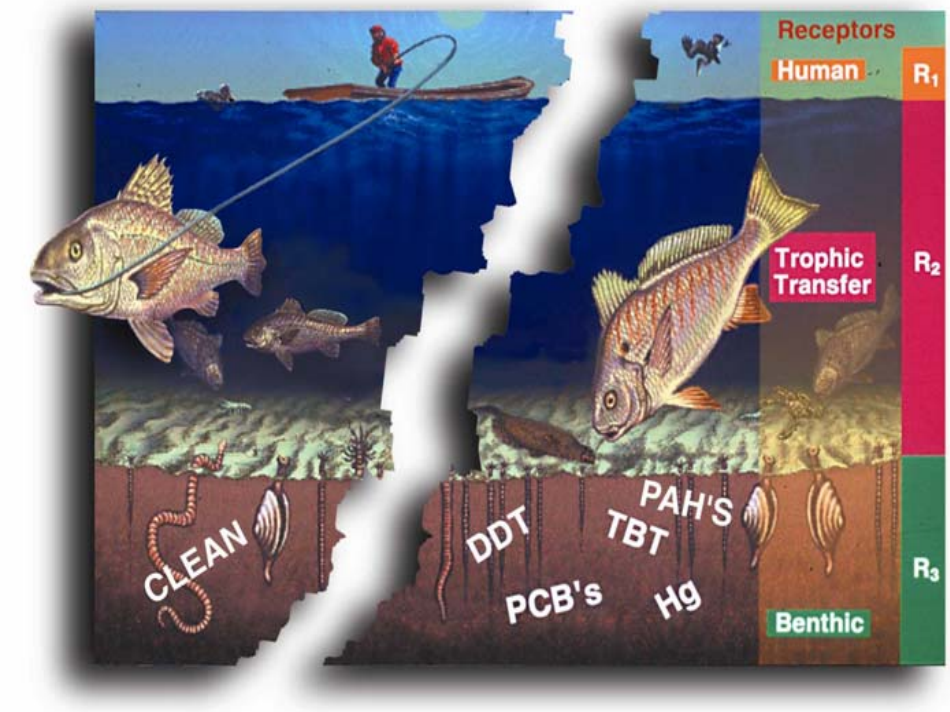
Office of Superfund Remediation and Technology Innovation
and
Office of Research and Development

Sediment Assessment and Monitoring Sheet (SAMS) #1

Using Fish Tissue Data to Monitor Remedy Effectiveness



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SEDIMENT ASSESSMENT AND MONITORING SHEET #1

Background and Purpose

This is the first fact sheet in the Sediment Assessment and Monitoring Sheet (SAMS) series prepared by the Office of Superfund Remediation and Technology Innovation (OSRTI). This sheet was prepared in collaboration with the Office of Research and Development. OSRTI anticipates that other SAMS will be completed, some in collaboration with other federal agencies.

This document provides technical guidance to the U.S. Environmental Protection Agency (EPA) staff on developing monitoring plans for contaminated sediment sites. It also provides information to the public and to the regulated community on how EPA intends to exercise its discretion in implementing monitoring plans. This document does not impose legally-binding requirements on EPA, states, or the regulated community, but suggests monitoring approaches that may be used at particular sites, as appropriate, given site-specific circumstances.

Introduction

Chapter 8 of the Contaminated Sediment Remediation Guidance for Hazardous Waste Sites (OSWER Directive 9355.0-85, December 2005), presents an approach for developing an effective monitoring plan (http://www.epa.gov/superfund/health/conmedia/sediment/guidance.htm). As stated in the Guidance, one of the goals of monitoring is to "evaluate long-term remedy effectiveness in achieving remedial action objectives (RAOs) and in reducing human health and/or environmental risk." The Guidance describes a successful remedy as one where "the selected sediment chemical or biological cleanup levels have been met and maintained over time, and where all relevant risks have been reduced to acceptable levels based on the anticipated future uses of the water body and the goals and objectives stated in the ROD." The information in the following text box is Highlight 8-1 from the Guidance.

