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ESTIMATION OF BIOTA SEDIMENT ACCUMULATION FACTOR (BSAF) FROM PAIRED OBSERVATIONS OF CHEMICAL CONCENTRATIONS IN BIOTA AND SEDIMENT

by

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INTRODUCTION

In March 2004, the Ecological Risk Assessment Forum (ERAF) submitted a request to ORD's Ecological Risk Assessment Center (ERASC) relating to the estimation of Biota-Sediment Accumulation Factors (BSAFs) (see Appendix). BSAF is a parameter describing bioaccumulation of sediment-associated organic compounds or metals into tissues of ecological receptors. The purpose of this report is to provide a response to the ERAF request. The *Problem Statement* in the request was "What is the most appropriate method to estimate the BSAF from paired observations of concentrations in biota and sediment?" The *Expected Outcome* asked for answers to specific questions regarding the use of regression analysis for estimating BSAFs for non-ionic organic compounds. The specific questions are addressed in the latter portion of this document. A statement on the most appropriate method to estimate the BSAF is provided below. This document is focused solely on the determination of BSAFs for non-ionic organic chemicals and is applicable to fish and benthic organisms, e.g., crabs and bivalves. The determination of BSAFs for metals is not discussed.

RECOMMENDATIONS

There are two methods for determining the BSAF from paired observations: (1) a regression approach, whereby the BSAF is estimated by determining the slope of the C_{soc}-C_ℓ line (C_{soc} is the concentration of chemical in the sediment on an organic carbon basis [µg/kg organic carbon] and C_ℓ is the concentration of chemical in the organism on a lipid basis [µg/kg lipid]) and (2) an averaging approach, whereby the BSAF is estimated by averaging the BSAFs from the paired observations across the site. Both approaches use the same data. The second approach is recommended as the more appropriate method for estimating the BSAF. Regression analysis has some limitations in comparison to the averaging approach:

1) The BSAF is, in essence, a thermodynamic (or fugacity) ratio for the chemical of interest between the organism and sediment. Determination of the individual BSAFs from appropriately paired observations will enable the detection of differences (if they exist) in partitioning behavior among the paired observations for the site. Regression analysis, which simply constructs a functional relationship between the C_{soc}-C_ℓ pairs, will not allow, very easily, the detection of the differences (if they exist). Remediation decisions often involve the use of sediment-organism relationships and if fundamental differences in partitioning behavior exist across the site, remedial actions might be different for the different portions of the site.

2) Regression analysis, whether model I (simple linear regression) or model II (geometric mean regression, major axis regression, Bartlett's three-group method, or Kendall's robust line-fit method [Sokal and Rohlf, 1995]), requires meeting parametric assumptions about the relationship between the X and Y variables.

3) Although regression analysis can be done on data sets with limited numbers of C_{soc}-C_{ℓ} pairs, determining the slope of the line fitting limited numbers of pairs can lead to highly uncertain slopes.

4) Computation of accurate confidence limits can be problematic when regression analyses are perform using log transformed C_{soc}-C_{ℓ} data pairs (Ricker, 1973); log transformation is often required to linearize the residue data.

In contrast, the averaging approach (estimating the BSAF by averaging the BSAFs from each C_{soc}-C_{ℓ} pair) largely avoids the above limitations. With the averaging approach, the distribution of the individual BSAFs (determined from each C_{soc}-C_{ℓ} pair) can be evaluated very easily; this evaluation is commonly done in statistical analysis of data. Knowing the underlying distribution of the BSAFs allows the selection of the most appropriate (unbiased) averaging technique. Further, with the individual BSAFs (C_{soc}-C_{ℓ} pairs), the homoscedasticity (equality) of the variances across the individual BSAFs can be assessed. In cases where the variances are heteroscedastic (unequal), an appropriate weighted averaging technique would be used, and in general, the weights would be the reciprocal of the variances for the individual BSAFs. The averaging approach can also be easily implemented with other weighting considerations such as portions of the site represented by individual BSAFs, e.g., some BSAFs might be reflective of three quarters of the site while the remaining BSAFs are reflective of the other quarter of the site. The averaging approach also provides the information on the final BSAF (grand mean) distribution and variance which are necessary for one and two stage Monte Carlo uncertainty analyses.

For Superfund sites and other sites, each C_{soc}-C_{ℓ} pair is location specific, and each pair incorporates all of the underlying ecological and chemical conditions existing at the sampling location (e.g., food web structure, sediment/water column concentration quotients, chemical bioavailability and diets/trophic levels of the organisms). Regardless of approach (regression or averaging), the conditions must be similar across all locations. For sites with highly heterogenous conditions, having similar underlying conditions can be problematic. Mixing of C_{soc}-C_{ℓ} paired observations with different underlying conditions is not recommended and will, in all likelihood, result in BSAFs with poor predictive accuracy.

There is great value in plotting the C_ℓ against C_{soc}; BSAFs against C_{soc}, C_ℓ, sediment organic carbon content, and organism lipid content; and C_ℓ, C_{soc} and BSAFs against geographical information. These plots should be done and evaluated for trends in the data! They may provide key insights and understanding of the complexities existing at the site of interest. If the BSAFs from the individual C_{soc}-C_ℓ pairs are independent of the C_{soc}, C_ℓ, sediment organic carbon content, organism lipid content values, and geographical location, this would be strongly suggestive of similar underlying ecological conditions for the C_{soc}-C_ℓ pairs. When discrepancies exist, the following evaluations are suggested: (i) the closeness of the measured residues or sediment contaminant concentrations to Method Detection Limits (MDLs), (ii) the characteristics of the sediment across the site (e.g., the types and amounts of organic carbon in the sediments [biogenic, coal, coke, soot, tars], grain size, etc.), (iii) the co-occurring contaminants, (iv) the diets/trophic levels of the organisms, (v) the lipid contents and health of the organisms (exceptionally low lipids often indicate the organisms are stressed), and (vi) past remedial actions. Depending upon what is learned from these evaluations, one would attempt to resolve the C_{soc}-C_ℓ pairs into units with similar underlying conditions. Potential actions could involve the segregation of the site into sub-units with C_{soc}-C_ℓ pairs having similar conditions, or possibly, discounting the importance of pairs where C_{soc} and/or C_ℓ in the pairs are just above MDL. The importance of resolving discrepancies within the data can not be overstated. Spending time and resources resolving these discrepancies will be well worth the effort since the uncertainties associated with remediation decisions will be smaller. Additionally, any discrepancies in the data at this level will be translated into higher and more complex analyses since these analyses use this information.

The following sections provide a description of the BSAF along with its underlying assumptions, a discussion on how to measure a useful BSAF, a discussion on the basis of the regression approach, and answers to specific questions related to regression analysis.

