Confounding Factors in Sediment Toxicology

David Lapota, PhD and Debbie Duckworth, SPAWAR Systems Center
Jack Q. Word, PhD, MEC Analytical Systems

Introduction

Bioassays, which are often required as part of the ecological risk assessment process as part of the remedial investigation, are demonstrating frequent toxicity that can lead to possibly inaccurate conclusions requiring potentially expensive remedial actions. This frequent toxicity in sediment can often be caused by natural factors termed "false positives" or "confounding factors" such as ammonia, sulfide, or grain size rather than actual contaminants of concern. The objective of this Issue Paper is to evaluate the natural factors that cause toxicity or false positives; to identify, discuss, and optimize methods to measure or eliminate these factors; and to identify and discuss standard and new cost-effective bioassays to conduct sediment toxicity tests.

Environmental managers concerned with sediment assessment, policymakers, and scientists previously have not been, and still may not be, aware of the factors that determine or contribute to confounding factors in sediment assessment. Focused interest in sediment assessment did not really become an issue until the early 1990s. ASTM and EPA published their first sediment bioassay guidelines in 1990. The California State Water Control Board, which sponsors the Bay Protection and Toxic Cleanup Program (BPTCP), is concerned with confounding factors and sediment assessment (Status of the Bay Protection and Toxic Cleanup Program, California State Water Resources Control Board (SWRCB), BPTCP, 1993). Sediment cleanup and dredging activities are by far the largest motivating influence on sediment assessment today and because sediments must be tested for toxicity before removal, new biological –chemical-geophysical information has been generated within the last decade, which impacts the decision-making process for managing sediments. Some of this information focuses on confounding factors, which can give rise to inaccurately characterized sediment and therefore inaccurate information about risk.

Water-quality criteria values are not applicable with respect to sediment quality assessments. Sediments, by their very nature, can contribute significantly to toxicity results, which can not be reduced to numerical values. For example, a reference toxicant used in amphipod bulk sediment tests may produce different toxicity responses depending on the pH, grain size, ammonia, salinity, total organic carbon, porewater volume, and ratio of simultaneously extracted metals/acid volatile sulfide (SEM/AVS). Clearly, there are multiple factors that which contribute to the potential toxicity of sediments. Therefore, each of these confounding factors is discussed and related to what factors a Remedial Project Manager (RPM) should be concerned with when assessing sediment issues. A series of questions, listed in this report, provide a means for RPMs and their clean contractors to address confounding factor issues during the initial phase of any site sediment investigation.

Whole Sediment - Pore Water Issues

Whole sediment tests are the standard when testing sediments for the obvious reason that organisms need to be exposed to the bulk sediment so that effects in survival and growth can be monitored. Water only tests have become standard laboratory practices as 96-hr reference tests. Ammonia is one of the most common reference toxicants used, followed by cadmium. Good control survival in the reference tests indicates that the animals should give a reasonable response when exposed to toxicants. The objective of running concurrent reference tests is to determine the health of the test population by observing the response from potentially confounding toxicants such as ammonia (Kohn et al., 1994; Gardiner et al., 1995).

Porewater is assumed to have common characteristics as the surrounding sediment primarily because of its physical proximity. Pore waters are known to contain elevated levels of ammonia and nitrites, as a result of biological decomposition activities from marine bacteria. Both ammonia and nitrites can be toxic to a wide variety of marine organisms (shrimp, fish, amphipods, and phytoplankton) and are normally found in all marine sediments. Some sediments, high in organic matter with a large percentage of fine grain sizes, contain high levels of ammonia (>2 PPM) while other marine sediments may contain low levels of total ammonia (<0.5 PPM) because of low organic content and large grain size (Middle Loch, Pearl Harbor, Hawaii). Porewaters can act as a pathway for chemicals in the water that eventually bind to the sediment. Some researchers have observed a strong relationship between toxicant levels in porewater and observed mortality in test organisms (Whiteman et al., 1996). There is also evidence that porewater, overlying water, and the sediment can be chemically different from each other which may indicate that porewater may not be in equilibrium with sediment or overlying water (Nipper et al., 1998). Salinity, dissolved oxygen, pH, and sulfide have also been observed to be different in porewater than in overlying water. Yet, others have observed the ability of porewater contaminants to bind to organics and become non-bioavailable resulting in little or no toxicity to test organisms (DeWitt et al., 1997).

Most Common Confounding Factors

Ammonia

Ammonia is usually reported as total ammonia and is the combination of the ammonium ion and un-ionized ammonia. Ammonia in sediments is a direct result of bacterial action on decaying organic matter and is a totally natural process. Because biological material sinks to the benthos, this is a major site for these natural degradation processes to occur. As the nitrifying bacteria degrade nitrogen into ammonia and nitrites, toxicity to other live biota also increases. Subsequently, only the hardiest of animals can tolerate these conditions within the sediments (amphipods, worms). Toxicity tests have been developed around these organisms to measure their response to “other” contaminants of concern while still being tolerant of high ammonia conditions.