As stated in the last two measures, fish tissue contaminant concentrations are often the key measures that need to be monitored. The Guidance, however, does not specify how, when, or where to collect fish tissue samples. There are many factors that can influence the measured concentrations of contaminants in biota tissues. The site manager and technical team need to be aware of these factors and consider them in developing the sampling plan. This will help ensure that the data collected can be used to evaluate remedy effectiveness and to evaluate the protectiveness of the remedy during the five year review process.

Sample Measures of Sediment Remedy Effectiveness

Interim Measures

1. Short-term remedy performance (e.g., Have the sediment cleanup levels been achieved? Was the cap placed as intended?)
2. Long-term remedy performance (e.g., Have the sediment cleanup levels been reached and maintained for at least five years, and thereafter as appropriate? Has the cap withstood significant erosion?)
3. Short-term risk reduction (e.g., Do data demonstrate or at least suggest a reduction in fish tissue levels, a decrease in benthic toxicity, or an increase in species diversity or other community indices after five years?)

Key Measures

4. Long-term risk reduction (e.g., Have the remediation goals in fish tissue been reached or has ecological recovery been accomplished?)

This SAMS provides general information on the collection and use of tissue residue data for monitoring the effectiveness of sediment remedies at Superfund sites. This information may also be useful in collecting baseline risk assessment data, but that is not the focus of this fact sheet. This fact sheet briefly discusses the factors that may be important and provides general recommendations for fish sampling at typical Superfund sites. Although this fact sheet focuses on finfish, much of the information is applicable to shellfish, *e.g.,* mussels and crabs. These recommendations are based on the experience and expertise of EPA researchers and program staff and are supported by peer-reviewed literature. Nevertheless, these recommendations may not apply at every site, and project managers are encouraged to make their own site-specific decisions concerning effective monitoring plans. Dependent upon the question(s) being asked, the data requirements may be relatively easy to meet, or could necessitate large and costly efforts.

Although this fact sheet focuses on collecting and using fish tissue contaminant data, surface sediment samples should also be collected at the same locations and same time as part of remedy effectiveness monitoring. It is important to try to understand the relationship between the contaminant levels in the surface sediment and the resulting levels in the fish. A biota sediment accumulation factor (BSAF) approach is often used to characterize this relationship and this approach is most useful if both fish tissue and sediment data are collected concurrently. Depending upon site specific conditions, it may also be important to collect surface water samples at the same locations to further understand the exposures resulting in contaminant uptake in fish.

Source: The Great Lakes National Program Office

Factors to Consider in Collecting Contaminant Residue Data in Fish and Other Aquatic Organisms

Contaminant Types

Contaminants accumulate in biota to varying degrees and at different rates. These variations are a function of the contaminant, the organism, and the environment. Contaminants found most frequently at Superfund sites may be divided into three chemical classes: organic compounds, metals, and organometallics like methylmercury and tributyltin. PCBs and pesticides have been the key organic contaminants of concern (COC) at over half of the Superfund sediment sites.

The most important characteristic of organic compounds that affects their ability to bioaccumulate is their hydrophobicity; *i.e.,* their resistance to be dissolved in water. A chemical's hydrophobicity is most often expressed or measured using the *n*-octanol/water partition coefficient, K_{ow}, often displayed as the logarithm, log K_{ow}. Fortunately, the grouping of compounds by their K_{ow} allows for some generalizations regarding the expected accumulation of these organic compounds in tissues.

The extent of bioaccumulation of a chemical is also fundamentally related to the rates of excretion and metabolism of the chemical in the organism. Organic compounds that are very slowly metabolized (if at all) are often highly chlorinated, such as PCBs, dioxins and furans, and DDTs. In general, organic chemicals that significantly bioaccumulate in fish are nonionic, have a K_{ow} greater than 10^5, and are not rapidly excreted or easily metabolized.

The second major class of contaminants in sediment at Superfund sites is metals. Unfortunately, there are no generalizations concerning bioaccumulation that can be made for metals. The chemical properties that affect the accumulation of metals can be different for different forms or chemical species of the same metal, *e.g.,* their oxidation state. The issues surrounding the accumulation and effects of exposures to metals are summarized in the Framework for Inorganic Metals Risk Assessment (U.S. EPA 2007).

Some metals can be transformed into organometallic compounds that accumulate in tissues to much greater levels than their inorganic counterparts. The best example of this is mercury. While inorganic mercury is not readily accumulated, the organic form, methylmercury, accumulates substantially.

