must be kept as simple as possible, and usually is determined by a color change in the medium (disappearance of substrate or appearance of product changes pH and a pH sensitive dye is present in the medium), by change in turbidity, or by the production of a precipitate or chemical whose presence can be assayed by spectrophotometry. Methods available include: dehydrogenase assay, ATP content and adenylate energy charge (AEC), incorporation of radiolabelled nucleic acids, and calorimetry (heat production). Activity is measured as a change in color (spectroscopy) or by calorimetry.

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**Logistical Considerations:****Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** MINIMAL.

**Sample Analysis:**

**Training:** MINIMAL to record data, MINIMAL to MODERATE to evaluate data.

**Time:** MINIMAL.

**Equipment:** A calorimeter or spectrometer is required.

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### Critique/Comments:

A much more extensive database is needed. Major factors whose effects must be considered in establishing a baseline for interpretation of these measurements are: 1) localization of enzymes, cells, substrates and nucleic acids in soil, 2) standardization of methodology, 3) sorption of substrates, products and cells by soil clay and organic fractions, 4) nutrient cycling during long incubation assays, and 5) sampling of field soils and incubation in the laboratory gives potential rates and not *in situ* rates. General enzymes are produced by a wide variety of microorganisms, requiring the toxicant to affect a general reduction in the activity of soil heterotrophs before a reduction in enzyme activity is evident. Therefore, toxicants with limited or targeted biological activity, e.g., non-heavy metal pollutants, will rarely show a general effect. The positive aspects of assaying enzyme activity are the well established, rapidly performed, inexpensive procedures, which can be performed on whole soils, as well as soil extracts. These tests should be performed after standard toxicity tests to delineate specific toxic effects. Either a suite of enzyme assays must be performed, or some knowledge of impact must be available in order to choose one or two indicator enzyme assays.

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### Key References:

- Christensen, G.M., D. Olson, and B. Reidel. 1982. Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. *Environ. Res.* 29:247-255.
- Dutka, B.J. and G. Bitton, eds. 1986. *Toxicity Testing Using Microorganisms*, Vol. 2. CRC press, Boca Raton, FL.
- Dutton, R.J., G. Bitton, and B. Koopman. 1988. Enzyme biosynthesis versus enzyme activity as a basis for microbial toxicity testing. *Toxicity Assess.* 3:245-253.
- Eiland, F. 1985. Determination of adenosine triphosphate (ATP) and adenylate energy charge (AEC) in soil and use of adenine nucleotides as measures of soil microbial biomass and activity. *Danish. J. Plant Soil Sci.* S:1777:1-193.
- Ladd, J.N. 1985. Soil Enzymes. IN D. Vaughan and R.E. Malcom (eds). *Soil organic matter and biological activity.* pp. 175-221. Martinus Nijhoff, Dordrecht, The Netherlands.
- Lenhard, G. 1968. A standardized procedure for the determination of dehydrogenase activity in samples from anaerobic treatment systems. *Wat. Res.* 2:161-167.
- Nannipieri, P., S. Grego, and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. *Soil Biochemistry* 6:293-355.
- Nannipieri, P., C. Ciardi, L. Badalucco, and S. Casella. 1986. A method to determine DNA and RNA. *Soil Biol. Biochem.* 18:275-281.
- Organics Ltd. 1985a. *The Toxi-chromotest, Version 2 (US)*. Organics Ltd., P.O. Box 360, Yavne 70650, Israel.
- Organics Ltd. 1985b. *The SOS Chromotest Blue Kit, TwoStep Version 3*. Organics Ltd.,

P.O. Box 360, Yavne 70650, Israel.

Sparling, G.P. 1981. Heat output of the soil biomass. *Soil Biol. Biochem.* 13:373-376.

Tyler, G. 1974. Heavy metal pollution and soil enzymatic activity. *Plant Soil* 41:303-311.

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**Technique Name: Soil Lipid Chemistry**

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**Technique Type:** Soil Biochemistry  
**Matrix Type:** Soil  
**Ecosystem Level:** Biochemical  
**Test Location:** Laboratory

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**Description:**

The chemical composition of soil lipids is the direct result of the nature and reactivity of the various compounds added to soil from plant litter, animals, insects and microorganisms. Analysis of soil lipid chemistry can be used to assess bacterial and fungal community composition shifts and quantify essential soil characteristics. Two approaches hold significant promise with respect to soil lipids. First, the identity of soil organism groups can be determined using lipid signatures of particular groups, such as families, genera and species. Lipid-structure signatures from particular microbial groups can indicate subtle shifts in the composition of affected soils. Second, past biodegradation processes, hydrophobic properties, reactivity, and soil development can be assessed by analyzing soil lipids. To develop the lipid signature library, soil is spread on plates, colonies which grow on the plate are chosen based on morphology, the organism grown in liquid cultures, tested for purity and a portion of that culture extracted for lipids. These lipids must then be analyzed for the specific signature compounds. Test soils are then extracted for lipids, the extracts analyzed, and compared to known lipid signatures. Recent advances in cross polarization magic angle spin nuclear magnetic resonance (NMR) and mass spectrometry have opened new horizons for the characterization of soil lipids, such that different types of carbon (aliphatic C, protein branching patterns, long alkyl chains, carbohydrates, OH-substituted aliphatics, aromatics, phenolic and carboxyl C) can be distinguished.

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**Logistical Considerations:****Sample Collection:**

**Training:** MODERATE to EXTENSIVE to extract lipids from soil.  
**Time:** MODERATE to extract lipids.  
**Equipment:** MODERATE to extract lipids.

**Sample Analysis:**

**Training:** MODERATE to EXTENSIVE to operate analytical equipment.

**Time:** MINIMAL to MODERATE.

**Equipment:** EXPENSIVE, a mass spectrometer or magnetic resonance imaging equipment is required.

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**Critique/Comments:**

Soil lipid biochemistry techniques have demonstrated a high correlation of soil lipids with heavy metal contamination. Changes in lipid chemistry have been used to assess changes in microbial diversity and changes in fungal distribution patterns in impacted soils. In order to incorporate lipid biochemistry into a reliable technique for ecological risk assessment, methods development is required including: better techniques and equipment to extract and characterize chemically highly complex (especially for organisms) and polymerized (especially for soils) lipids; improved knowledge about mechanisms of inhibitory action of certain lipids on microbial populations and seed germination; assessment of biodegradability of various types of lipids in cultivated and uncultivated soil; and evaluation of the effect of certain lipids on soil structure. Reliable extraction efficiency of lipids from the sample, whether soil or organisms in soil, remains a problem. Characterization of lipids is time-consuming and, if new structures occur, difficult. Effects of different soil communities on lipid expression by individual organisms is a completely unknown interaction at this time.

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**Key References:**

- Dinel, H., M. Schnitzer, and G.R. Mehuys. 1990. Soil lipids: Origin, nature, content, decomposition and effect on soil physical properties. *Soil Biochem.* 6:397-429.
- Nordgren, A.E., E. Baath, and B. Soderstrom. 1985. Soil microflora in an area polluted by heavy metals. *Can. J. Bot.* 63:448-455.
- Nordgren, A.E., E. Baath, and B. Soderstrom. 1983. Microfungi and microbial activity along a heavy metal gradient. *Appl. Environ. Microbiol.* 45:1829-1837.
- Vestal, J.R. and D.C. White. 1989. Lipid analysis in microbial ecology. Quantitative approaches to the study of microbial communities. *Bioscience* 39:535-541.

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**Technique Name: Nitrogen Cycling in Soil**

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**Technique Type:** Soil Chemical Bioassay  
**Matrix Type:** Soil  
**Ecosystem Level:** Biochemical  
**Test Location:** Laboratory

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**Description:**

One very simple method is to collect soil and assess nitrogen pools (ammonium, nitrate, nitrite) at time zero and after incubation in plastic "zip-lock" bags. The difference indicates the potential nitrogen cycling rate. A second method is to add N-15 labeled ammonium to the soil and determine the rate at which it appears as nitrate-nitrite. For nitrification rates, one approach is to compare nitrification rates in contaminated soils to rates in uncontaminated soils. An alternative method is to add the soil to be tested to a sensitive culture of nitrifying bacteria and test for continued function of the bacterial culture. An aqueous suspension of a toxic substance is added to a culture of *Nitrosomonas europaea*. The conversion of ammonium to nitrite is quantified. Although there are no reports to our knowledge of this approach being used to assess toxicity in soil samples, the results of Powell and Prosser (1986) suggest that the method has potential usefulness. The rate of ammonium conversion to nitrate and/or nitrite, and the rate at which nitrate is converted to nitrate are the endpoints measured. The concentrations of ammonium, nitrate and nitrite are determined colorimetrically, using either autoanalyzers or laboratory spectrophotometers.

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**Logistical Considerations:****Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** MINIMAL.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** Equipment is required to measure different oxidative states of nitrogen compounds.

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**Critique/Comments:**

Of the major nitrogen transformations mediated by microorganisms, nitrogen cycling is one of the most important and directly related to plant productivity. In addition, nitrification of ammonium to nitrite and then to nitrate appears to be the most sensitive transformation to a wide range of potential toxicants. A broad database of information has been published on nitrification and the effects of various toxic chemicals. A literature search is needed to summarize this information and improve the interpretation of hazardous chemical impacts in these organisms and this process. In general, biogeochemical transformation of nitrate has been shown to be highly sensitive to pesticides, herbicides, and heavy metals. The nitrifying organisms used in these tests were cultures from standard sources. No attempt was made to seek strains isolated from nonpolluted waters or soils that may be particularly sensitive to toxicants. Because nitrification is known to be sensitive to a wide range of toxicants, it should be relatively easy to select for strains that are particularly sensitive to different groups of toxicants. Knowing the composition of toxicants at a given site, technicians could select one or more strains that are known to be particularly sensitive to the toxicants present on the site. The resulting tests should be very sensitive, rapid, and easy to perform by relatively untrained personnel.

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**Key References:**

- Domsch, K.H. 1970. Effects of fungicides on microbial populations in soil. In: Pesticides in Soil Ecology; Degradation and Movement Symposium. E. Lansing State University, Mich.
- Parr, J.F. 1974. Effects of pesticides on microorganisms in soil and water. In: Pesticides in Soil and Water. Guenzi, W.D., J.L. Ahlrichs, M.E. Bloodworth, G. Chesters, and R.G. Nash (eds). Soil Sci. Soc. Amer. Inc. Madison. pp. 315-340.
- Powell, S.J. and J.I. Prosser. 1986. Effect of copper on inhibition by nitrapyrin of growth of *Nitrosomonas europaea*.
- Sato, C., S.W. Leung, and J.L. Schnoor. 1988. Toxic response of *Nitrosomonas europaea* to copper in inorganic medium and wastewater. *Water Res.* 22:1117-1127.
- Tu, C.M. 1970. Effect of four organophosphorus insecticides on microbial activities in soil. *Appl. Microbiol.* 19:479-484.
- Wainwright, M. 1978. A review of the effects of pesticides on microbial activity in soils. *J. Soil Sci.* 29:287-298.

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**Technique Name: Uptake and Utilization of Organic Compounds by Microbes**

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**Technique Type:** Uptake and Utilization of Chemicals  
**Matrix Type:** Soils  
**Ecosystem Level:** Biochemical  
**Test Location:** Laboratory

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**Description:**

Substrate uptake by bacteria and fungi can be used as a means of demonstrating toxic effects by following the fate of radiolabelled toxicant when added to a soil sample. This approach is most appropriate with organic toxicants that are broken down by organisms with relatively specialized function. Hydrocarbons, sugars or other substrates of interest are added to the soil. Their utilization is assayed by determining labeled CO<sub>2</sub> production, labeled biomass production, or disappearance of the labeled compound. Radiolabelled hydrocarbon uptake and incorporation into biomass have been used to demonstrate increased numbers of organisms capable of degrading crude oil and petroleum products in areas contaminated by those and related compounds. Thus, soils contaminated with degradable organics could be assayed for effects on the ability to utilize particular compounds by adding a particular radiolabelled compound and following its fate. Additionally, assaying for the enrichment of organisms capable of using the toxicant in the impacted soil as compared to a standard soil, analogous to assaying for resistant microorganisms, could be performed.

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**Logistical Considerations:****Sample Collection:**

**Training:** The laboratory must be licensed to use radiolabelled materials.  
**Time:** MODERATE to EXTENSIVE to determine extent of degradation/utilization, and to determine persistence of the organisms.  
**Equipment:** EXPENSIVE radiolabelled compounds are required.

**Sample Analysis:**

**Training:** MODERATE to learn analyses.  
**Time:** MINIMAL.  
**Equipment:** A scintillation counter is needed.

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### Critique/Comments:

Before this approach can be highly useful, we need to know how long after a pollutant enters the soil before enrichment of resistant, or degradatory organisms will occur, and how long the resistant/degradatory organisms persist in the environment after a pollutant has been degraded. This approach is beneficial for the remediation of impacted soil however. The organisms capable of degrading the pollutant can be isolated, high numbers grown in the laboratory, and used to inoculate the soil at the site. Since the organism was originally from the site, novel organisms are not being placed on-site. The organisms should be able to grow in the condition at the site, since they were originally isolated from the area. Testing is needed to make certain no changes in genetic capability of the organism occurs in laboratory culture. The main methodological drawbacks of this method are the need for relatively expensive radiolabelled isotopes, disposal of the radiolabelled test material, the specialized equipment needed for determining radiolabelled-compound degradation (a liquid scintillation counter) and the fact that no general-activity radiolabelled material is available. If a spectrum of effects is suspected, each substrate must be tested separately.

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### Key References:

- Atlas, R.M. 1991. Microbial hydrocarbon degradation - bioremediation of oil spills. *J. Chem. Tech.* 52:149-156.
- Dobbins, D.C., C.M. Aelion, and F. Pfaender. 1992. Subsurface, terrestrial microbial ecology and biodegradation of organic chemicals: A review. *In* *Critical Reviews in Environmental Control*. 22(1/2):67-136.
- McCormick, N.G., J.H. Cornell, and A.M. Kaplan. 1981. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-trianylene. *Appl. Environ. Microbiology*. 42:817-823.
- Nannipieri, P., S. Grego, and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. *Soil Biochem.* 6:293-355.

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**Technique Name:** Soil Respiration

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**Technique Type:** Chemical Assay  
**Matrix Type:** Soil Microorganisms  
**Ecosystem Level:** Biochemical  
**Test Location:** Laboratory, Field

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**Description:**

Soil respiration is a general indicator of microbial activity, easily measured with relatively simple tools using easy-to-follow protocols. One main advantage is that respiration can be determined non-destructively on intact soils. The same volume of soil in the same plot of ground can be followed over time, a distinct advantage when trying to assess recovery in a system. A standardized air-tight container is placed over a known volume of soil, either in the field, or in laboratory pots. After 1 to 24 hours, the accumulation of carbon dioxide in the collecting vessel is determined and compared to controls. Respired gases can be collected by trapping in alkali (KOH), by removing a known volume of gas from the headspace of the chamber and analyzing for CO<sub>2</sub> with a gas chromatograph or respirometer.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** A gas chromatograph or respirometer is required.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** A gas chromatograph or respirometer is required.

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**Critique/Comments:**

Considerable data are available on the effects of toxic chemicals on respiration rates, although this information needs to be compiled into one source. In general, pesticides and heavy metals have significant impacts on respiration (Nohrstedt, 1987). In work with heavy metal contamination, respiration has been shown to be a useful measure of impact most likely because heavy metals have such broad effects on organisms. The most important soil characteristic influencing the toxic response was the clay content for Cd, Fe content for Cu, Pb, and Zn toxicity, and pH for Ni toxicity. Inhibition was the greatest in sand and lowest in the clay soils.

As with soil enzymes, all the organisms in soil contribute to soil respiration rates, including roots. Chemicals may impact only one component part of all the organisms present in soil and the impact on total respiration may be small compared to the respiration of all organisms present. Pinpointing the impacted organism is not possible with this method. Thus, the toxicant must have broad effects in order to disrupt all soil organism components or there is little likelihood that an effect will be seen. This metric should be used as a general indicator of serious and far-reaching impact of soil contaminants.

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**Key References:**

- Doelman, P. and L. Haanstra. 1984. Short-term and long-term effects of cadmium chromium, cooper, nickel, lead, and zinc on soil microbial respiration in relation to abiotic soil factors. *Plant Soil* 79:317-337.
- Dumontet, S. and S.P. Mathur. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic acids. *Soil Biol. Biochem.* 21:431-435.
- Grossbard, F. and H.A. Davies. 1976. Specific microbial responses to herbicides. *Weed Res.* 16:163-169.
- Nohrstedt, H.O. 1987. A field study on forest floor respiration response to artificial heavy metal contaminated acid rain. *Scand. J. For. Res.* 2:13-19.
- Parr, J.F. 1974. Effects of pesticides on microorganisms in soil water. In: *Pesticides in Soil and Water*. Guenzi, W.E., J.L. Ahlrichs, M.E. Bloodworth, G. Chesters, and R.G. Nash (eds). *Soil Sci. Soc. Amer. Inc. Madison.* pp. 315-340.

