Massachusetts Department of ENVIRONMENTAL PROTECTION

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Freshwater Sediment Toxicity Tests

Update to: Section 9 of *Guidance for Disposal Site Risk Characterization – In Support of the Massachusetts Contingency Plan* (1996)

Introduction

Sediment toxicity tests are an important tool in a Stage II ecological risk assessment conducted as part of a Method 3 risk characterization under the Massachusetts Contingency Plan (MassDEP 1996). The objective of sediment toxicity tests is to determine whether sediment is harmful to benthic organisms. The tests measure interactive toxic effects of complex contaminant mixtures in sediment. Toxicity tests offer a direct method for assessing the toxicity of sediment contaminants.

A number of standardized and widely applied toxicity tests are available for evaluating toxicity to benthic organisms. Acute and chronic tests measure mortality and growth. In acute tests reproductive effects are not measured. The most commonly used test methods are published by the Environmental Protection Agency (USEPA 2000). These include acute and chronic tests using an insect, *Chironomus tentans*, and an amphipod, *Hyalella azteca*. These tests are described below.

Types of Toxicity Tests

USEPA (2000) describes four sediment toxicity test protocols:

- Test Method 100.1: *Hyalella azteca* 10-day (acute) Survival and Growth Test for Sediments
- Test Method 100.2: *Chironomus tentans* 10-day (acute) Survival and Growth Test for Sediments
- Test Method 100.4: *Hyalella azteca* 42-day (chronic) Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction
- Test Method 100.5: Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans*

These four test protocols are widely used for sediment toxicity testing. Brief descriptions of each of these tests are presented in attachment 1. These protocols are also discussed extensively in California EPA (2003).

For MCP risk assessments that include sediment toxicity testing, it has been common practice to conduct sediment toxicity tests using only one test organism, most often Hyallela azteca. Typically, short-term (acute) tests are used. This technical update recommends changes in toxicity testing practices for MCP risk assessments. Longer-term (chronic) tests are strongly recommended unless toxicity is clearly demonstrated using shorter-term tests. Further, for sites where contamination is relatively severe and/or widespread, toxicity tests with two different test organisms are recommended.

Recommendations for Sediment Toxicity Testing

<u>Test Length</u>.

Recommendation

MassDEP recommends that longer-term (chronic) toxicity tests be conducted at sites to evaluate the potential effects of sediment contamination. Chronic tests include reproductive endpoints as well as growth and survival. MassDEP considers reproductive endpoints ecologically significant. Reproductive endpoints may be more sensitive to some contaminants than growth and mortality endpoints, and therefore a test evaluating only growth and mortality may not be protective of reproductive effects. MassDEP recommends that chronic EPA protocols be run for their full test length so that reproductive endpoints can be evaluated unless there is evidence that reproduction is not the most sensitive endpoint for the contaminants at a site, or significant mortality and/or growth impairment are observed before the end of the 42 day test period.

Acute tests may be adequate for determining that there is significant risk at a site, but they are no longer considered adequate for demonstrating no significant risk. A risk assessor may opt to conduct an acute test initially, but if an acute test does not demonstrate toxic effects, follow-up with a chronic test would likely be appropriate. Given that a condition of “no significant risk” can only be demonstrated using chronic tests, it may be cost effective to proceed directly to chronic tests.

Discussion

While MassDEP strongly recommends the use of chronic toxicity tests, it is important to recognize that chronic tests do have certain drawbacks. Longer test periods used in chronic toxicity testing offer greater opportunities for random physical, chemical and biological factors to affect test results (Rand 1995). Therefore, results from chronic tests may be expected to be more variable (larger coefficients of variation than results from acute tests). Another drawback is the cost of chronic tests. Acute tests have the advantage of being cheaper and allow for collection of a greater amount of toxicity data for the same budget. In spite of the higher costs and variability associated with chronic tests, MassDEP believes that on balance chronic tests provide more definitive and reliable results, and are likely to be more cost effective in the long run. Chronic exposures more closely approximate the types of exposures for organisms in natural environments. Acute tests may fail to detect toxicity at a site where a longer chronic test may definitively determine there is ecological risk from exposure to contaminated sediments. In our experience acute test results have not shown a strong correlation with contaminant levels. The general consensus among regulatory agencies consulted has been that chronic tests tend to more reliably correlate with levels of contamination. Considering the strengths and weaknesses of both acute and chronic tests, the latter are strongly recommended.

<u>Test Organisms</u>

Recommendation

Chironomus tentans and *Hyallela azteca* are the two most common test species for freshwater sediment toxicity tests. MassDEP recommends that toxicity tests for two species generally be used to evaluate toxicity at a site. There are two types of situations that are exceptions to this rule:

- Sites with contaminants for which there is reliable information on the relative sensitivities of the two main test species.
- Small or moderate-sized sites with low to moderate levels of contamination.