Organism Type and Lipid Content

Different taxonomic groups of organisms or different life stages of the same organism can accumulate contaminants differently. This is a result of both the physiology and the life history of the particular organism. Different classes of organisms have different biochemical systems that vary in their ability to degrade or metabolize contaminants, and some classes of organisms have mechanisms to sequester and/or excrete the contaminant or detoxify it.

Because risks at Superfund sediment sites are often driven by the ingestion of fish and shellfish by humans or wildlife, these organisms are the ones that usually should be sampled. Since fish are mobile, they may be good integrators of varying sediment conditions and can be used to estimate typical exposures from ingestion of fish at a site. Some fish, however, have very large foraging ranges and, depending on site size, may not represent well the exposures attributable to just the site. Knowledge of the life history of the organism may be needed in order to limit your selection of fish species to those that have exposures reflective of site contaminants. Small non-game fish with high site fidelity and organisms with limited mobility, such as clams or mussels, can be very useful in estimating contaminant exposures from localized areas.

For organic compounds, the lipid content of the organism can greatly influence the degree of accumulation. Generally, the higher the lipid content, the greater the accumulation. Different species of fish (and other biota) have different lipid contents. Additionally, the age and physiological state, *e.g.,* gravid females, of the organism can also affect the lipid content.

Fish Species

The species selected for monitoring can have a substantial effect on the degree of bio-accumulation observed. Species can vary greatly in size, lipid content, feeding habits, and movement patterns, and these in turn influence bioaccumulation. Knowledge of the fish species present and their feeding behaviors can assist in the selection of the most appropriate species to meet the study objectives. However, since many sediment sites include a fish consumption advisory as a component of the remedy, the state public health agency that is implementing the advisory should be consulted on the selection of species. This consultation also can be valuable in determining where and how to best collect a particular species.

Sex of Organism

For some species, the tissue concentration can be influenced by the sex of the individual (Rypel et al. 2007). This can be because of inherent differences in the type and amounts of lipids between males and females, differences in feeding habits, spawning, and other life history parameters, or to differences in elimination rates of the contaminant. Spawning can alter lipid content and contaminant concentrations in females resulting in either biased data and/or increased variance in the data.

Source: The Great Lakes National Program Office

Sample Type and Size

Determining the most appropriate sample type and size depends on how the data are going to be used, the extent of the available baseline data, and several other site-specific factors. Within the Data Quality Objective process, decisions regarding the contaminant detection limit, fish size, number of fish, and location and number of sampling stations must be made. As with most investigations, the effort needs to be cost-effective, balancing the costs of fish collection and sample analyses against the need for increased accuracy and certainty in determining levels of risk reduction. Detection limits may need to be lower than typical if the remediation goal is low, especially if small species are to be sampled. For small species or small individuals of a species (*e.g.,* young of the year fish), the mass of each individual is often below the mass needed to meet standard analytical requirements; *e.g.,* 10 gram wet weight per organic scan and 0.5 gram for metal scan (*see* EPA's Contract Laboratory Program Web site http://www.epa.gov/superfund/programs/clp/target.htm). Micro extraction and analysis techniques exist but are not routinely available, and typically require additional expertise in the handling and storage of samples to avoid significant artifactual contamination.

One must decide whether to use samples composed of whole fish or fillet. If risks are driven by human ingestion of contaminated fish, then samples consisting of fillets are generally analyzed. If risks are driven solely by ecological risks, then samples consisting of whole fish are generally employed. At many Superfund sites, to reduce the number of fish collected and to obtain residue data compatible with both human and ecological risk evaluations, samples composed of the fillets and samples of their offal (the reminder of the carcass after filleting) are analyzed separately. Using the analytical data from the fillet and offal samples, whole body residues can be

estimated if needed using a tissue-weighted method.

Since most sediment remedies have been driven by human risks from ingestion of contaminated fish, monitoring plans for Superfund sites should collect data that are compatible with the data used by the state or other public health agency to set or modify fish consumption advisories. However, when evaluating ecological risks, it may be more useful to collect smaller, early indicator species as well.

