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**ISSUES RELATING TO SEDIMENT TOXICITY TESTING AND  
BIOACCUMULATION OF PERSISTENT CHEMICALS IN SRS SEDIMENTS**

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## **EXECUTIVE SUMMARY**

Many chemical contaminants that enter a water body in an aqueous form are ultimately deposited to the sediments. Over time, the concentrations of contaminants in sediments may build up to concentrations that are much higher than those found in the water column. However, not all chemicals present in sediments are toxic/bioavailable. Factors that affect bioavailability include aqueous solubility, pH, redox, and composition of the sediment matrix (grain size, mineral constituents, organic matter), and for metals, the quantity of acid volatile sulfides that are present in the sediments. Many sediments contain multiple chemical contaminants, which may interact synergistically or antagonistically with respect to toxicity.

Because the toxicity of a sediment cannot be determined simply by measuring the concentrations of chemical contaminants present in the sediment, laboratory toxicity testing methods have been developed to measure the toxicity and bioavailability of chemicals in sediment. Toxicity tests can be performed on bulk sediments and also on porewater (interstitial water) that is extracted from the sediments. Most screening level toxicity tests are performed on bulk sediments, but porewater testing is often used as an investigative tool to identify the specific chemical(s) responsible for the observed toxicity, since Toxicity Identification Evaluations (TIEs) are more readily performed on aqueous samples than on bulk sediments. Chemical bioavailability is generally estimated by performing bioaccumulation assays, which measure contaminant body burdens in benthic organisms. Bioavailability can also be estimated using Equilibrium Partitioning models to characterize the distribution of persistent contaminants between sediment and porewater.

Another approach that has been used to assess sediment quality is the Sediment Quality Triad approach, which is a weight-of-evidence approach that analyzes chemical, toxicity and biological (benthic invertebrate) data using multivariate statistics to provide a numerical rating of sediment quality.

Although SCDHEC does not currently require toxicity testing of contaminated sediments, both the U.S. EPA and EPA Region 4 have regulatory provisions for conducting sediment toxicity tests at Superfund sites that may have contaminated sediments.

The results of toxicity tests conducted on samples collected from SRS streams and seeps indicate that sediment grain size can greatly affect the outcome of the tests. Organisms exposed to silty sediments did poorly, even when no contaminants were present. Sediment toxicity testing, when combined with bioaccumulation studies, porewater TIEs and biological sampling, can be a powerful tool in assessing the health of SRS sediments.

## **1.0 INTRODUCTION**

Chemical contaminants most commonly enter a water body via industrial outfalls, runoff, or atmospheric deposition. However, once in the water, many chemical contaminants ultimately accumulate in sediments through chemical precipitation and/or adsorption to clays or organic particles. Sediment provides habitat for numerous species of aquatic life, which can be exposed to contaminants via ingestion or absorption. Of particular concern to aquatic life are heavy metals, (such as lead, mercury, copper and zinc) and

organic chemicals, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and some pesticides (i.e. chlordane, DDT). Sediments serve as both a sink and a reservoir for persistent chemicals. These chemicals can bioaccumulate in benthic organisms that inhabit sediments but many can also be remobilized into the water column under some conditions. However, not all chemicals present in sediments are toxic/bioavailable. In some instances concentrations of chemicals in the sediments may be several orders of magnitude higher than in the overlying water and not be toxic to the aquatic organisms that live in the sediments because the chemicals may be in a form that is not bioavailable to the organisms. Factors that affect bioavailability include aqueous solubility, pH, redox, and composition of the sediment matrix (grain size, mineral constituents, organic matter), and the quantity of acid volatile sulfides that are present in the sediments (U.S. EPA, 2000b).

The objective of a sediment toxicity test is to determine whether the chemicals that are present in the sediment are harmful to aquatic life, either by causing a toxic response or by bioaccumulating in the tissues of benthic organisms. Toxic responses can either be acute (resulting in death of the organism) or chronic (causing a reduction in growth or reproduction). Sediment toxicity tests can be used to: determine the relationship between toxic effects and bioavailability; investigate interactions among chemicals; compare the sensitivities of different organisms; determine spatial and temporal distributions of contaminants, rank areas for cleanup, and estimate the effectiveness of remediation (U.S. EPA, 2000b).

This paper provides an overview of factors that affect sediment toxicity and bioavailability, a summary of available test methods, and a discussion of potential sediment toxicity issues at SRS.

## **2.0 TECHNICAL BACKGROUND/ISSUES**

### **2.1 Factors Affecting Bioavailability of Contaminants**

A wide range of physical, chemical, and biological factors have the potential to influence the bioavailability of sediment contaminants. The bioavailability of contaminants in sediment is a function of the type of chemical and the chemical speciation, as well as the behavior and physiology of the organism. The two basic routes of exposure for organisms are transport of dissolved contaminants in pore water across biological membranes, and ingestion of contaminated food or sediment particles with subsequent transport across the gut. For upper-trophic-level species, ingestion of contaminated prey is the predominant route of exposure, especially for hydrophobic chemicals (U.S. EPA, 2000b). Uptake through ingestion of or direct exposure to water or sediment can also be important, depending on the trophic level of the organism and the physical-chemical characteristics of the contaminant.

**2.1.1 Physical Factors** - Sediments are dynamic environments with a wide range of interacting processes with variable rates. The rate of mixing in surficial sediment layers by physical processes such as turbulence and bioturbation competes with the rate of sedimentation to determine the depth to which contaminated sediment will be buried. Diffusion and resuspension can also have a large impact on the bioavailability of sediment associated contaminants either by re-exposing epibenthic filter feeders, such

as bivalves, to contaminated particulates or by increasing the aqueous concentration of a contaminant via desorption from the particulates within the water column.

**2.1.2 Chemical Factors** - The characteristics of a chemical, such as its molecular size and polarity, determine to a large extent the degree of association of the chemical with particles and thus have an effect on bioavailability. Large, nonpolar chemicals, such as highly chlorinated polychlorinated biphenyls (PCBs), have low aqueous solubilities and a strong tendency to be associated with dissolved and particulate organic matter; therefore, they are less bioavailable, at least to organisms that do not ingest sediments. Small ionic species, such as many metals, have higher aqueous solubilities and tend to be more bioavailable. Even between these extremes, chemical characteristics of contaminants have a large influence on bioavailability. The concentration of total metals in sediment is generally not predictive of the bioavailability of these elements. Metals concentrations in interstitial water (i.e., pore water) have been correlated with biological effects. For several divalent metals in sediments, acid-volatile sulfide (AVS) appears to have a strong influence on cationic metal activity and toxicity (DiToro et al., 1990, U.S. EPA 1994, Ankley et al., 1993a).