In either of these cases, one species would be considered sufficient. The decision diagram in Figure 1 depicts the decision process for identifying the appropriate

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number of tests (test species) based on site size and contaminant concentrations. A discussion of the decision criteria in the decision diagram follows.

Relative Sensitivities:

The relative sensitivity of *C. tentans* and *H. azteca* appears to vary from chemical to chemical. For example, chironomids are more sensitive to organophosphate and carbamate pesticides than amphipods, so it would be appropriate to choose *C. tentans* over *H. azteca* as a test species for those contaminants. Amphipods appear to be most sensitive to cationic metals and somewhat more sensitive to PAHs (Ankley, 2005), so it would be more appropriate to use *H. azteca* for these.

Size Criterion for Determining the Number of Test species:

For sites with extensive contamination, two tests are recommended. Two tests would be warranted at any site with contamination dispersed over an area greater than 10,000 square feet. When only one species is used, there is a substantial likelihood of failing to detect significant toxic effects. At large sites, the level of uncertainty inherent in using only one test is not acceptable. MassDEP believes that the cost of an additional test is justified when contamination is widely distributed.

Concentration Criterion for Determining the Number of Test Species:

MassDEP recommends using two test species for sites with high levels of contamination. Only one test is necessary for sites with moderate levels of contamination. For the purpose of this decision, high levels of contamination should be determined by reference to the Probable Effects Concentration (PEC, MacDonald et al. 2000) for each contaminant of concern. Two species should be used when either of the following criteria is met:

- Mean site concentrations averaged over no greater than 1,000 square feet exceed two times the PEC; or
- The maximum detected concentration exceeds five times the PEC.

Because it is difficult to establish hard and fast guidelines that are well suited to every situation, some discretion may be used in the decision to test only a single organism. If only one organism is used under conditions that would generally warrant two tests (i.e. high concentrations or large contaminated area), the risk assessor should present a very compelling case based on technical and/or practical considerations or limitations.

Hyallela azteca and *Chironomous tentans* are currently the most commonly used organisms for sediment toxicity testing, and are generally acceptable for MCP purposes. Other organisms, however, may be more sensitive indicators of toxicity at a site, considering the contaminants of concern, the nature of the site, and the emergence of newer information on relative toxicity in the scientific literature. The risk assessor may opt to use different organisms based on professional judgment supported by a strong technical justification for the choice. Relative sensitivity should be the main factor considered in the selection of test organisms. The known (or expected) presence of the test species in the indigenous invertebrate community at the site should not necessarily be a major consideration. In MCP risk assessments, toxicity test results from one or two test species are used as indicators of the potential for toxic effects on numerous indigenous species, the vast majority of which are never tested. Using the species most sensitive to the contamination will reduce the uncertainty about whether the testing program will detect significant effects on other invertebrates. Nevertheless, while other species may be more sensitive, *H. azteca* and *C. tentans* are acceptable as default test organisms.

Discussion

MassDEP performed literature searches but was unable to identify a comprehensive study or survey paper that compares the relative sensitivities of *C. tentans* and *H. azteca* for various chemical classes. Discussions with sediment toxicity testing experts also did not identify a comprehensive study. Phipps et al. (1995) tested five pesticides and five metals and found that the chironomid was generally more sensitive to the pesticides and the amphipod was generally most sensitive to the metals. In cases where only one species is tested, MassDEP recommends that the

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risk assessor collect additional sediment at all sampling locations. Samples that do not show toxicity for the first species can then be tested to determine if they are toxic to a second species.

Test Length vs. Number of Tests

Recommendation

For some small sites it may be appropriate to consider a tradeoff between length of test and number of species tested. In such cases, DEP recommends conducting chronic tests on one organism rather than acute tests on two. As stated above, if only one organism is used, the risk assessor should present a compelling case based on technical and/or practical considerations or limitations.

Discussion

For small sites, some sediment experts recommend conducting acute tests in two species rather than a chronic test in a single species. They believe that interspecies differences may be more important than acute versus chronic exposure differences, and that if something is sacrificed, it should be test length rather than number of species. However, two acute tests are significantly more expensive than a single chronic test, and a number of other experts believe that conducting a chronic test is critical. Further, all sediment experts that we consulted believed that chronic tests (28-days and longer) should be conducted whenever possible. Therefore, MassDEP recommends at small sites a risk assessment should include a chronic test in one species rather than acute tests in two species.

Minimum Number of Samples for Toxicity Testing

A sufficient number of samples should be collected and submitted for toxicity testing to allow for analysis of the spatial heterogeneity of toxicity at a site. The number of toxicity testing samples at a site will be determined by the size of the area of contamination, the variability in levels of contamination across that area, and variability in sediment properties such as grain size and percent organic carbon.

