

1 **Comparison of Bulk Sediment and Sediment Elutriate Toxicity Testing Methods**

2 Herman J. Haring¹ · Mark E. Smith¹ · James M. Lazorchak² · Philip A. Crocker³ · Abel Euresti⁴ ·
3 Melissa C. Wratschko¹ · Michael Schaub³

4

5 ¹The McConnell Group, c/o U.S. Environmental Protection Agency, Office of Research and
6 Development, National Exposure Research Laboratory, 26 W. Martin Luther King Drive,
7 Cincinnati, OH 45268

8 ²U.S. Environmental Protection Agency, Office of Research and Development, National
9 Exposure Research Laboratory, 26 W. Martin Luther King Drive, Cincinnati, OH 45268

10 ³U.S. Environmental Protection Agency, Region 6, Watershed Management Section, 1445 Ross
11 Avenue, Dallas, TX 75202

12 ⁴U.S. Environmental Protection Agency, Region 6, 10625 Fallstone Road, Houston, TX 77099

13

14 **Corresponding Author:**

15 James M. Lazorchak

16 U.S. Environmental Protection Agency

17 National Exposure Research Laboratory

18 26 W. Martin Luther King Drive

19 Cincinnati, OH 45268

20 Phone: 513-569-7076

21 Fax: 513-569-7609

22 Email: lazorchak.jim@epa.gov

Abstract Numerous methods exist for assessing the potential toxicity of sediments in aquatic systems. In this study, the results from 10-day bulk sediment toxicity test methods using *Hyalella azteca* and *Chironomus tentans* were compared to results from 96-hour *Pimephales promelas* and *Ceriodaphnia dubia* renewed acute toxicity tests conducted using elutriate samples prepared from the same sediments. The goal of the study was to determine if the results from the elutriate tests were comparable to those obtained from the bulk sediment tests. Of the 25 samples analyzed, 16 were found to be toxic to at least one of the species tested, in either elutriate or bulk sediment tests. The *C. tentans* 10-day bulk sediment test was the most sensitive, with 12 sediment samples exhibiting toxicity to this species, while the *H. azteca* bulk sediment test and *C. dubia* 96-hour elutriate test were the least sensitive, exhibiting toxicity in only 7 of the 25 sediments tested. The *P. promelas* elutriate test found 8 of the 25 sediments to be toxic. Based on the total number of sites found to show toxicity, results from testing indicate 96-hour elutriate tests show a level of sensitivity comparable to 10-day bulk sediment tests in assessing toxicity quantitatively. However, the methods did not always find toxicity at the same sites, suggesting that the ability of elutriate tests to predict toxicity (quantitatively) is not statistically correlated with bulk sediment methods. This would indicate that a suite of toxicity test methods would provide the most complete measure of site condition; however, in circumstances where bulk sediment testing is not feasible, elutriate tests can provide a practical and credible alternative for toxicity assessment.

Introduction

Bulk sediment toxicity tests are routinely used to assess the level and extent of contamination in

44 bottom sediments. While reliable, these tests can be resource intensive, requiring significant
45 time and materials. The purpose behind this study was to compare the results from bulk
46 sediment toxicity tests using *Hyalella azteca* and *Chironomus tentans* to the results obtained
47 from sediment elutriate tests using conventional *Ceriodaphnia dubia* and *Pimephales promelas*
48 ambient water toxicity testing methods. Sediment elutriate tests offer a considerable cost savings
49 and may have broad application where bulk sediment testing is not feasible. Such a case would
50 be an ambient toxicity monitoring program (ATMP) conducted by a U.S. Environmental
51 Protection Agency (USEPA) Regional Office or a state agency. The purpose behind most
52 ATMPs is to provide a measure of the baseline condition in an ecological system and to measure
53 any changes experienced in the system (Hall et al. 2000). These programs need to be cost
54 effective to be implemented. The resources required to include bulk sediment toxicity testing
55 preclude the use of this endpoint in most ATMPs. Use of the sediment elutriate test as a
56 surrogate endpoint could provide a cost-effective means to include sediment testing in an ATMP.