**Technique Name:** Seed Germination and Root Elongation

**Technique Type:** Germination and Growth  
**Matrix Type:** Vascular Plant Seed and Seedling  
**Ecosystem Level:** Individual  
**Test Location:** Growth Chamber, Greenhouse

**Description:**

Seed germination tests require exposure of size-graded seeds (e.g., Lactuca sativa (lettuce)) to a chemical in a soil slurry adjusted to pH 6-10. Screening tests should be completed on uncut, homogenized soil samples. For definitive tests,  $EC_{50}$  estimates require at least three replicates of at least five test soil concentrations. After planting, 16-mesh cover sand is poured over each plate, the petri dishes subsequently placed into plastic bags, sealed, and incubated at  $24 \pm 2^\circ C$  for 120 hours in an environmental chamber. The first 48 hours of incubation occurs in complete darkness, and the last 72 hours occurs under 16:8 light:dark cycle. The endpoint for screening tests is percent germination. If definitive tests are completed, median effective estimates ( $EC_{50}$ s) may be calculated.

Root elongation evaluations estimate the adverse biological effects of soil eluates to lettuce seedlings (Lactuca sativa) in a 120-hour test. Screening evaluations may be completed using uncut soil eluates; if definitive tests follow, at least three replicates must be included as part of the test design. Root lengths are measured from the transition point between the hypocotyl and root to the end of the root tip. Root elongation results in screening tests are reported as percent reduction in root lengths in treatments relative to controls; in definitive tests,  $EC_{50}$ s (the concentration which inhibits root elongation by 50% relative to controls) may be calculated. For both tests, three replicates of negative and positive controls are required for definitive tests.

**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL (120-hr).

**Equipment:** MODERATE. A refrigerator, pH meter, and supplemental lighting are required.

**Sample Analysis:**

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Critique/Comments:

Seed germination tests should be considered when field surveys suggest that plant communities have been impacted at a site, or when future land use may require a phytotoxicity evaluation as part of the soil contamination testing. The root elongation test measures biological activity of water soluble soil constituents, both contaminant and non-contaminant. The soil-derived eluate may be directly relevant to evaluations of soil contamination and groundwater quality relationships, or to evaluations of altered quality of surface water runoff from a contaminated site. Also, when soil contamination directly or indirectly impacts the plant rhizosphere, soil-derived eluates may provide information regarding interstitial water quality that potentially influences plants inhabiting contaminated soil. Both tests have been standardized and approved by USEPA and FDA.

The current data collection is heavily skewed toward north-temperate, agricultural species, particularly grasses and legumes; little information is available regarding less commercially important native plants and woody species.

Key References:

- AOSA (Association of Official Seed Analysts). 1990. Rules for testing seeds. J. Seed Tech. 12:1-122.
- CFR (Code of Federal Regulations). 1985. Rules and regulations; Section 797.2750, Seed germination/root elongation toxicity test. September 27, 1985. CFR 50 (188):39389-39391.
- FDA (Food and Drug Administration). 1987. Sections 4.06 (Seed germination and root elongation); 4.07 (Seedling growth). In Environmental Assessment Technical Assistance Handbook. NTIS, PB 87-175345. U.S. Food and Drug Administration, Washington, D.C.
- Linder, G., J.C. Greene, H. Ratsch, J. Nwosu, S. Smith, and D. Wilborn. 1990. Seed germination and root elongation toxicity tests in hazardous waste site evaluation: methods development and applications. In Plants for Toxicity Assessment, ASTM STP 1091. W. Wang, J.W. Gorsuch, and W.R. Lower, Eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 177-187.
- Ratsch, H. 1983. Interlaboratory root elongation testing of toxic substances on selected plant species. NTIS, PB 83-226. U.S. Environmental Protection Agency, Environmental Protection Agency, Washington, D.C.

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**Technique Name: Early Seedling Survival and Vegetative Vigor**

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**Technique Type:** Survival and Growth  
**Matrix Type:** Vascular Plant  
**Ecosystem Level:** Organismal  
**Test Location:** Growth Chamber or Greenhouse

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**Description:**

Seedlings are grown in soils collected on-site from identified sampling locations or are grown in soils dosed with known levels of contaminants. Plants identified for testing should be selected to meet the site-specific data needs (e.g., commercial seeds or native seeds), and should be grown under greenhouse or environmental chamber conditions specified by their species requirements. Supplemental lighting may be required to ensure sufficient photosynthetically active radiation under specified lighting regimens. Growth conditions, e.g., temperature and humidity, should be recorded daily as well as any additional exposure conditions that are critical to successful completion of the test. At test termination (usually 14 days), plant leaves and roots should be collected from each exposure and control replicate, and total biomass should be recorded as an endpoint for assessing plant vigor. Supplemental endpoints may also be defined during the problem formulation phase of an ecological effects study design (e.g., physiological and morphological indicators of plant health). In order to adequately interpret test endpoints, soil samples should be split after being prepared for testing and submitted for physicochemical characterization (e.g., soil moisture and pH, textural analysis, total nitrogen and total organic matter, and cation exchange capacity).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MODERATE (14-day).

**Equipment:** MODERATE to EXPENSIVE, a growth chamber or greenhouse is required.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** MINIMAL to MODERATE. A pH meter is required.

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**Critique/Comments:**

Vegetative vigor and early seedling survival tests are designed to extend the information gathered using short-duration phytotoxicity tests. For example, seed germination tests and root elongation tests may not adequately show chronic effects that are potentially associated with low concentration, environmental contaminant exposures. Vegetative vigor and early seedling survival tests may also be designed to address site-specific questions related to contaminant uptake into plant tissues, if chemical analytical data are collected concurrent with harvest data.

Within a regulatory setting, various agencies have outlined the requirements and specifications for vegetative vigor and early seedling survival tests (Holst and Ellwanger 1982; OECD 1984; FDA 1987). As with short-term phytotoxicity tests, the comparative data base is sparse, and testing with north-temperate, agricultural species, particularly grasses and legumes is emphasized. Little information is available for less commercially important native plants and woody species.

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**Key References:**

- CFR (Code of Federal Regulations). 1985. Rules and regulations; Section 797.2800, Early seedling growth toxicity test. September 27, 1985 CFR 50 (188):39391-39393.
- FDA (Food and Drug Administration). 1987. Sections 4.06 (Seed germination and root elongation); 4.07 (Seedling growth). In Environmental Assessment Technical Assistance Handbook. NTIS, PB 87-175345. U.S. Food and Drug Administration, Washington, D.C.
- Gorsuch, J.W., R.O. Kringle, and K.A. Robillard. 1990. Chemical effects on the germination and early growth of terrestrial plants. In Plants for Toxicity Assessment, ASTM STP 1091. W. Wang, J.W. Gorsuch, and W.R. Lower, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 49-58.
- OECD (Organization for Economic Co-Operation and Development). 1984. OECD guidelines for testing of chemicals. Director of Information, OECD. 2, rue Andre Pascal, 75775 Paris Cedex 16, France.

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**Technique Name:**            **Rooted Aquatic and Wetland Plants**

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**Technique Type:** Growth and Physiology  
**Matrix Type:**        Vascular Plants (Aquatic)  
**Ecosystem Level:**        Organismal  
**Test Location:**        Growth Chamber, Greenhouse, Test Ponds

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**Description:**

Standardized plant toxicity tests have been developed for species native to freshwater/estuarine and wetland environments. Aquatic species commonly tested include Hydrilla verticillata and Potamogeton pectinatus (sago pondweed). In the Hydrilla v. test, screening tests or definitive tests using soil or sediment dilutions may be completed with the standardized test system. Regardless of screening or definitive test application, each sediment or hydric soil sample should be evaluated in triplicate with three plants per jar following a 14-day exposure. Hoagland's nutrient is added to the jars and incubation occurs in an environmental chamber under controlled temperature ( $25 \pm 1^\circ\text{C}$ ) with continuous cool white fluorescent light ( $40 \mu\text{E m}^{-2}\text{sec}^{-2}$ ). Test endpoints may include estimates of shoot and root growth, as well as biological markers indicative of sublethal contaminant effects (Byl and Klaine 1991). P. pectinatus tests require a four-week test period.

Testing wetland sites includes the marsh plant, Echinochloa crusgalli. After 14-day exposure under controlled environmental conditions, plants are counted and weighed to measure survival and growth endpoints. For interpretation of ecological effects, methods are outlined for designing reference sediments or wetland soils, if natural reference materials are not available (Walsh, et al 1990).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to MODERATE.

**Time:** MODERATE (14-30 days).

**Equipment:** MINIMAL to MODERATE to regulate environmental factors.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** MINIMAL.

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**Critique/Comments:**

Plant tests with rooted aquatic plants have had previous application in evaluating contaminated sediments, and have a relatively well established toxicity data base in the literature for selected contaminants. Testing with marsh plants has only recently been fully developed, and the data base for hazardous waste site applications is limited. Technically, these tests, whether using aquatic rooted plants, or freshwater or estuarine wetland plants, are relatively straight forward, yet ecologically relevant contaminant information can be gained in a relatively short time period. Although the number of test species is relatively limited, the increasing awareness regarding the ecological significance of wetland habitats should support a consideration of site-specific laboratory testing.

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**Key References:**

- Byl, T.D. and S.J. Klaine. 1991. Peroxidase activity as an indicator of sublethal stress in the aquatic plant Hydrilla verticillata (Royle). In Plants for Toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 101-106.
- Fleming, W.J., M.S. Ailstock, J.J. Momot, and C.M. Norman. 1991. Response of sago pondweed, a submerged aquatic macrophyte, to herbicides in three laboratory culture systems. In Plants for Toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 267-275.
- Walsh, G.E., D.E. Weber, L.K. Brashers, and T.L. Simon. 1990. Artificial sediments for use in tests with wetland plants. Environ. Exper. Botany 30:391-396.
- Walsh, G.E., D.E. Weber, T.L. Simon, and L.K. Brashers. 1991. Toxicity tests of effluents with marsh plants in water and sediment. In Plants for Toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 517-525.

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**Technique Name: Plant Uptake Bioassay**

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**Technique Type:** Contaminant uptake in plants  
**Matrix Type:** Vascular Plant (nutsedge)  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

This bioassay is appropriate for estimating mobility of contaminants into the environment through plant uptake in wetland and marsh environments as well as drier upland sites. The test method was originally designed by the US Army Corps of Engineers Waterways Experiment Station (WES) to evaluate field-collected dredge materials. Field-collected sediment or wetland soils are physicochemically characterized, mixed and placed in pots. Cyperus esculentus (nutsedge) is planted in the contaminated sediment/soil and maintained under flooded and/or upland conditions for 45 days in a greenhouse or environmental chamber under controlled conditions (32±2°C daylight temperatures, 21±2°C night temperatures under 1,200  $\mu\text{E}/\text{m}^2$  photosynthetically active radiation (PAR) and > 50% relative humidity). Above-ground biomass is measured and contaminant content of leaves is determined.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to germinate and maintain plants.

**Time:** MODERATE (45-day test).

**Equipment:** MINIMAL.

**Sample Analysis:**

**Training:** MINIMAL to measure biomass. MODERATE to extract and analyze for heavy metals and organic compounds.

**Time:** MINIMAL to MODERATE to extract and analyze contaminants.

**Equipment:** MODERATE to EXPENSIVE for equipment to measure contaminant concentrations.

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**Critique/Comments:**

This method offers a technique for measuring uptake of a contaminant in a plant with a relatively short life-cycle. However, to date, the technique has only been applied to uptake of trinitrotoluene (TNT) in dredged material. Yellow nutsedge testing, although well-defined and applied in the U.S. Army Corps of Engineers dredge materials program, is unexploited for ecological assessments. Within an ecological risk assessment, various remedial options such as sediment dredging may suggest that testing with Cyperus esculentus be incorporated into site management plans. Also, the method could be adapted for soils.

Few technical support laboratories are currently providing tests with yellow nutsedge. Furthermore, equipment needed for extraction, purification, and analysis of contaminants in plant tissue is relatively expensive. Site-specific interpretation of ecological effects associated with potential responses in the test would center upon "laboratory to field" extrapolation error and interspecies variability with respect to contaminant-mediated adverse effects.

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#### Key References:

- Folsom, Jr., B.L. and R.A. Price. 1991. A plant bioassay for assessing plant uptake of contaminants from freshwater soils or dredged material. In Plants for toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 172-177.
- WES (U.S. Army Corps of Engineers Waterways Experiment Station). 1989. A plant bioassay for assessing plant uptake of heavy metals from contaminated freshwater dredged material. Technical Note EEDP-04-11. U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.

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**Technique Name:** TOXSCREEN

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**Technique Type:** Whole Plant Toxicity  
**Matrix Type:** Vascular Plant (mature)  
**Ecosystem Level:** Organismal  
**Test Location:** Greenhouse or Environmental Growth Chamber

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**Description:**

TOXSCREEN tests whole plant (non-seedling) response to hydroponically-applied chemicals. Plants, e.g. soybean (Glycine max) and barley (Hordeum vulgare) are grown in hydroponic culture in an environmentally-controlled greenhouse or growth chamber for 28 days, then exposed to chemicals in solution for 3-5 days. Test conditions are maintained under constant photoperiod (16/8 light/dark, light intensity of  $350 \text{ umolm}^{-2}\text{s}^{-1}$  at top of canopy) at  $25/21 \pm 2^\circ\text{C}$  and 50-70% relative humidity. When appropriate, solvent systems may be used as carriers, for example, when rhizosphere exposures are designed to reflect site-specific conditions. Soil eluates may also be used. Toxicity endpoints routinely include survival and growth, although exposure systems could be designed that allow additional measurements for estimating sublethal effects (McFarlane, et al. 1990).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to set up and maintain hydroponics system.  
**Time:** MODERATE to construct and calibrate hydroponics system. MINIMAL to perform experiments (3-5 days).  
**Equipment:** MODERATE to EXPENSIVE. Greenhouse or growth chambers required.

**Sample Analysis:**

**Training:** MINIMAL to test for growth endpoints, MINIMAL to MODERATE to test for physiological responses.  
**Time:** MINIMAL to measure most endpoints.  
**Equipment:** MINIMAL for growth endpoints. MINIMAL to EXPENSIVE to measure physiological responses.

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**Critique/Comments:**

TOXSCREEN should be considered primarily as a screening test, particularly if contaminants of concern are water soluble and conducive to hydroponic exposures. Depending upon site-specific characteristics, target analytes could be used in single compound or defined chemical mixture exposures, and if sufficient soil were collected, eluates could be used as the exposure medium. An advantage of the test over previous screening methods is that whole plants are used rather than seedlings. One disadvantage is that soils cannot be tested directly. TOXSCREEN was originally designed with regulatory applications being the central focus and therefore should be considered in developing sampling and analysis plans, depending upon site-specific contingencies. Few technical support laboratories are currently providing tests with these organisms; owing to its recent description, TOXSCREEN is not commercially available. If adequate facilities and technical support are available, the test exposure is relatively short; however, adequate technical considerations must be made to assure that plant materials are available for testing (e.g., hydroponic nursery facility or commercial sources).

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**Technique Name:** Vascular Plant Life-cycle Tests

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**Technique Type:** Plant Bioassay  
**Matrix Type:** Vascular Plant  
**Ecosystem Level:** Organismal  
**Test Location:** Greenhouse or growth chamber

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**Description:**

Two hydroponic test systems using short life-cycle plants are potentially applicable to ecological effects assessments for hazardous waste sites. Water-soluble constituents of waste site chemical mixtures may be evaluated with either Arabidopsis thaliana or Brassica rapa. Exposures occur in double-pot, static-replacement systems where a vermiculite-filled growth container is nested above a second larger pot that serves as a nutrient solution reservoir. Nutrients and water move from the nutrient reservoir to the vermiculite via polyester wicks that are draped between the two pots. Seeds are uniformly planted on the surface of the vermiculite, and greenhouse conditions or large growth chambers assure similar growing conditions for all plants. Depending upon the exposure period and growth conditions, plants will set seeds and mature. Exposure periods (approximate seed-to-seed life-cycle) are 28-36 days for A. thaliana and 36-44 days for Brassica rapa. Endpoints include total biomass, individual organ biomass (e.g., stems, leaves, roots, seeds, fruits), leaf and flower structure, and initial flowering date.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MODERATE, 28-44 days.

**Equipment:** MODERATE to EXPENSIVE, a hydroponic system and greenhouse or growth chamber are required.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:**

An analytical balance is required for biomass endpoints.

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**Critique/Comments:**

Both of these full life-cycle tests are intended to address toxicity endpoints that are inadequately considered in standardized plant tests measuring seed germination and root elongation. Exposures are hydroponic, and contaminant water solubility may limit exposures for some chemicals. If eluates are used in regard to soil contaminant as sources for potential groundwater and rhizosphere contamination, a direct measure of "worst case" can be addressed using these systems. Relatively large volumes of eluate may be required for these hydroponic systems relative to that volume used in the standard root elongation test, however; defined chemical mixtures similar to those found in site-soils could be incorporated into the test system's nutrient solution and used as an alternative exposure system. Few technical support laboratories are currently providing tests with these organisms; establishing this system in one's own facility may prove to be too costly and time-consuming. A book has recently been published describing detailed methods in *Arabidopsis* research (Koncy et al, 1992).