The EPA *Guidance for Assessing Chemical Contaminant Data in Fish Advisories, Vol. 1 – Fish Sampling and Analysis, Third Edition* (EPA-823-B-00-007, November 2000) states that programs monitoring contaminant levels in fish tissues for fishing advisories should pool individual fish into composite samples. This is done to improve estimates of the mean chemical residue in the fish population while reducing analytical costs. However, there may be cases where a more rigorous and more expensive sampling plan that analyses individual fish is warranted. At such sites, it may be useful to perform a power analysis[a] in order to determine the minimum number of fish to be collected and analyzed in order to detect a specified minimum significant differences in chemical residues over time and/or space; *e.g.,* have the residues in fish decreased by 50% in three years[b]?

If the sample size (*i.e.,* number of fish) is too small, the measurements will not have the precision needed to provide reliable detection of the differences in chemical residues over time. If the sample size is too large, resources will be wasted because too many fish were collected and analyzed. When fish are pooled into composite samples, there are tradeoffs between the number of composites (n) and number of fish per composite (m) in terms of their impact upon the estimate obtained for the population variance. EPA's guidance on fish advisories provides detailed information on the interchange between number of composites (n) and number of fish per composite (m) upon the measured variance, and further, provides look-up tables documenting statistical power of the hypothesis tests with a variety of specified assumptions. Power analysis can be performed using many statistical packages, *e.g.,* SAS, PASS, G*Power, R, and S-Plus.

The analysis of large numbers of individual fish at several locations can be costly for some contaminants. A second potential issue with the collection of large numbers of fish is that, depending on the site, it might not be possible to collect the target number of organisms within the specified size range. The field collection crew, no matter how good, can only collect what organisms are present.

a The power of a statistical hypothesis test is the probability that the test will not reject the null hypothesis (H_0: Residues in fish have not decreased by 20%) when the null hypothesis is false, *i.e.,* the residues have actually decreased by 20%. In other words, power is the probability that the test will not make a Type II error, *i.e.,* false negative (the apparent decrease is real, but is rejected as being not real). When one performs hypothesis tests, typically, one sets alpha to 5% (α=0.05), and this is the probability for the Type I error, *i.e.,* false positive (the apparent decrease is not real, but is accepted as being real). Setting alpha does not set the probability for the Type II error (β). The power of a test is calculated as 1- β. Power analysis defines beta (β), with a given alpha (α), for the statistical hypothesis test in either a prospective or retrospective applications. In prospective applications (before data are collected), power analysis will enable the refinement of your sampling designs (number of fish per composite and number of composites). See appendix for further information on power analysis.

b The 50% decrease in residues is illustrative. Other risk reduction goals can be used.

General Fish Sampling Recommendations for Superfund Sediment Sites

[Note: These recommendations are meant for a typical sediment site. Larger or more complex sites may require more sampling, while smaller or simpler sites may require less.]

Baseline Data

In order to implement a decision-oriented post-remediation monitoring plan, adequate baseline data (*i.e.,* pre-remediation) should be available so a statistical comparison of pre-and post-remediation data can be made. Where the baseline data were collected a long time ago, analytical methods improvements and changes might require resampling and analysis so that the pre- and post-remediation data are comparable. If adequate baseline data are not available from the RI, new data will need to be collected before the remedy is implemented.

The importance of having baseline data and post-remedy monitoring data that are compatible can not be over-emphasized. The fish collection methods should be as similar as possible; *e.g.,* same species, age classes/sizes, sexes, locations, timing, *etc.* By making the collections similar and minimizing variability, it will enhance your ability to detect statistically significant smaller reductions in tissue concentrations.

Sampling Frequency

When developing a fish or biota sampling plan, the study objective must be known and be clearly described; *e.g.,* determine if at the time of the first or second five-year review, the level of post-remedy risk reduction is acceptable and the remedy is expected to reach the remediation goal in the predicted time frame. If the observed rate of decline is less than the predicted rate, however, one needs to decide if any changes to the Record of Decision (ROD) are warranted. Additional remediation may be needed in order to achieve protection in a reasonable time frame. If the selected remedy includes dredging or capping, an expectation for an effective remedy could be that, five years after remedy completion, there has been a 50% decrease in the fish tissue levels and thus substantial progress towards meeting the RAOs and cleanup levels.

