20 Sampling, Analysis, and Assays

Measure if you can; model if you must.

Colin Ferguson quoted by Thomp and Nathanail (2003)

This chapter discusses in general terms the activities that comprise the sampling of environmental media and biological materials for analysis of contaminants. Sampling and contaminant analysis are obviously relevant to situations in which contamination already exists. However, it is also relevant to assessments of future contamination. In such cases, sampling and analysis are needed to support transport and fate modeling by determining background concentrations and relevant characteristics of environmental media such as pH, hardness, and temperature (Chapter 21). These data are also relevant to analysis of effects. For example, aquatic toxicity data for metals should be chosen based partly on the similarity of test water chemistry to ambient water chemistry.

The specifics of sample collection techniques, sample preparation and handling, and analytical techniques can be found in texts on environmental chemistry and in guidance documents from the US Environmental Protection Agency (US EPA), other government agencies, and standard organizations such as the American Society for Testing and Materials and the American Public Health Association. Analytical methods for chemicals, microbes, and physical properties of water and sediment in the United States are available at the National Environmental Methods Index (http://www.nemi.gov). However, most of the technical guidance for environmental sampling and analysis is intended to support human health risk assessments. These techniques may not be appropriate for the estimation of ecological exposures. For example, analytical guidance for contaminated sites in the United States calls for total extraction and analysis of water and soil, but total extractable concentrations are typically much higher than bioavailable concentrations. Ecological risk assessors should, when possible, obtain and process samples that are relevant to the exposures of endpoint receptors. When that is not possible, the concentrations should be converted to more relevant estimates of exposure. These issues are discussed in Chapter 22.

20.1 SAMPLING AND CHEMICAL ANALYSIS OF MEDIA

Most of the funds and effort expended on studies of contaminated sites are devoted to the collection and chemical analysis of the abiotic media: soil, water, and sediment. Similarly, most of the guidance for site studies is devoted to media sampling and analysis. These activities should be performed as specified in the analysis plan, and the quality of the data should be verified before it is used in the risk assessment (Chapter 9). The issues to be addressed here are the general approaches to media sampling and analysis, particularly the summarization, analysis, and interpretation of the resulting data. These issues are particularly

problematical when chemicals are detected in some, but not all, samples (Box 20.1). Specific issues with respect to using the measurements to estimate exposure are discussed in Chapter 22.

20.2 SAMPLING AND SAMPLE PREPARATION

Sampling should be performed in a way that produces a sample representative of the medium to which organisms are exposed and that does not modify the contaminant concentrations.

Soil samples should represent the range of depths to which organisms are exposed. As these vary greatly among taxa (Section 22.4), samples from multiple intervals should be obtained and analyzed so that the appropriate exposure can be estimated for each food web or endpoint species.

Sample preparation involves transforming raw samples into a form that can be chemically analyzed (Allen 2003). Initial sample preparation involves removing extraneous material (e.g., sieving or filtering), separation of phases (e.g., extracting pore water from sediment), stabilization (e.g., freezing to stop microbial processes or acidification of water to keep metals in solution), homogenization (e.g., mixing a soil sample or shaking a water sample), comminution

BOX 20.1
Handling Nondetects

Analytical data sets may include both reported concentrations (detects) and reported inability to detect the chemical (nondetects). Thus, the low end of the distribution of concentrations is censored. The problem is that nondetects do not signify that the chemical is not present, but merely that it is below the method detection limit (MDL) or quantitation limit. If a chemical is detected in some samples from a site, it is likely that it is also present at low concentrations in samples reported as nondetects. For screening assessments, this problem can be handled simply and conservatively by substituting the detection limit for the nondetect observations in order to estimate the distribution, or by using the maximum measured value as the estimate of exposure. However, for definitive assessments, such conservatism is undesirable. The most appropriate solution is to estimate the complete distribution by fitting parametric distribution functions (usually log-normal) using procedures such as SAS PROC LIFEREG or UNCENSOR (SAS Institute 1989; Newman and Dixon 1990; Newman et al. 1995). Alternatively, a nonparametric technique, the Product Limit Estimator, can be used to give more accurate results when data are not fitted well by the parametric functions (Kaplan and Meier 1958; Schmoyer et al. 1996). The US EPA provides guidance for analyzing data sets with nondetects that emphasizes simplicity of analysis (Quality Assurance Management Staff 2000).

The problem of censoring is exacerbated by the fact that method detection limits are not actually the lowest concentration that a method can detect, but rather the lowest concentration that can be reliably detected given a statistical criterion (Keith 1994). Therefore, an analytical laboratory may detect a chemical at 7, 9, and 11 μg/L in three samples but, if the MDL is 10 μg/L, the reported results are <MDL, <MDL, and 11 μg/L, respectively. Although the two lower concentrations in this example are more uncertain than the highest concentration, these measured values are clearly more accurate than the estimates generated by the methods discussed above. The best procedure from a risk assessment perspective would be to report all measured concentrations with associated uncertainties rather than allowing chemists to censor data that they deem to be too uncertain.