For nonionic organic chemicals, the most important factor determining bioavailability is sorption to dissolved and particulate organic matter. Sediment-pore water partitioning of nonionic organic compounds is influenced by the organic carbon content of the sediment. Hydrophobicity is the most important chemical characteristic determining the bioaccumulation behavior of organic chemicals in aquatic systems. Most polar organics do not bioaccumulate, while non-polar organics do tend to bioaccumulate, especially in lipids. Octanol-water partitioning has become a common method for evaluating the potential of a contaminant to bioaccumulate. It has been demonstrated that bioaccumulation can be predicted from octanol-water partitioning when the partition coefficient (log K<sub>ow</sub>) lies between 2 and 6 (U.S. EPA, 1993, 2002). There is also a relationship between the K<sub>ow</sub> of a chemical and its potential for biomagnification, with uptake efficiency increasing with increasing log K<sub>ow</sub> for values between 3 and 6 (U.S. EPA 2002). For compounds with a log K<sub>ow</sub> greater than 6, uptake efficiency begins to decrease. The predictive relationships between K<sub>ow</sub> and bioaccumulation or biomagnification potentials assume that the compound is not metabolized. If metabolism occurs, these correlations are not applicable, making interpretation more difficult.

**2.1.3 Biological Factors** - Bioaccumulation is a function of the bioavailability of contaminants in combination with species-specific uptake and elimination processes. Toxicity is determined by the exposure of an animal to bioavailable contaminants in concert with the organism's sensitivity to the contaminant. These processes have been shown to be a function of the organism's lipid content, size, growth rate, gender, diet, and ability to metabolize or transform a given contaminant, as well as the chemical conditions of the surrounding medium (U.S. EPA 2000b). Other biological factors that can affect contaminant bioavailability include the burrowing and feeding behavior. The depth to which an organism burrows, the type of feeding mechanism it uses (e.g., filter feeding, particle ingestion), the size range of sediment particles it consumes, and its diet all have a large influence on the concentration of contaminant to which the organism will be exposed (U.S. EPA 2000b)

## 2.2 Sediment Toxicity Testing

**2.2.1 Selection of Test Species** - The selection of the most appropriate test species has a major influence on the relevance, success, and interpretation of test results. It is important that test species selection be based on environmental relevance when possible. U.S. EPA (2000b) states that, ideally, a test species should meet the following criteria:

- be sensitive to the contaminants of concern
- be easily cultured or readily available from reputable sources
- be easily maintained in the laboratory
- be easily identified
- be benthic in nature such that it is intimately in contact with the sediment
- be ecologically relevant and/or economically important
- have a broad geographical distribution
- have a niche similar to indigenous organisms of concern
- be tolerant to a broad range of sediment types (based on grain size)

Test methods using a variety of species have been developed by the U.S. EPA and ASTM for assessing the effects of sediment contaminants on aquatic organisms (U.S. EPA 2000b; ASTM, 1998a, 1998b, 1998c). The most commonly used freshwater species are the amphipod, *Hyalella azteca*; the midges, *Chironomus tentans* and *Chironomus riparius*; and the earthworm, *Lumbriculus variegatus*. Less commonly, the amphipod, *Diporeia* spp., the tubificid worm, *Tubifex tubifex*, the mayfly, *Hexagenia* spp., and various species of mollusks have been used. For sediment elutriate testing, the cladocerans, *Daphnia* spp. and *Ceriodaphnia* spp. are generally the organisms of choice. Table 1 compares various selection criteria for nine test species.

**2.2.2 Sensitivity of Test Species** - Test species differ in their sensitivity to differences classes of contaminants (i.e. metals, vs. organics), and also in the sensitivity to individual contaminants within a class (i.e. copper vs. zinc). For example, Ankley et al. (1991b) reported that *H. azteca* and *C. dubia* responded very similarly to a variety of sediment elutriate and pore-water samples, while *L. variegatus* was much less sensitive. Table 2. lists 10-day LC50 ( $\mu\text{g/l}$ ) Values for *H. azteca*, *C. tentans*, and *L. variegatus* exposed to a variety of chemicals in water.

In a study of Great Lake sediments which tested the sensitivity of 24 species, *H. azteca*, *C. tentans*, and *C. riparius* were among the most sensitive species tested (Burton and Ingersoll, 1994; Burton et al., 1996a; Ingersoll et al., 1993). Kemble et al. (1994) reported the rank sensitivity of four species to metal-contaminated sediments to be (from most to least sensitive): *H. azteca* > *C. riparius* > *Onchorhynchus mykiss* (rainbow trout) > *Daphnia magna*. Similarly, in 10-day water-only and whole-sediment tests, *H. azteca* and *C. tentans* were more sensitive than *D. magna* to fluoranthene (Suedel et al., 1993). Thus it appears that the species most routinely used for sediment toxicity testing are at least as sensitive as those species typically used for aqueous testing.

West et al. (1993) found that with respect to copper, *H. azteca* was most sensitive, *C. tentans* was intermediate, and *L. variegatus* was least sensitive. *L. variegatus* has been reported in numerous studies to be less sensitive to a wide variety of chemicals (including metals, DDT and associated metabolites, and organophosphate insecticides).

The relative insensitivity of *L. variegatus* to many contaminants has been viewed by some researchers as a positive attribute when used in bioaccumulation studies, since this species can be exposed to sediments that may be lethal to more sensitive species.