57 In this study, USEPA Region 6 (Arkansas, Louisiana, New Mexico, Oklahoma, and
58 Texas) sought assistance from the USEPA Office of Research and Development (ORD), through
59 the Regional Methods Initiative (RMI) Program, to conduct side-by-side bulk sediment and
60 acute sediment elutriate toxicity tests. Region 6, in cooperation with states and tribes, has been
61 conducting aquatic toxicity tests with ambient water samples and sediment elutriate as part of a
62 regional ATMP since 1990
63 (<http://www.epa.gov/earth1r6/6wq/ecopro/watershd/monitrng/toxnet/index.htm>). Using the
64 sediment elutriate test has allowed USEPA Region 6 to include a cost-effective sediment toxicity
65 endpoint as part of the ATMP. The objective of the study was to assess the use of sediment

66 elutriate tests as a feasible alternative to conventional bulk sediment tests.

67 Numerous test organisms have been compared in the past to assess the toxicity associated
68 with contaminated sediments with varying degrees of success. Ten day exposures conducted by
69 Cairns et al. (1984) yielded 50% lethal concentrations (LC50s) of 38 and 39 µg/L, respectively,
70 for *Hyalella azteca* and *Chironomus tentans* using copper spiked sediments. Ten day exposures
71 performed by Suedel et al. (1993) revealed LC50s of 45 and 32 µg/L, respectively, for *H. azteca*
72 and *C. tentans* using fluoranthene spiked water. However, species' sensitivities have also shown
73 differences in the past. Water spiked with zinc displayed LC50s of 73 µg/L for *H. azteca* versus
74 1,125 µg/L for *C. tentans* in experiments conducted by Phipps et al. (1995). Both species were
75 tested to assess sediment toxicity in samples collected from 11 sites in the Keweenaw Waterway
76 in Michigan by West et al. (1993) and revealed only a marginal level of agreement at 55%.
77 These same types of discrepancies have been noted in 7-day *P. promelas* and *C. dubia* toxicity
78 test exposures from point-source discharge effluents in National Pollutant Discharge and
79 Elimination System (NPDES) permitting studies (Stewart et al. 1990). These limitations aside,
80 *Hyalella azteca* and *Chironomus tentans* are the species recommended by USEPA for use in
81 sediment toxicity testing (USEPA 2000), and *Ceriodaphnia dubia* and *Pimephales promelas* are
82 the species recommended for use in ambient water quality monitoring (USEPA 2002), therefore,
83 they were selected for use in this study.

84 **Materials and Methods**

85 Sediment Samples

86 The sediment toxicity samples provided by USEPA Region 6 for this study were collected by

87 state and tribal agencies that participate in the Region 6 Ambient Toxicity Monitoring Program.
88 USEPA Region 6 scientists pre-screened a number of waterbody sites that had been sampled
89 previously in the program, as well as other sites being sampled by state water quality agencies, to
90 ensure that the samples selected for use in this study were from sites observed as being toxic or
91 having a strong potential for toxicity.

92 The sediment samples were collected from freshwater lakes and streams located in Texas,
93 Oklahoma, and New Mexico over a two year period from early 2002 (01/02) through late 2003
94 (12/03). When the USEPA Region 6 Laboratory in Houston, Texas received the sediment
95 samples, they were homogenized and split into two sub-samples. One sub-sample was retained
96 by Region 6. A second 4-L sediment sub-sample was shipped overnight to the USEPA-National
97 Exposure Research Laboratory (NERL) in Cincinnati, Ohio for use in both bulk sediment testing
98 and acute toxicity testing with sediment elutriate samples. These sediment samples were logged
99 in at the Cincinnati facility and stored at 4°C until tested. Sediment samples were held for no
100 longer than two weeks before being used to prepare elutriate samples and for no longer than
101 eight weeks before being used in a bulk sediment toxicity test.

102 -

103 ~~SS~~Sediment Elutriate and Bulk Sediment Testing Water

104 Moderately hard reconstituted water (MHRW), with a hardness of 100 mg/L CaCO₃, was used to
105 prepare the sediment elutriate samples and as the control for the 96-h acute sediment elutriate
106 tests. MHRW was also used as the overlying water in the bulk sediment tests. MHRW was
107 prepared from a standard formula (USEPA 2002) using reagent grade chemicals and Super-Q®
108 (Millipore Corporation, Billerica, MA) ultrapure water. The water was prepared at least three

Formatted: Indent: First line: 0"

Formatted: Space Before: Auto, After: Auto

109 days prior to the start of the test to allow sufficient time for stabilization.