The main methods to measure ammonia are either with an ion selective (IS) probe or colorimetrically, measuring total ammonia by raising the pH so that un-ionized ammonia is converted to ionized ammonia before detection. An EPA model (1981) predicts toxicity in total ammonia based upon the percent of unionized ammonia and pH. This model illustrates the co-dependence of ammonia and pH in the sense that as pH changes; the concentration of un-ionized ammonia required to produce an EC50 changes. Various investigators have found that the toxicity of ammonia is dependent on pH and temperature, with the general trend that toxicity increases with increasing pH. The concentration of unionized ammonia increases at higher pH values. Thus far, the only real way to assess ammonia-related adverse effects from marine sediments is to perform an ammonia reference toxicity water only test. By running 4 or 5 dilutions, we can then identify at what concentrations the test organism responds to with some confidence. In practice, you could identify which sediment samples will cause some concern with "false positives" based on ammonia measurements from pore waters or leachates Gardiner et al., 1995; Kohn et al., 1997). Pore water ammonia can confound test interpretation. At issue is whether the observed toxicity is related to the COC's or a naturally occurring compound such as ammonia.

Sulfides in Sediments

The biological effects of sulfide in sediments are poorly understood, yet can be important in determining sediment toxicity to a wide range of organisms (Wang and Chapman 1999). Sulfide is produced by anaerobic decomposition of organic matter and can be an abundant constituent of aquatic sediments. Sulfide has been viewed as more toxic than ammonia under certain conditions. The USEPA fresh- and saltwater quality criterion for hydrogen sulfide is 2 μg/L, whereas that for unionized ammonia is 35 μg/L. Thus, sulfide, may well be more important than ammonia in determining sediment toxicity. Sulfide influences sediment toxicity in three ways: a toxicant by itself, by reducing metal toxicity by forming insoluble metal sulfide solids/or by forming metal sulfide complexes, and by affecting animal behavior, which in turn can alter the toxicity of not just the sulfide but also other sediment contaminants (Wang and Chapman 1999).

Sulfide production occurs I sediments containing large amounts of organic matter once oxygen is depleted by by aerobic bacteria. This occurs a few millimeters to centimeters beneath the sediment surface. In marine sediments, sulfate reduction by sulfate-reducing bacteria is the dominant process which produces large levels of sulfides by the decomposition of organic material. The toxicity of sulfide is pH dependent, as in ammonia toxicity. Sulfide exists primarily as unionized hydrogen sulfide and as sulfide ion. General toxicity thresholds have not been determined for benthic organisms because of the difficulty to obtain a reasonable dose-response relationship. In the first place, sulfide is volatile and oxidized, and because of these features, difficult to maintain a constant concentration during toxicity testing. Lowered oxygen levels generally accompany increased sulfide levels which can in turn contribute as a confounding effect. Still, effects data reveal a strong potential for sulfide to cause toxicity in many sediments as these concentrations are frequently higher in pore waters.

Grain Size

Grain size can add confounding effects through both its chemical and physical properties. For those organisms that require a certain type of substrate, grain size that is either too small or too large can be stressful to animals such as amphipods or worms because it can interfere with its ability to burrow and remain in contact with the sediment, and cause additional mortality. Amphipods are particularly sensitive to grain size and should be exposed to sediments with compatible grain size. Still, a second confounding factor associated with grain size is the availability of contaminants associated with sediment grain size. Fine grain sediments tend to be higher in clay content and contain higher levels of organic carbon, an indication of high bacterial activity. Fine sediments are also often-associated with anoxic, high sulfide, high ammonia conditions as well as elevated metals and organic contaminants associated with the high organic content.

High mortality has been observed in test organisms when fine sediments- are mixed. The mixing of these sediments can cause disassociation of many compounds from the sediment, increasing their bioavailability and toxicity (Ostrander 1996; Lawrence et al., 1997).

TOC

High levels of total organic carbon (TOC) is commonly associated with fine grain sediment. Bacteria feed on organic matter causing a chain of events which include oxygen depletion and elevated levels of sulfide and ammonia. These are natural processes and not to be confused with contaminants of concern, however, they do add to the complexity of natural toxicity from that which is man-derived. Consequently, test animals are not fed during acute toxicity test (10 day) as any added food to the test containers will be a source of increasing TOC which is yet another confounding factor added to the test results. However, when chronic tests are conducted (28 day), feeding is included in the protocol. There also appears to be some evidence that sensitivity to TOC may be species specific (Thompson et al., 1991).

Simultaneously Extracted Metals/Acid Volatile Sulfides (SEM/AVS)

Bacteria in the sediment reduce sulfate into sulfide. The presence of sulfide may be an indication that the TOC levels could also be high, typically resulting in depleted dissolved oxygen (DO) and high ammonia levels. Sulfide does not appear to be highly toxic, in fact, some marine worms (polychaetes) and fish are very tolerant to high levels of sulfide (Powell et al., 1979). Sulfide, because of its association with TOC and metals, has led to the ratio of SEM versus AVS. This ratio, presumably, is a predictor of toxicity by indicating the proportion of bioavailable contaminants. Values of SEM/AVS <1.0 typically show little toxicity in the tests (even when contaminant concentrations exceed the LC50), while values of >1.0 often show toxicity (O'Connor et al., 1998).

Dissolved Oxygen (DO)

DO is obviously important in maintaining a healthy test environment. DO may act synergistically with other toxicants to reduce toxicity by reducing the level of toxicants (eg. Ammonia) or in some cases to increase the availability of metals in

anoxic sediments. Low DO can increase toxic effects when hypoxia becomes an issue (Young-Lai et al., 1991).