**Table 1. Rating of Selection Criteria for Freshwater Sediment Toxicity Testing Species**

CRITERION	<i>Hyalella azteca</i>	<i>Diporeia</i> spp	<i>Chironomus tentans</i>	<i>Chironomus riparius</i>	<i>Lumbriculus variegatus</i>	<i>Tubifex tubifex</i>	<i>Hexagenia</i> spp	Mollusks	Daphnids
Relative sensitivity toxicity database	+	-	+	-	+	-	-	-	-
Inter-laboratory studies conducted	+	-	+	-	-	-	-	-	-
Contact with sediment	+	+	+	+	+	+	+	+	-
Laboratory culture	+	-	+	+	+	+	-	-	+
Ease of taxonomic I.D.	+/-	+/-	+/-	+/-	+	+	+	+	+
Ecological importance	+	+	+	+	+	+	+	+	+
Geographical distribution	+	+/-	+	+	+	+	+	+	+/-
Tolerance of sediment types	+	+	+/-	+	+	+	-	+	NA
Response confirmed with benthic populations	+	+	+	+	+	+	+	-	+
Peer reviewed	+	+	+	+	+	+	+	-	+/-
Endpoints monitored	S,G,M,R	S,B,A	S,G,E,R	S,G,E	B,S,R	S,R	S,G	B	S,G,R

+ or - rating indicates a positive or negative attribute.  
S=survival; G=growth; B=bioaccumulation; A=avoidance; R=reproduction; M=Maturation; E=emergence; NA=not applicable  
From: U.S. EPA, 2000b.

**2.2.3 Use of Indigenous Species** - The U.S. EPA currently allows the use of indigenous species in sediment toxicity testing only where state regulations require their

use or when state regulations prohibit importation of the recommended test species. Where state regulations prohibit importation or use of the recommended species, permission should be obtained from the appropriate regulatory agency before using indigenous species (U.S. EPA 2000b).

**Table 2. 10-day LC50 (µg/l) Values for *Hyalella azteca*, *Chironomus tentans*, and *Lumbriculus variegatus* in Water-only Exposures**

Chemical	<i>H. azteca</i>	<i>C. tentans</i>	<i>L. variegatus</i>
Copper	35	54	35
Zinc	73	1125	2984
Cadmium	2.8	NT	158
Nickel	780	NT	12160
Lead	<16	NT	794
p,p'-DDT	0.07	1.23	NT
p,p'-DDD	0.17	0.18	NT
p,p'-DDE	1.39	3.0	3.3
Dieldrin	7.6	1.1	NT
Chlorpyrifos	0.086	0.07	NT

NT – Not tested  
From Phipps et al. (1995)

**2.2.4 Test Duration** - The most common sediment toxicity tests use a short-term exposure (generally 10 days) and use survival as the endpoint. These short-term exposures can be used to identify high levels of chemical contaminants, but may not be able to identify moderately contaminated sediments (Sibley et al., 1996, 1997, 1998; Benoit et al., 1997; Ingersoll et al., 1998). Sublethal endpoints in sediment toxicity tests, including growth, behavior, and reproduction may provide a better estimate of the responses of benthic communities to long-term exposure of chemical contaminants. The decision to conduct a short-term or long-term sediment toxicity test depends on the goal of the assessment. In some instances, sufficient information may be obtained by measuring both survival and sublethal endpoints (such as growth) in short-term tests, or short-term tests may be used as a screening tool prior to initiating long-term tests. However, long-term tests are needed when the goal is to accurately assess the potential of a contaminated sediment to adversely impact the biota of a benthic ecosystem. Detailed methods for conducting short-term and long-term sediment toxicity tests can be found in ASTM 1998c and in U.S. EPA 2000b.

**2.2.5 Reference Sediments** - A reference sediment is used as the point of comparison for evaluating sediments that contain contaminants of concern (COCs). Reference sediments should have physical and chemical characteristics that are similar to the contaminated sediments of interest except that the COCs are not elevated above background concentrations in the reference sediments. Sediment attributes that should be evaluated in selecting a reference sediment include grain size, percent organic matter, pH, redox, and cation exchange capacity (Hunt et al., 2001). If the COCs are cationic metals, Simultaneously Extracted Metals (SEM) and Acid Volatile Sulfide (AVS) should be measured and the SEM/AVS ratio should be calculated (U.S. EPA 1994).

### 2.3 Porewater Toxicity Testing

Porewater is the water that resides in the interstitial spaces between sediment particles. It is well documented that porewater is a major route of contaminant exposure for benthic organisms (U.S. EPA 1993; DiToro et al., 1991; Adams et al., 1985) because contaminants associated with sediment particles reach an equilibrium concentration in the porewater, based on the physicochemical conditions of the sediment. Numerous methods have been developed for porewater extraction (Carr and Chapman 1995; Winger and Lasier 1991; Jahnke 1988; Hesslin 1976; Edmunds and Bath 1976; Presley et al. 1967) and toxicity testing of pore water with aquatic organisms (Carr 1998; Hooten and Carr 1998; Ankley et al. 1992a; Carr et al. 1989). However, numerous limitations have been identified for porewater toxicity testing. It is difficult and time consuming to extract the quantities of porewater needed for aqueous toxicity testing. In addition, it is impossible to extract a porewater sample from sediment and perform a toxicity test with the water with no resulting changes in the chemistry of the porewater. The moment that porewater is exposed to air, its chemical characteristics begin to change because changes in redox potential can greatly affect the solubility and chemical speciation of many toxicants. Despite these limitations, porewater toxicity tests can provide useful information on the bioavailability and toxicity of contaminants in sediments and can also be very instrumental in identifying the cause of the toxicity (see Section 2.5), since Toxicity Identification methods are not yet fully developed for solid phase sediment tests (Carr and Nipper, 2001).

Two broad categories of procedures exist for sampling sediment pore water: in situ methods, which involve the collection of pore water by the use of samplers (peepers) that are directly inserted into the sediment and left to equilibrate or by suction through the application of vacuum; and ex situ methods, where the sediment of interest is removed from the natural setting and the pore water isolated elsewhere, usually by pneumatic pressure or centrifugation, although extraction by vacuum can also be used (Carr and Nipper, 2001). Table 3 lists advantages and disadvantages of each collection method.

Porewater toxicity tests are performed using the same species (generally *C. dubia*, *D. magna* or *H. azteca*) and methods similar to standard aqueous tests, except that smaller volumes of water are generally used (usually 10 ml/test chamber). Generalized test methods can be found in APHA (1998). Porewater toxicity tests have also been conducted using the Microtox test, which uses luminescent bacteria (Adolphson, 2000).