DEFINITION OF BSAF

The BSAF is defined (Ankley et al., 1992) as

$$BSAF = \frac{C_o / f_\ell}{C_s / f_{soc}} \qquad \text{(Eq. 1)}$$

where C_o is the chemical concentration in the organism (µg/kg wet weight), f_ℓ is the lipid fraction of the organism (g lipid/g wet weight), C_s is the chemical concentration in surficial sediment (µg/kg dry weight) and f_{soc} is the fraction of the sediments as organic carbon (g organic carbon/g dry weight). In general, BSAFs should be determined from spatially and temporally coordinated organism and surficial sediment samples under conditions in which recent loadings of the chemicals to ecosystem are relatively unchanged (Burkhard et al., 2003). The BSAF definition does **not** invoke or include the assumption of equilibrium conditions for the chemical between the organism and sediment (Ankley et al., 1992; Thomann et al., 1992). As shown by Thomann et al. (1992), BSAFs are appropriate for describing bioaccumulation of sediment contaminants in aquatic food webs with non-equilibrium conditions between both the sediment and organism (fish in this case), and sediment and its overlying water. Equilibrium is regarded as a reference condition for describing degrees of disequilibrium, and thus, is not a requirement for measurement, prediction, or application of BSAFs.

With specific reference to benthic invertebrates, numerous investigators (Lake et al., 1984, 1990; McElroy and Means, 1988; Bierman, 1990; Ferraro et al., 1990) have invoked two assumptions regarding BSAFs: (1) equilibrium conditions and (2) no metabolism of the chemical. These assumptions when combined with EqP (equilibrium partitioning) theory (DiToro et al., 1991), lead to the conclusion that the BSAF, for these specific conditions, is equal to the partitioning relationship of the chemical between organic carbon in the sediment and lipids of the organism. Depending upon the affinities of the non-polar organic chemical for lipid and sediment organic carbon, the BSAF, under these specific conditions, should be in the range of 1 to 2 (McFarland and Clarke, 1986). For aquatic organisms tightly connected to the sediments like oligochaetes and other benthic invertebrates, experimental measurements (Lake et al., 1990; Tracy and Hansen, 1996) are generally consistent with the theoretical value, i.e., in the range of 1 to 2.

There are solid mechanistic reasons why fish should not be in equilibrium with their sediments (Thomann et al., 1992). For fish, BSAFs incorporate wide ranges of influences including biomagnification due to the trophic level of the fish, sediment-water column chemical disequilibrium, the diet of the fish and its underlying food web, the fish's foraging range and chemical metabolism within the fish and its food web (Burkhard et al., 2003). Suggestions that

BSAFs for fish should be in the range of 1 to 2 by combining the definition of the BSAF with the assumptions of equilibrium conditions and no metabolism are incorrect (Wong et al., 2001). As explained above, measured BSAFs above or below 1 to 2 are entirely reasonable for fish (Burkhard et al., 2003). BSAFs outside this range for fish do not violate the general definition of BSAFs nor invalidate the usefulness of BSAFs in predicting chemical residues in fish for sediment contaminants (Burkhard et al., 2004).

MEASURING USEFUL C_{soc}-C_ℓ PAIRS FOR CALCULATION OF BSAFs

Probably the most important factor in measuring a BSAF with predictive power is the requirement that the sediment samples analyzed be reflective of the foraging range of the fish. Depending upon the site, the degree of difficulty in defining the foraging range of the organism can vary widely. In situations where the movement of the organisms is confined by the geography of the site, e.g., dams or falls, the foraging range of the organisms can probably be defined fairly easily. When required, foraging ranges can be determined by tagging/recapture, radio-telemetry and/or ultrasonic telemetry studies at the site of interest. Estimates of home ranges (or foraging ranges) for freshwater fishes can be determined using the allometric relationship (Minns, 1995):

$$\ln H = -2.91 + 3.14\ HAB + 1.65 \ln L \quad \text{or} \quad \ln H = 3.33 + 2.98\ HAB + 0.58 \ln W$$

where H is the home range size (m^2), HAB is 0 for rivers and 1 for lakes, W is body weight (g) and L is body length (mm). For freshwater invertebrates (such as crabs), marine and estuarine ecosystems, allometric relationships for home range have not been reported.

Having a good understanding of the foraging range of the species is important. Organisms with smaller foraging ranges will, in all likelihood, be more representative of the study site than those with large foraging ranges that extend way beyond the study site. Just because a fish (or other aquatic organism) is caught at a sampling location, one can not infer that the chemical residue in the fish is due to the chemicals residing at the study site. Knowledge of the fish's foraging range is the only way that one can establish the connection of the fish to the sampling location. It is strongly recommended that local fisheries experts be consulted during the sampling design phase of the field study to help in determining the foraging range and

trophic level of the organisms at the site; local knowledge will be extremely helpful. It should be noted that the above allometric relationship provides only an estimate of the actual foraging range.

Once the foraging range of the species of interest is established, sediment samples reflective of the species foraging range need to be collected. It is important that the sediment samples collected be representative of the sediments where the species normally forages and not a homogenized sediment core representing the entire bed of contaminated sediment. For most organisms, the surficial sediments are most reflective of the organism's immediate exposure/foraging history, and generally, smaller depths of the surficial layer, e.g., 0 to 2 cm, are preferred over larger depths, e.g., 0 to 30 cm. For deeper burrowing organisms such as some clams and polychaetes, slightly larger surficial depths, e.g., 0 to 5 cm, might be more appropriate of their recent exposure history.

Beyond establishing the foraging range of the organism and the appropriate sediment samples, the collection and analysis of adequate numbers of organisms and sediment samples is necessary for deriving unbiased estimates of the mean concentrations of chemicals and their variances. This document will not address the subject of sample collection, compositing and analysis; see U.S. EPA (2002) for further information on these issues. With unbiased estimates of the mean concentrations, the BSAF for the specific site can be calculated using Equation 1.

In any study design, it is important that biota samples be collected and composited in size or age classes. For fish, dietary composition changes substantially with size and age, and these changes will result in differences in BSAFs among size and age classes. For forage fish, common classes are young-of-the-year, juveniles and adults, and for piscivorous fishes, common classes are year classes, e.g., 2, 3, 6 and 10 years old. Mixing of fishes of different size/age classes is not recommended because of the increased variance for the average chemical residue in the organisms.

Biota samples for chemical analysis should never be composited by mixing different fish species. Different fish species have different life histories and diets. BSAFs derived from composite samples composed of different species will be highly biased by the individual species. Further, resolving what the potential biases are for an individual species would require the collection and analysis of that species.