It should be noted that the methods for calculating detection limits and quantitation limits and even their conceptual definitions differ among users and can be highly contentious (Office of Science and Technology 2003).

(e.g., crushing or milling of solid materials), and subsampling. Initial preparation is followed by final preparation for the particular analysis, including extraction, digestion, and forming pellets. As with sampling, it is important to take steps to ensure that sample preparation does not significantly change the concentrations. This involves handling samples in a manner that is appropriate to the class of chemicals and method, and ensuring the cleanliness of equipment. Samples to be analyzed for volatile or labile chemicals should receive little preparation. Standard methods for chemical analyses often specify sample preparation methods, but they may not be appropriate for generating concentrations that are relevant to ecological exposures.

20.3 ENCOUNTERED DATA

At some contaminated sites, chemical concentrations in site media that were measured prior to the site investigation are available. The assessors must decide whether they should be used in the assessment. Although more data are generally better, encountered data may not be useful because of their age, quality, sampling techniques, or design. In general, the utility of encountered data must be determined by expert judgment. Considerations relevant to the age of the data include the rate of degradation of the contaminants, the rate of change in the rate of release of contaminants from the source, and the rate of movement of the contaminated media. Even if concentrations are declining, old data may be useful for screening assessments, because they provide conservative estimates of current concentrations. The quality of the data and the acceptability of the sampling methods must be judged in terms of the uncertainty that is introduced relative to the uncertainty from not having the data. For example, metal analyses that are performed without clean techniques may be acceptable if the contaminants of concern occur at such high concentrations that trace contamination of the sample is inconsequential. Another important consideration is the detection limits. Analyses with high detection limits may create misleading results in screening as well as definitive assessments.

20.4 SCREENING ANALYSES

Although the trend in practice is to employ specific analyses, screening analyses for classes of contaminants are still used which include analyses for total organic chlorine, total polycyclic aromatic hydrocarbons (PAHs), total hydrocarbons, gross alpha and beta radioactivity, and toluene-extractable organic matter (Thomp and Nathanail 2003). These can serve to identify hot spots and eliminate uncontaminated areas with respect to an entire class of contaminants. Hence, they can save effort and costs in analyses of specific chemicals. Some are conservative, which is acceptable in screening analyses. For example, toluene-extractable organic matter could include significant amounts of natural organic matter (Thomp and Nathanail 2003).

20.5 ANALYSIS OF COFACTORS

In addition to analyses of contaminant concentrations, analyses must be performed of the physical and chemical characteristics of the tested media that influence toxicity. These are particularly important when toxicity tests of the ambient media are performed, because the media may be unsuitable for the test organisms due to basic properties. For water, these include pH, hardness, temperature, dissolved oxygen, total dissolved solids, and total organic carbon. For sediments, they include particle size distribution, total organic carbon, dissolved oxygen, and pH. For soils, the same properties are measured, except that

dissolved oxygen is omitted and water content (e.g., field capacity) and major nutrients (e.g., N, P, K, S) are added. For example, differences in plant growth between contaminated and reference soils may be due to fertility, pH, or texture rather than toxicity. Without information on these properties, the case for toxic effects cannot be defended.

Exposure analyses for ambient media toxicity tests require analyses of chemicals of potential ecological concern (COPECs) from samples that are representative of the tested material. Therefore, results of analyses that are performed independently of the test should be used with great caution. Aqueous concentrations are highly variable over space and time. Storm events or episodic effluent releases may cause aqueous concentrations to change significantly over the course of a 7 d static replacement test, potentially making the analysis of only one of the three tested water samples inadequate for exposure characterization. Soil samples are variable over space both vertically and horizontally. Therefore, exposures in a soil toxicity test may not be well characterized by analyses of samples that were collected from "nearby" or from a different range of depths. Sediments may be relatively stable in time, like soils, or may be mobile and therefore temporally variable.

At most sites, abundant analytical data are generated which must be summarized and presented. The data summarization must meet the needs of the risk characterization. Depending on the effects and characterization models, the data may be presented as means and variances, distribution functions, percentiles, or other forms. Care must be taken in statistical summarization to avoid bias. For example, because many sets of environmental data have skewed distributions that approximate the log-normal distribution, the geometric mean is commonly recommended. However, this results in an anticonservative bias when the value is used in calculations or interpretations that involve mass balance (Parkhurst 1998). For example, if fish are exposed to varying concentrations in water, the best exposure metric for calculating their body burdens is the arithmetic mean concentration. Use of the geometric mean would improperly minimize the influence of high concentrations on uptake.