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**Key References:**

- Koncy, C., N. Chua, and J. Schell. 1992. *Methods in Arabidopsis Research*. World Scientific, River Edge, NJ.
- Ratsch, H.C., D.J. Johndro, and J.C. McFarlane. 1986. Growth inhibition and morphological effects of several chemicals in *Arabidopsis thaliana* (L.) Heynh. *Environ. Contam. Toxicol.* 5:55-60.
- Shimabuku, R.A., H.C. Ratsch, C.M. Wise, J.U. Nwosu, and L.A. Kapustka. 1991. A new plant life-cycle bioassay for assessment of the effects of toxic chemicals using rapid cycling *Brassica*. *In Plants for Toxicity Assessment: Second Volume*, ASTM.
- STP 1115. J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds. *American Society for Testing and Materials*, Philadelphia, PA. pp. 365-375.

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**Technique Name:** Plant Tissue Culture Tests

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**Technique Type:** Plant Bioassay  
**Matrix Type:** Plant Cell and Callus Tissue  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

For evaluating subacute effects, particularly chemical-related alterations in plant metabolism, plant cell and tissue culture techniques have become well developed over the past ten years. Suspension cultures of commercially important plant species e.g., soybean (Glycine max) and wheat (Triticum aestivum) are exposed to contaminants added to the culture nutrient medium. Exposures pertinent to an ecological effects assessment require that eluates be prepared from site-soil. The indirect effects of soil contaminants could then be evaluated by supplementing the test nutrient medium with eluate spikes. Alternatively, if the contaminant history for the site was reliable, or if analytical information regarding soil contaminants was available, defined chemical mixtures could be added as supplements to the nutrient medium. Endpoints include biomass, metabolic fate and biotransformation of chemicals. Similar test methods have been developed using callus cultures.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MODERATE to EXTENSIVE to learn tissue culture techniques.

**Time:** MODERATE (30-60 days) to grow cultures.

**Equipment:** MODERATE, tissue culture media and materials, and environmentally controlled chambers or rooms are required.

**Sample Analysis:**

**Training:** MODERATE to EXTENSIVE training in plant biochemistry is required to assess metabolic end products.

**Time:** MODERATE to purify and quantitate metabolites.

**Equipment:** MODERATE to EXPENSIVE analytical equipment is required to quantitate metabolites.

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**Critique/Comments:**

Both callus and cell suspension cultures of various plant species have been used in evaluating subacute chemical effects in plants, primarily by addressing the metabolic fate of xenobiotics (e.g., herbicides) in plants; but little correlative work has been completed to address the ecological interpretation of these in vitro plant cell and tissue culture methods. The method(s) outlined and summarized here are relatively early in the standardization process, and are not intended to be "stand alone" tests. Rather, the strengths of these method(s) lie in their contribution to evaluating phytotoxicity in species which are difficult to assess with whole plant tests. Metabolic effects of chemicals, ascertained by tissue-culture analysis, may be used to explain site-specific effects associated with soil exposures, e.g., diminished vigor in woody shrubs or poor reproductive performance in forbes, found during field surveys.

Tissue culture and analytical methods are costly and time-consuming. Few technical support laboratories provide tissue-culture techniques and chemical exposure/analyses. The cost-effectiveness of using tissue-culture techniques should be considered before implementing these methods in a risk assessment.

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**Key References:**

- Ebing, W., A. Haque, I. Schuphan, H. Harms, C. Langebartels, D. Scheel, K.T. von der Trenck, and H. Sanderman. 1984. Ecochemical assessment of environmental chemicals: draft guideline of the test procedure to evaluate metabolism and degradation of chemicals by plant cell cultures. *Chemosphere* 13:947-957.
- Harms, H. and C. Langebartels. 1986. Standardized plant cell suspension test systems for an ecotoxicologic evaluation of the metabolic fate of xenobiotics. *Plant Sci.* 45:157-165.
- Wickloff, C. and J.S. Fletcher. 1991. Tissue culture as a method for evaluating the biotransformation of xenobiotics by plants. In *Plants for Toxicity Assessment: Second Volume*, ASTM STP 1115. J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 250-257.
- Zilkah, S. and J. Gressel. 1977b. Cell cultures vs. whole plants for measuring phytotoxicity. III. Correlations between phytotoxicities in cell suspension cultures, calli, and seedlings. *Plant & Cell Physiol.* 18:815-820.

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**Technique Name:** Plant Community Structure Mesocosm

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**Technique Type:** Community Structure Analysis of Vascular Plants  
**Matrix Type:** Vascular Plant  
**Ecosystem Level:** Community  
**Test Location:** Greenhouse or Field

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**Description:**

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A mesocosm, representative of a larger system, is used to determine the impacts of the release of chemicals from hazardous waste sites on plant community structure. Seed for testing is obtained commercially from regional native seed supply sources, and the plant community to be tested can be defined. Alternatively, a seed bank is collected from a reference location and used in evaluations of site-soil. If seed bank sources are used, past land and chemical use should be documented and any confounding effects owing to the selection of reference area seed bank should be acknowledged. Raised beds are typically used as exposure containers and may be located in the field or in the greenhouse, depending upon the site-specific study design. Defined seed mixtures or seed bank are then incorporated into the soil, and depending upon the study design, irrigation and fertilization can be specified. Each site-specific study plan may differ in their details for analysis of plant community responses to contaminated soils, but for waste sites with similar contaminant histories and similar habitat settings, study designs may be nearly identical. Exposures will vary with respect to duration depending upon regional characteristics (e.g., native plant species composition when initiating test from seed bank). Target plant species may be identified for specific focus in the study. Or, ecological endpoints may be identified for analyzing community-level responses. For example, percent vegetative cover, total biomass, species diversity and richness may be determined. The level of analytical detail should be determined initially in the study design. Regardless of the study design, identification of a reference soil is critical in the evaluation of soil contamination and its effects on native plants.

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**Logistical Considerations:**

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**Sample Collection:**

**Training:** MINIMAL to construct and maintain mesocosm.

**Time:** EXTENSIVE (3-9 months) to establish a plant community within the mesocosm.

**Equipment:** A greenhouse may be required.

**Sample Analysis:**

**Training:** MINIMAL to MODERATE.

**Time:** MODERATE to EXTENSIVE for measuring community-level responses.

**Equipment:** MINIMAL.

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**Critique/Comments:**

Intended use: Although concentration-response relationships may be designed as part of the vegetation evaluation completed with a plant community study, the method may be more valuable as a screening method complementary to controlled plant test, e.g., vegetative vigor and early seedling survival. By using both an "ecotoxicity test" to measure plant community responses to contaminated soil and an organismic-level test like vegetative vigor and early seedling survival, uncertainty in the risk characterization for the site may be more adequately addressed on the basis of site-specific empirical information. As a field test, or greenhouse test, the plant mesocosm exposure beds are relatively easy to establish, but the test is time and labor intensive, owing to the real-time growth required for biomass measurements and data collection and reduction for evaluating community structure.

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**Key References:**

- Pfleeger, T. 1991. Impact of airborne pesticides on natural plant communities. *In* Plant tier testing: a workshop to evaluate nontarget plant testing in Subdivision J Pesticide Guidelines. 600/9-91/041. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.
- Weinstein, L.H. and J.A. Laurence. 1989. Indigenous and cultivated plants as bioindicators. *In* Biologic markers of air-pollution stress and damage in forests. Committee on Biologic Markers of Air-Pollution Damage in Trees, G.M. Woodwell (Chair). National Research Council. National Academy Press. Washington, D.C. pp. 195-204.
- Weinstein, L.H., J.A. Laurence, R.H. Mandl, and K. Walti. 1990. Use of native and cultivated plants as bioindicators and biomonitors of pollution damage. *In* W. Wang, J.W. Gorsuch, and W.R. Lower (eds.). Plants for Toxicity Assessment. ASTM STP 1091. American Society for Testing and Materials, Philadelphia, PA. pp. 117-126.

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**Technique Name:** Phytotoxicity Testing with Ambient Air  
**Exposure Systems**

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**Technique Type:** Visual Injury, Physiological Analysis  
**Matrix Type:** Vascular Plant (terrestrial)  
**Ecosystem Level:** Organismal, Community  
**Test Location:** Field, Greenhouse, Growth Chamber

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**Description:**

Field, greenhouse, and growth chamber exposure systems, originally designed to examine the effects of gaseous air pollutants and acidic deposition on vegetation can be adapted for ecological risk assessments. Known concentrations of pollutant gases (e.g., O<sub>3</sub>, SO<sub>2</sub>, volatile organics) and/or wet deposition contaminants (e.g., acidic precipitation) have been applied to crop plants, forest trees, and native vegetation using these systems. Additionally, field systems can be used to regulate pollutant exposure on-site by selectively filtering contaminants. Measurement endpoints include foliar injury, biomass growth, physiological measurements (e.g., photosynthesis, respiration) and bioaccumulation of contaminants in plant tissues.

Recently, a field method was developed to test the impact of smokes/obscurants used by the U.S. Army in training exercises on native vegetation (Sadusky, et. al., 1993, Skelly, 1990). Open-top field exposure chambers were adapted to expose tree seedlings to hexachloroethane smoke. Following four exposures at two-week intervals, particulate deposition was estimated and visual injury was quantified. This method may be adapted for use with other air pollutants found at military installations.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to set up and maintain systems.

**Time:** MODERATE (7-30 days) to study acute effects,  
EXTENSIVE (2 months-2 years) to study chronic effects.

**Equipment:** EXPENSIVE to install and maintain exposure systems.

**Sample Analysis:**

**Training:** MINIMAL for most endpoint measurements.

**Time:** MINIMAL.

**Equipment:** MINIMAL to MODERATE. Analytical equipment maybe necessary for measurement of physiological endpoints.

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**Critique/Comments:**

The method(s) outlined and summarized here are well developed but have not been standardized. The strengths of these method(s) lie in their potential contribution to evaluating exposure pathways that generally have not been considered within an ecological effects assessment (e.g., exposures to military smokes/obscurants). In determining whether ambient air exposures are critical to the ecological effects assessment, various elements influencing exposure should be considered. In general these elements may be categorized as: contaminant physicochemical attributes in the atmosphere; soil, habitat, and atmospheric conditions that may influence exposure and non-exposure periods; and biological attributes of receptors - plant or animal - that may be exposed via ambient air pathways. These systems are very expensive to establish and maintain and technical support laboratories with established systems may not be equipped to adapt their systems to tests with military chemicals.

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**Key References:**

- Heagle, A.S., D.E. Body, and W.W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* 2:365-368.
- Hogsett, W.E., D. Olszyk, D.P. Ormord, G.E. Taylor, Jr., and D.T. Tingey. 1987. Air pollution exposure systems and experimental protocols. Volume 1: A review and evaluation of performance. 600/3-87/037a. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.
- Hogsett, W.E., D. Olszyk, D.P. Ormord, G.E. Taylor, Jr., and D.T. Tingey. 1987. Air pollution exposure systems and experimental protocols. Volume 2: Description of facilities. 600/3-87/037b. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.
- Sadusky, M.C., J.M. Skelly, M. Simini, R.T. Checkai, and R.S. Wentzel. 1993. Hexachloroethane obscurant: Assessing tree foliage injury. *Environ. Tox. Chem.*, 12(4)685-694.
- Skelly, J.M. 1990. Open-top chambers for ecological assessments. Evaluation of the effects of Army smokes/obscurants on forest tree species and "natural" vegetation. Final Report. Battelle Research, Research Triangle Park, NC.

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**Technique Name:** Chlorophyll Fluorescence Bioassay

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**Technique Type:** Photosynthesis Inhibition Bioassay  
**Matrix Type:** Terrestrial and Wetland Plants  
**Ecosystem Level:** Organismal  
**Test Location:** Growth Chamber or Field

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**Description:**

Impaired photosynthetic function caused by stresses due to soil contamination may be indicated by abnormal fluorescence patterns relative to plants inhabiting uncontaminated soils. A transportable fluorometer dedicated to analysis of photosynthesis is used in the field or at a fixed laboratory. Intact leaves or leaf segments are placed with the adaxial surface facing an actinic light source in the fluorometer. After dark adaptation (generally less than two minutes), fluorometric analyses are initiated. Fluorescence profiles may be plotted on an X-Y recorder, or electronic data may be stored in a data logger for later analysis. Variable and maximum fluorescence values [ $F_v$  and  $F_{max}$ ] are measured from these fluorescence profiles, and plant health is in part described on the basis of derived ratio estimators based on  $F_v$  and  $F_{max}$  for short-term (0-30 sec) analyses. Longer-term tests (30 seconds to six minutes) have been performed to determine fluorescence decay over time. Fluorescence bands with maxima at 440 nm, 685 nm, and 740 nm have been measured.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** A portable fluorometer and oscilloscope, datalogger, or computer is required.

**Sample Analysis:**

**Training:** MINIMAL to extrapolate and calculate endpoints.

**Time:** MINIMAL.

**Equipment:** A computer is required to calculate endpoints.

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**Critique/Comments:**

Chlorophyll fluorescence analysis is a fast, simple method for intact plants. If considered alone, photosynthesis or fluorescence may express sufficient variability (i.e., diurnal, seasonal, age-related, and non-contaminant related effects) to confound interpretations regarding adverse ecological effects. However, if completed in conjunction with whole-plant studies, altered fluorescence profiles in exposed plants may lend credence to interpretations of adverse ecological effects on the basis of site-specific empirical data. Also, compensatory mechanisms common to plants, e.g., reallocation of resources between plant organs in response to contaminant stress, may make extrapolation from leaf-level measurements to whole-plant and plant community-level interpretations difficult. Good control plants are essential.

The method(s) outlined and summarized here are early in the standardization process, and are not intended to be "stand alone" tests. Rather, the strengths of these method(s) lie in their contribution to weight of evidence arguments that are supportive

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**Technique Name:** Aquatic Organism Toxicity Tests

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**Technique Type:** Aquatic Bioassay  
**Matrix Type:** Groundwater, surface water, soil or sediment eluate  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Various aquatic organisms from many different trophic levels have been used to assess the effects of contaminants in aquatic ecosystems. Species of fish, macroinvertebrates, algae, and zooplankton are commonly used in bioassays. Organisms are added to a dilution series of site surface water, groundwater, or soil/sediment eluate. At the end of a given exposure period (usually 24, 48, or 96 h, depending on the species) for acute exposure or seven days for chronic exposure, relevant endpoints are measured and statistically calculated. Algal toxicity tests are conducted by adding cells of Selenastrum capricornutum to a series of concentrations of site surface water, groundwater, or soil/sediment eluate. Test chambers are incubated for 96 h under specific lighting conditions. At the end of the test period, cells are counted to determine measures of algal biomass and mean cell volume.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL

**Equipment:** MINIMAL for tests with surface water. A centrifuge is required for eluate extraction from sediment and soil.

**Sample Analysis:**

**Training:** MINIMAL

**Time:** MINIMAL

**Equipment:** A microscope, spectrophotometer, or electronic particle counter is needed to quantify algal cells.

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## Critique/Comments:

These tests have been extensively researched and validated for use in the field of environmental assessments. A large body of information is available documenting the ability of these tests to confirm the existence of adverse ecological effects. Unicellular algae function as primary producers and as such are important components of the aquatic ecosystem. Algal communities may be inhibited or stimulated by water quality changes. Cladoceran species -- e.g. Daphnia magna, Daphnia pulex, Ceriodaphnia dubia -- are the most common invertebrate species used. Fish tests typically involve species such as fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus), or rainbow trout (Oncorhynchus mykiss). These tests have been developed for use with a broad range of organisms beyond the few listed here. The American Society for Testing and Materials and the American Public Health Association have established testing guidelines for many species and for variations of the described methods.

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## Key References:

- APHA, AWWA, WPCF. 1989. Part 8000 Toxicity test methods for aquatic organisms, In L.S. Clesceri, A. E. Greenberg and R.R. Trussel, eds., Standard Methods for the Examination of water and wastewater, 17th ed. American Public Health Association, Washington, D.C. pp. 8-1 through 8-143.
- American Society for Testing and Materials (ASTM). 1994. Standard practice for conducting acute toxicity tests on aqueous effluents with fishes, macroinvertebrates, and amphibians, ASTM Committee E-47, American Society for Testing and Materials, Philadelphia, PA.
- Blanck, H., and B. Bjornsater. 1989. The algal microtest battery: a manual for routine test of growth inhibition. KEMI Science and Technology Department Report, No. 3/89.
- Environment Canada. 1992. Biological test method: growth inhibition test using the freshwater alga Selenastrum capricornutum. Conservation and Protection. Ottawa, Ontario, Canada. Environmental Protection Series, Draft Report (Jan.) 42p.
- Horning, W.B., and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/60/4-85/014. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.
- Linder, G., J. Wyant, R. Meganck, and B. Williams. 1991. Evaluating amphibian responses in wetlands impacted by mining activities in the western United States. In R.D. Comer, P.R. Davis, S.Q. Foster, C.V. Grant, S. Rush, O. Thorne, and J. Todd (eds.). Issues and technology in the management of

impacted wildlife. Thorne Ecological Institute. Boulder, CO. Pp. 17-25.