110

111 Sediment Elutriate Preparation

112

113 Sediment elutriate samples were prepared based on procedures described in the American
114 Society for Testing and Materials Guide E 1391 (ASTM 2000) and USEPA-U.S. Army Corps of
115 Engineers (USEPA-USACOE 1998). The samples were mixed in a 1:4 (v/v) ratio of sediment to
116 water and placed on a rotary shaker table for 1 h, at a speed of 100 rpm. After mixing for an
117 hour, the samples were centrifuged at 3000 rpm for 20 min, to separate the water from the
118 sediment. The aqueous fraction (elutriate sample) was poured off and stored in a cubitainer at
119 4°C for use in acute toxicity testing within 24 h of preparation. The remaining subsample was
120 retained and stored at 4°C for use in the corresponding bulk sediment toxicity tests. This
121 sediment elutriate mixing procedure differs from that described for use in the Region 6 ATMP.
122 The procedure used here is the standard procedure described by USEPA-USACOE 1998 and is
123 more widely used. The decision was made to use a standard elutriate preparation method for this
124 comparison. Another paper will compare the USEPA-USACOE elutriate method used here with
125 the method described in the Region 6 ATMP.

126

127 Toxicity Tests with Sediment Elutriate Samples

128

129 Ninety-six (96)-h static-renewal acute toxicity tests were conducted with the elutriate samples,

Formatted: Space Before: Auto, After: Auto,
Line spacing: Double

130 using standard USEPA methods (USEPA 1988, 2002). A test temperature of $25 \pm 1^\circ\text{C}$ and
131 photoperiod of 16 h light:8 h dark were maintained during testing with both *C. dubia* and *P.*
132 *promelas*. Routine chemical parameters (pH, dissolved oxygen, conductivity, and temperature)
133 were measured in fresh test solution at test startup (0 h) and test solution renewal (48 h), and on a
134 composite aliquot of old test solution (at 48 and 96 h), for each test treatment and control
135 sample.— No additional chemical analyses were performed with bulk sediment samples or
136 elutriate water samples.

137 The *C. dubia* test procedure used four replicate test chambers (30-ml plastic cups) for each
138 test treatment, with five animals per replicate (20 organisms per control or treatment), and a test
139 solution volume of 25 ml. The less than 24-h old *C. dubia* neonates were obtained from in-house
140 cultures maintained at the USEPA-Cincinnati facility. The *C. dubia* were fed 0.1 ml each of
141 *Selenastrum capricornutum* (freshwater algae) and YCT (yeast, trout chow, cerophyll mixture)
142 during holding, 2 h prior to test start, and 2 h prior to test solution renewal at 48 h.

143 The *P. promelas* acute toxicity test used four replicate test chambers (250-ml plastic cups)
144 for each test treatment, with 10 animals per replicate (40 organisms per control or treatment),
145 and a test solution volume of 200 ml. The *P. promelas* used in testing were supplied from an in-
146 house culture maintained at the USEPA Cincinnati facility. The *P. promelas* were 2- to 10-days
147 old (\pm 24-h age range) at the start of the test. This age range is more restrictive than the 1 to 14
148 day old range recommended in USEPA 2002 and was selected as a means to reduce test
149 variability. The fish were fed 0.2 ml newly hatched brine shrimp (GSL Brine Shrimp, Ogden,
150 UT) during holding, 2 h prior to the start of the tests and 2 h prior to test solution renewal at 48
151 h.