In addition, the chemical data must be summarized for presentation to other members of the assessment team, risk managers, and stakeholders. The goal of these presentations should be to make important patterns in the data apparent. The best general approach to displaying relationships between parameters (e.g., stream flow and contaminant concentrations) is the conventional x–y scatterplot. Although maps are generally not as good as scatterplots for showing potentially causal relationships, they provide an important means of presenting spatially distributed data. The difficulty comes in converting data that are associated with points to areal representations. The simplest approach is to present the results on a map at the point where the sample was taken. The results may be in numeric form or as a glyph such as a circle with area proportional to the concentration. Alternatively, various geospatial approaches can be used to associate concentrations with areas. These may be discrete areas (e.g., Theissen polygon), isopleths (e.g., Kriging interpolation), or gradients (e.g., polynomial interpolation) (Figure 20.1). Discussions of data presentation for contaminated sites can be found in Stevens et al. (1989) and Environmental Response Team (1995). More technical guidance may be found in Goovarts (1997). This is an area in which a little creative thought can be useful. Good general guidance for data visualization is provided by Edward Tufte (1983, 1990, 1997).

Toxicity normalization provides a means of summarizing exposure data for numerous chemicals in an interpretable form. This is done by converting the concentrations (C) to toxic units (TUs), which are proportions of a standard test endpoint such as the *Daphnia magna* 48 h EC_{50}.