Peltier, W. and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. Third Edition. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

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**Technique Name:** Sediment Toxicity Tests

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**Technique Type:** Aquatic Bioassay  
**Matrix Type:** Freshwater Sediment  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Test organisms are added to chambers containing contaminated sediment and control water in a 1:4, v/v, ratio of sediment and water. At the end of the test, one of a wide variety of endpoints is measured. Test organisms commonly used in sediment toxicity tests include bacteria, rotifers, nematodes, periphyton, pelecypods, oligochaetes, cladocerans, isopods, amphipods, insects, fish, amphibians, and macrophytes. Sediment toxicity assessments can be conducted using acute or chronic exposures using one of a variety of endpoints depending on exposure period and test organism. Typical endpoints include survival, growth, molting frequency, reproduction, enzyme activity, avoidance, embryo-larval survival, adult emergence, and luminescence.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** MINIMAL

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MINIMAL  
**Equipment:** MINIMAL

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**Critique/Comments:**

A serious need exists for the development of standard sediment toxicity assessment procedures. The procedure described above varies considerably from some other accepted methods currently used. Test species should be selected based on their behavior in the sediment, their sensitivity to chemical and physical

parameters of the sediment, their availability, their sensitivity to the contaminant, and sediment phase tested. A large amount of literature deals with the subject of species selection. Benthic organisms serve as excellent overall indicators of aquatic contaminant effects for several reasons. Benthic organisms are integrally associated with the sediment and interstitial waters. The sensitivity of many species to common pollutants is well documented. Several well developed assays have proven effective in detecting sediment toxicity. One of the most important aspects of sediment toxicity assessment is selection of the proper sediment phase to test. Phases of sediment are extractable phase, elutriate phase, interstitial water phase, and whole sediment. The elutriate phase contains contaminants extractable by water. Since many contaminants are not removed by water, other solutes may be employed to extract contaminants in the extractable phase. The interstitial water phase is the interstitial water from the sediment. The whole sediment is the sediment sample collected from the contaminated waste site with as little manipulation as possible.

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#### Key References:

- American Society for Testing and Materials. 1994. ASTM standards on Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials, Philadelphia, PA.
- Burton, G.A., Jr. 1991. Assessing the toxicity of freshwater sediments. *Environ. Toxicol. Chem.* 10:1585-1627.
- Burton, G.A., Jr., M.K. Nelson and C.G. Ingersoll. 1992. Freshwater benthic toxicity tests, In G.A. Burton, ed., *Sediment Toxicity Assessment*. Lewis Publishers, Boca Raton, FL, pp 213-240.
- Giesy, J.P. and R.A. Hoke. 1989. Freshwater sediment toxicity bioassessment: rationale for species selection and test design. *J. Great Lakes Res.* 15:539.
- Hill, I.R., Matthiessen, P, and F. Heimbach, eds. 1994. Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments. Society of Environmental Toxicology and Chemistry-Europe. 105 pp.
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**Technique Name: Amphibian Test Methods**

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**Technique Type:** Survival, Growth, Teratogenesis  
**Matrix Type:** Amphibian (embryos)  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

Amphibian testing uses FETAX (frog embryo teratogenesis assay: Xenopus laevis). Tests may be completed with surface waters, groundwater, or soil/sediment-derived eluates. To initiate exposures, less than eight-hour old frog embryos are placed in Petri dishes containing aqueous test solutions for either screening or definitive tests. In definitive tests triplicate exposure series are set up with a maximum of five to six concentrations plus controls in each replicate. For screening purposes, triplicate Petri dishes may contain 100% site-samples. Once exposures have been initiated, the 96-hour static-replacement exposures are renewed at 24-hour intervals at 22±2°C. Endpoints include survivorship, growth (e.g., length), and malformations observations. Survivorship data (LC<sub>50</sub> or percent survival in 100% site-sample) is determined at the end of four-day exposures. Similarly, EC<sub>50</sub>s for malformation are recorded in definitive tests, or percent malformations is recorded in screening tests. Subacute response data will reflect numbers of gross terata (e.g., scoliosis, lordosis, and kyphosis) developed in exposed embryos.

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**Logistical Considerations:****Sample Collection:**

**Training:** MINIMAL to expose and maintain organisms.

**Time:** MINIMAL (96-hr).

**Equipment:** MINIMAL to maintain embryos.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** MINIMAL.

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**Critique/Comments:**

Amphibian test systems are standardized through ASTM (American Society for Testing and Materials). Early embryos of the African clawed-frog (Xenopus laevis) are used in the standardized test; however, much work has been completed with alternative test species and should be considered on a site-specific basis. The test method was originally designed for testing surface waters and water column exposures with sediments. At present the method is most directly applicable to wetland evaluations that may be required as part of an ecological effects assessment.

Care must be taken to correctly determine the degree of ecological significance that may be derived from these tests. Unless in situ methods are also included as part of the ecological effects assessment "laboratory to field" extrapolation error may confound biological assessments within an ecological risk context. While more laboratories are offering testing services with amphibians, only a limited number of technical support laboratories are currently providing tests with these organisms.

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**Key References:**

- ASTM E729. 1991. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA.
- ASTM E1439. 1991. Standard guide for conducting the frog embryo teratogenicity test: Xenopus. Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA.
- Adamus, P.R. and K. Brandt. 1990. Impacts on quality of inland wetlands of the United States: A survey of indicators, techniques, and applications of community level biomonitoring data. (EPA/600/3-90/073). U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oregon, 97333.
- Linder, G., J. Wyant, R. Meganck, and B. Williams. 1991. Evaluating amphibian responses in wetlands impacted by mining activities in the western United States. In R.D. Comer, P.R. Davis, S.Q. Foster, C.V. Grant, S. Rush, O. Thorne, and J. Todd (eds.). Issues and technology in the management of impacted wildlife. Thorne Ecological Institute. Boulder, CO. Pp. 17-25.

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**Technique Name:           Static Microalgae Toxicity Test**

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**Technique Type:** Growth Assay  
**Matrix Type:** Green Algae  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

Algae are cultured in nutrient media. Culture in log phase growth ( $\geq 2 \times 10^4$  cells/mL) are placed into erhlenmyer flasks containing reagent grade laboratory chemicals. The preferred solvent is dilution water. However, if an organic solvent is necessary, triethylene glycol is recommended because of its low volatility and high ability to dissolve organic chemicals. Methanol, ethanol, and acetone may also be used, but they might stimulate undesirable growth of algae and microorganisms. The concentration of an organic solvent should be  $\leq 0.5$  mL/L. Treatments consist of one or more controls and a geometric series of at least five concentrations of test material. Temperature and illumination should be controlled and will differ depending on the test organism. Several organisms are recommended depending upon the type of habitat being studied. For freshwater studies, the green algae Selenastrum capricornutum is most widely used, however other green and blue green algae, and diatoms have been used successfully. The diatom Skeletonema costatum is most commonly used for saltwater samples. Test endpoints are biomass and 96-hour IC<sub>50</sub> based on reduction of growth.

A modified version of this test uses microplates (220 ul, 10,000 cells/mL) rather than flasks, incorporates 9 concentrations, and has a duration of 72 hrs (Environment Canada, 1992. Blanck and Bjornsater, 1989). This test may be more amenable to testing effluent receiving water, leachates, and elutriates. Endpoints include LOEC and NOEC as well as IC<sub>50</sub>.

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**Logistical Considerations:****Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** Controlled temperature and lighting are required.

**Sample Analysis:**

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

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**Critique/Comments:**

These tests provide information on the toxicity of test materials to an important component of the aquatic biota and might indicate whether longer term tests are desirable. The tests may also be used to study biological availability of, and structure-activity relationships between, test materials. These procedures are applicable to many chemicals, either individually or in formulations, commercial products, or known mixtures. With appropriate modifications, these tests can be used to measure the effects of temperature, dissolved oxygen, and pH on such materials as aqueous effluents, leachates, soils, particulate matter, sediments, and surface waters. Static tests might not be applicable to materials that have a high oxygen demand, are highly volatile, are rapidly transformed in aqueous solutions either biologically or chemically, or are removed from test solutions in substantial quantities by the test chambers or organisms during the test.

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**Key References:**

- ASTM. 1992. Standard guide for conducting static 96-hour toxicity tests with microalgae, designation E1218-90. p 874-885. In Annual Book of ASTM Standards, Section 11, Water and Environmental Technology, Volume 11.04.
- Blanck, H., and B. Bjornsater. 1989. The algal microtest battery: a manual for routine test of growth inhibition. KEMI Science and Technology Department Report, No. 3/89.
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- U.S. Environmental Protection Agency. 1974. Marine Algal Assay Procedure: Bottle Test. National Environmental Research Center, Corvallis, OR.
- Weber, C.I., ed. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. Environmental Monitoring and Support.

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**Technique Name: Early Life Stage Fish Toxicity Test**

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**Technique Type:** Survival, Growth Assay  
**Matrix Type:** Freshwater and Saltwater Fish  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

The fish in this test are newly fertilized (uneyed) embryos ( $\leq 24$  h after fertilization). Recommended species for such tests include: salmon, trout, char, Northern pike, fathead minnow, white sucker, channel catfish, bluegill, sheepshead minnow, and silversides. Fish are cultured in flow-through tanks and incubation cups may be used. Temperature and aeration should be monitored and controlled, with air filtration using a 0.22  $\mu\text{m}$  bacterial filter recommended. To reduce stress, organisms should be shielded with partitions or curtains. Use of a "non-toxicant" test in which organisms are placed in dilution water to determine survival and growth survivability is recommended. The dilution water source is recommended to be reconstituted water or uncontaminated natural dilution water for early-stage toxicity tests. The test material should be dissolved in the dilution water before adding.

The tests usually consist of at least one control treatment and a geometric series of at least five concentrations of test material. The length of the tests will vary greatly dependent primarily upon the time required for hatching which varies with the species. Tests are generally terminated within 28 days after exposure to the test material, depending upon the purpose of the test. The most common endpoints of these tests are determination of mortality, number, and growth. The mortality is commonly measured at selected times using  $LC_{50}$  values, while the growth endpoint uses NOEC, LOEC,  $IC_p$  values. Endpoint values such as  $EC_{10}$ ,  $EC_{25}$ , and  $EC_{50}$  may be used for both.

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**Logistical Considerations:****Sample Collection:**

**Training:** MODERATE, experience in obtaining egg and sperm from adult fish may be required for some fish species as well as handling and monitoring of health may be needed. Such training typically would require several weeks.

**Time:** MODERATE, up to 47 days for some species.

**Equipment:** MODERATE cost to setup, possibly in the \$10,000 range including monitoring equipment. MINIMAL if a lab already possesses equipment.

**Sample Analysis:**

**Training:** MINIMAL to record survivability and weight.

**Time:** MINIMAL to MODERATE depending upon the species of fish.

**Equipment:** MINIMAL for most visual endpoints, an analytical balance is needed to record weights.

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**Critique/Comments:**

These tests are generally used to provide data on the toxicity of test materials of varying concentrations compared to controls. They can be used to determine embryo survival, fry survival, overall survival, and weight of the survivors in each treatment. These tests are applicable to all chemicals, either individually or in formulations, commercial products or known mixtures, that can be measured accurately at the necessary concentrations of water. With appropriate modifications, these procedures can be used to conduct tests on the effects of temperature, dissolved oxygen, and pH, and such materials as aqueous effluents, leachates, oils, particulate matter, sediments, and surface waters. Results of these tests may also be used to predict long-term effects likely to occur on fish in field situations, except that mobile organisms might avoid exposure when possible. Another possible use of these tests is to assess hazards to aquatic organisms when deriving water quality criteria.

Some species of fish, particularly striped bass, silversides, and trout, are difficult to handle without proper training and may have high mortality rates after hatching. The validity of test results may be placed in question if the rate of survivability after hatching exceeds 70%. Thus, much time in setup and determining ideal living conditions for the fish may be needed.

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**Key References:**

Keddy, C., J.C. Greene, and M.A. Bournell. The National Contaminated Sites Remediation Program, October 1992. Prepared for CCME Subcommittee on Environmental Quality Criteria for Contaminated Sites.  
Annual Book of ASTM Standards, Section 11, Water and Environmental Technology, Vol. 11.04. Designation E1241-92.

Standard Guide for Conducting Early Life-Stage Toxicity Tests  
with Fishes, pp. 886-913.

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**Technique Name:** Conducting Three Brood, Renewal Toxicity Tests with Ceriodaphnia dubia

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**Technique Type:** Survival, Growth, Reproductivity, and Physiological Response  
**Matrix Type:** Invertebrate, Crustacea, Ceriodaphnia dubia  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

Survival, growth, reproductivity, and physiological response endpoints can be obtained from Ceriodaphnia dubia exposed to the effects of an effluent or test material added to test material, but not food, during a portion of the organisms life. C. dubia are easily cultured in small (30-50 ml) covered test chambers of glass or plastic construction. Natural freshwater from an uncontaminated source or reconstituted water may serve as the dilution water. Aeration (between 40 and 100% saturation) and temperature must be controlled and monitored. Organisms should be less than 12 h old and are easily obtainable from commercial supply houses. A three brood toxicity test intended to allow calculation of an endpoint (usually a reduction in number of live neonates produced by first-generation C. dubia) usually consists of one or more control treatments and a geometric series of at least five concentrations of test material or effluent. Point estimates such as EC10, EC25, and EC50 may be used when using regression analysis. LC<sub>50</sub> may also be an applicable endpoint for survivability.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL for culturing and feeding.  
**Time:** MINIMAL (<3 days).  
**Equipment:** MINIMAL for culture of C. dubia.

**Sample Analysis:**

**Training:** MINIMAL to determine endpoints.  
**Time:** MINIMAL to determine endpoints.  
**Equipment:** MINIMAL, sensitive balance for determining weight, dissection

microscope for detecting physiological response and measuring length.

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**Critique/Comments:**

Daphnids such as C. dubia are easily obtained and cultured. Their sensitivity to a variety of test materials makes them ideal organisms for freshwater aquatic tests. Applicability to field conditions indicate a high correlation with laboratory test. C. dubia is preferable to Daphnia magna because of its shorter generation time (tests can be carried out in only 4-7 days as opposed to 21 days required for D. magna). The 7-day test is preferred by Oris et al (1991) because of its sensitivity to both individual substances and complex effluents. One drawback to using C. dubia, is that behavioral and developmental effects are difficult to quantify and may not provide meetable endpoints.

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**Key References:**

- ASTM E1295. 1989. Standard Guide for Conducting Three-Brood, Renewal Toxicity Tests with Ceriodaphnia dubia. Annual Book of ASTM Standards. Vol. 11.04. Philadelphia, PA.
- Mount, D.I., and T.J. Norberg. 1984. "A Seven-Day Life-Cycle Cladoceran Toxicity Test," Environmental Toxicology and Chemistry, Vol. 3, pp. 425-434.
- Oris, J.T., A.T. Hall, and J.D. Tylka. 1990. "Humic Acids Reduce the Photo-Induced Toxicity of Anthracene to Fish and Daphnia." Environmental Toxicology and Chemistry, Vol. 9, pp. 575-583.

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**Technique Name:** Earthworm Survival and Sublethal Effects

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**Technique Type:** Survival, Growth, Physiological Response  
**Matrix Type:** Invertebrate, Earthworms  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory, Field

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**Description:**

Methods directly evaluate the biological effects of contaminated soils on a representative macroinvertebrate (Eisenia foetida, E. andrei, or Lumbricus terrestris). In 14 day screening tests, percent survival is recorded at day 7 and day 14. Mortality is the most frequently measured end point in definitive tests; although growth, behavioral, and pathogenic observations may also be recorded. Median lethal concentrations (LC<sub>50</sub>'s) and 95% confidence intervals are calculated at day 7 and day 14. Positive control LC<sub>50</sub>'s (with 2-chloroacetimide) should be completed for definitive survival tests. Sublethal endpoints include one or more of the following: weight loss, dermopathologic responses, muscular responsiveness, presence or rate of burrowing, and reproduction. Analysis of covariance (ANCOVA) is recommended for weight loss studies. A similar test with an alternative soil annelid (Enchytraeus albidis) should be considered when the physicochemical properties of a test soil (e.g. moisture fraction, temperature) are not conducive to a successful test with E. foetida. Four-week tests with E. albidis measure mortality and biomass end points; eight-week tests measure offspring production.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to raise earthworms, treat soils, and record growth and survival.

**Time:** MODERATE for earthworms (14-30 days) and enchytraeids (30-60 days).

**Equipment:** Cost is MINIMAL. Tests can be purchased or materials bought separately. Coolers, media and feed are required to raise earthworms. Tests can be performed in jars or beakers in temperature and light controlled chamber or room.

## Sample Analysis:

**Training:** Training is MINIMAL to record survivability, weight, and cocoon production, MINIMAL to MODERATE for recognizing physiological responses.

**Time:** Time is MINIMAL to measure necessary endpoints.

**Equipment:** Equipment is MINIMAL for most visual observations. An analytical balance is required for weight endpoints.