When a C_{soc}-C_ℓ pair (or BSAF) is measured for a specific chemical, the measured value incorporates all conditions and parameters existing at the location of interest. The major conditions and parameters incorporated into the C_{soc}-C_ℓ pair (or BSAF) are (1) the distribution of the chemical between the sediment and water column, (2) the relationship of the food web to water and sediment and (3) the trophic status (or position in the food web) of the species.

CALCULATION OF BSAFs

The BSAF is calculated from four measured variables (see Equation 1, and Equation 2 below): concentration of the chemical in the organism on a wet weight basis (C_o), the lipid content of the wet tissue (f_ℓ), the concentration of the chemical in the sediment on a dry weight basis (C_s) and the organic carbon content of the dry sediment (f_{soc}).

$$BSAF = \frac{C_o / f_\ell}{C_s / f_{soc}} = \frac{C_\ell}{C_{soc}} \qquad \text{(Eq. 2)}$$

Depending upon the species and field sampling design, a C_{soc}-C_ℓ pair might be composed of only one sediment sample and one tissue sample. However, a C_{soc}-C_ℓ pair might be composed of multiple composite tissue samples and/or multiple sediment samples spanning the foraging range of the organisms. In either of these cases, samples could be analyzed in duplicate/replicate in the laboratory, and thus, determining the C_{soc} and/or C_ℓ values could involve the averaging of results from replicate analyses and/or results from a number of different samples.

Tissue Samples

For a specific tissue sample, the lipid normalized concentration of the chemical is determined using the following equation

$$C_\ell = \frac{(\Sigma C_o)/n}{(\Sigma f_\ell)/m} \qquad \text{(Eq. 3)}$$

where n is the number of replicate chemical analyses and m is the number of replicate lipid analyses. The variance of the C_ℓ can be estimated using the equation (Mood et al., 1974):

$$s_{C_\ell} = C_\ell \sqrt{\frac{(s_{C_o})^2}{\left[\sum C_o/n\right]^2} + \frac{(s_{f_\ell})^2}{\left[\sum f_\ell/m\right]^2} - \frac{2rs_{C_o}s_{f_\ell}}{\left[\sum C_o/n\right]\left[\left(\sum f_\ell\right)/m\right]}} \qquad \text{(Eq. 4)}$$

where s_{C_ℓ}, s_{C_o} and s_{f_ℓ} are the standard deviations for the C_ℓ, C_o and f_ℓ, respectively; and r is the correlation coefficient between C_o and f_ℓ. In cases where both lipid and chemical contents are measured once, s_{C_ℓ} can not be computed.

For a group of tissue samples for a specific C_{soc}-C_ℓ pair, their individual C_ℓs (computed using Equation 3) are averaged using an appropriate statistical technique, dependent upon the distribution of the individual C_ℓs. The resulting average is the average lipid normalized concentration in the tissue for the specific C_{soc}-C_ℓ pair. If the tissue samples have different numbers of organisms in each composite, e.g., three fishes in one sample and five fishes in the second sample, the determination of a weighted average concentration is suggested. Typically, residues in aquatic organisms are normally or log-normally distributed.

Normally Distributed Residues: For a group of tissue samples for a specific C_{soc}-C_ℓ pair with normally distributed residues, the weighted average lipid normalized concentration is computed using the following equation, illustrated numerically using the two fish sample example from above:

$$C_{\ell-avg} = \sum\left(w_i \times C_{\ell-i}\right)/\sum w_i = \left(3 \times C_{\ell-one} + 5 \times C_{\ell-two}\right)/\left(3+5\right) \qquad \text{(Eq. 5)}$$

where w_i is the number of organisms in composite i, $C_{\ell\text{-}i}$ is the lipid normalized concentration of the chemical in composite i and $C_{\ell\text{-avg}}$ is the weighted average lipid normalized concentration in the tissues. The standard deviation of a weighted average ($s_{C_{\ell-avg}}$) equals

$$s_{C_{\ell-avg}} = \sqrt{\sum\left[w_i \times \left(C_{\ell-i} - C_{\ell-avg}\right)^2\right]\Big/\left(\sum w_i - 1\right)} \qquad \text{(Eq. 6)}$$

Note, a non-weighted average and standard deviation would be determined by setting the weights, w_i, equal to 1.0.

Log-Normally Distributed Residues: For a group of tissue samples for a specific C_{soc}-C_ℓ pair with log-normally distributed residues, a weighted average lipid-normalized concentration is suggested with the number organisms in the samples as the weights. The minimum variance unbiased (MVU) estimators as described by Gilbert (1987) is suggested for log-normally distributed residues. However, there are other appropriate estimators for log-normally distributed data. MVU estimators are calculated by estimating the mean and variance of the log-normal distribution of the chemical concentrations:

$$\bar{y} = \sum_{i=1}^{n} w_i y_i \Big/ \sum_{i=1}^{n} w_i \qquad \text{(Eq. 7)}$$

$$s_y^2 = \sum_{i=1}^{n} [w_i \times (y_i - \bar{y})^2] \Big/ [\left(\tfrac{n-1}{n}\right) \sum_{i=1}^{n} w_i] \qquad \text{(Eq. 8)}$$

where $\bar{y}$ and s_y^2 are the arithmetic mean and variance of the n transformed values $y_i = \ln C_\ell$, and w_i are the weights for the individual samples (NIST, 1996).

The minimum unbiased estimator for the mean ($\hat{\mu}$), $C_{\ell\text{-}avg}$ in this application is

$$C_{\ell-avg} = \hat{\mu} = [\exp(\bar{y})]\psi_n\left(\frac{s_y^2}{2}\right), \qquad \text{(Eq. 9)}$$

where exp($\bar{y}$) is the sample geometric mean, and $\psi_n(t)$ (with $t = s_y^2/2$) is the infinite series:

$$\psi_n(t) = 1 + \frac{(n-1)t}{n} + \frac{(n-1)^3 t^2}{2!n^2(n+1)} + \frac{(n-1)^5 t^3}{3!n^3(n+1)(n+3)} + \cdots \qquad \text{(Eq. 10)}$$

and this infinite series converges quickly, e.g., four to six terms are normally required for convergence.

The minimum unbiased estimator for the variance of $\hat{\mu}$ ($C_{\ell\text{-avg}}$ in this application) is:

$$s^2(C_{\ell-avg}) = s^2(\hat{\mu}) = \exp(2\bar{y})\left\{\left[\psi_n\left(\frac{s_y^2}{2}\right)\right] - \psi_n\left[\frac{s_y^2(n-2)}{n-1}\right]\right\} \quad \text{(Eq. 11)}$$

Note, a non-weighted average and standard deviation would be determined by setting the weights, w_i, equal to 1.