$$TU = C/EC_{50} \tag{20.1}$$

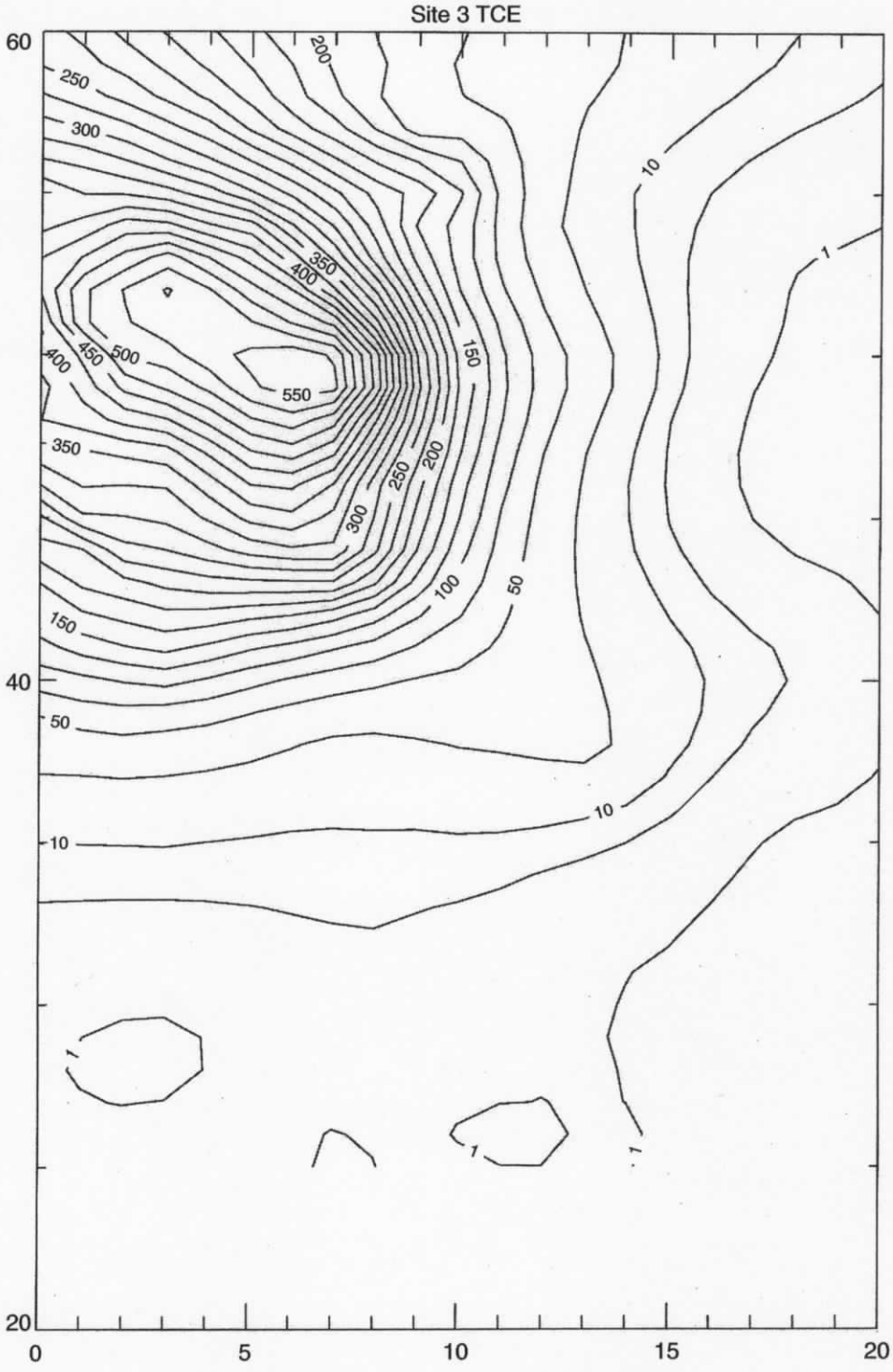

FIGURE 20.1 An example of a map generated by Kriging which, given a spatial array of chemical measurements, defines areas estimated to have chemical concentrations within prescribed ranges. (Provided by Yetta Jager, ORNL, and previously published in Suter, G.W., II, Efroymson, R.A., Sample, B.E., and Jones, D.S., *Ecological Risk Assessment for Contaminated Sites*, Lewis Publishers, Boca Raton, FL, 2000. With permission.)

TUs may be plotted as the values for each reach, subreach, transect, or other unit (Figure 20.2). The height of the plot is the sum of toxic units (ΣTU) for that location. The advantage of this approach is that it displays the contaminant concentrations in units that are indicative of potential toxicity rather than simply mass per unit volume. Therefore, one can

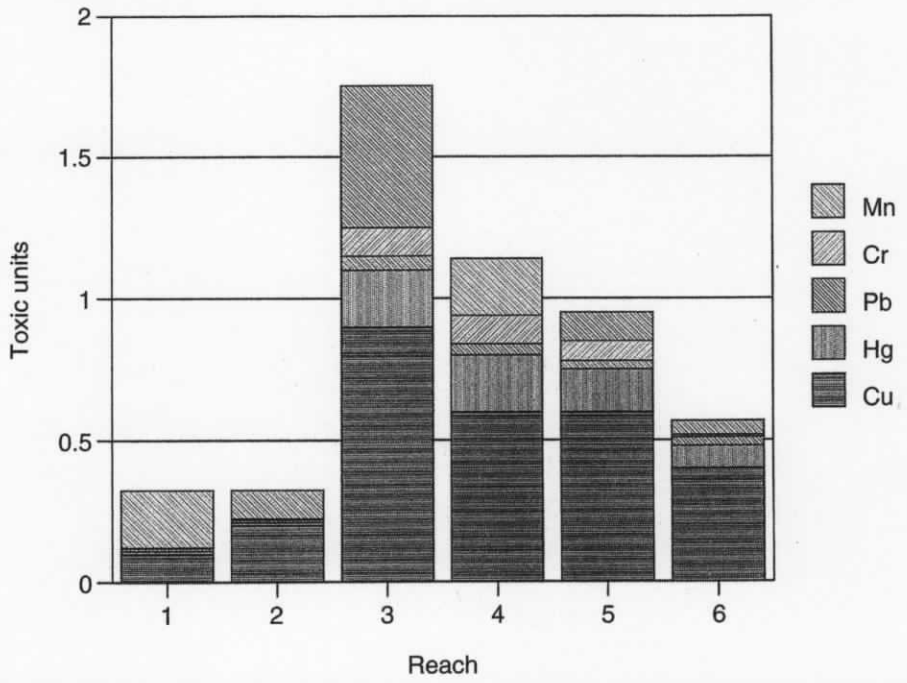

FIGURE 20.2 A display of the toxicity-normalized concentrations of metals in six stream reaches. The height of each bar is the sum of toxic units. (Previously published in Suter, G.W., II, Efroymson, R.A., Sample, B.E., and Jones, D.S., *Ecological Risk Assessment for Contaminated Sites*, Lewis Publishers, Boca Raton, FL, 2000. With permission.)

see which locations are most likely to pose significant risks, and which chemicals are likely to be major contributors to the toxicity. The purpose of this analysis is heuristic.

20.6 WATER

A common issue in analysis of ambient waters is the low concentrations of many chemicals, particularly when dissolved rather than total concentrations are desired. Detection of low concentrations requires not only high-quality analytical techniques, but also great care in sampling and sample handling to avoid trace contamination. Because of the general lack of ultraclean sampling and handling techniques, aqueous metal concentrations reported prior to the mid-1990s are generally unreliable (Benoit 1994).

20.7 SEDIMENT

Sediment samples may be obtained by a variety of dredges, coring devices, and, in wadeable streams or intertidal areas, by hand-held trowels or other devices. As with soil, the samples should be taken to a depth that is relevant to the organisms, usually a few centimeters. Another important consideration is the multiphasic nature of sediments. Apart from the basic distinction between the aqueous and solid phases, it may be important to distinguish sorbed material in the aqueous phase from the freely dissolved phase. The importance of these distinctions depends on the assumptions and models that are used to estimate exposures (Section 22.3).