The sensitivity of porewater toxicity test methods has been compared with that of solid-phase tests (Carr et al. 2000; Nipper et al. 1998; Carr et al. 1996a, 1996b; Sarda and Burton 1995; Carr and Chapman 1992). Because of the complexity of the interactions between contaminants and biota in benthic systems, generalizations about concordance between solid-phase and porewater toxicity tests are difficult. There is no single acceptable level of concordance between tests. Rather, the degree of concordance should be a function of the study objectives and the sample characteristics. Situations where porewater and solid-phase toxicity tests would be expected to give similar results include studies of sediments from highly-contaminated sites, reference sites, situations where pore water is the primary route of exposure, and studies where the same species is tested in both porewater and solid-phase matrices. Low concordance is commonly observed when there are considerable differences in the relative sensitivities of the

different species tested and/or the endpoints used in the tests, or when there are different exposure pathways (Carr and Nipper, 2001)

**Table 3. Advantages and Disadvantages of Several Methods for Isolating Sediment Porewater for Toxicity Testing.**

Method	Advantages	Disadvantages
Peeper (in situ)	<ul style="list-style-type: none"> <li>- Porewater chemistry is measured without significant disturbance of the in situ equilibrium conditions.</li> <li>- Reduced sample manipulation</li> <li>- Reduced sampling influences on the oxidation state of metals</li> <li>- Eliminated potential for loss of volatile substances, such as H<sub>2</sub>S, and high Henry's law constant HOCs, which occur with ex situ methods</li> <li>- Use of a dialysis membrane eliminates the post-retrieval pore water filtration</li> <li>- pH and redox conditions are relatively unaltered, minimizing changes in pH and oxygen-sensitive species (such as metals)</li> </ul>	<ul style="list-style-type: none"> <li>- Operates well for inorganic constituents (e.g., divalent metals), but their utility for accurately sampling highly hydrophobic organic compounds is poorly defined (i.e., sorption of hydrophobic compounds onto the sampler, the dialysis membrane, or onto the fouling organisms associated with the membrane, depending on the length of deployment, could artificially reduce pore water contaminant concentrations).</li> <li>- An extended equilibration time in the field is required (generally 15 to 20 days), resulting in the need for 2 field trips: 1 for peeper deployment and 1 for peeper retrieval.</li> <li>- Sample volumes are limited, generally to less than 10 ml. Larger peepers are limited to very porous substrates.</li> <li>- Uncontaminated water inside newly deployed peeper cells could effectively dilute pore water contaminant concentrations in low porosity sediments.</li> <li>- Samples must be collected from peepers immediately upon retrieval, resulting in a longer holding time for pore water outside of its natural matrix prior to toxicity testing.</li> <li>- A high degree of technical competence and effort is required for proper use. Use in deeper water requires diving.</li> <li>- In situ methods are often not practical for deep waters or high-energy situations</li> </ul>
Suction (in situ)	<ul style="list-style-type: none"> <li>- Easy and low-technology operation; use of inexpensive equipment</li> <li>- Is suitable for use with a wide variety of sediment textures</li> <li>- Procedure can generate large volumes of pore water</li> </ul>	<ul style="list-style-type: none"> <li>- Potential sorption of metals and HOCs on 'filter'</li> <li>- Some clogging may occur in small-to-medium particle-sized sediments and slow down the porewater extraction process.</li> <li>- Collection of pore waters from non-targeted depths (e.g., overlying water) may occur when collection is conducted in situ.</li> <li>- Degassing of pore water may occur.</li> </ul>
Centrifugation (ex situ)	<ul style="list-style-type: none"> <li>- Several variables (e.g., duration, speed) can be varied to optimize operation</li> <li>- Procedure can generate large volumes of pore water</li> <li>- Functions with fine-to-medium particle-sized sediments</li> <li>- Easy operation</li> </ul>	<ul style="list-style-type: none"> <li>- Labor intensive (e.g., sediment loading); requires a refrigerated centrifuge with large tube capacity</li> <li>- Lack of a generic methodology</li> <li>- Potential sorption of HOCs to centrifuge tube</li> <li>- Lysis of cells during spinning</li> <li>- Does not function in sandy sediments</li> </ul>
Pressurization (ex situ)	<ul style="list-style-type: none"> <li>- Can be used with highly bioturbated sediments without lysis of cells</li> <li>- Procedure can generate large volumes of pore water</li> <li>- Can be used with a wide variety of sediment textures</li> </ul>	<ul style="list-style-type: none"> <li>- Potential loss of HOCs on filter</li> <li>- Changes in dissolved gasses may occur</li> </ul>

From Carr and Nipper, 2001

In 2001, a SETAC technical workshop was held to discuss issues relating to porewater toxicity testing (Carr and Nipper, 2001). The major conclusions of the workshop participants were that:

“• Concordance between the results of solid-phase and porewater toxicity tests should not always be expected, and discordance is indicative of different routes of exposure and/or species sensitivity, rather than inaccuracy in the results of 1 type of test.

- It is important to conduct both porewater and solid-phase tests whenever possible, which enhances the ability to discriminate sediment quality.
- The toxicity data from porewater tests should be used along with the parallel data from tests of other sediment phases to form a weight of evidence and to determine concordance among the triad components.
- Sampling, extraction, and storage techniques are critically important for achieving the most field-representative samples of pore water. Several sampling methods were suggested, and method selection should be based on the objective of the study.
- It is nearly impossible to avoid artifacts and chemical changes when removing pore water from sediment and using it in a toxicity test. Since artifacts are always introduced to some extent, the determination of chemical concentrations in the pore water is recommended, in addition to the regular contaminant measurements conducted in the whole sediment, as a means of providing information on routes and levels of exposure, aiding in the interpretation of test results, and identifying sources of toxicity.
- The measurement of several porewater features, a number of which can act as confounding factors (e.g., salinity, alkalinity, pH, conductivity, DO, NH<sub>3</sub>, H<sub>2</sub>S, Eh), should be recorded shortly after porewater collection and after storage. This would help in interpreting test results, understanding the contribution of these factors to concordance/discordance between solid-phase and porewater test methods, and contributing to TIE procedures.
- From the statistical point of view, confounding factors are best accounted for by normalization in univariate tests and by simply including the confounding variables in a multivariate analysis.
- Some potential confounding factors (e.g., salinity) should be adjusted prior to testing to assure that test conditions are compatible with the needs of the test species.
- The use of a variety of test species was recommended, in order to enrich the database and help account for different modes of action and species sensitivity.
- The use of indigenous species is not recommended or suggested as important for the understanding of potential biological impacts as identified from the results of porewater toxicity tests. The use of water column organisms for porewater toxicity tests was considered scientifically appropriate.