Bulk Sediment Toxicity Tests

The 10-day static-renewal bulk sediment toxicity tests with *H. azteca* and *C. tentans* were conducted using standard USEPA sediment testing methods (USEPA 2002). The tests for both species were conducted at $23 \pm 1^\circ\text{C}$, using 100 ml of sediment and 175 ml of MHRW as the overlying water. The control sediment was a commercially available washed-grade 40 white silica sand that is typically used as a landscape material. The sand was acid washed then rinsed with deionized water prior to use. It was, supplemented by the addition of 1% liquid alfalfa for use in testing. This control sediment has been extensively used in testing conducted for the Environmental Monitoring and Assessment Program-Great Rivers Ecosystems project (EMAP-GRE) as well as in the assessment and remediation of contaminated sediments (Tabak et al. 2005). Each control or sediment treatment used six replicate 400-ml beakers as test chambers, with 10 animals in each replicate (60 organisms total per concentration). The photoperiod during testing was 16 h light:8 h dark and a water change of two volume additions (350 ml) was performed daily, using a modified Zumwalt renewal system (Zumwalt et al. 1994). Temperature was measured on a daily basis and routine physical/chemical parameters (pH, dissolved oxygen, conductivity, and temperature) were measured on initial setup (day 0) and final takedown (day 10) for each test concentration or control sample. The sediment testing conducted under the EPA Region 6 TOXNET program provides a screening level assessment. Therefore the majority of the toxicity results do not have accompanying chemical analysis data. Such data would be generated as part of an intensive special study to evaluate spatial and temporal characteristics of toxicity at a designated location.

The *C. tentans* and *H. azteca* used in testing were from in-house cultures maintained at the

USEPA-Cincinnati facility. Prior to testing, both species were held at $25\pm 1^{\circ}\text{C}$ and fed daily. At the start of testing, the *C. tentans* were third instar larvae (10-day old) and the *H. azteca* were 7- to 10-days old. The feeding regimes for both species followed standard USEPA guidance (USEPA 2000). Each species was fed 1.0 ml YCT (yeast, trout chow, cerophyll mixture) daily throughout the duration of the test.

Data Analysis

The endpoint values for these tests were determined using procedures and statistical methods established by USEPA (2000, 2002). Mortality differences in the samples compared to the corresponding test controls were analyzed for both the sediment elutriate and bulk sediment tests, using the t-test function ($\alpha=0.05$) in Lotus 1-2-3 (IBM, Armonk, NY). Each sample tested included a corresponding control sample for comparison. Any sample with survival significantly less than the control ($p<0.5$) was classified as being toxic.

Statistical analyses were performed on the results of these toxicity bioassays to establish associations between the results from elutriate test methods and bulk sediment methods to determine if the use of elutriate testing is just as applicable in determining toxicity as bulk sediment methods. Comparisons between the elutriate versus bulk sediment methods were made by McNemar's test of symmetry ($p=0.05$) in Systat 11 (Systat Software, San Jose, CA) to derive whether the probability of rating a sample as toxic is similar between the two methods being compared. Cohen's Kappa measure of agreement (values between 0 and 1) in Systat 11 was also performed on elutriate versus bulk sediment methods to determine the strength of association between each method's ability to detect toxic effects.

195 Results

196 A total of 25 sediment samples were tested for toxicity in this study (Tables 1 and 2). All arrived
 197 in good condition and the tests were started for each sample within the established time
 198 parameters. Of the 25 samples tested, 16 were found to be toxic to at least one of the species
 199 tested in either the elutriate tests or the bulk sediment tests (Table 2, Figs 1 and 2). Of these 15
 200 samples, six (NM3-A, TX1-A, TX2-A, TX2-C, TX4-A, and TX7) were found to be toxic to just
 201 those species used in the bulk sediment tests (*C. tentans*, *H. azteca*, or both); three (TX1-B,
 202 TX1-D, and TX3-C) were found to be toxic to just those species used in the elutriate tests (*C.*
 203 *dubia*, *P. promelas*, or both); and seven (NM1-A, NM1-B, TX1-C, TX3-A, TX4-B, TX8-A, and
 204 TX8-B) showed toxicity to at least one species used in both the elutriate and bulk sediment tests.
 205 The remaining samples (NM2, NM3-B, OK1-A, OK1-B, TX2-B, TX3-B, TX5-A, TX5-B, and
 206 TX5-C) showed no toxicity to the species tested in either method.