The aqueous phase of sediments, pore water, is important as it is the most bioavailable phase for many chemicals and organisms. Extracting pore water from sediment samples can be labor-intensive, can require large amounts of sediment in order to obtain sufficient sample

volume for multiple analyses, and can alter the form and speciation of the chemicals measured. The advantage is that measured pore water concentrations can be evaluated using the same techniques and effects data used for surface water. Measuring pore water concentrations is particularly useful for metals and ionic organic chemicals, because the particle–pore water partitioning mechanisms are complex and difficult to model.

20.8 SOIL

The concentration of contaminants in soil may be characterized in either bulk soil or soil solution. Sampling and analytical methods should be selected that are precise and easily related to measures of effects. The most direct and common approach is the collection and analysis of the bulk medium. Total concentrations of metals may be obtained by total digestion (e.g., $HF/HNO_3/HClO_4$), which removes elements bound to silicates, or by partial digestion (*aqua regia*), which removes all metals not firmly bound to silicates (Thomas 2003). Similarly, thorough extractions using organic solvents and heat allow the estimation of organic compounds (Hatzinger and Alexander 1995; Hendriks et al. 1995). These analyses have the reassuring feature of including the full extent of contamination as well as background concentrations of elements.

Aqueous extractions of soil may be designed to simulate the extraction processes of soil organisms. That is, the mass of the chemical extracted by an aqueous solution (somewhat less than the total) divided by the mass of the soil would approximate the bioavailable concentration. Appropriate procedures would depend on the organisms for which exposure is being estimated. Relatively mild extractions would be appropriate for root uptake, and stronger extractions would be expected to correlate with uptake by earthworms. Although many extraction procedures have been proposed, none has been demonstrated to be reliable for a variety of organisms, soils, and contaminants. For example, although concentrations of diethylenetriamine pentaacetic acid (DTPA)-extracted contaminants from soils sometimes correlate with those taken up by plants (Sadiq 1985) and earthworms (Dai et al. 2004), this estimate of bioavailability has been observed not to be valid for some metals (Sadiq 1985, 1986; Hooda and Alloway 1993; Dai et al. 2004) and for soils of varying pH (Miles and Parker 1979). As another example, three very different approaches for extracting bioavailable PAHs have been proposed in recent years, but none has demonstrated to be superior (Cuypers et al. 2000; Loibner et al. 2000; Liste and Alexander 2002).

Extractions intended to simulate mammalian gastrointestinal processes have been developed for human health risk assessment, but their range of applicability is unclear (Kelly et al. 2002). Because these extractions are believed to overestimate bioavailable concentrations in ingested soil, their results are referred to as bioaccessible concentrations.

20.9 BIOTA AND BIOMARKERS

Analysis of abiotic media provides a measure of external exposure to contaminants, but not internal exposure or exposure through trophic transfers, which require estimates of uptake from media and transfer between biotic compartments. In the absence of reliable models of uptake and transfer, internal exposures and trophic transfers can be estimated by collecting and analyzing biota from the contaminated site or from laboratory exposures to contaminated media. This approach has the advantage of avoiding the use of highly variable empirical models or unvalidated mechanistic models. However, analytical chemistry is expensive, and some chemicals are rapidly metabolized or may not accumulate to detectable levels. Similarly, body burden analyses are not feasible for some species such as those designated as threatened and endangered or those that do not currently occur on the site.

Care must be taken to ensure that the body burden analysis is relevant to the assessment. One issue is the treatment of unassimilated material. For example, if soil or sediment oligochaetes are not purged, the analysis may be dominated by chemicals in the gut contents that have not been incorporated. This may either overestimate or underestimate internal exposure of the worms and dietary exposure by vermivores, depending on whether the uptake factor (organism concentration/soil concentration) is less than or greater than one. However, for chemicals that are rapidly depurated following assimilation, long holding times for purging may result in underestimation of exposure. Although 24 h is the standard holding time to evacuate gut contents, as little as 6 h may be sufficient (Mount et al. 1999). The issue of unassimilated material also arises with contamination of the surfaces of leaves, and fur, feathers, and gut contents of wildlife. Decisions concerning this issue should be based on careful consideration of the actual mode of exposure of the endpoint organisms and of the exposure model used in the assessment. For example, if soil ingestion is included as a separate route in the exposure model, care should be taken to avoid incorporating soil into the chemical analysis of endpoint organisms or their food.

A second aspect of ensuring relevance of analyses to the risk assessment is selection of appropriate species, higher taxa (e.g., insects), or assemblages (e.g., benthic invertebrates) for sampling and analysis. This depends on the purpose of the sampling. In general, the purpose is either to estimate the dietary exposure of consumers (i.e., analyzing plants to estimate exposure of herbivores) or to estimate the internal exposure of endpoint organisms. In the first case, sampling should focus on the primary food organisms and on the parts that are consumed. In the latter case, the sampling should focus on the endpoint species or, if that is impractical, on a closely related species with similar habits. If the endpoint entity is a community or higher taxon, one may choose a representative species, representative set of species, or the entire group. To the extent that they can be identified and are relevant, the species that have the highest level of accumulation should also be selected. When other criteria are satisfied, organisms may be chosen on the basis of practical considerations such as ease of collection and body size.