Salinity

Salinity can be an important factor in sediment quality because it affects ammonia toxicity. Ammonia appears to be less toxic under higher salinity conditions. It is believed that the increased sodium ions available "out compete" ammonia ions for Na+/NH4 transport sites (Miller et al., 1990). Porewaters may have a different salinity from overlying water, so both should be measured to determine if there might be a potential confounding influence.

Proceed with Caution

Many labs promote ammonia reduction prior to testing by several water changes and they do not like homogenizing the sediment samples prior to testing. The main reason for these views: eliminate potential factors, which will give "false positives." The problem with any field sampling centers around the way sediment samples are collected. Any sampling will disrupt the natural field structure of the sample and will liberate certain compounds throughout the entire sample. This is in contrast to the limited distribution of certain compounds, which probably would not have seen the "light of day" with respect to causing toxicity to our test organisms. Any toxicity test has limitations and introduces problems into our assessment. These laboratory artifacts must be recognized before we start to conduct the collection of the samples and the testing protocols.

Presently, the method for sediment assessments varies widely. While guidelines do exist for conducting toxicity tests with amphipods and worms, all regulatory agencies acknowledge that there are problems with conducting these tests as well as the interpretation of the test results. The choice of test organisms has an important influence on how the test will come out due to species differences and sensitivities to confounding factors and to contaminants.

The Plan – Methods to Address Confounding Factors

The Navy's CERCLA/BRAC Program requires assessment of risk from persistent chemicals of concern contained in sediment. Toxicity testing over-rides can modify interpretation of risk relative to sediment quality criteria guidelines. Sediments can be and have been inappropriately classified as toxic. **Sediments with contaminants of concern that are biologically unavailable but which exceed sediment quality criteria values can be classified as having acceptable risk through appropriately conducted toxicity tests, however, this is often a negotiation point with the regulators.**

Before putting any plan in motion, the environmental project manager must decide what are the appropriate tests to conduct? The tests must address persistent chemical contaminants of potential ecological concern that are greater than trace quantities.

Acceptable Procedures to Address Confounding Factors

The main question to ask is are there acceptable procedures to address potentially confounding factors? We can satisfy this perplexing problem by (1) using the appropriate test species; (2) account for influence by the use of standards; (3) compare effects to test specific reference evaluations; (5) remove the sediment sample and retest; and (6) remove/retest sediment and then replace and retest the sediment. **Do not try and explain away observed toxicity in tests as a confounding factor issue without supporting data; this has not worked in the past and results in agency distrust.**

The steps to address confounding factors are:

(1) **Determine** the specific question being addressed.

(2) **Identify** the potential for confounding factor influence:

(a) Is the sediment in an area with freshwater?

(b) Is there a source of recent organic enrichment?

(c) Is the assessment addressing sediments that are buried deeper than 10 cm?

(d) Is the assessment addressing a deepening project that has sediment that has not been at the sediment/water interface during man's residence in the area?

(e) What is the sediment grain size?

(f) What are the heavy metals of the mineral fraction of the sediment?

(g) Is the assessment evaluating the effects of the persistent COC's in-place?

(h) Is the assessment evaluating the effects of the persistent COC's during removal?

(i) Is the assessment evaluating the effects of the persistent COC's during disposal or placement of the materials at another site?

(3) **Before** sampling occurs address the means of assessing these factors with resource agencies and the interpretation framework that will be implemented.

(4) **Develop** sampling and analysis plan with confounding factors in mind

(5) **Obtain** interpretation framework agreement with resource agencies.

(6) **Perform** tests; **follow** interpretation framework guidelines and **present** to resource agencies.

Suggestion for Successful Toxicity Tests

- Select the appropriate species
- Ship quickly, coolly, and with sediment
- Minimize handling stress
- Avoid chemical contamination
- Avoid temperature or salinity shock
- Acclimate test organisms to test conditions, discard animals that do not meet survival criteria: > 15-20% mortality, discard batch, and send for another
- Feed if necessary during holding and acclimation
- Tests should be conducted within an established time period following animal receipt (2-3 days for *A. abdita*)
- Use the right life stage for testing
- Always run ALL of the necessary controls
- READ THE MANUAL

Controls: The Essential Ingredient

Improper use of controls, which may lead to false conclusions, include the following:

- Using inappropriate controls
- Using controls outside of tolerance limits
- Using reference toxicant control charts that shows mean ±2 SD

Types of Controls

- Population control (negative control) assess general health of test organisms
- Experimental control (negative control) assess response of test conditions
- Carrier controls assess effects of chemical carrier
- Reference-toxicant positive controls (positive controls) assess sensitivity to known toxicants
- Reference treatment "control" assess characteristics of disposal or receiving site.

Example Evaluation of Confounding Factors (from Word, August 29, 1999)

(The following is reproduced in its entirety from the Draft Ecological and Human Health Risk Assessment Work Plan under Contract No. N62474-94-D-7609, U.S. Navy, Engineering Field Activity West, NAVFAC, San Bruno, CA.

The initial step in evaluating confounding factors includes a step-wise progression through a series of 22 questions to assist in isolating the potential for confounding

factors. These questions assist in examining the biological, chemical, and physical laboratory data and the constituent quality assurance and quality control records. The following provides an example of this evaluation.

Question 1. Were there correlations between COPCs and biological effects?