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## Critique/Comments:

Earthworm tests are commercially available, cost-effective, simple, relatively short-term, and reliable. Methods for survival and/or growth have been published by International Standards Organization (ISO), OECD and EPA. Requirements for routinely using earthworm test methods are outlined in USEPA (1989), as well as the applied ecology literature (e.g. Callahan et al, 1985 and Neuhauser, et al 1986). These tests may be useful in integrated studies that include laboratory and in situ toxicity evaluation. Assessments with earthworms can help address site-specific issues related to bioavailability of contaminants. Survival and growth endpoints are relatively easy to implement, but behavioral and pathological endpoints require special training. Soil characteristics (e.g. strongly acidic, strongly alkaline, wetlands, nutrient deficient) at some sites may be incompatible with earthworms. The selection of test species must be given ample consideration during the problem formulation phases of the ecological assessment. Adequate characterization of the soil matrix prior to toxicity testing can minimize "false negatives" that may result from selection of an inappropriate test species.

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## Key References:

- Callahan, C.A., L.K. Russell, and S.A. Peterson. 1985. A comparison of three earthworm bioassay procedures for the assessment of environmental samples containing hazardous wastes. *Biol. Fert. Soils*. 1:195-200.
- Callahan, C.A., C.A. Menzie, D.E. Burmaster, D.C. Wilborn, and T. Ernst. 1991. On-site methods for assessing chemical impact on the soil environment using earthworms: a case study at the Baird and McGuire Superfund site, Holbrook, Massachusetts. *Environ. Toxicol. Chem.* 10:817-826.
- Lofs-Holmin, A. 1980. Measuring growth of earthworms as a method of testing sublethal toxicity of pesticides. *Swedish J. Agric. Res.* 10:25-33.
- Neuhauser, E.F., P.R. Durkin, M.R. Milligan, and M. Anatra. 1986. Comparative toxicity of ten organic chemicals to four

- earthworm species. *Comp. Biochem. Physiol.* 83C(1):197-200.
- Rombke, J. 1989. Enchytraeus albidus (Enchytreidae, Oligochaeta) as a test organisms in terrestrial laboratory systems. *Arch. Toxicol., Suppl.* 13:402-405.
- US EPA. 1989. Protocols for short term toxicity screening of hazardous waste sites. J.C. Greene, C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, S.A. Peterson, and W.E. Miller (eds.). EPA/600/3-88/029, U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.
- Wentsel, R.S. and M.A. Guelta. 1987. Toxicity of brass powder in soil to the earthworm Lumbricus terrestris. *Environ. Toxicol. Chem.* 6:741-745.

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**Technique Name:** Free-Living Nematode Survival and  
**Sublethal Effects**

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**Technique Type:** Survival, Growth, Physiological Response  
**Matrix Type:** Invertebrate, Nematodes  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

Survival, growth, reproduction and mutagenicity of Panagrellus redivivus or Caenorhabditis elegans exposed to contaminated soils can be measured using a short-term (4-7 day) test. P. redivivus, an aquatic species with a well-developed database in aquatic toxicity testing, has been applied to sediment, and can be applied to soil toxicity testing. A more recent test using C. elegans, a soil-dwelling nematode, may be applicable for ecological effects assessments. Tests with this nematode may more accurately reflect soil contaminant effects in terrestrial habitats. Comparative analysis of LC<sub>50</sub>'s of C. elegans, Daphnia magna, and sediment macroinvertebrates exposed to aqueous solutions from soil sediments contaminated with metals showed acute toxicities among the three tests. Methods using free-living nematodes were designed as complimentary to earthworm tests.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL for culturing and treating nematodes.

**Time:** MINIMAL ( $\leq$  7 days).

**Equipment:** MINIMAL for culture of nematodes.

**Sample Analysis:**

**Training:** MINIMAL to determine endpoints.

**Time:** MINIMAL to determine endpoints.

**Equipment:** MINIMAL TO MODERATE. A compound light microscope is required.

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### Critique/Comments:

Nematodes afford many advantages for toxicity testing from a laboratory perspective. Nematodes frozen in liquid nitrogen can be preserved (-80°C) and rehydrated prior to testing. Standardized sampling strategies have been established and published by ASTM. Although developed for efficacy evaluations for nematode control agents, methods could easily serve the needs of ecological effects assessment. For soil contamination evaluations, relatively little work has been completed to address questions related to "laboratory to field" extrapolation errors, and few applied studies have been published regarding the effects of contaminant mixtures on soil community structure. P. redivivus is an aquatic species and would be applicable to indirect (i.e. assessing soil eluates) tests. C. elegans although a free living soil nematode, may not inhabit all soil types.

### Key References:

- ASTM E629. 1991. Standard guide for field evaluation of nematode control agents - determination of nematode population responses to control agents. Annual book of ASTM standards. Volume 11.04. Pesticides; Resource Recovery; Hazardous Substances and Oil Spill Responses; Waste Disposal; Biological Effects. American Society for Testing and Materials (ASTM). Philadelphia, PA 19103.
- Samoiloff, M., S. Schulz, Y. Jordan, K. Denich, and E. Arnott. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode Panagrellus redivivus. Can. J. Fish. Aquat. Sci. 37:1167-1174.
- Samoiloff, M., J. Bell, D. Birkholz, G. Webster, E. Arnott, R. Pulak, and A. Madrid. 1983. Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediment. Environ. Sci. Technol. 17:329-333.
- van Kessel, W., R. Brocades Zaalberg, and W. Seinen. 1989. Testing environmental pollutants on soil organisms: a simple assay to investigate the toxicity of environmental pollutants on soil organisms, using CdCl<sub>2</sub> and ematodes. Ecotoxicol. Environ. Safe. 18:181-190.

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**Technique Name: Terrestrial Arthropods (insects)**

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**Technique Type:** Survival, Growth, Reproduction  
**Matrix Type:** Invertebrate, Insect  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory or Field

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**Description:**

Various methods have been developed for evaluating chemical effects on terrestrial insects, especially pesticide effects on nontarget species. Survival is the most commonly measured endpoint, although other acute and chronic endpoints have been used. One standardized method for evaluating acute and subacute chemical effects on terrestrial insects was initially developed for agrichemical evaluations, especially for evaluating the effects of insecticides on non-target insects (e.g., honey bees (apis mellifera); see US EPA 1982). Crickets and harvester ants have been used on a limited basis within an ecological effects assessment. Adult house crickets (Acheta domesticus) were exposed to acridine via the diet, and following 18-day exposures lethality and sublethal effects were determined. House crickets and field crickets (Gryllus pennsylvanicus), have been used to show lethal effects (LC<sub>50</sub>) and bioaccumulation of PCB's in a field situation (Burrow, et. al., 1993). Harvester ants (Pogonomyrmex owyheeii) have proven sensitive to some organic contaminants (i.e., pesticides) and complex chemical mixtures (e.g., wood preservative sludge, drilling fluid, and slop oil). Genotoxicity screening with Drosophila melanogaster (fruit fly) may be applicable if it is considered to be a representative hymenopteran.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MODERATE (7-21 days).

**Equipment:** MINIMAL to culture insects and treat soils for laboratory tests and MINIMAL to construct traps for field tests.

**Sample Analysis:**

**Training:** MINIMAL to record endpoints.

**Time:** MINIMAL.

**Equipment:** MINIMAL TO MODERATE. A dissecting light microscope is required for some assays.

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**Critique/Comments:**

As ecological indicators of soil contamination, terrestrial insects, and soil arthropods in general, are potentially critical organisms. These test guidelines were all designed with agrichemicals or other challenging agents (chemical or biological) as potential hazards. However, in evaluating adverse biological effects, test design may be similar regardless of the agent. And, while toxicity estimates may be derived from modifications of these existing tests, the interpretation of the toxicity information should be weighted by site-specific information gathered, for example, during field surveys. It is critical that issues regarding the interpretation of toxicity test data be addressed early in the problem formulation phase of the ecological effects assessment.

Few technical support laboratories are currently providing tests with insects, particularly within the context of hazardous waste sites, but technical support may be gained on a site-specific basis, e.g., through local or regional testing services available at land-grant colleges. Until adequate technical support is available, implementing this biological assessment within an ecological effects assessment may be difficult. Additionally, the data base is relatively sparse; use of adequate reference soils for site-specific comparisons is critical.

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**Key References:**

- Croft, B.A. 1990. Arthropod biological control agents and pesticides. John Wiley & Sons, New York, NY. 723 pp.
- Gano, K.A., D.W. Carlile, and L.E. Rogers. 1985. A harvester ant bioassay for assessing hazardous chemical waste sites. PNL-5434, UC-11. Pacific Northwest Laboratory, Richland, WA.
- OECD (Organization for Economic Co-Operation and Development). 1984. OECD guidelines for testing of chemicals. Director of Information, OECD. 2, rue Andre Pascal, 75775 Paris Cedex 16, France.
- US EPA. 1982. Pesticide assessment guidelines, Subdivision L, Hazard Evaluation: Non-target insects. 540/9-82/019. Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C.
- Walton, B.T. 1980. Differential life-stage susceptibility of Acheta domesticus to acridine. Environ. Entomol. 9:18-20.

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**Technique Name:** Terrestrial Arthropods (non-insect) and isopods

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**Technique Type:** Survival, Reproduction, Genotoxicity  
**Matrix Type:** Invertebrate, Arthropod (non-insect), and Isopod  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

Test systems are well characterized although exposures are generally not associated with soil exposure directly. Within an ecological effects assessment, however, test systems may be easily modified to assure that exposures occur directly via site-soil (for example, contained in Petri dishes). Alternatively, exposures could occur via glass plates or Petri dishes coated with dried films of single-compound, defined chemical mixture, or soil eluate. Acute toxicity has been the most easily measured endpoint following exposure periods that range from nearly one week to four weeks. Additional endpoints should also consider reproductive success in the test species; most frequently achieved by counting the number of eggs laid during the exposure period. A springtail (Folsomia Candida) test has been adopted as a draft test method by the International Standards Organization (ISO). Endpoints for this 4-week test include adult survival, offspring number, NOEC and LOEC. Testing of pure chemical or soil eluates occurs in whole artificial soil under controlled temperature, light intensity, and photoperiod.

Although the distribution of microarthropods has not been routinely used to assess soil toxicity, this approach could be used in hazardous waste site assessments. The microarthropods are extracted from the contaminated and uncontaminated soils using Berlese funnels or high intensity Tullgren extractors. The extracted arthropods should be preserved for effects-based comparisons (e.g., total numbers and identification).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to maintain organisms in soil.

**Time:** MINIMAL to MODERATE (7-day to 4-wk) extractors can be built with MINIMAL expense.

**Equipment:** MODERATE. An environmentally-controlled room or chamber is required to regulate

temperature and light throughout the test.

**Sample Analysis:**

**Training:** MINIMAL to MODERATE for inexperienced personnel. However, training should be performed by someone with EXTENSIVE training.

**Time:** Sampling time is MINIMAL to MODERATE depending on experience of sampler and the number of samples.

**Equipment:** Sampling equipment is MODERATE. A dissecting light microscope is required.

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**Critique/Comments:**

Because of their role in the environment, terrestrial arthropods and isopods should receive consideration as an ecological "receptor" during the ecological assessment within the RI/FS process. By comparison of populations in contaminated and uncontaminated site soils, it will be possible to demonstrate population shifts corresponding to soil toxicity. In addition, information on specific community responses to specific chemicals and chemical mixtures must be developed.

Determination of the impacts of specific chemicals on specific populations of microarthropod or isopods have generally been limited, although the applied literature indicates that species-specific sensitivities may be expressed, e.g., copper effects on isopods. Expertise in the identification of microarthropods, and determination of their numbers exists within most land-grant universities, the USDA, and the extension service, either at the Federal or State level. Regional centers of expertise have been suggested although few technical support laboratories currently provide these tests. From a technical perspective, terrestrial arthropods in general and non-insects in particular have a poorly established comparative effects and toxicity database. While the potential strengths associated with toxicity evaluations and effects measurements are numerous (e.g., more ecologically relevant, amenable to laboratory and field assessment), the lack of commercial availability may also limit the routine application of these methods in an ecological effects assessment.

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**Key References:**

- Anderson, J.M. 1988. Spatiotemporal effects of invertebrates on soil processes. *Biol. Fertil. Soils.* 6:216-227.  
Hassan, S.A. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed

- by the IOBC/WPRS work group 'Pesticides and beneficial organisms.' Bull. OEPP/EPPO 15:214-255.
- Hassan, S.A., R. Albert, F. Bigler, P. Blaisinger, H. Bogenschutz, E. Boller, J. Brun, P. Chiverton, P. Edwards, W.D. Engloert, P. Huang, C. Inglesfield, E. Nation, P.A. Oomen, W.P.J. Overmeer, W. Rieckmann, L. Samsøe-Petersen, A. Staubli, J.J. Tuset, G. Viggiani, and G. Vanwetswinkel. 1987. Results of the third joint insecticide testing programme by the IOBC/WPRS-working group "Pesticides and beneficial organisms." J. Appl. Ent. 103:92-107.
- Hopkin, S.P. 1986. The woodlouse Porcellio scaber as a 'biological indicator' of zinc, cadmium, lead and copper pollution. Environ. Pollut. (Series B) 11:271-290.
- Moldenke, A.R. and B.L. Fichter. 1988. Invertebrates of the H.J. Andrews Experimental Forest, Western Cascade mountains, Oregon: IV. The oribatid mites (Acari; Cryptostigmata). USDA Forest Service. PNW-GTR-217.

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**Technique Name: Invertebrate Immunotoxicity**

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**Technique Type:** Immunotoxicity  
**Matrix Type:** Invertebrate, Earthworm  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Although immunotoxicity test methods are more widely described for vertebrates, similar methods have been applied to terrestrial invertebrates under controlled laboratory conditions. For example, methods have been developed for evaluating the immunocompetence of earthworms (generally Lumbricus terrestris). In these subacute tests, earthworms are exposed to single-chemicals or complex mixtures. The majority of work has been completed using filter-paper contact tests, but soil exposed-earthworms could be tested in conjunction with laboratory or in situ tests. A biomarker, altered immune function in earthworms, should be considered as supporting data in an integrated study that addressed, for example, organismic-level measurements (e.g., standardized 14-day earthworm test; and field survey information. Although not sufficiently developed at present, the future value of these methods centers upon the comparative toxicity data base that can be developed relative to other receptors (e.g., mammals and birds).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MODERATE to EXTENSIVE.  
**Time:** MODERATE (7-14 days).  
**Equipment:** MODERATE to collect leucocytes.  
EXTENSIVE to collect and purify enzymes.

**Sample Analysis:**

**Training:** MODERATE to EXTENSIVE.  
**Time:** MODERATE to conduct enzyme assays and immunoassays.  
**Equipment:** MODERATE to EXTENSIVE.

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**Critique/Comments:**

At present, the interpretation of altered immune function in soil

macroinvertebrates should be guarded, particularly within the context of an ecological effects assessment. Only through integrated studies using organismic-level tests and field surveys will results from these invertebrate immune function tests be ecologically relevant. However, unlike the immunotoxicity information garnered from terrestrial vertebrates, an evaluation of the immunocompetence of soil macroinvertebrates, such as earthworms, may directly reflect the long-term adverse biological effects associated with soil contaminants. For terrestrial vertebrates, the majority of exposure routes are indirect, except for direct soil ingestion, but for soil-dwelling invertebrates the routes of exposure are more direct due to the close contact between receptor and contaminant source. Cutaneous or dermal uptake of contaminants in earthworms are equal, if not greater, than direct soil ingestion depending upon physicochemical properties of the contaminant mixture at a site.

Measuring immunocompetence requires specialized training and the techniques can be time-consuming. Few technical support laboratories are currently providing tests with these organisms, and implementing these methods within an ecological effects assessment may be difficult due to an absence of experienced testing services.

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#### Key References:

- Chen, S.C., L.C. Fitzpatrick, A.J. Goven, B.J. Venables, and E.L. Cooper. 1991. Nitroblue tetrazolium dye reduction by earthworm (Lumbricus terrestris) coelomocytes: an enzyme assay for nonspecific immunotoxicity of xenobiotics. Environ. Toxicol. Chem. 10:1037-1043.
- Enyambe, G.S., A.J. Goven, L.C. Fitzpatrick, B.J. Venables, and E.L. Cooper. 1990. A non-invasive technique for sequential collection of earthworm (Lumbricus terrestris) leukocytes during subchronic immunotoxicity studies. Lab. Animals 25:61-67.
- Mohrig, W., E. Kanschke, and M. Ehlers. 1984. Rosette formation by coelomocytes of earthworm Lumbricus terrestris L. with sheep erythrocytes. Devel. Comp. Immunology 8:471-476.
- Rodriguez-Grau, J., B.J. Venables, L.C. Fitzpatrick, and E.L. Cooper. 1989. Suppression of secretory rosette formation by PCBs in Lumbricus terrestris: an earthworm assay for humoral immunotoxicity of xenobiotics. Environ. Toxicol. Chem. 8:1201-1207.
- Stein, E.A. and E.L. Cooper. 1988. In vitro agglutinin production by earthworm leukocytes. Devel. Comp. Immunol. 12:531-547.