A third aspect of ensuring relevant analyses is selection of appropriate components of the organisms for analysis. This requires first a decision as to whether to analyze the whole organism, some organ, or another component. Once again, the primary consideration is the relationship of the analysis to the mode of exposure. If one is interested in the dietary exposure of a grazing or browsing animal, the leaves of plants should be analyzed; for beavers, the bark and cambium of small branches; for granivores, the seeds. If the analysis is performed to estimate internal exposure of an endpoint receptor, one should perform the analysis that is appropriate for the exposure–response model.

If internal measures of exposure are to be employed in an assessment and site-specific field data are to be collected, it is important to know something of the toxicokinetics of the chemical of interest. Toxicokinetic data will provide an indication of whether the exposures are likely to result in detectable concentrations and which tissues should be sampled and analyzed. Tissue types most frequently sampled include liver and kidney, as they are the primary organs for metabolism and excretion, and are therefore likely to be adversely affected by contaminants. Brain is frequently analyzed for contaminants that are neurotoxic and accumulate in lipid. Chemical concentrations in eggs are widely used to evaluate the exposure of birds to contaminants that may be transferred through eggs (lipophilic chemicals occur in yolk) or are known to have adverse effects on development.

Some tissues are analyzed not because they are clearly associated with effects but because they are reservoirs for contaminants. Tissues such as bone and fat become reservoirs because of the chemical-specific affinities. Because most organochlorine contaminants are hydrophobic and lipophilic, they tend to accumulate in fatty tissues. Similarly, because lead

and strontium are analogs for calcium, these inorganic contaminants tend to accumulate in bones. Contaminants in reservoir tissues may be mobilized episodically. Examples include mobilization of organochlorine chemicals from fat during starvation (e.g., hibernation or migration) or mobilization of lead associated with calcium mobilization during pregnancy or egg formation.

Other tissues, such as hair and feathers, are contaminant sinks. Chemicals in sink tissues cannot be reabsorbed, and are generally restricted to inorganic contaminants. Advantages of analyzing hair and feathers are that both tissues can be sampled nondestructively (without killing the animal) and that they may be sampled repeatedly from the same individual so that contaminant exposure may be tracked over time. Chemical concentrations in hair and feathers from various locations have been compiled and summarized (Huckabee et al. 1972; Jenkins 1979), but few data relate the concentrations to effects. Exceptions are provided by studies of lead and mercury in feathers (Burger 1995; Burger and Gochfeld 1997).

It is also necessary to consider the relevance of analyses of mobile organisms. Mobile organisms collected on a site may have spent little time on that site. To the extent that it is consistent with the endpoints of the assessment, organisms that are most associated with the site should be preferred, such as less mobile organisms and organisms with small home ranges. However, if the organisms of concern are not confined to the site, body burden analyses can still be relevant in that they realistically represent the proportional exposure of those organisms to the site and its contaminants. This rationale is applicable only if the organisms are not significantly exposed to sources of the contaminant outside the site.

In some cases, analysis of organisms from a site is not practical because the site is small or highly disturbed. In such cases, organisms can be exposed to the contaminated site media under controlled conditions. For example, at the Naval Weapons Station, Concord, California, plants and earthworms were exposed to site soils in the laboratory, and caged clams were exposed to site waters in the field (Jenkins et al. 1995). Similarly, earthworms were exposed in containers of soil at the Baird and McGuire Superfund Site in Holbrook, Massachusetts (Menzie et al. 1992). While providing consistent bioconcentration data, such studies can also provide information on toxicity.

An alternative to body burden analysis is analysis of biochemical biomarkers such as hepatic mixed function oxidase enzymes (Huggett et al. 1992). Biomarkers may be detected when the contaminant cannot be detected, and in some cases they may be measured without sacrificing the animal. For example, blood aminolevulinic acid dehydratase (ALAD) was used to estimate lead exposure in birds on the contaminated floodplain of the Coeur d'Alene River, Idaho, and liver lead concentrations were determined in a subsample of birds (Johnson et al. 1999). However, biomarkers tend to be nonspecific, to increase nonlinearly with increasing exposure levels (e.g., to decline at high exposures due to inhibited protein synthesis), and to vary with extraneous variables such as the animal's breeding cycle or nutritional state. In addition, few reliable exposure–response functions are available to relate biomarker levels to effects on organisms. For these reasons, biomarkers have been used much less than analysis of contaminant burdens in ecological risk assessments. However, one potentially important use is as bioassays (Section 20.10).