- The sediment depth to be sampled for pore water should match the depth of interest for each particular survey.
- The need for appropriate reference sites was discussed and it was concluded that reference sites should have similar sediment (and therefore porewater) characteristics and be selected from a location near the study site, or at least in the same ecoregion. It was also suggested that if a suitable reference sediment cannot be found, the performance control could be used for the statistical comparison.
- It is also important to know the tolerance levels of the test species to major confounding factors.
- Regulatory aspects of the use of porewater toxicity tests included the need for prior determination of the purpose for the testing in a specific regulatory program and the need to determine what question(s) are being asked, ensuring that the question(s) are appropriate for the specific regulatory application. Porewater tests were considered suitable for several types of frameworks, but unsuitable for others, e.g., as stand-alone pass/fail methods or as a substitute for a solid-phase test. This corroborates the findings that the 2 tests represent different routes of exposure and that the feeding mode of a test species can be of critical importance for the exposure to certain chemicals.
- Among the desirable attributes for porewater toxicity tests used in the Sediment Quality Triad approach, the ability to identify causality seemed to take precedence over other aspects, although it was recognized that non-specific assays are useful exploratory tools to identify toxicity." (Carr and Nipper, 2001)

## **2.4 Bioaccumulation of Contaminants from Sediments**

Two basic approaches exist to assess bioaccumulation: the first consists of methods that directly measure bioaccumulation, and the second consists of methods that model bioaccumulation (Ingersoll et al., 1995). The selection of the appropriate approach is dependent on what questions are being asked, the type of environment, the species, and the contaminants of concern

**2.4.1 Direct Measure** - Direct measurement, the simplest approach to assessing bioaccumulation in aquatic organisms, can be conducted using either laboratory-exposed or field-collected organisms. This approach minimizes or eliminates many of the problems associated with modeling. Important issues associated with laboratory measurements of bioaccumulation of chemicals from sediment include selection of an appropriate test species, sediment sampling and handling methods, conditions during exposure to the sediment, exposure duration, and statistical analyses. Measuring bioaccumulation at a particular site requires consideration of which test species to use, whether to examine natural populations or use transplanted populations, and how to compare bioaccumulation occurring under conditions at a potentially contaminated site with that occurring at a reference site (Besser et al 1997).

Bioaccumulation assays can be very useful in determining the bioavailability of contaminants. In these tests, overlying water is renewed daily and the test organisms are not fed during the tests, so that they must ingest sediment in order to feed.

Bioaccumulation assays have become an important part of sediment quality assessments. Standard methods for bioaccumulation assays can be used to identify environmental risks from persistent, bioaccumulative sediment contaminants, which may express toxicity via food-chain transfer to higher trophic levels rather than by direct toxicity to sediment-dwelling invertebrates (Ingersoll et al. 1995). Sediment quality criteria for such compounds can be derived by combining data from sediment bioaccumulation assays with models of contaminant transfer in aquatic food-chains, and thresholds for dietary toxicity to consumers such as fish eating birds and mammals. Bioaccumulation studies are also used for investigations of physicochemical and biologic factors controlling contaminant bioavailability.

The importance of bioaccumulation processes in mediating contaminant availability in aquatic ecosystems has resulted in the development of standard bioassay procedures for measuring bioaccumulation from sediments. Standard bioassays for assessing bioaccumulation from freshwater sediments generally use the oligochaete worm, *Lumbriculus variegatus* (ASTM 1998a). Oligochaetes have several advantages for bioaccumulation assays, including: burrowing habits, relative insensitivity to toxicity, and limited ability to metabolize contaminants.

Studies of metal bioaccumulation from sediments have also proved to be useful tools for assessing ecological risks from metal-contaminated sediment and for investigating influences on bioavailability and mobility of metals in sediments. For example, diets containing metal-contaminated invertebrates from the upper Clark Fork River in Montana have been found to reduce survival and growth of juvenile trout (Woodward et al. 1994). Laboratory bioaccumulation studies with Clark Fork sediments found that uptake of Cu and Zn from sediment by the amphipod, *Hyalella azteca*, corresponded closely to metal concentrations in field-collected invertebrates (Ingersoll et al. 1994). Bioaccumulation tests with larvae of the midge, *Chironomus tentans*, found that bioavailability of Cu was negatively associated with concentrations of AVS and organic carbon in sediments (Besser et al. 1995), and that changes in AVS concentrations were an important factor affecting spatial and temporal variation in metal bioavailability in Clark Fork sediments (Besser et al. 1996). Methods for bioaccumulation studies can be found in ASTM 1998a and U.S. EPA 2000.

**2.4.2 Bioaccumulation Models** - The two main approaches to bioaccumulation model development are (1) an empirical approach in which laboratory or field data are interpreted to calculate parameters such as bioaccumulation factors (BAFs) and biota-sediment accumulation factors (BSAFs) and (2) a deterministic modeling approach that employs kinetic or equilibrium models in which the mechanistic aspects of bioaccumulation are considered, usually referred to as food web models (Ankley et al., 1994). Empirical models include bioconcentration factors, BAFs, BSAFs, food chain multiplier, and theoretical bioaccumulation potential. Mathematical models or food web models can be grouped into two categories - equilibrium-based and kinetic approaches. Equilibrium-based models assume steady-state conditions between organisms and the environment. In contrast to equilibrium-based models, kinetic models describe bioaccumulation as the net effect of rate processes (uptake and loss of contaminant). General assumptions of kinetic models include constant uptake rate(s), instantaneous mixing, and a negative exponential depuration process for all compartments. More recently, a critical body residue approach has been proposed, which links body burdens in an individual organism to toxicological effects in that organism (Landrum et al (2003).