207 Sediment Elutriate Tests

208 Test acceptability for the *C. dubia* and *P. promelas* sediment elutriate test methods is defined as
 209 $\geq 90\%$ survival in the controls (USEPA 2002). All elutriate tests conducted in this study met or
 210 exceeded the control survival acceptability criteria. All elutriate tests met or exceeded this
 211 control survival acceptability criterion (Fig. 3). Control survival for the *C. dubia* tests ranged
 212 from 90% to 100%. Control survival for the *P. promelas* tests ranged from 95% to 100%.

Formatted: Font: Italic

Formatted: Font: Italic

213 The *C. dubia* elutriate tests revealed 7 of the 25 samples were toxic (Table 2, Fig. 1), and
 214 the *P. promelas* elutriate tests revealed 8 of the 25 samples were toxic (Table 2, Fig 1). Five
 215 samples were determined to be toxic by both methods (NM1-A, NM1-B, TX1-B, TX8-A, and

TX8-B). Samples TX1-D and TX4-B were found to be toxic to just *C. dubia*, while samples TX1-C, TX3-A, and TX3-C were determined to be toxic to only *P. promelas*. In total, the combined elutriate tests determined 10 of the 25 samples tested were toxic to at least one of the species tested, with little difference in the overall sensitivity between the two species (Table 2, Fig. 1).

Bulk Sediment Tests

Test acceptability for the *C. tentans* test method is defined as $\geq 70\%$ survival in the control, while the acceptability for the *H. azteca* test method is defined as $\geq 80\%$ survival in the control (USEPA 2000). All bulk sediment tests conducted in this study met or exceeded the control

survival acceptability criteria. ~~(Fig. 3) Control survival in the *C. tentans* tests ranged from 70% to 85%. Control survival in the *H. azteca* tests ranged from 90% to 100%.~~

Formatted: Font: Italic

Formatted: Font: Italic

The *H. azteca* bulk sediment tests revealed 7 of the 25 samples were toxic, while *C. tentans* bulk sediment tests yielded toxicity for 12 of the 25 samples (Table 2, Fig. 2). One sample (TX1-A) was found to be toxic to only *H. azteca*, and six samples (NM3-A, TX2-A, TX3-A, TX4-A, TX4-B, and TX7) were found to be toxic to only *C. tentans*. The remaining six samples (NM1-A, NM1-B, TX1-C, TX2-C, TX8-A, and TX8-B) were toxic to both species. In total, of the 25 samples tested using the bulk sediment method, 13 were found to be toxic to at least one of the species tested (Table 2, Fig. 2). The *C. tentans* appear to be slightly more sensitive than the *H. azteca*.

McNemar's test of symmetry indicated no significant differences in designating a site toxic between elutriate and *H. azteca* bulk sediment tests ($S=1.2857$, $p=0.4531$) or between elutriate

and *C. tentans* bulk sediment tests ($S=0.50$, $p=0.7266$). Cohen's Kappa test revealed no significant agreement between elutriate and *H. azteca* bulk sediment tests ($K=0.386$, $p=0.0618$) or between elutriate and *C. tentans* bulk sediment tests ($K=0.3548$, $p=0.0820$).

Discussion

The purpose of this study was to assess the utility of using sediment elutriate tests as a tool for monitoring sediment condition as part of a long-term ambient toxicity monitoring program. Results from this study indicate that the 10-day bulk sediment toxicity tests conducted with *C. tentans* were the most sensitive, with 12 of the 25 sediment samples exhibiting toxicity to this species (Table 2, Fig. 2). The *H. azteca* bulk sediment tests (Table 2, Fig. 2) and *C. dubia* elutriate tests (Table 2, Fig. 1) were the least sensitive, exhibiting toxicity in only 7 of the 25 sediments, while the *P. promelas* elutriate tests found 8 of the 25 sediments to be toxic (Table 2, Fig. 1). Two of the sites that were sampled and tested multiple times (OK1 and TX5) did not exhibit toxicity with either the bulk sediment or elutriate tests.