There are three basic ways of evaluating the potential influence of COPCs in sediment. In the first case, correlation of contaminants to biological responses provides a direct assessment of the relationship. In the second case, the concentration of a contaminant is compared to a known effects based concentration in terms of Toxic Units (TUs). The third procedure relies on dosing sediment at various concentrations of a particular contaminant and observing the effects related to that concentration. Each of these procedures have strengths and weaknesses that related to the availability of a particular contaminant and the influence of complex mixtures on increasing or decreasing the level of effect of a particular chemical. For the evaluations that we are pursuing with the Navy's data we have instituted a TU approach to assessing the relationship of COPCs to biological effects. The process uses ER-M values. The ER-M value is the median concentration associated with effects measured at many stations throughout the country. This approach permits an evaluation of the relative level of exceedence of each chemical or by summing the TU's of a mixture of chemicals. The TU's are calculated by dividing the concentration of the chemical (when detected) by the ER-M value (when available).

Question 2. Was the appropriate species selected to address the specific assessment type?

Test species should be selected based upon the assessment question (Questions 3-5) and the behavior of the test organism and its relationship to the available fraction of the COPCs in the appropriate environmental compartment. Improper selection of species reduces the value of the test in predicting the consequences of leaving sediment in place, during its removal or during its disposal and may create a need for selection of a new and more appropriate assessment endpoint.

Question 3. Was the assessment evaluating the effects of COPCs in-place?
Question 4. Was the assessment evaluating the effects of COPCs during removal of sediment?
Question 5. Was the assessment evaluating the effects of COPCs during sediment placement?

The first assessment type seeks to evaluate the availability and effects of COPCs when sediment is left in place (**Question 3**). If sediment is to be left in place the testing scenario should attempt to represent the in-situ conditions of the site as much as possible. This means that sampling and handling procedures need to minimize disturbance of the chemical/physical relationships that have been established in the environment as much as possible. Therefore, collection methods should disrupt sediment as little as possible. Sediment mixing and composting of sediment treatments is not appropriate for this assessment objective. This also means that test species that will be exposed to these

sediments should satisfy the following criteria. They should be likely surrogate organisms for living under the salinity and sediment conditions of the site (i.e., sediment modifications should not occur and test organisms used should have characteristics that permit them to live without sediment adjustments being made). They should also represent the exposure pathways that are present under in-situ conditions. These exposure pathways generally include organisms that live within the sediments and have direct exposure to contaminants within the pore waters and on the buried sediment grains. Organisms that live at the sediment/water interface and are exposed to COPCs that flow by in the water column, near the sediment/water interface, and that are released from the sediment are another category of test organism. The final groups of test organisms are the ones that live in the water column and are only exposed to COPCs in the water. The actual number of these exposure pathways at a particular site may vary depending on the physical limitations imposed on the organisms that might occur at the site. In all cases organisms should be selected based on their tolerance to the conditions of the site and the testing environment should not be modified to accommodate an inappropriate species. Surrogates of each of the exposure types present at the site should be a part of the environmental assessment. Modification of the testing regime to accommodate inappropriate species selection negates the value of this assessment type in two ways. Sediment disruption occurs, maximizing the release of potentially available COPCs, and modification of the sediment regime to accommodate the selected species alters the potential availability of the COPCs. Both attributes strongly influence the outcome of the tests and can provide inaccurate assessments of the risk of leaving sediment in-place.

The second assessment type seeks to evaluate the availability and effects of COPCs that are released during the removal process (**Question 4**). If sediment is to be removed then disturbance of the chemical/physical relationships that have been established at the site is reasonable and acceptable. Therefore, collection methods can be more disruptive of the sediment. Mixing and composting of sediments are entirely appropriate. This means that the test species that will be exposed to these sediments should satisfy the following criteria. They should be likely surrogate organisms for living under the salinity and sediment conditions of the site (i.e., sediment disruption and mixing can occur and test organsisms used should have characteristics that permit them to live under the conditions of the removal site). They should also represent the exposure pathways that are present and most likely influence by the removal process. These exposure pathways do not include the organisms that live within the sediments because they will be removed from the site and lost. Organisms that live at the sediment/water interface and are exposed to COPCs that flow by in the water column, near the sediment/water interface, and that leak from the sediment is a category of test organism that is likely influenced in the areas surrounding the removal area. The final groups of test organisms are the ones that live in the water column and are exposed to COPCs that are resuspended into the water column during the removal process. In all cases, organisms should be selected based on their tolerance to the conditions of the site and the testing environment should not be modified to accommodate an inappropriate species. Again, inappropriate selection of species and modification of the test environment to accommodate those species can reduce the value of the estimation of the risk of removing COPC bearing sediment from a particular location as a result of modifying the availability of the COPC.