## 2.5 Toxicity Identification Evaluations for Freshwater Sediments

Toxicity Identification Evaluations (TIEs) were originally developed by the U.S. EPA in the 1980s to identify the source(s) of toxicity in aqueous samples that contained complex mixtures of contaminants (U.S. EPA 1991). TIEs employ a series of physical and chemical manipulations, each of which is designed to remove a specific class of toxicant from the sample. The treated sample is then retested to determine if its toxicity has been altered. This approach, when combined with chemical analyses to determine what toxicants were removed from the sample has proven to be a very effective tool in identifying the source of toxicity in aqueous samples. More recently, similar approaches have been used to identify sediment toxicants. One approach has been to extract porewater from sediments and use a sequence of treatment steps similar to that used for aqueous TIEs (Figure 1; SAIC, 2003).

A second approach has been to develop TIE methods that can be used with whole sediments. These methods have focused on the use of selective treatments to selectively reduce the bioavailability of three classes of sediment contaminants: nonpolar organics, cationic metals, and ammonia. Methods for selective reduction of the bioavailability of nonpolar organics and cationic metals are based on the same principles used for development of sediment quality criteria. Non-polar resins, such as Amborsorb have been used successfully to reduce the toxicity of nonpolar organic compounds, and addition of excess Acid Volatile Sulfide (AVS) or complexing agents (e.g. EDTA) have been used with mixed success to reduce bioavailability of cationic metals (Besser et al., 1997). Efforts to selectively reduce the toxicity of ammonia in sediments have focused on sorption of the ammonium ion using a natural zeolite mineral, clinoptilolite. Addition of clinoptilolite to whole sediment substantially reduces porewater ammonia concentrations and reduces or eliminates toxicity to amphipods and midges (Besser et al. 1996).

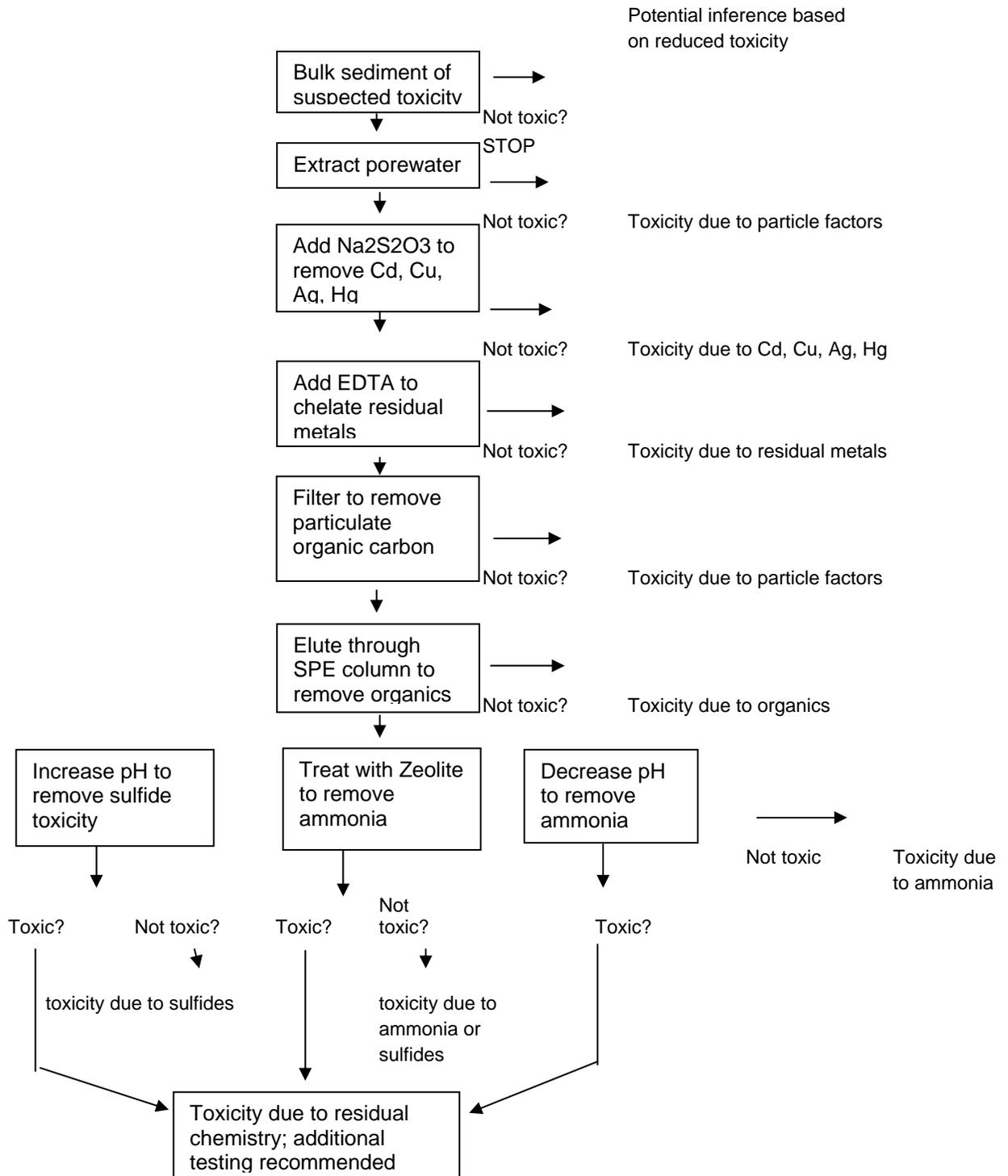
The most recent approach to be used for sediment TIEs are in situ TIEs (iTIEs). iTIEs use an exposure chamber in the field that is placed on top of the sediment. Porewater from the chamber is then suctioned out of the sediment and passed through a variety of sorptive materials, including Amborsorb 563 for nonpolar organics, Chelex for metal adsorption, and zeolite for ammonia removal. The treated porewater samples are then pumped into a test chamber containing *Daphnia magna*. Preliminary results indicate that the iTIE method provides a more accurate and sensitive evaluation of porewater toxicity than the laboratory TIE method (Burton and Nordstrom, 2004a, 2004b)

Although much progress has been made in conducting TIEs on whole sediments, sediment TIEs are still in a dynamic state of development and methods need to be refined before sediment TIEs are used routinely and produce consistent results. At present, porewater TIEs (either laboratory or iTIEs) are probably a better choice than whole-sediment TIEs, since the methods are better defined, and the contaminants present in porewater are in the most bioavailable form.