The time frame needed to demonstrate reductions can vary greatly depending on the type and scope of remedy implemented. For some capping remedies, reductions in fish tissue levels may begin shortly after completion of the capping. For some dredging remedies, however, due to resuspension and release of contaminants throughout the dredging project and the formation of a residual sediment layer, there may be a short-term increase in fish tissue levels before reductions are observed. For monitored natural recovery (MNR) remedies, the rate of reduction should be similar to the rate observed before remedy selection. To improve the confidence in evaluating reductions in risk, at least two sampling events should be conducted by the time of the first five-year review.

Source: USEPA, Region 9

Species

Tissue residue data should always be analyzed on a species-specific basis; *i.e.,* tissues of different species should not be combined. The species should have a limited foraging range in order to be representative of the exposures caused by the contaminated sediment in the study area. This increases the likelihood that the contaminant tissue level is representative of the sediment and/or water exposure level at the particular sampling location.

Generally, at least two species that are commonly caught by local anglers or subsistence fishers and represent different trophic levels in the food chain should be collected and analyzed for contaminant tissue

levels. For sites where the risk is based only on ecological risk, important prey species for the receptors of concern should be collected. The species should be reasonably easy to collect in the future. If it might be difficult to obtain a sufficient number of a selected species of a specified size range, an alternate species or a different size range should be identified before the sampling team is mobilized.

In inland waters, one bottom feeder and one commonly sought after predator/game fish species should be collected. To develop a comparative national set of data for Superfund remedies, site managers are encouraged to use a bottom-feeder, such as channel catfish, brown, black or yellow bullhead, and a game fish such as smallmouth bass, largemouth bass, or walleye. Depending on the location, and the needs of state and other trustee agencies, it may also be important to sample a trout or salmon in addition to, or instead of, one of the other fishes. In most saltwater bodies, two fish species or one fish and one shellfish species that are commonly sought after by recreational or subsistence anglers should be collected.

Size/Age

To minimize variability, fish from the same age class should be collected. As a surrogate for age class, in order to save the expense of aging fish, fish of similar length can be collected. The relationship between length and age varies greatly for each species and for each location and depends greatly on water temperature, population density, and food availability. A good goal, however, is that the smallest fish sampled is no less than 75% the length of the largest fish (EPA-823-B-00-007, November 2000). State fish and game agencies will often have data on this relationship, but for many of the preferred species discussed above, a 4 year old fish is often about 12 inches in length. Fish should also be within the legal size limit. Fish of this size/age are often more abundant, easier to catch, and may respond sooner to reductions to exposure concentrations after remedy implementation than older, larger fish. Although older fish often have higher contaminant levels, the objective is to measure changes in tissue levels, not to estimate maximum concentrations. For sites where ecological risk drove the remediation, sizes should be consistent with the size of the prey typically consumed by the receptor(s) of concern.

Sex

It is recommended that collections of fish be done in a manner to avoid sampling unequal numbers of males and females as much as possible. Consult your local fisheries experts on the life history of the species of interest. To minimize variability, relatively similar numbers of males and females should be analyzed.

Sample Locations

The optimum number of sample locations varies depending on site size and on the extent of variations in sediment conditions, habitat types, and hydrology. Because of the typical variation observed historically in fish tissue residues from different locations within the same water body, fish should be collected from at least three site locations. At large sites, or at riverine sites that contain more than one impoundment, it may be necessary to collect fish from more than three sampling stations. If there are areas that are preferred fishing locations, these areas should be given special consideration for sampling.

Sample Time

Fish should typically be collected at the same time of year and under similar stream flow conditions. Since the lipids content can be low in the spring, spring sampling should be avoided. At sites where methylmercury is driving the need for remediation, fish should be collected during times of active methylation, typically in late summer or early fall. To help minimize variability between individuals, sampling should not be done 2-4 weeks before or after the spawning season.