The third assessment type seeks to evaluate the the availability and effects of COPCs when sediment is removed and disposed of at another locality (**Question 5**). If sediment is removed and placed at a new location the sediment will be highly disturbed and testing scenarios should attempt to maximize disturbance of the chemical/physical relationships that have been established in the original location. Therefore, collection methods can be highly disruptive. Again, sediment mixing and compositing is entirely appropriate for this assessment objective. This also means that the test species that will be exposed to these sediments should satisfy the following criteria. They should be likely surrogate organisms for living under the salinity and sediment conditions of the disposal site. That is, sediment modifications should occur and test organisms used should have characteristics that permit them to live at the disposal and thus sediment adjustments must be made to accommodate the receiving site. These exposure pathways include organisms that live within the sediments and have direct exposure to contaminants within the pore waters and on the buried sediment grains. Organisms that live at the sediment/water interface and are exposed to COPCs that flow by in the water column, near the sediment/water interface, and that leak from the sediment are another category of test organism. The final group of test organisms is the ones that live in the water column and are only exposed to COPCs in the water during the disposal operation. In all cases organsims should be selected based on their tolerance to the conditions of the disposal site and the testing environment should be modified to accommodate the conditions of the removal site without modifying the test sediments will provide inaccurate estimations of the risk of placing sediment containing COPCs in a new location. A supplement to this type of evaluation is the disposal of sediment in confined facilities on land or in water. Under these conditions the types of biological tests would need to be defined based upon the potential for particular organism types that might come into contact with confined COPCs.

Question 6. What were the acclimation conditions and rates of acclimation for test organisms?
Question 7. What was the mortality to test organisms prior to test initiation (greater than 10% in last 24h)?
Question 8. What were the water quality conditions during the conduct of the test?
Question 9. How variable were the test result replicates? In excess of 30%?
Question 10. How variable were the reference toxicant test results (a factor of 3 or 10?) compared within the lab conducting the test. How similar are the test results compared to other labs.

Answers to these questions relate to the health and sensitivity of the test population prior to initiation of the test. Organisms that are captured in the field and brought to the laboratory are introduced to a variet of stresses that they do not normally encounter. Testing stressed populations can increase the sensitivity of the population and result in adverse biological effects that are greater than would have occurred if the organisms were in an unstressed condition prior to testing. To limit these types of stresses, handling protocols have been established as well as assessment methods indicated prior to use of a test population. These include standard methods of collecting the test organisms that

vary depending on the species being collected and handling of the organisms during capture and shipping to test labs. They also include acclimation schedules that limit the degree of change that test organisms are exposed to during preparation for testing conditions. The success of these procedures is then initially assessed just prior to testing to assure the analyst that they have reduced the chance of using stressed organisms during test initiation (<10% mortality observed within 24 hours of test initiation).

Tests that do not follow these methods run the risk of having increased population sensitivity with increased mortality and abnormalities appearing in test treatments. Lack of adverse biological effects resulting from compromised collecting, handling, and acclimation procedures do not negate the test results. In fact the lack of adverse biological effects occurring in stressed organisms indicates an even lower risk associated with COPCs in sediment. If adverse biological effects are identified in samples where collecting, handling, or acclimation procedures are compromised, the value of these results in evaluating risk is also compromised and resampling and analyses should be considered.

Test organism collection and handling prior to use on any program is difficult to evaluate. It is based on estimating the mortality or behavior of test organisms shortly before test initiations. This is a part of the art of toxicity testing and relies on the laboratory staff to notice aberrant behavior or excess mortality in holding tanks. Since toxicology labs often deal with variable numbers of test organisms for particular test setups the estimation or pre-mortality conditions under holding conditions is difficult and open to error during the estimation process. Often the pre-test mortality is not completely known until after a test has been set-up and all organisms in the holding tanks are used or accounted for. Aberrant behavior is more easily noticed by experienced personnel than increased mortality but it is still a non-quantitative assessment of the health of the initial population of test organisms. Another factor that influences the decision by laboratory staff is that deciding not to initiate a test on a specific date because of the apparent health of the test organisms is a hard decision to make. It necessitates delaying a program that is often on a tight time schedule and can mean the purchase and acclimation of a new batch of test organisms. Both create cost and time delays that are difficult to support with hard data. It is rare that a test will be delayed due to perceived health of test organisms but we may see the result of their use with increased variability of response as well as greater than expected mortality. This hindsight is often perfect but not very useful when deciding whether to conduct a test or not.

Acclimation of test organisms is an easier procedure to document. Standard practices are that temperature should not change by more than 3°C/day and salinity changes must be less than 5 percent /day. Acclimated organisms should then be held for a minimum of two days under these conditions prior to their use. A laboratory that reduces the time for these acclimation schedules runs the risk of testing more sensitive organisms. Survival of test organisms in native control sediment and measurement of the sensitivity of a test species to a reference toxicant provides an indication of the relative sensitivity of the test population. Successful survival in the native control sediment is a necessity but it does not always indicate that the population was completely healthy. It simply means that

under the most ideal conditions the test organisms chosen for testing (the better appearing organisms available) will survive at a high level (generally less than 10% mortality over the test period).

Comparison of the control chart limits for reference toxicant exposure within a laboratory indicates whether that laboratory consistenly handles the test organisms (narrow control limits) but it does not indicate whether Lab A would have a different result than Lab B. Multiplying the standard deviation of the response by two and adding and subtracting that amount from the mean creates the control chart ranges within a laboratory. Typical control chart ranges for reference toxicant responses are generally <~3-fold, indicating a standard deviation with a 25% coefficient of variation. Ranges of 10-fold or more should be viewed with concern that the populations being tested are either naturally more variable (e.g., seasonal trends in sensitivity) or that the organisms are not being handled in a consistent manner. These larger test ranges also do not indicate whether an increased sensitivity brought about acclimation rates caused marginal responses to become greater during the testing of sediment COPCs or CFs. A challenge toxicity test is often used in drug efficacy studies to determine the ability of a drug to aid stressed organisms. If acclimation schedules are not performed in accordance with standard protocols and increased range of replicate responses (range in excess of 30%) are noted then one of the expected CFs in these types of data are increased sensitivity due to internal (seasonal effects) or external stresses than can result from the handling of test organisms.