Using the appropriate statistical averaging technique, the average lipid normalized concentration of the chemical ($C_{\ell\text{-avg}}$) is determined for a group of tissue samples for a specific C_{soc}-C_ℓ pair.

Sediment Samples

The sediment sample(s) associated with a specific C_{soc}-C_ℓ pair would treated like the tissue samples as described above and the overall average organic carbon normalized concentration of the chemical in the sediment(s) ($C_{soc\text{-avg}}$) would be determined. Weights in the averaging process should be set to equal to 1.

Calculating the BSAF

For a specific C_{soc}-C_ℓ pair, the BSAFs for the pair would be determined by dividing its weighted average lipid normalized concentration of the chemical in the tissue ($C_{\ell\text{-avg}}$) by its average organic carbon normalized concentration of the chemical in the sediment ($C_{soc\text{-avg}}$).

$$BSAF = C_{\ell-avg} / C_{soc-avg} \quad \text{(Eq. 12)}$$

The variance for the specific BSAF can be estimated using the equation (Mood et al., 1974):

$$s_{BSAF} = \frac{1}{C_{soc-avg}}\sqrt{(s_{C_{\ell-avg}})^2 + BSAF^2(s_{C_{soc-avg}})^2 - 2rs_{C_{\ell-avg}}s_{C_{soc-avg}}BSAF} \quad \text{(Eq. 13)}$$

where s_{BSAF}, $s_{C_{soc-avg}}$ and $s_{C_{\ell-avg}}$ are the standard deviations for the BSAF, $C_{\text{soc-avg}}$, and $C_{\ell\text{-avg}}$, respectively; and r is the correlation coefficient between $C_{\text{soc-avg}}$ and $C_{\ell\text{-avg}}$.

Calculating the Average BSAF

For each specific C_{soc}-C_{ℓ} pair, a BSAF is determined. As discussed previously, the average BSAF would subsequently be determined from the set of individual BSAFs using the most appropriate (unbiased) averaging technique based upon the underlying distribution of the BSAFs.

Variability/Uncertainties of BSAFs

The BSAF is calculated from four measured variables, i.e., C_{o}, f_{ℓ}, C_{soc} and f_{soc}. Analytical variances are captured using Equation 4 and across sample variances are captured using Equations 6 and 11 for normally and log-normally distributed data, respectively. Equation 13 captures the across sample variances in the BSAF but does not include the analytical variances captured by Equation 4. The variance across a group of BSAFs (each BSAF resulting from a specific C_{soc}-C_{ℓ} pair) does not include the analytical or across sample variances.

Propagation of error and Monte Carlo techniques can be used to estimate total variances associated with the BSAFs. A good description of propagation of error technique is provided by Campbell (1982) and an application of the technique is provided by Clarke and McFarland (2000). Monte Carlo techniques are discussed in Superfund's risk assessment guidance (U.S. EPA, 2001).

BASIS FOR BSAF REGRESSION APPROACH

Equation 1 can be rearranged as

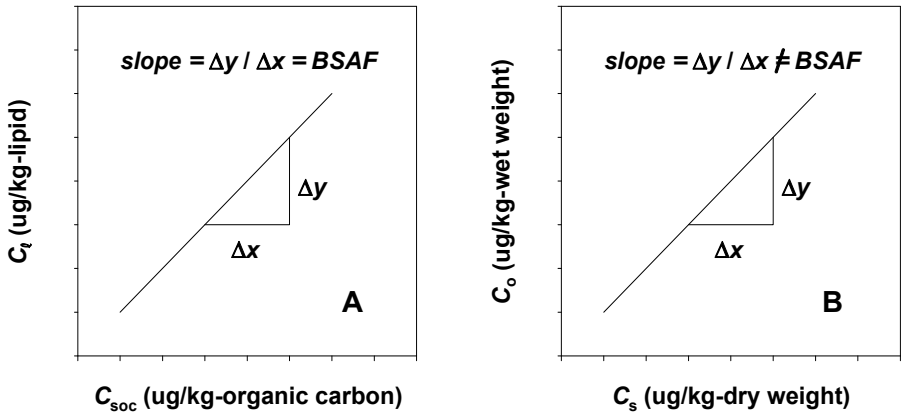
$$C_o / f_{\ell} = BSAF \times C_s / f_{soc} \qquad \text{(Eq. 14)}$$

By substitution, Equation 14 can be expressed as

$$C_{\ell} = BSAF \times C_{soc} \qquad \text{(Eq. 15)}$$

where C_{soc} is the concentration of chemical in the sediment on an organic carbon basis (µg/kg organic carbon) and C_ℓ is the concentration of chemical in the organism on a lipid basis (µg/kg lipid).

Plotting of C_{soc} against C_ℓ results in the following illustrative plot (Graph A), where the slope of the line is the BSAF. However, the slope of C_s plotted against C_o (Graph B) is not the BSAF because these two measures of chemical concentrations are **not** organic carbon and lipid normalized. Use of the regression approach to derive the BSAF incorporates an implicit assumption above and beyond those required for measuring a BSAF at a specific location. The implicit assumption of the regression approach is that all C_{soc}-C_ℓ pairs must have or incorporate the same underlying ecological conditions and parameters. Further, the regression approach assumes that the relationship between C_{soc} and C_ℓ is linear.



For a Superfund site, it is common to collect samples across the site with a number of different sampling locations. For example, consider a New England stream with a series of three dams, and assume that 2-year-old carp and sediment are collected and analyzed in each reservoir. Further assume that representative and unbiased mean concentrations of the chemical were determined for fish and sediment in each reservoir. Thus, three sets of paired carp-sediment observations would be determined, one for each of the three reservoirs.