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**Technique Name:** Mollusks (Terrestrial and Wetland)

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**Technique Type:** Survival, Transformation  
**Matrix Type:** Invertebrates, Mollusks  
**Ecosystem Level:** Organismal  
**Test Location:** Laboratory

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**Description:**

Anodonta imbecilis was initially selected as a representative unionid mollusk; however, the techniques should be applicable for testing mussels with similar reproductive strategies. Exposures are static or renewal, and depending upon endpoint (e.g., survival or transformation), the exposures are 24-hr or 9 to 11 days. All tests involve the early developmental stages of the mussel, or glochidia, and juvenile mussels, depending upon endpoints being measured. The method is applicable to assessments of wetland or aquatic habitats.

A similar method, originally developed for efficacy tests can be modified to assess terrestrial habitats. Laboratory reared snails (e.g., Derocera reticulatum) or slugs are exposed to contaminated soils in test boxes or glass aquaria for 24 to 48-hr. Endpoint is mortality, but the test duration could be lengthened to measure sublethal endpoints.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to prepare media.  
**Time:** MINIMAL to MODERATE (24-hr to 11-day).  
**Equipment:** MINIMAL.

**Sample Analysis:**

**Training:** MINIMAL to measure growth and survival.  
**Time:** MINIMAL.  
**Equipment:** MINIMAL.

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**Critique/Comments:**

Aquatic toxicity tests with freshwater mussels may be critical to a wetland evaluation if complex chemical mixtures characteristic of hazardous waste sites were impacting the habitat. Guidance for developing the test with freshwater mussels followed ASTM E729 (1991), and while not widely used at this time, toxicity assessments with freshwater mussels should be considered within an ecological effects assessment. Similarly, the test with terrestrial snails and slugs could complement a field survey. Although originally designed as an efficacy test, the method can be applied to evaluations of the effects associated with soil exposures.

Additionally, when threatened or endangered fresh water mussels are potential receptors at a Superfund site, these test methods should seriously be considered. No comparative toxicity data base has been developed, although the mollusk literature is widespread with an increasing amount of work being reported that summarize ecological effects associated with exposures involving freshwater and terrestrial mollusks. Only a few technical support laboratories are currently providing tests with these organisms.

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**Key References:**

- ASTM E729. 1991. Standard guide for conducting acute toxicity test with fishes, macroinvertebrates, and amphibians. Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA.
- Crowell, H. 1979. Chemical control of terrestrial slugs and snails. Station Bulletin 628. Agricultural Experiment Station, Oregon State University. Corvallis, OR.
- US EPA. 1985. Hazard evaluation division, Standard evaluation procedure. Acute toxicity test for freshwater invertebrates. 540/9-85/005. Office of Pesticide Programs. Washington, D.C.

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**Technique Name:** Use of Small Mammals to Assess Exposure and Effects

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**Technique Type:** Indicator Species  
**Matrix Type:** Mammals  
**Ecosystem Level:** Individual  
**Test Location:** Field, Laboratory

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**Description:**

Many species of small mammals have used to assess contaminant exposure potential or to assess contaminant effects. Three of the more commonly used small mammals are species of the genus Microtus (voles), Peromyscus leucopus (white-footed mouse), and Sigmodon hispidus (hispid cotton rat). Parameters that may be evaluated include the effects of exposure on reproductive effort, behavior, physiological parameters, immune system functioning, and DNA alterations. The mammals listed above have ranges that cover considerable portions of the US and North America. Some species develop intricate burrow systems. The behavioral, morphological, and physiological characteristics of these organisms have been extensively studied.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL - EXTENSIVE depending on the sample size required and study design.

**Equipment:** All the species listed can be easily captured using live traps or snap traps.

**Sample Analysis:**

**Training:** Not applicable. Depends on the analysis to be performed.

**Time:** Not applicable. Depends on the analysis to be performed.

**Equipment:** Not applicable. Depends on the analysis to be performed.

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### Critique/Comments:

The ubiquitous nature of rodents, their foraging habits, prolific reproductive potential, adaptability to laboratory setting, and the extensive database available in the literature make rodents excellent indicator species to model contaminant effects on small mammal communities and to assess trophic transfer of contaminants.

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### Key References:

- Elangbam, C.S., C.W. Qualls, R.L. Lochmiller and J. Novak. 1989. Development of the cotton rat (Sigmodon hispidus) as a biomonitor of environmental contamination with emphasis on hepatic cytochrome P-450 induction and population characteristics. *Bull. Environ. Contam. Toxicol.* 42:482-488.
- Health, V., D.J. Schaeffer, T.R. Seastedt, D.J. Gibson, D.C. Hartnett, B.A.D. Hetrick, S.W. James, D.W. Kaufman, A.P. Schwab, E.E. Herricks and E.W. Novak. 1990. Field bioassessments for selecting test systems to evaluate military training lands in tallgrass prairie ecosystem. *Environ. Manage.* 14:81-94.
- McBee, K., J.W. Bickham, K.W. Brown and K.C. Donnelly. 1987. Chromosomal aberrations in native small mammals (Peromyscus leucopus and Sigmodon hispidus) at a petrochemical waste disposal site: I. Standard karyology. *Arch. Environ. Contam. Toxicol.* 16:681-688.
- McBee, K. and J.W. Bickham. 1989. Mammals as bioindicators of environmental toxicity, In H.H. Genoways, ed., *Current Mammalogy*. Plenum Press, New York, pp. 37-88.
- Thompson, R.A., G.D. Schroder and T.H. Connor. 1988. Chromosomal aberrations in the cotton rat, Sigmodon hispidus, exposed to hazardous waste. *Environ. Molec. Mutagen.* 11:359-367.
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**Technique Name:** Use of Avian Species to Assess Exposure and Effects

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**Technique Type:** Indicator Species  
**Matrix Type:** Avian Species  
**Ecosystem Level:** Individual  
**Test Location:** Field, Laboratory

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**Description:**

Many bird species have been used in ecological risk assessments to assess exposure potential for other species or to assess possible contaminant effects.

The great blue heron (Ardea herodias) can be used to evaluate effects of exposure to contaminants on wetland bird populations. Parameters that may be evaluated include the effects of exposure on reproductive effort, behavior, physiological parameters, immune system functioning, DNA alterations, and contamination of food items. The genus is ubiquitous throughout wetlands of North America. The diet of the great blue heron--composed of frogs, fish, and crustaceans--places it high in the food chain and increases its utility as a sentinel species for wetland birds.

The European starling (Sturnus vulgaris) can be used as a biomonitor for the effects environmental contaminants. Effects of toxicants can be evaluated on such variables as reproductive effort, growth and development, gross pathological abnormalities such as tumors and lesions, physiological impairments, and survival of adults and nestlings. Food items fed to nestlings can be examined to determine toxicant exposure through the diet. The species is particularly suited for field evaluations as the species readily utilizes artificial nesting boxes, allowing populations to be established in areas of known or suspected contamination, and is tolerant or repeated visitation to boxes by field personnel. The species is ubiquitous across North America.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL - EXTENSIVE depending on the sample size required and study design.

**Equipment:** The equipment required differs greatly among species. Many studies involve use radiotelemetry equipment.

## Sample Analysis:

- Training:** Not applicable. Depends on the analysis to be performed.
- Time:** Not applicable. Depends on the analysis to be performed
- Equipment:** Not applicable. Depends on the analysis to be performed.

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## Critique/Comments:

Searching areas for natural nests is time-consuming, and needs to be repeated over several weeks, especially if data on renesting attempts and second clutches is needed. Nests of some species may also be relatively inaccessible, requiring the use of mirror-poles or ladders to examine the nests. Establishing nest boxes may be labor-intensive, as are platforms for raptors, although to a lesser extent as raptors are rarely abundant on study sites. Once avian nests have been identified or boxes and platforms established, an immense amount of data can thereafter be collected with relative ease. Through use of the artificial nesting structures, populations can be established on areas of known or suspected contamination and data collected to address various risk assessment objectives with minimal time and equipment.

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## Key References:

- Grue, C.E. and C.C. Hunter. 1984. Brain cholinesterase activity in fledgling starlings: implications for monitoring exposure of songbirds to ChE inhibitors. Bull. Environ. Contam. Toxicol. 32:282-289.
- Grue, C.E. and L.P. Franson. 1986. Use of captive starlings to determine effects of environmental contaminants on passerine reproduction: pen characteristics and nestling food requirements. Bull. Environ. Contam. Toxicol. 37:655-663.
- Kendall, R.J., L.W. Brewer, T.E. Lacher, B.T. Marden and M.L. Whitten. 1989. The use of starling nest boxes for field reproductive studies: provisional guidance document and support documents. EPA/600/8-89/056. U.S. Environmental Protection Agency, Washington, D.C.
- Kessel, B. 1957. A study of the breeding biology of the European starling (*Sturnus vulgaris*) in North America. Am. Mid. Nat. 58(2):257-331.
- Lower, W.R. and R.J. Kendall. 1990. Sentinel species and sentinel bioassays, In J.F. McCarthy and L.R. Shugart, eds., Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, pp. 168-179.

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**Technique Name:** Avian Eggshell Thinning

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**Technique Type:** Physiological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Field

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**Description:**

The thinning of avian eggshells has been observed as a response to various stressors including DDT metabolites, dieldrin, chlordecone, lindane, polychlorinated biphenyls, mercury, and aluminum. In the past, eggshell thickness has been assessed using a thickness index. Recently, a more sensitive and highly quantifiable technique has been developed in which the breaking strength of the egg is assessed. Eggshell thinning is a widely used tool in field assessments.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL - MODERATE  
**Equipment:** No special equipment is required.

**Sample Analysis:**

**Training:** MINIMAL  
**Time:** MINIMAL for individual samples.  
**Equipment:** Typical laboratory equipment is required.

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**Critique/Comments:**

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**Key References:**

Bennett, J.K., R.K. Ringer, R.S. Bennett, B.A. Williams and P.E. Humphrey. 1988. Comparison of breaking strength and shell thickness as evaluators of eggshell quality. Environ. Toxicol. Chem. 7:351-357.

Carlisle, J.C., D.W. Lamb and P.A. Toll. 1986. Breaking strength: an alternative indicator of toxic effects on avian eggshell quality. Environ. Toxicol. Chem. 5:887-889.  
Ratcliffe, D.A. 1967. Decrease in eggshell weight in certain birds of prey. Nature (London) 215:208-210.

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**Technique Name:** Fish Survey

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**Technique Type:** Survey of biota  
**Matrix Type:** Freshwater  
**Ecosystem Level:** Community/Individual  
**Test Location:** Field

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**Description:**

Fish are sampled using electrofishing techniques and/or various types of nets, and the number of each species in the samples is determined. Typical analyses of the data include relative abundance, species richness, and size structure. Population estimates may be determined if repeated samples are taken. Fish communities can be assessed using the Index of Biological Integrity, which was developed specifically to determine the effects of decreased habitat quality. Fish samples can also be taken for residue analysis for contaminants that bioaccumulate. Residues can be compared to limits for consumption set by the Food and Drug Administration. Other methods of contaminant effects include percentage of tumors, vertebral anomalies, disease and parasites, and fin erosion.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** An electrofishing unit is required if that technique is to be employed.

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MODERATE  
**Equipment:** MINIMAL

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**Critique/Comments:**

Information from residue analysis should be interpreted with caution, as many contaminants to which fish have been exposed will not show in the analysis due to degradation of the compound. Furthermore, it is difficult to relate body burdens of a

contaminant to potential biological effects. Findings of physical abnormalities should also be interpreted cautiously due to mobility of fish, statistical errors in inferences, differential species sensitivity, and subjectivity in observations.

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**Key References:**

Baumann, P.C., W.K. Smith and W.K. Parland. 1987. Tumor frequencies and contaminant concentration of brown bullheads from an industrialized river and a recreational lake. *Trans. Am. Fish. Soc.* 116:251-253.

Bengtsson, B.E. 1975. Vertebral damage in fish induced by pollutants, In J.H. Kowman, J.J. Strik, eds., *Sublethal Effects of Toxic Chemicals on Aquatic Animals*. Elsevier Scientific, Amsterdam.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. *Illinois Natural History Survey Special Publication No. 5*, Illinois Natural History Survey, Champaign, IL.

Overstreet, R.M. and H.K. Howse. 1977. Some parasites and diseases of estuarine fishes in polluted habitats of the Mississippi. *Ann. N.Y. Acad. Sci.* 298:427-462.

Sherwood, M.J. and A.J. Mearns. 1977. Environmental significance of fin erosion in polluted habitats of the Mississippi. *Ann. N.Y. Acad. Sci.* 298:427-462.

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**Technique Name:** Plankton Survey

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**Technique Type:** Survey of flora and fauna  
**Matrix Type:** Freshwater  
**Ecosystem Level:** Community  
**Test Location:** Lab

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**Description:**

Samples of the plankton community are taken from the water column at a different depths using one or several of a variety of techniques. Plankton samples are then preserved for taxonomic identification. Species richness, relative abundance, and community indices can be determined from the taxonomic data.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** MINIMAL

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MODERATE  
**Equipment:** A dissecting microscope may be needed for taxonomic evaluation.

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**Critique/Comments:**

The choice of sampling technique, sample size, and sample numbers will depend on the characteristics of the habitat.

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**Key References:**

American Society for Testing and Materials (ASTM). 1987. Standard practice for sampling phytoplankton with water-sampling bottles, In Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA, pp. 53-54.  
American Society for Testing and Materials (ASTM). 1987. Standard

practice for sampling phytoplankton with pumps, In Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA, pp. 45-46.

American Society for Testing and Materials (ASTM). 1987. Standard practice for sampling phytoplankton with conical tow nets, In Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA, pp. 42-44.

DeBernardi, R. 1984. Methods for the estimation of zooplankton abundance, In J.A. Downing and F.H. Rigler, eds., A Manual on Methods for the Assessment of Secondary Productivity of Fresh Waters, IBP Handbook 17. Blackwell Scientific Publications, Oxford, England, pp 59-86.

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**Technique Name:** Periphyton Survey

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**Technique Type:** Survey of microflora  
**Matrix Type:** Freshwater  
**Ecosystem Level:** Community  
**Test Location:** Lab

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**Description:**

Changes in lotic systems resulting from contaminants can be assessed by surveys of the periphyton community. A sample of the periphyton community is obtained from natural substrate or artificial substrate implanted specifically for the purpose of colonization by periphyton. Samples are analyzed for taxonomic composition such as cell number, species richness, and relative abundance. Community indices such as diversity and community similarity and other productivity-related indices can also be determined.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** MINIMAL to MODERATE

**Sample Analysis:**

**Training:** MODERATE  
**Time:** MINIMAL  
**Equipment:** A microscope is needed for identification.

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**Critique/Comments:**

Periphyton surveys should be supported by additional physical and chemical information, which sometimes influences periphyton production and dynamics. Enough cells must be counted to ensure that rare cells are counted.

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**Key References:**

- American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.
- Crossey, M.J. and T.W. La Point. 1988. A comparison of periphyton community structural and functional responses to heavy metals. *Hydrobiologia* 162:109-121.
- Stevenson, R.J. and R.L. Lowe. 1986. Sampling and interpretation of algal patterns for water quality assessments, In B.G. Isom, ed., *Rationale for Sampling and Interpretation of Ecological Data*. ASTM STP 894. American Society for Testing and Materials. Philadelphia, PA. pp 118-149.
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**Technique Name:** Analysis of Benthic Macroinvertebrate Populations

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**Technique Type:** Survey of benthic biota  
**Matrix Type:** Benthic habitat  
**Ecosystem Level:** Community  
**Test Location:** Field

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**Description:**

Benthic macroinvertebrates are the most common fauna used in ecological assessments of contaminants. Typically, macroinvertebrates are sampled from the natural benthic habitat using one or several of a variety of techniques. An alternative sampling technique is to place artificial substrate into the water and then to collect the substrate after a period of colonization (usually about 6 weeks). Various information can be gleaned from macroinvertebrate surveys including relative abundance; species richness; guild structure; and indices of diversity, evenness, and community similarity.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MODERATE  
**Equipment:** MINIMAL

**Sample Analysis:**

**Training:** MINIMAL  
**Time:** MODERATE  
**Equipment:** MINIMAL

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**Critique/Comments:**

Numerous excellent references deal with the collection, identification, and analysis of benthic invertebrate populations. It is essential that the sampling technique chosen be adequately suited for the target taxa and habitat type. The amount of time and training required for sample analysis varies depending on the taxonomic resolution desired.