Question 11. Was the sediment near a source of freshwater? (Surface or groundwater).
Question 12. What is the interstitial water salinity concentration? (Before sediment compositing and mixing, after compositing and mixing and just before the test initiation, during and at the end of the test)
Question 13. What is the interstitial water ammonia concentration? (Before sediment compositing and mixing, after compositing and mixing and just before the test initiation, during and at the end of the test)
Question 14. What is the interstitial water sulfide concentration? (Before sediment compositing and mixing, after compositing and mixing and just before the test initiation, during and at the end of the test)

These four questions relate to the potential influence of these non-persistent contaminants on the survival of test organisms. All three of these water quality characteristics (salinity, ammonia, and sulfides) can individually or in combination cause adverse biological effects if the parameters exceed critical no observable effects concentrations (NOEC). They may be natural components of the sediment or they can also be produced in the laboratory during handling of the sediment.

If the sediment is near a source of freshwater, the interstitial water salinity concentrations may be very low. The source of the freshwater may be rivers and streams, effluent discharges, or even underwater seeps of freshwater from submerged aquifers. Proximity to known sources of fresh water cannot always be ascertained accurately, especially with underwater seeps. Therefore, the only sure way of determining the salinity of interstitial

water is to measure that characteristic upon collection of the sediment. If the interstitial water salinity is different than the proposed test conditions then additional measurements should be made. These measurements should be made after appropriate compositing and mixing, just before test inititiation, during the test and at the end of the test. This will document any changes to the interstitial water environment during handling of the sediment. These measurements may also reveal potential causes for increases in ammonia or sulfides due to disruption in the indigenous populations of microbes that normally handle the processing of these material to less toxic forms.

Modifying the salinity of the interstitial water to accommodate selected test species may be necessary to evaluate potential effects at a disposal site. This needs to be done carefully while addressing other CFs that may be created as a result of modifying the salinity of the interstitial water environment. Salinity should not be modified to assess the effects of COPCs that may be present in sediment when the proposed option is to leave that sediment in-place. It should also not be modified to evaluate the influence of disturbed COPCs during the process of removing the sediment from the site. Instead, the appropriate species that can accommodate the salinities in these environments should be selected. Inappropriate selection of species or modification of the sediment to accommodate those species will compromise the potential value of these data.

Biological processes mediated by bacteria, influence ammonia and sulfide production. In a well-balanced aquatic system the direct release of ammonia of the production of ammonia are parts of the nitrogen cycle that regenerate nitrogen for plant uptake from biological waste products. In this well-balance system the bacterial populations are capable of altering the available ammonia as it is being produced into relatively less toxic nitrites and nitrates. Unbalance systems are not as capable of this process and as a result ammonia will increase in concentration, often to very high levels that are toxic to many different species. This imbalance can occur naturally. Examples include:

-Seasonally flushed water bodies that push large quantities of organic material into environmental collection locations (organic oversupply and the indigenous microflora populations cannot handle the increased supply of organic materials).

-Areas that are typically fresh or marine in origin and then inundated by water of a different salinity (seasonal cycles of flow require microbial populations to shift from forms with different salinity tolerance).

-Aquatic sediment environments near river discharges, which are tidally influenced by contact with both fresh and marine water (salinity disturbed microbial community interactions).

-Sediment samples from deep cores that do not have a recent history of contact with biogenic zones and microbial populations that regenerate nitrogen (atypical microbial community replacements).

This imbalance may also occur as a result of laboratory manipulation of environmental samples. In the simplest case, sediment that has a microbial based community that is fresh water dependent when placed under marine conditions will die and increased ammonia concentrations will occur. Ammonia increases will then continue until the marine microbial community has replaced the fresh-water based community that was killed. This process occurs over extended periods of time and can take up to 6-8 weeks to complete. As a result, typical bioassays which are performed on sediment or elutriates of sediment that has had modified salinity conditions will still have unbalanced bacterial communities and elevated ammonia concentrations are to be expected. These artifacts of laboratory processes need to be evaluated during the data review.

Question 15. Was there a source of recent organic enrichment?

Question 16. Were the sediments collected from sediment depths in excess of 10 cm?

Question 17. Were the sediments being evaluated collected from sediment depths not at the sediments surface during mans residence in the area?

These questions relate to the available quantity and quality of organic food materials contained in sediment and to the distribution of those organic materials different vertical horizons within the sediment. Sediment generally contains sufficient quantity of high enough quality detrital food materials to support the test organisms that are used. We have discovered in some instances, however, that the amount of useable detrital foods is insufficient to maintain certain test organisms, especially over longer testing periods. Examples of these include *Nephtys caecoides*, a sedimet dwelling and engulfer polychaete, when exposed to low TOC environments (<0.1%) from deeply buried sediment. Other examples include longer-term tests that require supplementation of additional sediment or food to maintain the test population (bioaccumulation tests with *Macoma nauta* and *Nephyts caecoides* or long term chronic tests using the amphipods – *Leptocheirus plumulosus* or *Ampelisca abdita* or the polychaete – *Neanthes arenaceodentata*). Low survival in sediment with low TOC (e.g., <0.5%) and low concentration of COPCs should be viewed with suspicion, especially in tests that ≥10 days in length.