Body burdens and biomarkers of exposure must, in most cases, be related to concentrations in media to which the organisms are exposed. The derivation of such relationships requires sampling and analysis of the exposure media colocated with the sampled biota. A series of such analyses of colocated biological and media samples can be used to develop a site-specific uptake factor or other model. If the range of sites encompasses the range of contaminant levels, and if the uncertainty in the site-specific factor or model is sufficiently low, the factor or model can be used to predict body burdens or biomarker levels at locations where media samples, but not biological samples, have been analyzed. As media and biota concentrations

may vary, samples should also be colocated in time. The acceptable interval between samples depends on the rate of variance of the biota and media, but the samples should not be taken in different seasons.

A wide variety of methods are available for the collection of biota samples for residue analyses, with sampling methods generally being medium- or taxon-specific. Common collection methods for taxa generally of interest in risk assessments are outlined in Appendix A of Suter et al. (2000) and in many other sources. General guidance on biota sampling is presented in Box 20.2.

20.10 BIOASSAYS

Bioassays are measures of biological responses that may be used to estimate the concentration or determine the presence of some chemical or material. Bioassays are seldom used since the development of sensitive analytical chemistry. One valuable use of bioassays is to determine the effective concentration of chemicals with a common mechanism of action. For example, the H4IIE bioassay provides a toxicity-normalized measure of the amount of chlorinated diaromatic hydrocarbons in the food of an organism (Tillitt et al. 1991; Giesy et al. 1994b). This use is analogous to the use of biomarkers to estimate internal exposure (Section 20.9), except that the goal is to estimate external response-normalized concentrations.

It has also been proposed that activity of contaminant-degrading microbes be used as a bioassay for bioavailable contaminant constituents (Alexander et al. 1995). A weak interpretation of this bioassay is that, if biodegradation has stopped, there is no more bioavailable chemical to cause toxicity. This conclusion requires the assumption that biodegradation has stopped because the residue is unavailable rather than because it is resistant to biodegradation. A stronger interpretation would be that bioavailable concentration is a function of biodegradation rate so that one could estimate exposure from measures of degradation. This idea requires the assumption that the availability of a chemical for degradation by microbes is proportional to availability for uptake by endpoint plants and animals. The use of microbial toxicity tests as measures of bioavailability or ecological effects is beyond the current state of practice.

Bioassays may be used more generally to screen contaminated media for toxicity (Loibner et al. 2003). That is, toxic responses may be used in place of chemical analyses in screening

BOX 20.2
Rules for Sampling Biota

- Take enough samples to adequately represent the variability at the site.
- Sample endpoint taxa for which internal measures of exposure are useful.
- Sample organisms or parts of organisms that represent the food of assessment endpoint species.
- Take samples of biota and contaminated media at the same locations and at effectively the same time.
- Take samples at reference and contaminated locations or on contamination gradients.
- Take samples from all sites at approximately the same time because chemical concentrations in organisms may vary seasonally.
- Be aware of the information that is lost when samples are composited.

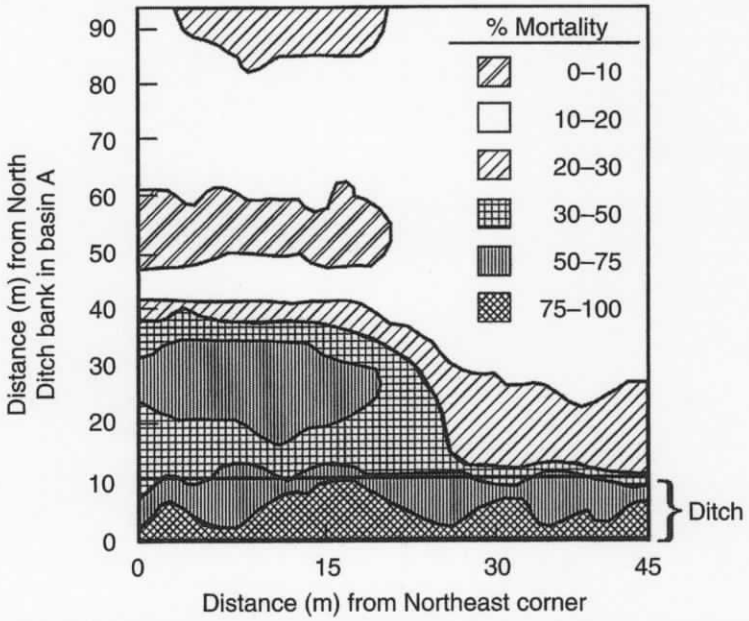

FIGURE 20.3 A map derived by Kriging of percentage of lettuce seed mortality in surface soil at the Rocky Mountain Arsenal. (From Thomas, J.M., Skalski, J.R., Cline, J.F., McShane, M.C., Simpson, J.C., Miller, W.E., Peterson, S.A., Callahan, C.A., and Greene, J.C., *Environ. Toxicol. Chem.*, 5, 487, 1986. With permission.)