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**Key References:**

- American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, D.C.
- Downing, J.A. 1984. Sampling the benthos of standing waters, In J.A. Downing and F.H. Rigler, eds., A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17. Blackwell Scientific Publications, Oxford, England, pp. 87-103.
- Merritt, R.W. and K.W. Cummins, eds. 1984. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publ., Dubuque, IA.
- Peckarsky, B.L. 1984. Sampling the stream benthos, In J.A. Downing and F.H. Rigler, eds., A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17. Blackwell Scientific Publications, Oxford, England, pp. 131-160.
- Southwood, T.R.E. 1978. Ecological Methods. John Wiley and Sons, New York, NY.
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**Technique Name:** Presence/Absence of Indicator Species

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**Technique Type:** Survey of biota  
**Matrix Type:** Aquatic  
**Ecosystem Level:** Individual  
**Test Location:** Field

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**Description:**

The indicator species concept was based originally on the premise that an increase in anthropogenic organic matter provides the food energy required by "tolerant" species, while the numbers of "sensitive" species declines in response to increased competition, predation, or decreased dissolved oxygen. Under this concept, the presence of sensitive species at a contaminated site led to the conclusion that there was little impact on the aquatic community. However, this approach holds limited applicability to contaminants other than organic matter. Currently, the indicator species concept is utilized by comparing changes in taxa numbers between a control site and hazardous waste sites. Effects of the contaminants can be assessed by assuming that an adverse effect on the community will be reflected by a decline in the number of members in more sensitive taxa.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL

**Time:** MINIMAL

**Equipment:** An electroshocking unit is recommended for fish censusing.

**Sample Analysis:**

**Training:** MODERATE

**Time:** MODERATE

**Equipment:** MINIMAL

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**Critique/Comments:**

The use of indicator species to assess the effects of toxicants can be a useful tool if care is taken to carefully limit its

application. Communities do not respond similarly to different toxicants, so it is necessary to carefully consider the toxicant, mode of exposure, and community at risk when designing the study.

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**Key References:**

- Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. Illinois Natural History Survey Special Publication No. 5, Illinois Natural History Survey, Champaign, IL. 28pp.
- Plafkin, J.L., M.T. Barbour, K.K. Porter and S.K. Gross. 1988. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. Draft Report RT182A, from EA Engineering, Science and Technology Inc. to the U.S. Environmental Protection Agency, Monitoring, and Data Support Division, Washington, DC.
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**Technique Name: Remote Sensing of Vegetation**

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**Technique Type:** Remote Sensing  
**Matrix Type:** Vascular Plant (terrestrial)  
**Ecosystem Level:** Organismal, Community  
**Test Location:** Field

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**Description:**

Remote sensing may be used advantageously in a number of ways to assess vegetation of hazardous waste sites. Extensive efforts are underway in the U.S. National Aeronautics and Space Administration (NASA) and to a limited extent in EPA to characterize regional patterns in vegetation. Primary sources of radiometric data are the Landsat Multi Spectral Scanner (MSS), the Thematic Mapper (TM), and the French Systeme Probatoire d'Observation de la Terre (SPOT) data banks. Resolution is the major limitation of these satellite imaging systems. For improved resolution, the satellite images may be supplemented with fixed-wing aircraft (including ultralights) utilizing comparable sensing equipment. The flights may also employ infrared and conventional photography. Coordinated work at individual sites for verification ("ground truthing") or for additional resolution can be performed from "cherry picker" booms with field model sensors. These different levels of resolution provide the following opportunities: relatively unlimited accessibility; safe, non-intrusive assessment and monitoring; and the opportunity to assess large-scale seasonal and annual vegetational patterns. Radiometric data have been used effectively to map vegetational boundaries (detecting shifts in dominant canopy species within a given forest type), estimate net photosynthesis and net primary production, estimate foliar nitrogen content, detect drought stress, detect effects from pest epidemics such as gypsy moth, and assess forest decline due to air pollutants.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MODERATE to EXTENSIVE.

**Time:** MINIMAL to EXTENSIVE depending on size of area.

**Equipment:** Very EXPENSIVE.

**Sample Analysis:**

**Training:** MODERATE to EXTENSIVE.

**Time:** MINIMAL to EXTENSIVE.

**Equipment:** A computer with digitizing board, or a radiometer is needed.

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**Critique/Comments:**

Remote sensing and radiometry techniques can be useful in identifying areas of a hazardous waste site that have been negatively impacted by contaminants. However, verification or "ground truthing" (i.e., direct visual observation, chemical analysis of plant tissue and soils) must also be performed to insure that the impacted area is being affected by the contaminant(s) in question. The methods, whether satellite images, aerial photos or hand-held radiometers are used, are time consuming and expensive. Also, extensive training is often required to accurately collect and interpret the data. A cost-effect analysis should be performed before these techniques are implemented in a risk assessment program.

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**Key References:**

- Daughtry, C.S.T. and L.L. Biehl. 1985. Changes in Spectral Properties of Detached Birch Leaves. Remote Sensing of Environment 17:281-289.
- Duinker, P. and S. Nilsson. 1988. Proceedings: Seminary on remote sensing of forest decline attributed to air pollution. International Institute for Applied Systems Analysis, Luxenburg, Austria.
- Hardisky, M.A., M.F. Gross, and V. Klemas. 1986. Remote Sensing of coastal wetlands. BioScience 36:453-460.
- Rock, B.N., J.E. Vogelmann, D.L. Williams, A.F. Vogelmann, and T. Hoshizaki. 1986. Remote detection of forest damage. BioScience 36:439-445.
- Roller, N.E.G. and J.E. Colwell. 1986. Coarse-resolution satellite data for ecological surveys. BioScience 36:468-475.
- Waring, R.H., J.D. Aber, J.M. Melillo, and B. Morre, III. 1986. Precursors of change in terrestrial ecosystems. BioScience 36:433-438.

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**Technique Name:** Digital Imaging Analysis of Vegetation

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**Technique Type:** Digital Imaging  
**Matrix Type:** Vascular Plant  
**Ecosystem Level:** Organismal, Community  
**Test Location:** Field or Laboratory

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**Description:**

Images of plant communities, individual plants, or individual leaves, acquired from satellite, aerial, or hand-held cameras or sensing equipment, are analyzed for relative light reflectance. After the image is captured, it is digitized using an array of gray levels (pixels). Damaged areas of plant communities, individual plants, or leaves can be distinguished from "healthy" areas based on pixel intensity. Varying degrees of damage within an image can be further delineated using a pseudocolor system that assigns a color to each level of intensity. Damage can be quantified by measuring total pixel intensity of an image or by determining the difference in surface area between "damaged" and "healthy" areas of the vegetative tissue. Sensitivity can be increased by using near-infrared film or filters or by using filters with wavelengths similar to chlorophyll (i.e., 680 nm or 730 nm).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** A digital imaging analysis system (DIAS), including video camera, digitizing board, computer, video monitor, and supplemental lighting is required.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** A DIAS system is required.

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**Critique/Comments:**

Digital imaging analysis can be a quick, accurate method for determining foliar injury in individual leaves, whole plants or small field plots. The method provides more accurate estimates of injured tissue surface area, and homogeneity among rating times and evaluators than visual injury estimations. Lighting, camera settings (i.e., F-stop, focus, contrast) and pixel intensity levels must be optimized prior to analyses. Set-up and calibration of the system is critical. By using selective filters, it may be possible to detect injury at the cellular level that may not be detected visually. Analysis can easily be incorporated into standard 14-day early seedling growth and vigor tests to detect subtle, chronic effects of contaminants. Imaging of areas acquired by satellite or aerial photography can determine differences in vegetation growth or canopy cover, but additional visual surveys, biomass measurements, or chemical analyses may be required to verify contaminant effects.

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#### Key References:

- Hader, D.P. 1988. Computer-assisted image analysis in biological sciences. Proc. Indian Acad. Sci. 98:227-249.
- Stutte, C.A. and G.W. Stutte. 1988. An interactive image capture and analysis system (ICAS) for research and crop management, p. 151-159. In P. Mausel (ed.). Videography: First workshop. Amer. Soc. Photogram. and Remote Sens., Falls Church, VA.
- Stutte, G.W. 1989. Quantification of net enzymatic activity in developing peach fruit using computer video image analysis. HortScience 23:113-115.
- Stutte, G.W. 1990. Analysis of video images using an interactive image capture and analysis system. HortScience 25(6):695-697.
- Stutte, G.W., R. Bors, and C.A. Stutte. 1990. Quantification of nutrient stress in horticultural crops using videography. Volume 16, Number 3. Journal of Imaging Technology 16:124-127.

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**Technique Name:** Plant Ecological Surveys

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**Technique Type:** Survey  
**Matrix Type:** Terrestrial and Wetland Ecosystems  
**Ecosystem Level:** Community  
**Test Location:** Field

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**Description:**

Plant communities are surveyed in the field using plot (grid), transect, and/or point-quarter sampling techniques. The plot technique involves dissecting an area into a grid system. Cells are selected randomly within each grid, plots are positioned within each cell through some unbiased "random process" (e.g., a random number of paces north and west of a designated point within each cell). Transect sampling involves establishment of a line following a compass bearing. Sampling occurs at pre-determined regular or random intervals along the line. In point-quarter sampling, a number of randomly-determined points are selected within a stand. Each point represents the center of four compass directions (N,S,E,W), that divide the sampling site into four quadrants. In each quadrant, the distance from the center point to the center of the nearest individual is measured. Endpoints include community structure (i.e., species identification, plant form, foliage density, frequency, biomass, coverage, community similarity, and ecological succession) species diversity within a community, community similarity, and ecological succession.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to establish plots and collect data.

**Time:** MODERATE to EXTENSIVE (a few weeks to a few years) depending on the size of the area and amount and type of sampling required.

**Equipment:** MINIMAL.

**Sample Analysis:**

**Training:** MODERATE to EXTENSIVE to identify species and calculate ecological endpoints.

**Time:** MODERATE to EXTENSIVE.

**Equipment:** A computer is required for data compilation and statistical analyses.

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**Critique/Comments:**

Implementation of these methods often requires considerable training and time consumption. These methods should only be used when a detailed analysis of the plant ecology is required. These methods should also be done in conjunction with short-term toxicity tests. Extreme caution must accompany any interpretation of synthetic indices, e.g., community structure and species diversity since natural selection and stress effect the diversity of a community in non-linear patterns. A good characterization of the soils should be made, as well as an analysis of other confounding factors (i.e., ambient air pollutants such as O<sub>3</sub> and SO<sub>2</sub>) to delineate site effects from contaminant effects. Furthermore, diversity may increase or decrease at a hazardous waste site. Qualitative values of harm or benefit cannot be assigned to fluxes in diversity without careful ecological analysis of the underlying features affecting a given change.

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**Key References:**

- Bonham, C.D. 1989. Measurements for terrestrial vegetation. John Wiley & Sons, Inc. New York, NY. 338 pp.
- Cox, G.W. 1985. Laboratory Manual of General Ecology. W.C. Brown, Dubuque, IA.
- Green, R.H. 1979. Sampling design and statistical methods for environmental biologists. Wiley Interscience.
- Greig-Smith, P. 1983. Quantitative Plant Ecology. Third Edition. University of California Press, Berkeley. 359 pp.
- Meyers, W.L. and R.L. Shelton. 1980. Survey Methods for Ecosystem Management. John Wiley & Sons, New York, NY.

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**Technique Name:** Soil Fauna Microcosm

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**Technique Type:** Community and Trophic Level Analysis  
**Matrix Type:** Soil Microfauna  
**Ecosystem Level:** Community  
**Test Location:** Laboratory

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**Description:**

This technique evaluates the effects of contaminants on community structure of soil-borne nematodes and microarthropods using a soil microcosm. A field soil is collected, sieved, and mixed with contaminant solutions at various treatment levels. The treated soils are placed into plastic leach tubes and incubated at room temperature (18-21°C) for 7-14 days. Soil is then removed from each tube, gently mixed, and then subsampled for chemical concentration, percent moisture and soil nematodes and arthropods. To extract nematodes, soil fractions are placed on Baermann funnels for 48 hours at room temperature. Nematodes are counted live, identified taxonomically, and sorted into fungivore, bacteriovore, herbivore, and omnivore-predator trophic groups. Hatchlings are also counted. For extraction of microarthropods, soil fractions are extracted into 95% ethanol from Merchant-Crossley high-gradient tullgren extractors for 7 days at 42°C. Microarthropods are sorted into acarine suborders Prostigmata, Mesostigmata, and Oribatida, the insectan order Collembola, and "other" miscellaneous arthropods. Community structure and trophic level analysis is performed and differences among treatment levels is determined.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL  
**Time:** MINIMAL  
**Equipment:** MINIMAL

**Sample Analysis:**

**Training:** MINIMAL to recognize and count microfauna.  
MODERATE to conduct community and trophic level analysis.  
**Time:** MODERATE.

**Equipment:** A 140-power dissecting microscope is required to identify and count microfauna.

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**Critique/Comments:**

The soil fauna microcosm is a simple inexpensive assay to measure contaminant effects on soil invertebrates using trophic structure and community analysis. This method has an advantage over single-species tests because it can be used for site-specific ecotoxicological studies of pollutants on communities of native species occupying many trophic levels in the soil system. Therefore, higher resolution of ecotoxicological effects in complex soil systems can be obtained by this approach, rather than by single-species based methods. This is a new method that has been tested with only a few chemicals and, therefore, no database has been established. Furthermore, considerable training is needed to conduct community and trophic level analysis and only a few laboratories have the expertise to do these studies. However, the test has a lot of potential for use in ecological risk assessments.

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**Key References:**

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- Moore, J.C. and P.C. De Ruiter. 1990. Temporal and spatial heterogeneity of trophic interactions within belowground food webs. In: Crossley, D.A. Jr. (ed.), *Modern Techniques in Soil Ecology*. Elsevier, Amsterdam. pp 371-398.
- Parmelee, R.W. and D.G. Alston. 1986. Nematode trophic structure in conventional and no-tillage agroecosystems. *J. Nematol.* 18:403-407.
- Parmelee, R.W., R.S. Wentsel, C.T. Phillips, M. Simini, and R.T. Checkai. 1993. A soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. *Environ. Tox. Chem.* (In press).
- Petersen, H. and M. Luxton. 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* 39:287-388.

**Technique Name:** Bacterial Biomass in Soils

**Technique Type:** Soil Survey  
**Matrix Type:** Soil Biota  
**Ecosystem Level:** Kingdom, Community  
**Test Location:** Field, Laboratory

**Description:**

Tests include direct estimates of total and active bacterial numbers, and community composition. Estimates of active bacteria in a sample involve extraction by shaking in buffer solution and staining with a solution of fluorescein diacetate (FDA). Number and diameter of all fluorescent bacteria are measured using epi-fluorescent microscopy at 1000X or greater total magnification. To estimate total bacterial numbers, each sample is diluted and stained with fluorescein isothiocyanate, filtered, de-stained, and counted using epi-fluorescent microscopy. Bacterial community composition assays test the effect of contaminant(s) on the number of sensitive species (if tagged with an immunofluorescent stain) and on bacterial ecosystems. Dilutions of soil are spread on a variety of different agar media in a variety of different abiotic conditions. The colonies of bacteria which appear are then isolated and identified. Bacterial identity is usually determined by ability to grow and catabolize specific test nutrients (e.g., various sugars, carbohydrates) or to produce specific enzymes (dehydrogenase, oxidase, etc.).

**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to grow, extract, and isolate bacteria.

**Time:** MINIMAL (< 7 days).

**Equipment:** MINIMAL for estimates of active and total bacterial numbers. An incubator is required for community composition tests.

**Sample Analysis:**

**Training:** MINIMAL.

**Time:** MINIMAL.

**Equipment:** A light microscope equipped to view

fluorescent bacteria is required.

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**Critique/Comments:**

Several research publications have suggested that changes in bacterial activity and biomass indicate possible changes in decomposition rates, soil fertility, and general ecosystem function. However, total bacterial biomass is relatively constant over all ecosystem and soil types, suggesting that this metric is less sensitive to disturbance with respect to other soil foodweb determinations. Few studies have measured active bacterial numbers using FDA. In combination with information on active and total fungal biomass, protozoan numbers and community structure and nematode numbers and community structure, nutrient cycling, energy flow, foodweb structure and diversity can be estimated. Regulatory standards exist for numbers and types of bacteria in water and wastewater. These measurements are performed by plating on a general medium (total aerobic bacteria) and media specific to coliforms. In soil, there is no known group of bacteria with a comparable indicative function such as coliforms have in water. Much more work is needed in soils to relate species presence, function and total numbers to a regulatory role.

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**Key References:**

- Babiuk, L.A. and E.A. Paul. 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of a grassland soil. *Can. J. Microbiol.* 16:57-62.
- Coleman, D.C. 1985. Through a ped darkly: an ecological assessment of root-soil-microbial-faunal interactions. In A. H. Fitter, D. Atkinson, D.J. Read, and M.B. Usher (eds.), *Ecological Interactions in Soil*. Blackwell Scientific Publications, Cambridge, U.K. pp. 1-21.
- Domsch, K.H. and G. Jangnow. 1990. Soil bacteria. pg. 1-48 In Dindal, D. 1990. *Soil Biology Guide*. John Wiley and Sons. 1349 pp.
- Nannipieri, P., S. Grego, and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. *Soil Biochemistry* 6:293-355.

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**Technique Name:** Fungal Biomass in Soils

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**Technique Type:** Soil Bioassay  
**Matrix Type:** Soil Fungi  
**Ecosystem Level:** Kingdom, Community  
**Test Location:** Laboratory

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**Description:**

Three methods are primarily used to determine fungal biomass in soils: 1) Homogenization of substrate, dilution with agar solution, gelling of agar to make a thin film, and phase-contrast microscopic counting of hyphae; 2) Homogenization of substrate, staining with fluorescing dye, membrane filtration, and microscopic counting of fluorescent hyphae; 3) chemical clearing of litter substrate, application of a stain, and microscopic counting of stained hyphae within intact substrate. Various combinations of, and modifications to these techniques have been implemented. Active and total fungi can be determined. Lengths and diameters of fungal hyphae are determined by using a pre-calibrated grid. Total hyphae biomass is then calculated per sample. Community composition may also be determined by spreading soil dilutions on a variety of agar media, isolating colonies of fungi that appear, and identifying these fungi.