## 2.6 Sediment Quality Triad Approach

The Sediment Quality Triad (SQT) was developed by Long and Chapman (1985) as a weight-of-evidence approach to assess sediment quality. This approach analyzes the results of sediment chemistry, sediment toxicity, and benthic invertebrate data using multivariate statistical analyses to provide a numerical rating of sediment quality.

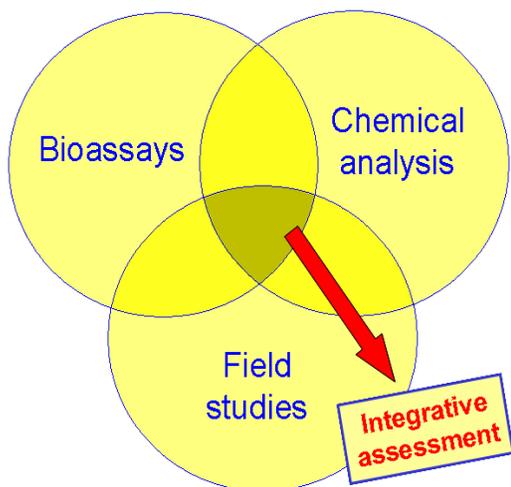
**Figure 1. Flow Diagram for Sequential Porewater TIE**



From SAIC, 2003

Sediment chemistry data is compared to numerical quality guidelines to determine the presence and degree of anthropogenic contamination as well as the spatial extent of the problem. Sediment toxicity tests are performed using laboratory test organisms to investigate whether or not anthropogenic substances in the sediment interfere with normal biological functioning. Test species are selected to assess a variety of trophic levels and potential exposure pathways. The sediments of interest are sampled to assess the health of resident biological communities using benthic community structure analysis and/or bottom fish histopathological abnormalities. In addition, sometimes bioaccumulation studies are performed in the laboratory or body burdens are measured in resident organisms to evaluate potential hazards to higher trophic levels via the food chain. The data are then analyzed using multivariate techniques, including cluster analysis, and a site is rated as high quality, intermediate/high quality, intermediate/degraded quality or degraded quality depending where degradation was detected in none, one, two, or all three of the test parameters (sediment chemistry, sediment toxicity, and benthic biota; Figure 2). Details of the Sediment Quality Triad approach can be found in Long and Chapman (1985), Chapman (1996; 2000), and Chapman et al. (1997)

**Figure 2. Sediment Quality Triad Approach**



The main challenge facing investigators who have applied the SQT approach to aquatic impact assessment is how to interpret the array of data from the different measurement endpoints (sediment chemistry, benthic community structure, and toxicity tests). There have been numerous suggestions proposed in the literature, including summary indices (Chapman 1992a, 1990; Alden 1992), tabular decision matrices (Carr et al., 1996a, 1996b; Chapman et al. 1996; Chapman 1992b), scaled ranking factors (Carr et al. 2000; Canfield et al. 1996; Carr, Chapman, Howard et al. 1996), or multivariate analyses (Chapman et al. 1996; Green and Montagna 1996; Green 1993a, 1993b; Green et al. 1993). All of these approaches require an appropriate reference station or group of stations, and all of these approaches also involve a “weight of evidence” (WOE) interpretation, or a way to draw conclusions based on congruent or conflicting lines of evidence. As with multivariate techniques for data analysis where there is no single “right” way to relate sets of variables (Green 1993a), a recurring theme in these papers

(Chapman et al. 1997; Chapman 1996) is that there is no single "best" way to depict or use the SQT.

## **2.7 Modeling Bioavailability of Sediment-Associated Contaminants**

Recent research on the bioavailability of sediment-associated contaminants has used simple Equilibrium Partitioning models to characterize the distribution of persistent contaminants between sediment and porewater. The goal of this approach has been to calculate sediment quality criteria (SQC), which estimate concentrations of contaminants in sediment which are protective of sediment-dwelling organisms (Besser et al. (1997).

For nonpolar organic compounds, SQCs have been derived based on thresholds for toxicity of compounds in water and the relative hydrophobicity of individual compounds. Water quality criteria, based on results of laboratory toxicity tests, are assumed to represent toxicity thresholds in porewater. Although direct measurement of concentrations of nonpolar organics in porewater is often difficult, porewater concentrations can be modeled by assuming that these compounds partition between sediment organic carbon (SOC) and porewater. The SOC:water partitioning behavior of individual compounds is assumed to be represented by octanol:water partitioning coefficients (DiToro et al. 1991).

Efforts to derive criteria for cationic metals have taken a different approach, largely because no single sediment component controls metal bioavailability across a variety of sediment environments. The most widely-used approach to model metal bioavailability in sediments is based on the tendency of many toxic metals to form highly-insoluble metal sulfides in the presence of acid-volatile sulfide (AVS). Metals are predicted to be unavailable (and sediments non-toxic) if the molar sum of the concentrations of metals is less than the molar concentration of AVS (Ankley et al. 1996a). This model defines a conservative "no-effect" condition, rather than a true SQC. The model does not consider the sorption of metals to sediment components other than AVS, notably organic carbon and hydrous metal oxides. An alternative approach, based on direct measurement of metals in porewater, is limited by difficulties of defining and analyzing bioavailable forms of aqueous metals.

## **3.0 REGULATORY ISSUES**

At present, SCDHEC does not require toxicity testing of contaminated sediments. SCDHEC's only regulations that pertain to sediments can be found in SCDHEC (2003), but these are related to erosion and sedimentation issues, rather than to sediment toxicity.

The U.S. EPA and EPA Region 4 both have regulatory provisions in their Ecological Risk Assessment Guidance for requiring sediment toxicity tests at Superfund sites that may have contaminated sediments (U.S. EPA, 1997; U.S. EPA Region 4, 2001).