These paired observations of C_{soc} and C_ℓ can be plotted (Graphs C & D). In Graph C, the pairs form a nearly linear relationship suggesting that the underlying conditions for the C_{soc}-C_ℓ pairs are consistent across the samples and thus allow estimation of the BSAF using the regression approach. In Graph D, the pairs form no easily defined linear relationship, and in this case, there is too little variability in the C_{soc}-C_ℓ pairs for the regression approach to be useful in

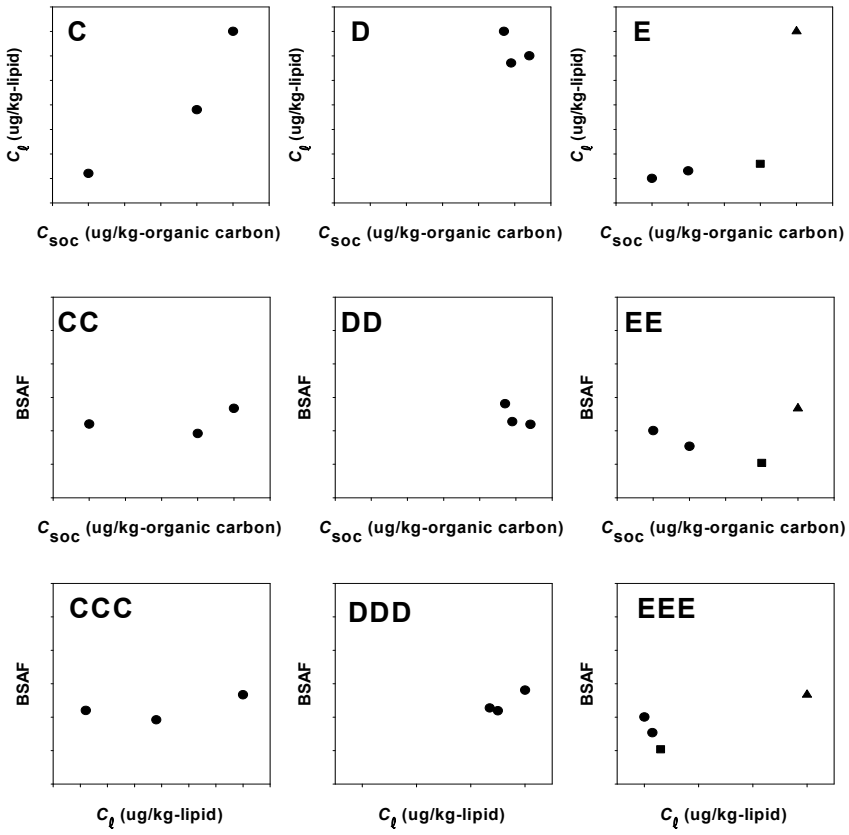
estimating the BSAF. In Graph E, a situation where four sets of paired carp-sediment data were determined, three of the pairs form a nearly linear relationship, but one pair is different from the other pairs. Depending upon how one draws the line, either the triangle or square data in Graph E could be the different (or outlier) C_{soc}-C_ℓ pair. In this case, one or more of the C_{soc}-C_ℓ pairs likely have different underlying conditions, and, thus, it would be inappropriate to estimate the BSAF using the regression approach.

As discussed above, each carp-sediment pair is location specific and each pair incorporates all of the major conditions and parameters existing at the location. In order to use the regression approach with pairs of C_{soc}-C_ℓ observations, the major conditions and parameters must be the same for all locations. This requirement is the implicit assumption incorporated into the regression approach. Mixing of C_{soc}-C_ℓ paired observations with different conditions and parameters will result in C_{soc}-C_ℓ plots where the C_{soc}-C_ℓ pairs will form a non-linear relationship (e.g., possibly Graph E), and in all likelihood, a BSAF with poor predictive power.[1]

For the above examples, if the BSAF for each pair of C_{soc}-C_ℓ observations is plotted against C_{soc}, the following graphs are obtained (Graphs CC, DD and EE). The relationships among the C_{soc}-C_ℓ pairs in the above graphs remain in the graphs based upon the BSAFs; compare Graphs C to CC, D to DD and E to EE. In essence, by calculating the BSAF, one has mathematically removed the concentration dependence shown in Graphs C, D and E. For further comparison purposes, the BSAF for each pair of C_{soc}-C_ℓ observations is also plotted against C_ℓ (Graphs CCC, DDD and EEE).

The graphs, i.e., C, D, E, CC, DD, EE, CCC, DDD and EEE, are some of the plots recommended for evaluating trends and underlying conditions associated with the C_{soc}-C_ℓ pairs. We recommend that these plots be completed prior to performing the final calculations for determining the site-specific BSAF. These plots will help in identifying sources of variation and error in the individual C_{soc}-C_ℓ pairs and BSAF values.

[1]The mixing of C_{soc}-C_ℓ paired observations with different conditions and parameters is not recommended for the averaging approach as well. BSAFs with poor predictive power (i.e., accuracy) will, in all likelihood, result when different conditions and parameters exist across the individual C_{soc}-C_ℓ pairs used in the analysis.



THE REGRESSION APPROACH

A key consideration in using the regression approach is to realize that both C_{soc} and C_ℓ are measured with error. With the simple linear regression least-squares technique, one variable (the *Y*'s) are measured with error while the other variable (the *X*'s) are fixed and have no error. Simple linear regression is referred to as model I regression analysis. When *X*'s and *Y*'s are both measured with error, one of a number of model II regression techniques will be more appropriate and unfortunately "the appropriate method depends on the nature of the data" (Sokal and Rohlf, 1995). Sokal and Rohlf (1995) provide an excellent discussion on model II regression and the techniques of geometric mean regression (also called reduced major axis, standard major axis, or relation d'allometrie), slope of the major axis, Bartlett's three-group method and Kendall's robust line-fit method. Additionally, Sokal and Rohlf (1995) discuss the *Berkson case* of model II regression where model I regression is appropriate.

It is suggested that the determination of the slope of C_{soc}-C_ℓ pairs be performed using the geometric mean regression technique (Halfon, 1985; Sokal and Rohlf, 1995) because with this technique the slope of the regression is not dependent upon the scale of the *X*'s and *Y*'s used in

the analysis. Additionally, Ricker (1973) has recommended that the geometric mean regression technique be used for determining functional relationships (i.e., slope) when "the variability is mostly natural ... in *X* and *Y*"; the case when sediment samples representative of the organism's actual exposure history are collected.

For the geometric mean regression technique, the slope of the geometric mean regression line is the geometric mean of the slopes of the following two linear regression least-squares lines:

$$y = a + b''x \qquad \text{(Eq. 16)}$$

and

$$x = c + dy \qquad \text{(Eq. 17)}$$

The slope of the geometric mean regression line is computed as the geometric mean of b'' and $1/d$:

$$b = (b''/d)^{1/2} \qquad \text{(Eq. 18)}$$

The intercept *a* is computed as done in linear regression:

$$a = \hat{y} - b\bar{x} \qquad \text{(Eq. 19)}$$

For further details on the geometric mean regression technique, the reader is referred to Halfon (1985) and Sokal and Rohlf (1995). In addition, a Microsoft® Office Excel add-in function for geometric mean regression can be downloaded from the following URL.
http://www.lpc.uottawa.ca/data/scripts/index.html.