assessments to discriminate areas that are significantly contaminated and require more study from areas that are not toxic and may be ignored. Bioassays used for this purpose must be sufficiently sensitive, but need not bear a defined relationship to the assessment endpoints. For example, tests of soil extracts using *Daphnia* or *Ceriodaphnia* spp. (sensitive organisms to many chemicals) may be good screening bioassays, but would not, in general, be accepted as predictors of effects on terrestrial organisms. However, tests that serve to estimate risks to assessment endpoints may further serve to define areas that require remediation without identification of the toxic chemicals (Thomas et al. 1986) (Figure 20.3). For example, it might be agreed in the problem formulation that any sediment that is acutely lethal to an amphipod would be dredged without further characterization or assessment, sediments with sublethal effects would be further characterized, but sediments with no effects would not be considered further. Tests used for this purpose must be sufficiently sensitive to the contaminants, reliable, robust to characteristics of the site media, and inexpensive relative to a full suite of chemical analyses.

20.11 BIOSURVEYS

Surveys of organisms (biosurveys) are used in ecological epidemiology to identify impaired communities (Chapter 4) and in ecological risk assessments primarily as a means of determining effects of contaminants (Chapter 25). However, they may also play a role in the analysis of exposure. Specifically, they can be used to determine whether a species or taxon is present in contaminated areas, what life stages are present, their abundance, and how long a migratory or otherwise transient species is present on the site. Without biological surveys, these presence and abundance parameters must be estimated using habitat models or assumptions. Biosurveys of contaminated sites provide estimates of these parameters for the current condition. Biosurveys of uncontaminated reference areas can provide estimates of these parameters for precontamination or postrestoration scenarios. Biosurveys may be

conducted in conjunction with the collection of organisms for chemical analysis (Section 20.9), but care must be taken to ensure that sampling designs are adequate for both purposes.

20.12 SAMPLING, ANALYSIS, AND PROBABILITIES

In risk assessments, it is necessary to consider the distribution of the exposure metrics with respect to variability and uncertainty (Chapter 5). For example, if a data set consists of a set of cadmium concentrations in water, they may be time-averaged or instantaneous concentrations, at one or several points, at one or more times, which may extend over a season or a year, etc. Spatial or temporal variability may dominate the distribution of concentrations, or there may be significant uncertainty due to lack of information about the form of the measured cadmium. Distributions derived from these data need to be created and interpreted in such a way as to correspond to the distributions needed to estimate exposure of the endpoint receptors.

The most common probabilistic treatment of these data is to fit a distribution to the individual observations. However, these distributions often do not make sense as expressions of exposure. Assessors must determine the appropriate temporal and spatial units of exposure and how they are distributed given the definition of risk to the endpoint in question.

Concentrations in water may be treated as spatially constant (i.e., a fish community is assumed to occupy a pond or stream reach), and the critical variable is time. For effects of observed pulse exposures such as those that occur during storm events which flush pollutants into the system or failures of treatment or containment systems, the distribution of aqueous concentrations with respect to duration of the event should be derived. Choosing an appropriate duration for chronic exposures is more difficult. A default value is 7 days, the duration of the standard subchronic toxicity test developed by the EPA and employed at wastewater discharges and contaminated sites. The time period is based on the time required for most chemicals to induce effects on survival and growth of larval fish and survival and reproduction of planktonic crustaceans. Some chemicals induce effects on those organisms much more quickly, and some organisms, particularly larger ones, respond much more slowly. Ideally, the durations should be set at the time required to induce the endpoint response. The selection of that interval requires a careful study and analysis of the original toxicological literature. Once the duration has been chosen, the appropriate measure of variability is the variance in the x day moving average concentration, where x is the duration of the episodic exposure or the time required to induce chronic effects in routine exposures.

An additional concern is lack of correspondence between the measures of exposure and the appropriate exposure metric for the risk model. While it is generally recognized that measures of effects must be extrapolated, exposure extrapolations are less often acknowledged. Examples from ecological risk assessment include the following:

- Estimation of forage fish contamination from game fish analyses
- Estimation of whole fish concentrations from fillets
- Estimation of dissolved concentrations from whole water concentrations
- Estimation of undetected concentrations from limits of detection
- Estimation of pore water concentration from sediment concentration
- Estimation of annual average concentration from summer samples

In some cases, data and techniques are available to estimate the uncertainties associated with these extrapolations. Examples include maximum likelihood estimators for concentrations below detection limits and statistical models of the fillet to whole fish extrapolation (Bevelhimer et al. 1996). In other cases, expert judgment must be employed.

20.13 CONCLUSIONS

Sampling and analysis methods for contaminated environmental media are well developed and documented and routinely applied. However, their results are often less than optimal for use in ecological risk assessments. Ecological assessors need to be more involved in the design of sampling and analysis activities (Chapter 18) and must be insistent that their needs be met.