Sample Size and Type

The goal is to collect enough fish and analyze enough fish samples to be able to determine reliably whether adequate decreases in tissue concentrations are occurring. For example, the hypothesis test may evaluate whether there has been a 50% reduction in chemical residues in fish five years after completion of the remediation. For typical sites, in order to control costs and help minimize variability, a sampling plan should consider pooling fish into composite samples rather then analyzing individual fish. A sampling plan that specifies a minimum of 5 composites consisting of at least 5 fish per composite is often adequate to determine with a confidence level of 90-95% if the post-remediation concentrations have decreased at least 50%. Although it depends on the variance, if a smaller decrease needs to be detected, more composites will typically be needed. Increasing the number of composite samples analyzed provides greater improvements than increasing the number of fish per composite in the ability to detect smaller significant reductions in chemical residues in fish (see *EPA Guidance for Assessing Chemical Contaminant Data in Fish Advisories, Vol.1- Fish Sampling and Analysis, Third Edition*, EPA-823-B-00-007, November 2000). However, compared to the additional cost for contaminant analyses of more composites, the cost of collecting more fish per composite is less.

Source: The Great Lakes National Program

In such cases where little or no baseline data are available, fish should be collected during remedial design in order to have a basis for future comparisons of fish tissue residues. Since there is no estimate of variance to use in a power analysis, it may be beneficial to collect more than five composites and/or more than five fish per composite. Then, based upon the measured population variance of the initial samples, the design can be changed to collect the most appropriate number of composite samples that would allow adequate determination of mean residue concentrations. This will allow site managers to maximize certainty in their conclusions about any changes in the mean residue levels as a result of the remediation.

When contaminant concentrations in tissue are measured, lipid contents of the tissue should be determined. Lipid determination allows for lipid normalization of the data and assists in data interpretation and calculation of BSAFs. When BSAFs are used, it is just as important that the contaminant concentrations in sediment are normalized on a percent organic carbon basis. This is particularly important when evaluating non-polar compounds and strongly bioaccumulating compounds that have an affinity for lipids. However, lipid data can also be used to evaluate the relative health or status of an organism. Individuals with low lipid contents may be unhealthy, starved, or may have recently lost lipid-soluble contaminants due to egg laying, thereby transferring contaminants and biasing the bioaccumulation data. Data from fish with unusually low lipids relative to data from other sampling events should be reported but discounted in the analyses.

When measuring lipid contents, the specific analytical technique should be considered. Total non-polar lipids is an acceptable determination, and EPA (2000) recommends using the dichloromethane extraction solvent method.

Contact Information

For questions on this fact sheet, please contact
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Appendix: Significance and Power of Residue Comparisons; Refinement Techniques

To determine the most appropriate number of individual fish per composite, and the number of composite samples to analyze, statistical precision and power analyses may be performed. Many statistical packages, *e.g.,* SAS, PASS, G*Power, R, and S-Plus, provide tools for sample size estimation, hypothesis testing, and power analysis.

For readers desiring detailed information on statistical power analysis, consult Cohen (1998). For a less detailed, but well-written and informative description of power analysis as it pertains to sampling design, see EPA (1987) (EPA/430/09-87-003, Bioaccumulation Monitoring Guidance: Strategies for Sample Replication and Compositing – Volume 5). Note, this guidance document was written to detect significant differences in chemical residues between sampling stations. Further note, the analysis for detecting differences between sampling stations is same as that for detecting differences between sampling dates.

Other Federal Agencies have documents and information on statistical power analysis and the determination of sample sizes. The U.S. Fish and Wildlife Service (USFWS) has a Web site, http://www.umesc.usgs.gov/ltrmp/stats/statistics.html, that provides detailed information on *Sampling Design and Statistics* for their Long Term Resource Monitoring Program (LTRMP). The site discusses these techniques by using primarily population endpoint examples. However, these techniques are appropriate for examining changes in chemical residues in fish. The U.S. Army Corps of Engineers (US-ACE) has a document that discusses these techniques in the context of evaluating dredged sediments (Clarke and Brandon). Additionally, EPA and US-ACE have jointly published a document on sediment evaluation that includes an extensive section on statistical methods (EPA 1998) covering power analysis and determination of sample size.

Sampling plans should use a Type I error rate (false positives; i.e., the apparent decrease is not real) of α=0.05 or 0.10, while minimizing the Type II error rate (false negatives; i.e., the decrease although not detected is real), and maximizing the statistical power, (i.e., 1-β). In order to perform statistical precision and power analyses, a good/accurate estimate of the population variance from baseline data is required.