There are two different ways in which a regression model can be used. First, the regression model can be used to determine the BSAF for the chemical and species of interest. Second, the regression model can be used to predict residues in biota given a residue in sediment or vice-versus. When geometric mean regression and model I regression techniques are performed using log transformed data and residues in biota or in sediment are predicted using the

ln-ln regression equation, the back transformed arithmetic values of the predictions (in log space) **are bias low** (Newman, 1993). As shown in Equation 9, the variance of the log transformed data needs to be included in the back calculation. The MVU estimators, previously discussed, or "less efficient but simpler estimators" as presented by Gilbert (1987) are two approaches for deriving potentially unbiased predictions of the residues.

Monte Carlo uncertainty techniques (U.S. EPA, 2001) are suggested for determining uncertainties associated with BSAFs and/or predictions from the regression model approaches. With this approach, uncertainties from measurement error in biota and sediment residues, biota lipid content and sediment organic carbon content and from the fit of the regression model can be incorporated into the BSAFs and/or predictions from the regression model.

The fit of the regression model/line to the data can be assessed using cross-validation techniques (Neter et al., 1996). Cross-validation involves the splitting of the data into a training set and prediction set, and the prediction set is used to evaluate the reasonableness or predictive ability of the model developed with the training set of data. Fairly close agreement between the PRESS statistic (prediction sum of squares) and SSE (error sum of squares) for a regression model would suggest that its MSE (error mean square) would be a reasonable indicator of the model's predictive ability (Neter et al., 1996).

RESPONSES TO QUESTIONS RAISED IN EXPECTED OUTCOMES

Do I fit a straight line through the data?

Yes. If the C_{soc}-C_{ℓ} observations do not form a straight line, then one must figure out why data diverge from the linear relationship. Reasons for the C_{soc}-C_{ℓ} observations diverging from a straight line include (Note, there are many more causes than those listed)

- The organisms in different C_{soc}-C_{ℓ} pairs reside at different trophic levels in the food web.
- The organisms in different C_{soc}-C_{ℓ} pairs have dramatically different diets even though they reside at the same trophic level. For example, for one pair, the organisms might consume primarily zooplankton while for other pairs, the organisms might consume primarily benthic invertebrates.
- The bioavailability of the chemical in the contaminated sediment varies substantially across the C_{soc}-C_{ℓ} pairs.
- Across the sampling locations, inputs of the chemicals to the site differ substantially. For example, consider a harbor where organisms residing in the lower parts of the harbor are exposed to runoff and ground water seepage from an old industrial site

while organisms residing in the upper parts of the harbor are not exposed this to discharge.

- Different populations of the same species. For example, in the Hudson River, there are resident and migratory striped bass fish populations, and chemical residues in the populations differ widely.

Do I plot my data on a log-log scale?

It is suggested that data should be plotted and examined on both arithmetic and log scales in the interest of fully understanding the data set in exploratory analysis. Plotting of the data with arithmetic-arithmetic scales might be preferred because in arithmetic-arithmetic space, the slope of the line is the BSAF when C_{soc}-C_ℓ pairs are used.

In log-log scales, the slope of the regression line (log C_ℓ regressed against log C_{soc}) is not the BSAF. The logarithm of the BSAF is the intercept of the regression line:

$$\log C_\ell = \log \left[C_{soc} \times BSAF \right] \quad \text{(Eq. 20)}$$

$$\log C_\ell = \text{slope} \times \log C_{soc} + \log BSAF \quad \text{(Eq. 21)}$$

Do I force the line through the origin?

It is suggested that with arithmetic data, regression be performed initially with an intercept, and this intercept should be checked to determine if it is significantly different from zero. If the intercept is not significantly different from zero, the regression should be redone with no intercept. When the intercept is significant, the slope of the regression line is not equal to the BSAF. In these cases, the regression line can be used to predict residues in biota or sediment given a residue in sediment or biota, respectively. When the intercept is not used (regression line is forced through the origin), the slope of the regression line is the BSAF.

If one is performing the regression with log-log scales, it is suggested that regression be performed with an intercept. The intercept is equal to the log of BSAF.

How do I handle non-detects?

Non-detects will be interpreted as analytes reported as being below the method detection limit (MDL) of the analytical method. Generally, these analytes are flagged with the "U" code and the amount reported is the MDL. For concentration values greater than the MDL and less than the practical quantification limit (QL), the concentration values are reported and these values are flagged typically with the "J" code. The "U" and "J" flags are most often defined as unknown/not-detected and estimated, respectively. The QL is 3–5 times the MDL (U.S. EPA, 1989).

For cases where the amount reported is flagged with the "U" code, Superfund most often uses ½ of the MDL in subsequent calculations with the data (U.S. EPA, 1989). For values greater than the MDL and less than the QL, these values are generally reliable and can be used directly. In both cases, one should carefully track the effects of the flagged data points through their subsequent calculations. Calculation of BSAFs using estimates derived from the MDL for concentrations in sediment and/or biota (i.e., simple-substitution methods with left-censored data [Helsel, 2005]) can result in spurious and non-predictive BSAFs (Lee and Helsel, 2007).

When plotting of the different C_{soc}-C_{ℓ} pairs is done, different symbols/colors should be used for the above two flagged data types. Examine this plot to see if the flagged data aligns with the general trend of the C_{soc}-C_{ℓ} pairs that are not flagged. Chemicals with the "U" and "J" flags should be treated separately. This comparison/evaluation should be performed by doing the regression analysis without the flagged data, without the "U" flagged data, and with the flagged data alone. Significance testing of the slopes (asking whether the slopes are different) should be done and these comparisons should help in determining whether to include or exclude the flagged data in the final regression. Examination of the residual plots should be done and will help greatly in determining whether to include or exclude chemicals with unknown concentrations ("U" flagged) and/or with concentration between MDL and PQL ("J" flagged).

There are statistical approaches for averaging with censored data, i.e., non-detects/unknown concentrations (El-Shaarawi and Dolan, 1989; Newman et al., 1989;

Newman, 1995). In a recent publication, Helsel (2005) provides a clearly written and very thorough presentation on practical solutions to this issue. These approaches can be used with normally and log-normally distributed data.

It is recommended that unbiased means be calculated only if less than 20% of the reported values are reported as being non-detect (Berthouex and Brown, 1994).

Some of the techniques/approaches suggested above are dependent upon the number C_{soc}-C_{ℓ} pairs. With a limited number C_{soc}-C_{ℓ} pairs, e.g., 3–5, these approaches for handling data with unknown concentrations will be limited.

How do I estimate the confidence interval around a prediction?