Too often a single reference sample is submitted for toxicity testing and no estimate is made of natural heterogeneity in the reference area. MassDEP recommends that at a minimum, sediment toxicity tests should be conducted for three sample locations in a reference area. Further, if the site encompasses separate areas with different types or levels of contamination, three samples should be collected from each area.

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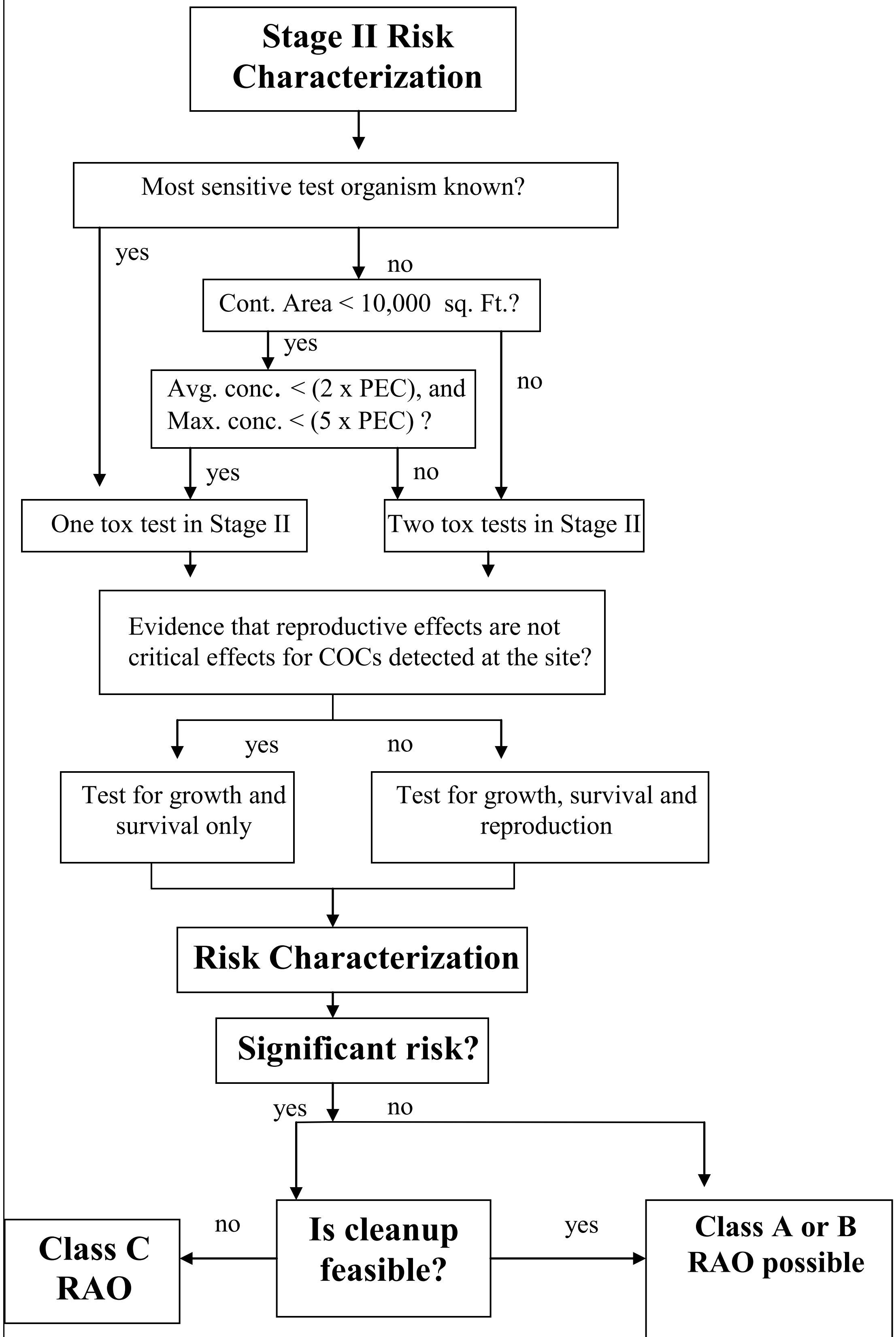
Sediment Toxicity Testing Decision Diagram

Number of Test Organisms

Figure 1



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Attachment 1
Description of Sediment Toxicity Test Protocols (USEPA 2002)

Test Method 100.1: *Hyalella azteca* 10-day Survival and Growth Test for Sediments