## **4.0 SRS ISSUES RELATED TO SEDIMENT TOXICITY TESTING**

Although aqueous toxicity testing has been conducted on many SRS effluents and surface waters, application of sediment toxicity testing has been limited. In 1996, sediments were collected from three clean SRS surface waters (Mill Creek, Tinker

Creek, and Fourmile Branch at Road F and one clean seep (UTR-029). 10-day toxicity tests were conducted on all samples, using *H. azteca* as the test organism and survival and growth as the test endpoints. The results were compared to the results from a reference sediment from Resurrection Creek, located in Greenville County, SC. ETT Environmental had used sediment from this location as a reference sediment in other toxicity tests and determined that the test organisms had good survival and growth when exposed to this sediment. A 28-day toxicity test was also performed on one of the sediments (Fourmile Branch at Road F). In the 10-day tests survival was not significantly lower than the reference sediment for any of the SRS sediments, but growth was significantly lower in two of the four sediments (Tinker Creek and UTR-029; Table 4). In the 28-day test, survival in the clean Fourmile Branch sediment was 0%, while survival in the reference sediment was >90%. These results indicated that *H. azteca* may not do as well in uncontaminated SRS sediments as in the reference sediment, particularly in long-term exposures. However, the data also suggested that short-term tests (10-day) using survival as the endpoint might be a useful screening tool for assessing sediment toxicity.

**Table 4. Results of Sediment Toxicity Tests on Uncontaminated SRS Sediments, December 1996.**

Location	Test Duration	Survival (%)	Length (mm)
Resurrection Creek	10 days	90%	3.54
FMB Road F	10 days	84%	3.43
Mill Creek	10 days	89%	3.44
Tinker Creek	10 days	93%	3.31*
Seep UTR-029	10 days	79%	3.20*
Resurrection Creek	28 days	91%	5.08
FMB Road F	28 days	0%*	N/A*

\*significantly different from control (p=0.05)

In 1997, 10-day *H. azteca* toxicity tests were conducted on sediments from four locations in Fourmile Branch and 10 seeps that outcrop to Upper Three Runs or Fourmile Branch. Two of the seeps were clean (BG-001 and UTR-029). The remaining 8 seeps had elevated concentrations of metals. Fourmile Branch Road F is upstream from all inputs of contaminated seep water into the stream; Road 4 is downstream from the H-seeps; Road C is downstream from the F-seeps, and Road C-4 is about 1 km downstream from Road C. The results of the tests indicated that three of the SRS sediments had significantly lower survival than the control and that two of the sediments had significantly slower growth than the control (Table 5). Two of the three locations with poor survival were in Fourmile Branch, and both of the locations with poor growth were in Fourmile Branch.

The results of the sediment toxicity tests often differed from the results of aqueous toxicity tests conducted earlier, and some of the most toxic sediments were found in Fourmile Branch, rather than in seeps that were known to have much higher concentrations of metals. Porewater samples were not analyzed for metals, so bioavailability of the metals was not determined. However, the results suggested that factors other than contaminant concentrations probably had more of an effect on the

**Table 5. Results of Sediment Toxicity Tests Conducted on SRS Seep and Stream Sediments, July, 1997.**

Location	Test Duration	Survival (%)	Length (mm)
Resurrection Creek	10 days	88.3	1.99
BG-001	10 days	90	1.81
FMB-Rd F	10 days	82.5	1.91
FMB-Rd 4	10 days	56.3*	2.27
FMB Rd C	10 days	37.5*	1.44*
FMB Rd C-4	10 days	87.1	1.71*
FSP 204	10 days	70	1.95
HSP 008	10 days	88	1.98
HSP 029	10 days	78	1.80
HSP 060	10 days	74	1.97
HSP 103	10 days	86	1.96
FSP 032	10 days	44*	1.96
FSP 102	10 days	88	1.83
FSP 290	10 days	80	2.08
UTR 029	10 days	74	2.35

\*significantly different from control (p=0.05)

viability of the test organisms than did COC concentrations. FMB Road C had very silty sediments and also had the lowest survival and growth, suggesting that small grain size can adversely affected the outcome of the tests. There was also little concurrence between the degree of contamination at a seep and the outcome of a toxicity test. Therefore, it was concluded that sediment toxicity tests probably had little application at SRS, other than possibly as a preliminary screening tool. However, there have been significant advances in methodologies for determining bioavailability of COCs in sediments since the testing was performed in the mid-1990s. Since most of sediment contamination issues at SRS are metals-related, it would be useful to conduct some sediment toxicity tests on sediments that contain elevated concentrations of metals, and measure metal concentrations in porewater, as well as in the sediments. It would also be useful to measure SEM and AVS and calculate SEM:AVS ratios to estimate metal bioavailability. Additionally, performing sediment toxicity tests using a different species might be of value, to compare their performance in SRS sediments to that of *H. azteca*. The literature indicates that *H. azteca* and *C. riparius* are somewhat more tolerant of various sediment types than *C. tentans* (U.S. EPA 2000); therefore *C. riparius* would probably be the more promising species to try. It would also be useful to conduct daphnid aqueous toxicity tests on sediment porewater samples.

The areas of SRS where sediment toxicity testing would be of most use are the F- and H-Area seeplines, and the floodplain of Fourmile Branch adjacent to the seeplines; the Tims Branch/Steeds Pond system; and areas near D Area that have received inputs of coal ash that contain metals. Tims Branch received effluent containing elevated concentrations of metals for many years and there are several depositional areas in the stream channel, including Steeds Pond that contain elevated concentrations of metals in the sediments. The D-Area wetland, located southwest of the ash basins received large deposits of coal ash in the past and has elevated concentrations of arsenic, selenium and other metals associated with the coal ash.

## 5.0 FUTURE DIRECTIONS IN SEDIMENT TOXICITY TESTING

Research is continuing in the areas of: (1) chronic sediment toxicity methods, (2) field validation of laboratory toxicity and bioaccumulation tests, and (3) Toxicity Identification and Evaluations (TIE). Other active research areas include the evaluation of factors controlling the partitioning or sorption of a compound between water, colloids, and sediment including: aqueous solubility, pH, redox, affinity for sediment organic carbon and dissolved organic carbon, grain size of the sediment, sediment mineral constituents (oxides of iron, manganese, and aluminum), and the quantity of acid volatile sulfides in sediment.

If funding becomes available to investigate sediment toxicity issues at SRS, studies related to the effect of organic carbon and AVS on contaminant availability, and the effect of grain size would be useful.

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