It is also recognized that buried sediment can produce ammonia and sulfides that are not conducive to the survival of test organisms. When testing sediment that is buried to greater than typical biogenic depths there are also problems related to the quality of organic material and microbial communities that can efficiently process ammonia or sulfides during the test period. Sediment that is buried beneath the typical biogenic zone of ~10 cm may not have a sufficient microbial stocking density to handle the production of these materials when sediment treatments are initiated. Measurement of interstitial and overlying water concentrations of ammonia and sulfides can establish whether the production of these compounds occurred at sufficient concentrations to influence test results. If test results are influenced by these factors then the degree of effect needs to be addressed.

If there is a source of recent organic enrichment, either naturally from plankton bloom collapses, river discharges, seasonal cycling of plant materials in estuary systems or from constructed sources of effluent discharges, habitat destruction, etc. then there are generally sufficient supplies of organic materials. However, an oversupply of organic material can overwhelm microbial populations that are used to a specific loading rate. As a result, increased organic materials may cause increased ammonia and sulfide production in test containers until the microbial populations have attained population levels that can handle the supply of organics. Therefore, while the organic foods for test organisms are sufficient, the oversupply of organics may result in increased ammonia and sulfides that will influence test organism survival. Measurement of interstitial water ammonia and sulfide concentrations during the conduct of the test should reveal potential problems associated with organic imbalance with microbial populations.

If the sediment comes from deeper depths the organic materials may be relatively unavailable to organisms because the available fractions may have been used by organisms when the sediments were near the sediment water interface or by anaerobic organisms over many years of burial. North San francisco Bay sediments have buried peat layers that have high TOC content. The peat however, does not seem to have much nutritive value for test organisms and in fact seems to contribute to the ammonia related effects during testing. Monitoring interstitial and overlying water ammonia concentrations addresses the issue of microbial population imbalances. The nutritive value of peat in conjunction with more recent supplies of organic materials at the surface of the sediment has not been adequately addressed. However, the presence of peat in samples of sediment that is being used for toxicity testing should be indicated.

Question 18. Were the sediments being evaluated highly compact?
Question 19. What was the sediment grain size?
Question 20. Were sharp angles present on sediment grains?

These are all persistent features of sediment that are not directly related to COPCs but which have the potential for creating adverse biological effects. Highly compact sediment that has very low sediment water content limits the ability of infaunal test organisms to burrow. This will occur naturally in the field if these sediments are near the sediment-water interface as well as in the laboratory. The choice of modifying the sediment to meet the needs of test organisms or selecting a test organism that woud not be effected by this factor relates to the question that is being asked of these data. It is entirely appropriate to test organisms that reside on the surface of these materials and which do not need to burrow into the substrate. This type of assessment goal can establish the likely biological impact that might occur as a result of exposure to COPCs in this material at a disposal site or during the process of sediment removal.

Question 21. Was the sediment elutriate cloudy to preclude observations of test organisms during the test or during post-test assessments?

A non-persistent feature created by the amount of low density, fine-grained sediment materials. This feature interferes with observations made during daily water quality

monitoring and can also interfere with measurements and counts made after completion of the test. Lack of observation ability during testing is permitted to be adjusted by centrifugation to remove suspended particles, which interfere with behavioral observations. While this is not addressed in terms of larval tests (since larvae are microscopic) the suspended materials can still have an effect. The CF effects most likely encountered in elutriate tests include fouling of test organism cilia and the resultant loss from the water of developing larvae and inability of counting and seeing larvae in flocculated masses of larval cells and suspended particulate matter after settling. In both cases, the influence of excess suspended materials is sufficient to interfere with the interpretation of adverse biological effects.

Question 22. What were the heavy metal concentrations in the mineral fraction of the sediment and the bioavailable fraction?

This question relates to a persistent feature of the sediment that can be interpreted to have a potential for an adverse biological effect. The concentration of heavy metals in the mineral fraction of sediments is not a direct reflection on the bioavailable fraction of metals in sediment. Sediment quality guidelines (such as ER-L, ER-M, severe effect level [SL], minimum effect level [ML], and apparent effect threshold[AET], etc.) have been obtained using a variety of extraction techniques. These techniques range from full acid digestion of the internal matrices of sediment to weaker acid digestions of sediment that do not attack the mineral matrices of the sediment. These are not equivalent digestion techniques and as such guidance values based on a mixture of these multiple methods of extraction have marginal value in establishing no or lowest observable effects levels. Observations of exceedences of sediment guidance values should be set aside when these levels are not coupled with adverse biological effects.