For normally distributed data: The standard error of the geometric mean regression slope can be approximated by the standard error of the linear least-squares regression slope (Sokal and Rohlf, 1995). (Note, the slope is the BSAF). Most linear least-squares regression programs (SAS) or spreadsheets (IBM® Lotus® 1-2-3 and Microsoft® Office Excel) calculate the standard error of the slope. The 95% confidence intervals on the slope would be calculated using Student t-value:

$$\text{Upper 95\% CI} = b + s_b \times t_{0.05\,[n-2]} \qquad \text{(Eq. 22)}$$

$$\text{Lower 95\% CI} = b - s_b \times t_{0.05\,[n-2]} \qquad \text{(Eq. 23)}$$

where b is the geometric mean regression slope, s_b is the standard error of the geometric mean regression slope, n is the total number of data points used in the geometric mean regression, and $t_{0.05}$ is the two tailed Student-t for an $\alpha = 0.05$.

For log-normally distributed C_{soc}-C_{ℓ} data, the intercept of the geometric mean regression of the log transformed data ($y = \ln(x)$) is the ln(BSAF) (Equation 21). The variance of the geometric mean regression intercept can be found using the equation of (Ricker, 1973):

$$s^2_{y_{2-\text{int}}} = s^2_{y_2}(1-r^2) + b(1-r)^2\left(y_{1-avg}\right)^2 \qquad \text{(Eq. 24)}$$

where y_1's are the natural logarithm of C_{soc} values, y_2's are the natural logarithm of C_ℓ values, $s^2_{y_2}$ is the variance of the y_2 values, $s^2_{y_{2-\text{int}}}$ is the variance of the intercept, y_{1-avg} is the arithmetic mean of the y_1 values, r is the Pearson correlation coefficient between y_1 and y_2 and b is the geometric mean regression slope. Calculation of the confidence limits for the intercept are problematic because "no methods of computing accurate confidence limits appears to have been developed" (Ricker, 1973). It is suggested that the method of Land as reported by Gilbert (1987) be used to calculate the confidence limits. Land's equation for the upper one-sided 100(1-α)% and lower one-sided 100α% confidence limits for the arithmetic space average are

$$UL_{1-\alpha} = \exp\left(\overline{y} + 0.5s^2_y + \frac{s_y H_{1-\alpha}}{\sqrt{n-1}}\right) \qquad \text{(Eq. 25)}$$

$$LL_{\alpha} = \exp\left(\overline{y} + 0.5s^2_y + \frac{s_y H_{\alpha}}{\sqrt{n-1}}\right) \qquad \text{(Eq. 26)}$$

where $H_{1-\alpha}$ and H_α are obtained from tables provided by Land (see Gilbert, 1987). However, Singh et al. (U.S. EPA, 1997) reports that for small sample sizes, Land's method can result in unacceptably large (and potentially small) values for the upper (and lower) one-sided confidence levels when the coefficient of variation is larger than 1.0. Singh et al. (U.S. EPA, 1997) recommend that other methods should be used for computing the confidence limits.

If the regression model is used to predict residues in biota for known residues in sediment or vice-versa, confidence limits on the predicted residues can be estimated. For regression lines developed using Model I regression techniques in arithmetic space, confidence limits can be readily derived using standard statistical software, e.g.,

SAS/STAT software. For geometric mean regression performed in arithmetic space, the confidence limits are determined using the equations:

$$95\%Cl = y_I \pm s_I \times t_{0.05[n-2]} \quad \text{(Eq. 27)}$$

$$s_I^2 = s_Y^2(1-r^2) + b(1-r)^2(X_I - X_{avg})^2 \quad \text{(Eq. 28)}$$

where y_I is the predicted value, s_I is the standard deviation of the predicted value, s_y is the standard deviation of the Y variables used in the regression, X_{avg} is the average of the X variables used in the regression, r is the Pearson correlation coefficient, X_I is the observation which predicts the y_I value and b is the slope of the geometric mean regression line. As noted above, for geometric mean regression, calculation of the confidence limits are problematic because "no methods of computing accurate confidence limits appear to have been developed" (Ricker, 1973), and thus, the symmetrical approximation is used (Equation 27).

For geometric mean regression performed with log transformed data, confidence limits would be calculated using Land's method (Equations 25 and 26) with the variance of the predicted value (in log space) calculated using Equation 28 with the log transformed data.

Do I normalize by organic carbon and lipid?

Yes. The BSAF is the ratio of the concentration in the biota on a lipid basis to the concentration in the sediment on an organic carbon basis.

By working with C_{soc}-C_ℓ pairs (which are organic carbon and lipid normalized), one places these concentrations on a thermodynamic basis. By expressing the concentrations on a thermodynamic basis, the concentrations of the chemicals in sediment and tissue are corrected for differences in bioavailability and partitioning behavior. By using the thermodynamic based expressions, the C_{soc}-C_ℓ pairs are expressed equivalently.

Do I use weighted regression?

There are two general cases. First, when the C_{soc} and C_{ℓ} are individual observations (not averages), then individual C_{soc}-C_{ℓ} pairs should be given equal weights. Second, if the C_{soc} and C_{ℓ} are averages, then individual C_{soc}-C_{ℓ} pairs should be given equal weights except if the C_{soc} and C_{ℓ} variances are highly heterogeneous ($p < 0.001$). If the variances are highly heterogeneous (very dissimilar), then perform both weighted (by the inverse of the variance) and unweighted regression and compare slopes. The heterogeneous variances might or might not have any appreciable effect on the slope. If appreciable effects exist on the slope, then the weighted regression model is preferred.

If I transform the data, do I need to use weighted regression?

See answer to previous question. The variances would need to be evaluated in log space for heterogeneity.

How do I take into account the home range of the biota whose tissue I measured?

As explained earlier, one should have knowledge of the organism's foraging range. With this information, sediment samples across the foraging range should be collected and analyzed, and the sediment samples should be representative of the organism's immediate life history. Accounting for the foraging range of the organism is done by averaging the analytical results for sediment samples collected within the organism's foraging range.

What if my r^2 is low and my data do not plot with the appearance of an increasing linear function?

Occurrence of this type of behavior in the plot of C_{soc}-C_{ℓ} pairs strongly suggests that different sampling locations have either different underlying conditions and parameters (e.g., different food webs, different organism populations, differences in chemical bioavailability, different diets, etc.) or a very limited dynamic range. In these cases, one will need to determine the factors causing these differences. If one can not resolve these difference, the same problems will also exist with other methods for predicting chemical residues, e.g., food web models, because these methods require this knowledge as well. In general, when this type of behavior is observed, the problem is in the data itself, and

no statistical analysis method will circumvent the problem. Without resolving these differences, the data should not be used.

How do I deal with outliers?