In Figure A-1, sampling precision curves are provided for three sampling designs for four different population variances (expressed in terms of coefficients of variation (CVs)). Using an α=0.05, five fish per composite will provide low relative errors (*i.e.,* adequate sampling precision to obtain the mean) with the collection of five or more replicate composites when the coefficient of variation (CV) between fish is below 75% (Figure A-1). In other words, 95% of the time, the error in estimation of mean tissue concentration will be less than 30%, 20%, and 10% when the CV is less than or equal to 75%, 50%, and 25%, respectively. Figure A-1 was determined using the equation (Snedecor & Cochran, 1989): $\varepsilon^2 = (CV \cdot z_{1-\alpha/2})^2/(mn)$ where ε is the relative error in estimation, *CV* is the coefficient of variation, $z_{(1-\alpha/2)}$ is z-value from a normal distribution, m is the number of fish per composite, and n is the number of composites. Based upon EPA's analyses on the interchange between number of fish per composite and total number of composites taken, increasing the number of replicate composite samples will have a greater impact than increases to the number of individual fish per composite upon precision of the estimate (EPA-823-B-00-007, November 2000). Increasing either the number of fish per composite or the number of composite samples taken will further improve precision.

In Figure A-2, the Power to detect a minimum detectable decrease (MDD) in tissue concentrations is shown to range widely depending on the desired MDD and the CV between sample means. Higher levels of Power can be achieved for MDDs between 35-50% for sample sizes (*i.e.,* number of replicate composites) between 5 and 10 when CV=25%. Similarly, for CVs as high as 50%, reasonable levels of Power can be expected when sample sizes approach 10 replicate composites. The precision and Power analyses are closely interrelated in that when one evaluates Power for a composite sampling program, the total number of individual fish to be collected for the composite samples shouldn't become exceeding large and not manageable from a collection and/or ecosystem capacity standpoints.

To illustrate, the following example is provided.

Assume:

Baseline data: 5 composite samples with 5 fish per composite
Average of 5 composites = 1.5 mg/kg (ww)
Standard deviation of average of composites = 0.3

Compute Population standard deviation (σ)

var ($\bar{z}$) = $\sigma^2/(nm)$
var ($\bar{z}$) = $(0.3)^2$
n = 5 5 composite samples
m = 5 5 fish per composite
Population standard deviation = $((0.3)^2 \text{ x } (nm))^{½} = (0.3^2 \text{ x } 5 \text{ x } 5)^{½}$
Population standard deviation = 1.5
Population coefficient of variation = 100%

Determine Minimum Detectable Decrease (MDD) with 80% Power (1-β)

$\alpha = 0.10$
$\beta = 0.20$
Power = 1-β = 0.80
$\alpha' = 0.10$ (one-sided test) where α' is α for a one-sided test and $\alpha/2$ for a two-sided test.
$z_{1-\alpha'} = 1.282$
$z_{1-\beta} = 0.8416$
n = 5
m = 5

$$n \geq \frac{s^2 (z_{1-\alpha'} + z_{1-\beta})^2}{(\mu_1 - C)^2} + \frac{z_{1-\alpha'}^2}{2}$$

$$5 \geq \frac{1.5^2 (1.282 + 0.8416)^2}{MDD^2} + \frac{1.282^2}{2}$$

$$MDD = \sqrt{\frac{5 - (1.282^2/2)}{1.5^2 (1.282 + 0.8416)^2}} = \sqrt{0.4118} = 0.64$$