Table 1 Recommended Protocol Modifications to Minimize False Positives Resulting from Interfering Factors for Tests of the Effects of Contaminated In-Situ Sediment

Interference	Solid-Phase Tests
Disturbance	*Minimize sediment manipulation
Salinity	*Measure interstitial water salinity
	*Use species that are tolerant to salinity in-situ
	*Compare survival to established dose/response curves. Only consider mortalities that are not accounted for by salinity

	*Use species that are not exposed to interstitial water
Ammonia	*Measure interstitial water prior and during test
	*Temperature increases during sample collection and holding should be avoided
	*Ammonia reference toxicant tests should be performed on same population at same time as testing
	*Manipulate ammonia in interstitial water by NOEC in overlying water by waiting until NOEC is attained
	*Manipulate ammonia in interstitial water by EPA approve protocol *2-exchanges/day until NOEC is attained)
	*Use test organisms that are less intimately associated with interstitial water
	*All measurements of ammonia should include measurements of temperature, salinity, and pH in the sample
Grain Size	*Compare established dose-response curves. Only consider mortality that exceeds that relationship
	*Use more tolerant species
	*Compare to reference
Hydrogen Sulfide	*For all tests, analytical difficulties confound separating the effects of sulfide from anoxia. Procedures should be developed to measure hydrogen sulfide and its potential

	effects in interstitial and overlying water for both toxicity tests and for benthic community evaluations
Storage	*Test storage should be minimized in order to reduce the potential for introduction of sediment changes that will influence test organisms survival

Points-of-Contact

SPAWAR Systems Center San Diego
Environmental Quality Branch, Code D362
53475 Strothe Road
San Diego, CA 92152-6310
Phone (619) 553-2798
Email: lapota@spawar.navy.mil

References

(DeWitt, T.H., Swartz, R.C., Hansen, D.J., Berry, W.J., McGovern, D., 1997, "Interstitial Metal and Acid Volatile Sulfide Predict the Bioavailability of Cadmium During a Full Life Cycle Sediment Toxicity Test Using the Estuarine Amphipod *Letocheirus plumulosus*," Batelle Marine Sciences Laboratory, 1529 West Sequim Bay Rd., WA 98382.

Gardiner, W.W., Antrium, L.D., and Word. J.Q., 1995, "An Evaluation of Ammonia Toxicity in Suspened-Particulate Phase Testing," Poster Presented at SETAC 16th Annual Meeting, Vancouver, British Columbia, Canada, November 5-9, 1995.

Kohn, N.P., Word, J.Q., Niyogi, D.K., Ross, L.T., Dillon, T., and Moore, D.W., 1994, "Acute Toxicity of Ammonia to Four Specie of Marine Amphipod," Mar. Environ. Res. 38(1): 1-5.

Kohn, N.P., Barrows, E.S., Word, J.Q., Gruendell, B.D., Rosman, L.B., 1997, "Ammonia levels in Sediment Toxicity Tests with the Marine Amphipods *Ampelisca abdita* and *Rhepoxynius abronius*," Battelle Marine Sciences Laboratory, 1529 West Sequim Bay Rd., WA 98382.

Lawrence, C., Duh, D., Myers, J., and Pallop, T., 1997, "The Effects of grain Size and TOC on Marine Amphipods in Whole Sediment Bioassays," SETAC, 18th Annual Meeting, IT Corporation, 2200 Cottontail Ln, Somerset, NJ, 08873.

Miller, D.C., Poucher, S., Cardin, J.A., and Hansen, D., 1990,"The Acute and Chronic Toxicity of Ammonia to Marine Fish and a Mysid," Archives of Environmental Contamination and Toxicology 19(1): 40-48.

Nipper, M.G., Roper, D.S., Williams, E.K., and Martin, M.L., 1998, "Sediment Toxicity and Benthic Communities in Mildly Contaminated Mudflats," Environmental Toxicology and Chemistry 17(3): 502-510.

O'Connor, T.P., Daskalaskis, K.D., Hyland, J.L., Paul, J.F., and Summers, J.K., 1998, "Comparisons of Sediment Toxicity with Prediction Based on Chemical Guidelines," Environmental Toxicology and Chemistry 17(3): 468-471.

Ostrander, G.K., 1996, "Techniques in Aquatic Toxicology, Assessment of Sediment Toxicity at the Sediment-Water Interface," Techniques in Aquatic Toxicology 33: 609-624.

Powell, E.N., Crenshaw, M.A., and Rieger, R.M., 1979, "Adaptations to Sulfide in the Meiofauna of the Sulfide System. 1. ^{35}S-Sulfide Accumulation and the Presence of a Sulfide Detoxification System," Journal of Experimental Marine Biology and Ecology 37(1): 57-76.

Status of the Bay Protection and Toxic Cleanup Program, California State Water Resources Control Board (SWRCB), BPTCP, 1993; Workplan for the Development of Sediment Quality Objectives for Enclosed Bays and estuaries of California, Lorenzato, S.G., Wilson, C.J., 1991, Water Resources Control Board, California, P.O. Box 100, Sacramento, CA 95812, 91-14-WQ.

Thompson, B., Bay, S., Greenstein, D., and Laughlin, J., 1991, "Sublethal Effects of Hydrogen Sulfide in Sediments on the Urchin *Lytechinus pictus*," Marine Environmental Research 31(4): 309-321.

Wang, F. and Chapman, P.M., 1999, "Biological Implications of Sulfide in Sediment – A Review Focusing on Sediment Toxicity," Environmental Toxicology and Chemistry 18(11): 2526-2532.

Whiteman, F.W., Ankley, G.T., Kahl, M.D., Rau, D.M., Bulcer, M.D., 1996, "Evaluation of Interstitial as a Route of Exposure for Ammonia in Sediment Tests with Benthic Macroinvertebrates," Environmental Toxicology and Chemistry 15 (5): 794-801.

Young-Lai, W.W., Charmantier-Daures, M., and Charmantier, G., 1991, "Effect of Ammonia on Survival and Osmoregulation in Different Life Stages of the Lobster *Homarus americanus*," Marine Biology 110:293-300.