For C_{soc}-C_{ℓ} pairs that are very different from the general population of C_{soc}-C_{ℓ} pairs (i.e., appear to be outliers), always make sure that the data are not miscalculated, transposed, or misidentified. Further, ensure that no other types of methodological errors are associated with the data. If the data pairs appear to be numerically and methodologically correct, statistical techniques are available for the testing of the data pairs to determine if they are outliers. If a data pair is found to be significantly different from the general population of C_{soc}-C_{ℓ} pairs, the outlier should be excluded from the regression analysis, because it is likely indicative of different underlying ecological conditions.

Snedocor and Cochran (1980, p 167–168) and Neter et al. (1996, p. 374–375) present a statistical method for linear regression where the regression is performed without the outlier, and then the outlier is tested as to whether it is within sampling error of the population. The test criterion is a t-value. Because the outlier is not chosen randomly, to ensure a $1 - \alpha$ confidence, the calculated t-value is compared to the t-value from the t-table using α'; where α' equals α divided by n. Probability values for testing for outliers should generally be conservative, e.g., $\alpha = 5\%$ or $\alpha = 1\%$. With an n of 20, the critical t-value for an α of 5% would be found using an α' of 0.25% with the t-table.

There are other statistical techniques for outlier detection beyond those described above, i.e., m-estimators, s-estimators, least median of squares, and least trimmed squares, that provide significant advantages in the detection of outliers and leveraged data pairs. Although a bit dated in terms of the software applications used, Rousseeuw and Leroy (1987) provides an excellent introduction and discussion of these techniques. A brief but helpful description and application of these techniques has been provided by Chen (2002). These advanced outlier detection algorithms are in numerous statistical packages including SAS/STAT software (SAS Institute, Inc.), SYSTAT software (SyStat Software, Inc.) and R Statistics software (freeware GNU package).

Do I develop a separate regression for each compound in a mixture?

Yes. This is most desirable because different chemicals have different chemical properties. For example, differing behavior is observed with PCBs where fish appear to be slightly enriched with the higher chlorinated PCB congeners relative to the distribution existing in the sediments.

When the value of x (i.e., exposure point concentration in sediment) is uncertain (e.g., when biota migrate), how do I account for this in my regression?

The best method of accounting for organism migration is to design your sampling plan for the organism such that the organisms are collected just before they migrate back out of the site. This approach maximizes time the organism spends at the site of interest, and provides the best estimate of the residue in the organism based upon the organism's exposure in its foraging range at the site.

Sampling design simulations (Burkhard, 2003) for the measurement of BSAFs (or C_{soc}-C_ℓ paired observations for determinations of BSAFs) suggest that spatial variability in the concentrations of the chemical does not add large uncertainties into the measured BSAF beyond those caused by temporal variability of the chemical concentrations in the water. Further, random walk migration simulations suggested that BSAFs (or C_{soc}-C_ℓ paired observations for determinations of BSAFs) can be measured with low uncertainty even when extreme variability in spatial concentrations exist at the field site, provided the measurements are performed in more contaminated locations of the site for higher K_{ow} chemicals, i.e., $>10^5$ (Burkhard, 2003). The requirement of performing the field measurements at the more contaminated locations within the site will limit the analysis of BSAF because the range of C_{soc}-C_ℓ pairs will be small.

If the organisms spend a very short time at the site, e.g., the fish migrate through the site in a few days to a week, determination of BSAFs is not recommended even though the BSAF can be measured. The sediments from the site would not be reflective of the fish's recent exposure history.

Are there ways to improve my study design knowing what I know now about regression?

First, the importance of collecting sediment samples that are reflective of the organism's foraging range can not be overstated. Spending time and resources to better define the relationship of the organisms to the sediments will greatly decrease the uncertainty associated with the resulting BSAFs. In addition, predictions using food web models, both steady-state or dynamic, will greatly improve because of the improved knowledge on the underlying relationship between the sediment and organism.

Second, it is important that composite samples reflective of the biota at the site of interest be collected. Clearly, collection and analysis of more organisms will provide a better measure of the average residue in the biota. However, biota samples consisting of mixed age classes is not recommended, e.g., juvenile and adult minnows, or 1-year-old and 3-year-old largemouth bass. Minimizing the differences in age (or size) will improve the quality of the biota samples and ultimately provide smaller variances for the biota residues. Typically, fishes of given size (e.g., the weight of the smallest fish is not less than 75% of the weight of largest fish) or age group (e.g., 3-year olds) are collected.

After sample collection and analysis, plans should be made to visually examine the data by making plots of C_{soc}-C_ℓ paired observations and plots of BSAFs against C_{soc}. The C_{soc}s, C_ℓs and BSAFs should be plotted on a GIS type plot to determine if the values are correlated with geographical trends and conditions, e.g., the BSAFs increase with increasing distance away from the source on a river. Any additional information or understanding one can glean for the site will be advantageous in the remediation decision process.

As part of the overall study plan for successfully measuring a BSAF, time and resources should be allocated for resolving causes of non-linearity (when they exist) in the C_{soc}-C_ℓ paired observations. Resolving why will greatly aid in understanding the complexities of the site, and provide decision makers and risk assessors a much better basis for assessing and evaluating remediation options.

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APPENDIX
ECOLOGICAL RISK ASSESSMENT SUPPORT CENTER REQUEST FORM

Problem Statement: What is the most appropriate method to estimate the Biota Sediment Accumulation Factor (BSAF) from paired observations of concentrations in biota and sediment?

Requestors: Sharon Thoms and Al Hanke, Region 4

Background: BSAF is a parameter describing bioaccumulation of sediment-associated organic compounds or metals into tissues of ecological receptors. In a typical experiment to measure bioaccumulation the researcher collects colocated sediments and tissues over a gradient of contamination. Simple compared to bioaccumulation and trophic transfer models, it finds its use at Superfund sites to estimate progress toward achieving a protective tissue concentration as sediments become cleaner.

Expected Outcome: The expected outcome is a white paper addressing the following questions regarding the use of regression to obtain the most accurate estimate of BSAF:

Do I fit a straight line?
Do I plot my data on a log-log scale?
Do I force the line through the origin?
How do I handle non-detects?
How do I estimate the confidence interval around a prediction?
Do I normalize by organic carbon and lipid?
Do I use weighted regression?
If I transform the data, do I need to use weighted regression?
How do I take into account the home range of the biota whose tissue I measured?
What if my r^2 is low and my data do not plot with the appearance of an increasing linear function?
How do I deal with outliers?
Do I develop a separate regression for each compound in a mixture?
When the value of x (i.e., exposure point concentration in sediment) is uncertain (e.g., when biota migrate), how do I account for this in my regression?
Are there ways to improve my study design knowing what I know now about regression?

Where the topics are covered by standard books or web sites on statistics, they may be referenced. A few case studies may be useful to illustrate the concepts.

Additional Comments: Requestor can provide case studies.