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to extract, stain, and culture fungi.

**Time:** MINIMAL.

**Equipment:** MINIMAL.

**Sample Analysis:**

**Training:** MINIMAL for fungal biomass measurements. MODERATE to EXTENSIVE for identification of fungi.

**Time:** MINIMAL for biomass measurements, MODERATE to EXTENSIVE for fungal identification and community composition.

**Equipment:** A compound light microscope with phase-contrast or fluorescence capabilities is required.

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### Critique/Comments:

Any soil, sediment, litter or plant material can be tested using this method. Sensitive species can be added and assayed using this approach, as long as survival and growth requirements for the particular species is known for the material being tested. Determination of total and active fungal length and biomass indicates effects of toxics on fungal activity, function and total biomass. Reductions in toxicant-impacted soil as compared to controls, or expected levels given the soil type and organic matter level, indicate a negative effect on fungal activity and biomass. Development is needed to determine the quantitative levels which delineate impacts with different toxic chemicals in different soil types.

For many fungi so isolated, the requirements for it to fruit (sexual or asexual reproductive structures) are not known, and thus the fungus can not be identified. Additionally, the culture requirements for many soil fungi are not known and the percentage of the actual fungal community present in soil which grow on the agar media chosen cannot be determined.

Development of the database for a variety of soil types is currently underway and research is needed to determine the application of this test to Superfund and general regulatory settings. Soil biomass testing services are available at several land-grant institutions.

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### Key References:

- Ingham, E.R. and D.A. Klein. 1984. Soil fungi: Relationships between hyphal activity and staining with fluorescein diacetate. *Soil Biol. Biochem.* 16:273-278.
- Jones, P.C.T. and J.E. Mollison. 1948. A technique for the quantitative estimation of soil micro-organisms. *J. General Microbiology* 2:54-69.
- Kendrick, W.B. and D. Parkinson. 1990. Soil Fungi. pg. 49-68. In Dindal, D. 1990. *Soil Biology Guide*. John Wiley and sons. 1349 pp.
- Newell, S.Y. and R.E. Hicks. 1982. Direct-count estimates of fungal and bacterial biovolume in dead leaves of smooth cordgrass (*Spartina alterniflora* Loisel). *Estuaries* 5(4):246-260.
- Olson, F.C.W. 1950. Quantitative estimates of filamentous algae. *Trans. Am. Microscopy Soc.* 69:272-279.
- Paul, E.A. and R.L. Johnson. 1977. Microscopic counting and adenosine 5'-triphosphate measurement in determining microbial growth in soils. *Appl. Environ. Microbiol.* 34(3):263-269.

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**Technique Name: Protozoan Numbers and Diversity**

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**Technique Type:** Soil Survey  
**Matrix Type:** Soil, Protozoans  
**Ecosystem Level:** Community  
**Test Location:** Field, Laboratory

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**Description:**

Several methods are available to determine protozoan number and diversity in contaminated soil, soil eluates, sediment, litter, and ground water. The most commonly used methods are either direct observation following dilution or extraction, or separation techniques. These methods include: turbidity based on protozoan feeding rates; most probable number (MPN) using a compound microscope following dilution; direct observation of watered soil suspensions; staining and fixation, membrane filtration to enumerate testate amoebae; high resolution microscopy (phase contrast, differential interference, scanning electron microscopy); and density centrifugation followed by staining and fixation. End points are usually total numbers and community structure in contaminated vs. control substrates. Determination of numbers of each protozoan group, i.e., flagellates, testate amoebae, naked amoebae, and ciliates indicates effects of toxics on protozoan function and total biomass. Reductions in toxicant-impacted soil as compared to controls, or expected levels given the soil type and organic matter level, indicate a negative effect on protozoan biomass. Additional approaches for assessing protozoan numbers, especially of particular protozoan groups, are available from the American Society of Agronomy (Stout, et. al., 1992).

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**Logistical Considerations:**

**Sample Collection:**

**Training:** MINIMAL to learn extraction, dilution, staining, and fixation techniques.

**Time:** MINIMAL for direct soil extractions, MINIMAL to MODERATE (a few weeks) for tests requiring incubation.

**Equipment:** MINIMAL for extraction and culture materials.

**Sample Analysis:**

**Training:** MODERATE to EXTENSIVE to identify and accurately count types of protozoa.

**Time:** MINIMAL to MODERATE for community analysis.

**Equipment:** Requires EXPENSIVE compound or electron microscope.

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**Critique/Comments:**

As indicator organisms, protozoa are perhaps unsurpassed. Protozoa can be classified to genus and often to species based on morphology alone (Lee et. al. 1985). Protozoa occur in large numbers in natural ecosystems and "capture" of a representative picture of the entire community is not a problem, unlike mammals or birds. To improve the use of protozoa as indicators, however, a greater understanding of: 1) their response to disturbances beyond the normal seasonal cycle, 2) their habitat-specificity, and 3) their prey-preferences in specific habitats is needed. Efforts should be directed towards understanding changes in protozoan community composition in terrestrial systems. Soil sediment, litter or plant material can be tested using these methods. Sensitive species can be added and assayed, as long as survival and growth requirements for the particular species is known for the material being tested. Considerable training is required to identify genera and species, and to accurately assess community structure. Interferences in soil matrixes (i.e., poor visual resolution, adsorption of organisms to soil particles) decreases extraction efficiency and identification accuracy compared to aquatic samples. Furthermore, several useful keys for aquatic protozoa exist; however, a comprehensive taxonomic guide to soil protozoa is lacking. Development of the database for a variety of soil types is currently underway.

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**Key References:**

- Bamforth, S.S. 1991b. Enumeration of soil ciliate active forms and cysts by a direct count method. *Agric. Ecosyst. Environ.* 34:209-212.
- Basel, R.M., E.R. Richter, and G.J. Banwart. 1983. Monitoring microbial numbers in food by density centrifugation. *Appl. Environ. Microbiol.* 45:1156-1159.
- Darbyshire, J.F., R.E. Wheatley, M.P. Greaves, and R.H.E. Inkson. 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. *Ecology* 61:764-771.
- Foissner, W. 1986. Soil protozoa: fundamental problems, ecological significance, adaptations, indicators of environmental quality, guide to the literature. *Prog. Protist.* 2:69-212.
- Griffiths, B.S. and K. Ritz. 1988. A technique to extract, enumerate and measure protozoa from mineral soils. *Soil*

- Biol. Biochem. 20:163-174.
- Lee, J.J., S.H. Hutner, and E.D. Bovee. 1985. An Illustrated Guide to the Protozoa. Soc. of Protozoologists, Lawrence, Kansas. 629 pp.
- Stout, J.D., S.S. Bamforth, and J.D. Lousier. 1992. Protozoa, p. 1103-1120. In A.L. Page et al. (ed.), Methods of Soil Analysis. Part 2. 3rd. ed. American Society of Agronomy, Madison, WI.

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## APPENDIX C

### TECHNICAL TEST METHODS UNDER DEVELOPMENT FOR ECOLOGICAL RISK ASSESSMENT

#### INTRODUCTION

This appendix includes a variety of assays or techniques presently used or under development by various experts. The methods are primarily biochemical, concern DNA adducts or metabolic processes. Although the techniques listed herein have been published and show great promise in assessing the effects of contaminants at the cellular and sub-cellular levels, they have not yet been adapted for use in ecological risk assessment. For these procedures to be useful in the regulatory arena of CERCLA, research is needed which integrates these methods to supplement and complement information on populations and community responses to hazardous wastes.

The fact that these techniques have not been applied in assessment of hazardous waste sites should not preclude their continued development nor hinder efforts to link sublethal responses to population responses (mortality, changes in reproductive status, emigration, etc.). Therefore, the authors feel these techniques should be included in this volume.

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**Technique Name:** Adenosine Triphosphatase Activity

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**Technique Type:** Enzyme Inhibition  
**Matrix Type:** Biological  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Adenosine triphosphatase is a  $Mg^{2+}$ -activated enzyme that uses the energy of ATP to transport  $Na^+$  and  $K^+$  across cellular membranes. Effects on enzyme activity may occur by a decrease in ATP concentrations or by a direct toxic effect on the enzyme. Adenosine triphosphatase is inhibited in vitro by a variety of organochlorine compounds and heavy metals. More field research needs to be conducted before this procedure can be utilized in field studies.

The majority of research with this enzyme has been conducted using aquatic organisms in controlled laboratory environments.

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**Key References:**

- Saunders, R.L., E.B. Henderson, P.R. Harmon, C.E. Johnston and K. Davidson. 1983. Physiological effects of low pH on the smolting process in Atlantic salmon, In R.H. Peterson and H.H.V. Hord, eds., Workshop on Acid Rain. p. 49.
- Watson, T.A. and F.W.H. Beamish. 1980. Effect of zinc on branchial ATPase activity in vivo in rainbow trout, Salmo gairdneri (Richardson). J. Wildl. Dis. 13:263-270.
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**Technique Name:** Adenylate Energy Charge

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**Technique Type:** Physiological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Adenylate energy charge (AEC) is a measure of the metabolic energy available to an organism from the adenylate pool, i.e. adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Concentrations of these molecules are highly regulated and have a strong influence on many metabolic processes. AEC is a direct calculation based on concentrations of ATP, ADP, and AMP. AEC has been used extensively to assess responses of a variety of organisms to various toxicants and conditions under laboratory conditions, but its potential value as a biomarker in environmental studies has not been determined.

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**Key References:**

- Ivanovici, A.M. and W.J. Wiebe. 1982. For working definition of "stress": a review and critique, In G.W. Barrett and R. Rosenberg, eds., Stress and Natural Ecosystems. John Wiley & Sons, Inc., New York, p.13-27.
- Giesy, J.P., C.S., Duke, R.D. Bingham and G.W. Dickson. 1983. Phosphoadenylate concentrations and adenylate energy charge as an integrated biochemical measure of stress in invertebrates. The effects of cadmium on the freshwater clam Corbicula fluminea. Toxicol. Environ. Chem. 6:259-295.
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**Technique Name:** Plant Enzyme Activity

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**Technique Type:** Physiological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Activities of various plant enzymes have been used to assess the effects of air pollutants. The activity of peroxidase has been used as a nonspecific marker of general metabolic shift while enzymes such as ribulose-1,5,-bisphosphate carboxylase/oxygenase have been used because of their importance in critical metabolic reactions. Other enzymes have been selected because of their high sensitivity to a particular contaminant. Plant enzymes that mediate detoxification of a contaminant or its products have the highest potential value as biomarkers of stress.

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**Key References:**

- Alscher, R., M. Franz and C.W. Jeske. 1987. Sulfur dioxide and chloroplast metabolism, In J.A. Saunders, L. Kosak-Channing and E.E. Conn, eds., *Phytochemical Effects of Environmental Compounds*. Plenum Publishing, New York, pp.1-28.
- Byl, T.D., and S.J. Klaine. 1991. Peroxidase activity as an indicator of sublethal stress in the aquatic plant Hydrilla verticillata (Royle), In Gorsuch, J.W., W.R. Lower, M.A. Lewis, and W. Wang, eds., *Plants for Toxicity Assessment: Second Volume*. American Society for Testing and Materials, Philadelphia, PA. pp. 101-106.
- Heath, R.L. 1988. Biochemical mechanisms of pollutant stress, In W.W. Heck, O.C. Taylor and D.T. Tingey, eds., *Assessment of Crop Loss from Air Pollutants*. Elsevier Applied Science, London, pp. 311-328.
- Scholz, F., H.R. Gregorius and D. Rudin, eds., 1989. *Genetic Effect of Air Pollutants in Forest Tree Populations*. Springer-Verlag, Berlin, p. 201.
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**Technique Name:** Scope for Growth

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**Technique Type:** Physiological -- Energetics  
**Matrix Type:** Whole Organism  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Scope for growth (SFG) is an integrative approach to assessing the energy status of an organism. Energy available for growth and reproduction can be assessed by measuring energy absorbed from food, energy lost via respiration, and energy lost via excretion. This test requires that organisms be taken from the field and transported to a laboratory for measurements. SFG is a highly developed method that has been rigorously tested under field conditions. Research has shown that SFG is a good indicator of general ecosystem health.

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**Key References:**

- Bayne, B.L., D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingstone, D.M. Lowe, M.N. Moore, A.R.D. Stebbing and J. Widdows. 1985. The effects of stress and pollution on marine animals. Praeger Publishers, New York, p. 384.
- Warren, G.E. and G.E. Davis. 1967. Laboratory studies on the feeding, bioenergetics and growth of fish, In S.K. Gerhuy, ed., The Biological Basis of Freshwater Fish Production. Blackwell Scientific Publications, Oxford, England, pp. 175-214.
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**Technique Name:** Protein Synthesis

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**Technique Type:** Physiological  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

By measuring protein synthesis rate in certain tissues, the growth rate of an organism can be assessed. Protein synthesis is generally considered to be a nonspecific biomarker. Synthesis rate is assessed by exposing the organism to radiolabeled amino acids that will be incorporated in de novo synthesized proteins. By examining various tissues for radiolabeled amino acid residues, rates of protein synthesis can be assessed. This approach has been validated in field studies. Protein synthesis is generally considered to be a nonspecific biomarker.

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**Key References:**

- Aldeman, I.R. 1987. Uptake of radioactive amino acids as indices of current growth rate of fish: a review, In R. Summerfelt and G. Hall, eds., Age and Growth of Fish. Iowa State University Press, Ames, IA, pp. 65-80.
- Lied, E. and G. Rosenlund. 1984. The influence of the ratio of protein energy to total energy in the feed on the activity of protein synthesis in vitro, the level of ribosomal RNA and RNA-DNA ration in white trunk muscle of Atlantic Cod (Gadus moshua). Comp. Bioch. Physiol. 77A:489-494.
- Viarengo, A., M. Pertica, G. Mancinelli, R. Capelli and M. Orunesu. 1980. Effects of copper on the uptake of amino acids, on protein synthesis and on ATP content in different tissues of Mytilus galloprovincialis L. Mar. Environ. Res. 4:145-152.
- Viarengo, A., M. Pertica, G. Mancinelli, S. Palmero, G. Zanicchi and M. Oranesu. 1981. Evaluation of general and specific stress indices in mussels collected from populations subjected to different levels of heavy metal pollution. Mar. Environ. Res. 6:235-243.
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**Technique Name:** Oncogene Activation

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**Technique Type:** DNA Modification  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Oncogene activation analysis can be used as a biomarker of specific DNA mutations associated with the formation of cancerous tumors. Oncogenes are specific genetic sequences that may be activated when a chemical carcinogen directly alters the base-pair sequence of the gene. Altered protein function, resulting from translation of the mutation, inhibits the ability of a cell to properly regulate growth and can lead to tumor formation. Several families of oncogenes have been described. Of those, the c-ras family is detected most often in animal tumors. Genetic mutations at other loci may serve to inactivate certain tumor suppressors. Several techniques have been used to detect genetic mutations at specific sites of concern within amplified DNA strands. Methods include restriction analysis, oligotide hybridization, direct DNA sequencing, RNase mapping, gel retardation, plaque screening assay, liquid hybrid selection, and nonradioactive restriction fragment length polymorphism. The time and costs associated with detection of oncogene activation varies with the procedure. Studies of oncogene activation have been conducted using environmental fish species.

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**Key References:**

- McMahon, G., L.J. Huber, M.J. Moore, J.J. Stegeman and G.N. Wogan. 1990. Mutations in c-Ki-ras oncogenes in diseased livers of winter flounder from Boston Harbor. Proc. Natl. Acad. Sci. U.S.A. 87:841-845.
- Wirgin, I.I., D. Currie, C. Gorunwald and S.Y. Garte. 1989. Molecular mechanisms of carcinogenesis in a natural population of Hudson River fish. Proc. AACR Mtg. 30:194.
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**Technique Name:** Genetic Mutation Rates

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**Technique Type:** DNA Modification  
**Matrix Type:** Biological Tissue  
**Ecosystem Level:** Individual  
**Test Location:** Laboratory

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**Description:**

Certain highly conserved genes exhibit very little variation in base-pair sequences even between species in distantly related phyla. DNA sampled from species environmentally exposed to contaminants can be amplified. By analyzing these samples for variant base sequences, inferences can be drawn concerning the mutagenic effect of contaminants. Various procedures can be used to analyze samples.

Techniques that can be used to analyze samples are costly. Many laboratories are assessing background levels of genetic variation within and among natural populations of many species. This procedure has the advantage of detecting genetic mutations that have occurred through generations of organisms exposed to low levels of a mutagen. Extensive research needs to be conducted using environmental species before the value of this technique as a biomarker can be assessed.

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**Key References:**

- Appels, R., and R. L. Honeycutt. 1986. rDNA: evolution of a billion years, In S.K. Dutta, ed., DNA Systematics. CRC Press, Boca Raton, FL, pp 81-135.
- Moritz, C., T.E. Dowling and W.M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann. Rev. Ecol. Syst. 18:269-292.
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