Hyalella azteca is a freshwater amphipod species that inhabits lakes, ponds, and streams throughout North America. It is an epibenthic detritivore that burrows into the upper two centimeters of sediment. It is tolerant of a wide range of physico-chemical conditions (e.g., variations in grain size, TOC, and conductivity), but is sensitive to chemical contaminants. The 10-day test with H. azteca has endpoints of survival and growth. The protocol requires eight replicates for each sample with ten 7 to 14-day old amphipods. The amphipods are exposed to 100 mL of sediment in 300 mL beakers, with 175 mL of overlying water at a temperature of 23 ^{o}C. The overlying water is renewed twice daily and 1.0 mL of food is added to each test container. After 10 days, the amphipods are removed by sieving, and the number of surviving animals is recorded. Growth is measured as dry weight per individual amphipod, or as length per animal. Studies have shown that growth is often a more sensitive indicator than survival. For the test to be considered valid minimum mean control survival must be 80% and growth of test organisms in the control sediment must be measurable.

Test Method 100.2: *Chironomus tentans* 10-day Survival and Growth Test for Sediments

The midge, *Chironomus tentans* is a dipteran fly species whose larval and pupal stages are found in eutrophic ponds and lakes in northern latitudes. The life cycle of *C. ten*tans can be divided into four stages: (1) an egg stage (3 days in length), (2) a larval stage, consisting of four instars (18 days in length total), (3) a pupal stage (3 days in length), and (4) an adult stage (3-5 days in length). The larval stages of *C. tentans* occur in the upper few centimeters of sediment, and are tolerant of a range of physico-chemical parameters, but are relatively sensitive to chemical contaminants. The relatively short life cycle of this species makes it suitable for both short-term and long-term tests.

The 10-day *C. tentans* test is conducted using 10 second and third-instar larvae (less than 10 days old). The test conditions, containers, sediment, and overlying water are the same as those used for the *H. azteca* test. The larvae are fed 1.5 mL of food daily. During the test the larvae burrow into the sediment and construct tube cases. They feed on particulate material drawn into either end of the open-ended tubes. After 10 days, the surviving animals are sieved from the sediment, and survival is recorded. Growth may be measured as length or dry weight. For the test to be considered valid, minimum mean control survival must be 70%, with minimum weight/surviving control organism of 0.48 mg.

Test Method 100.4: *Hyalella azteca* 42-day Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction

In addition to the acute 10-day test that measures survival and growth, there is a chronic test for *H. azteca* that evaluates a reproductive endpoint, in addition to survival and growth. The chronic test follows a procedure similar to the acute test. The primary differences are that the chronic test is initiated with 7 to 8-day old amphipods, and the test starts with 12 replicates per sample rather than eight. On day 28, four of the replicate chambers are sacrificed and growth and survival are measured. The remaining eight replicate chambers are used to evaluate reproduction. Offspring are typically released between day 28 and day 42. On day 28, amphipods from the remaining eight replicates are transferred to beakers containing clean water (no sediment) so that released young may be counted. Water is renewed twice daily and amphipods are fed once per day. Growth can also be measured in these remaining eight replicates. Minimum mean control survival of 80% on day 28 is required for the test to be acceptable. This protocol can also be used for in situ toxicity tests.

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Test Method 100.5: Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans*

The life cycle test with *C. tentans* is started with larvae less than 24 hours old, and continues through emergence and reproduction of the adults, and hatching of the F1 generation. The test incorporates a lethal and several sublethal endpoints: survival (20-day), growth (20-day), emergence (23-day and on), and reproductions as number of eggs per female (23-day and on), and percent hatching success (23-day and on). Survival is determined at 20 days and at the end of the test (as long as 60 days for the hatching of F1). From day 23 to the end of the test, emergence and reproduction are monitored daily. The number of eggs in each egg case is determined. Egg cases are incubated for six days to determine hatching success. Each treatment of the life cycle test is ended separately when no additional emergence has been recorded for seven consecutive days. The test is started with sixteen replicates, each with twelve larvae. Four replicates are used for the 20-day survival and growth endpoints, and eight replicates are used for determination of emergence and reproduction. Because C. tentans males typically begin emerging 4 to 7 days before the females, additional males are needed during the prime female emergence period. The additional males are provided by the four additional replicates. Emergent insects are removed from the chambers and are paired for mating. Females usually oviposit a single primary egg case within one day of fertilization. Hatching success is quantified after six days of incubation. In order for the test to be acceptable, the average size of *C. tentans* in the control sediment at 20 days must be at least 0.6 mg per surviving organism. Pupae survival in control sediment should be >83% and the adult survival should be >96%. Mean time to emergence in controls should be <6.5 days for males and <5.1 days for females. The mean number of eggs per egg case in control sediment should be $\geq$800 and the percent hatch should be $\geq$80%.

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