Relative precision of estimated mean concentration

$$\varepsilon^2 = (CV * z_{1-\alpha/2})^2 / (mn)$$

$$\varepsilon^2 = (1.00 * 1.645)^2 / (5 * 5) = 0.11$$

$$\varepsilon = 0.33$$

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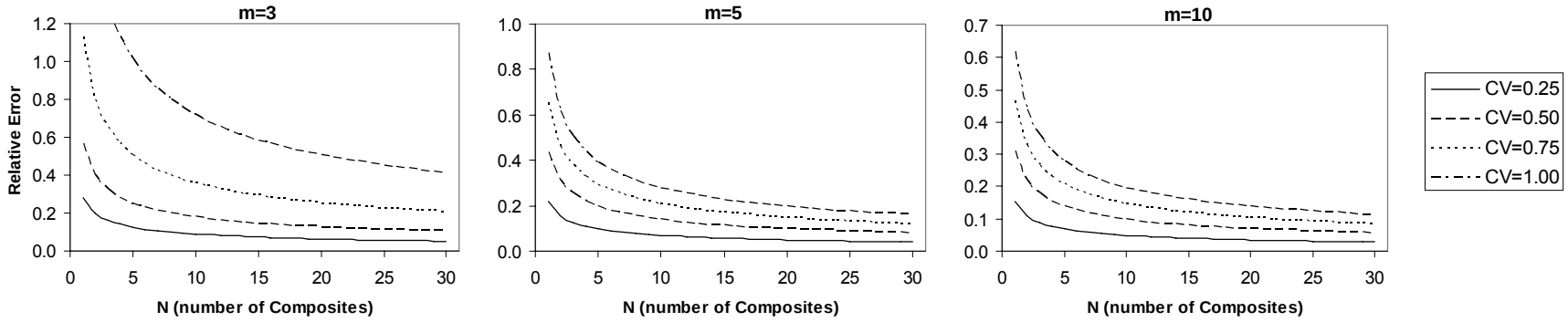

Figure A-1. Precision curves of relative error versus number of composites (N) for various values of the coefficient of variation (CV) when $\alpha = 0.05$. Calculations based m= 3, 5, or 10 individual fish per composite.

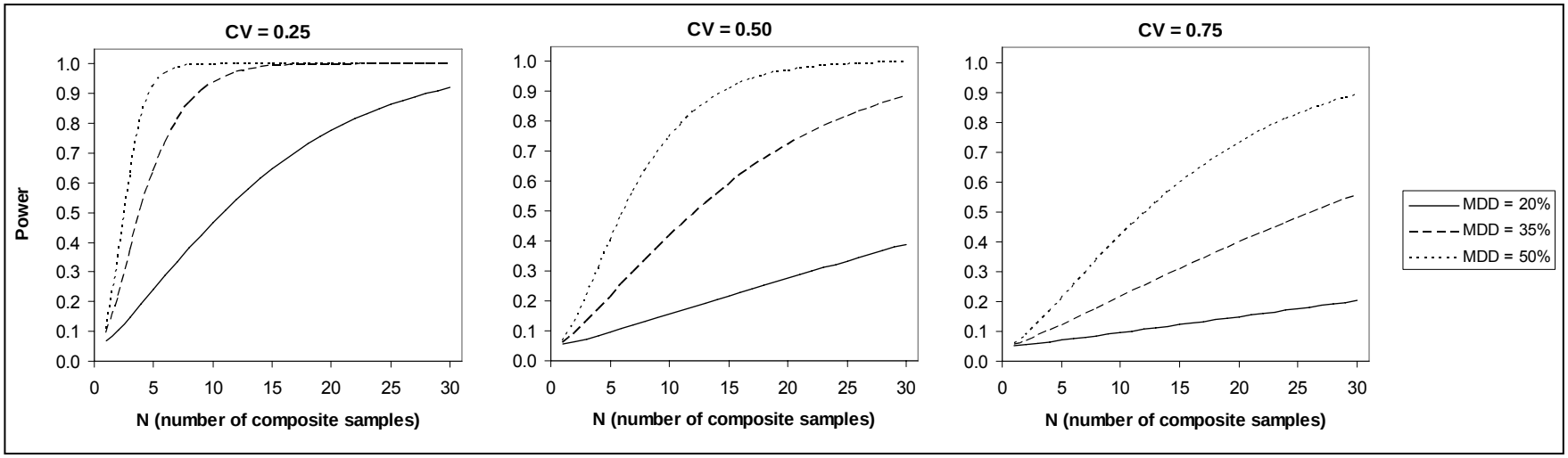

Figure A-2. Power (1-β) curves for a 25%, 35%, or 50% minimum detectable decrease (MDD) in contaminant concentrations between means at $\alpha = 0.05$, two-tailed, when the coefficient of variation (CV) for between-sample variability is 0.25, 0.50, and 0.75.