The results from the statistical comparison of elutriate testing data and bulk sediment testing data reveal interesting trends. McNemar's test of symmetry indicated there was no significant difference between the ability of an elutriate test or bulk sediment test to predict the toxicity of a sample. Cohen's Kappa measure of agreement suggested that both elutriate tests and bulk sediment tests alone lacked the qualitative ability to predict toxicity in a given sample. This could be due to a variety of factors, including the sensitivity of the species tested and the toxic components found in the samples. Based on the results of both data analysis methods, this data set indicates limited differences between the use of a sediment elutriate test or a bulk

258 sediment test in predicting the toxicity in a given sediment sample. The 64% agreement
259 between the test methods in detecting toxicity –would seem to validate the performance of the
260 elutriate test method. The lack of agreement in the remaining samples could simply be due to
261 interspecies differences in sensitivity to various toxicant components of the sediments.

262 As Burton et al. (1996) note, all methods have inherent variability which must be taken into
263 account when interpreting test results. The methods used in this study did not always find
264 toxicity at the same sites, indicating that both elutriate and bulk sediment tests have built-in
265 biases. The decision becomes which method provides data adequate for the scope of the project
266 or ATMP (Hall et al. 2000, OSPAR Commission 1997). The elutriate tests can be effective in
267 identifying acutely toxic sites; however, the assessment of elutriate toxicity alone is not
268 sufficient to assess the overall potential hazards of contaminated sediments in some cases
269 (Burton et al. 1996, Liß and Ahlf 1997, Ahlf and Wild-Metzko 1992, Burton 1992). Research
270 has shown the results from elutriate tests can correlate well to bulk sediment metals
271 contamination (Finlayson et al. 2000, Callier et al. 2009) and bulk sediment organic
272 contamination (Karbe 1992). In a major study conducted as part of the Bremerhaven Workshop,
273 sediments were collected from 16 sites located in the North Sea and a total of 11 different
274 toxicity tests were conducted, with 20 toxicity endpoints being measured (Chapman et al. 1992).
275 The results from those toxicity tests indicated that the 10-day amphipod test with bulk sediment
276 and the 48-h oyster larval abnormal development test with sediment elutriate most clearly
277 reflected the toxicity gradient across the samples and best corresponded with the chemical
278 analysis and in-situ community data (Chapman et al. 1992). Other researchers have shown that
279 the results from elutriate tests correspond well to impacts noted in the in-place benthic

community (Callier et al. 2009). Based on the ability of elutriate tests with *Daphnia magna* to determine toxic sites in Izmir Harbor in western Turkey, the elutriate test with *D. magna* has been proposed as a low-cost, efficient method to screen for sediment toxicity (Yegane et al. 2008). Others have made this same proposal, based on the ease of conducting elutriate tests and the associated resource savings (Marin et al. 2001).

The original purpose behind the design of the sediment elutriate test is another factor to consider when determining which method to use. This test method was originally designed to assess the impact of re-suspension of sediment contaminants due to dredging and the release of the dredged material back into an aquatic environment (ASTM International 2000, USEPA 1988). The re-suspension of sediments is not limited to dredging. Flood events can cause significant quantities of bottom sediment to be re-suspended (Mucha et al. 2004), as can boat and ship traffic in harbors, rivers, and recreational lakes and reservoirs (Sousa et al. 2007). The water bodies tested in this study are all large enough to support recreational use, including boat traffic. They are also subject to high flow levels and flood events. These factors indicate the Elutriate tests would be an appropriate screening tool to use to monitor for the effects of this type of activity as well in these systems.

The data presented in this study indicate that sediment elutriate and bulk sediment tests show a comparable level of sensitivity, based on the total number of sites found to be toxic with each method. Other researchers have reached these same conclusions (see Finlayson et al. 2000, Chapman et al. 1992). The two methods did not always find toxicity at the same sites, and both were shown to have built-in biases. One probable cause for these biases would be interspecies differences in tolerance to toxicants or combinations of toxicants present in the sediments. This

not only exists between the bulk and elutriate test species, but between the species used in each type of test as well. *C. dubia* are considered to be more sensitive to many types of toxicants than are *P. promelas*. The sensitivity roles change when the toxicant is ammonia or hydrogen sulfide, in which case *P. promelas* is more sensitive than *C. dubia*. The same differences can be seen with *C. tentans* and *H. azteca*. As a burrowing species *C. tentans* has an increased level of contact with the sediment and therefore with the toxic components of the sediment. *H. azteca* is more epibenthic, so it would be affected by those toxic components that are absorbed into the water column. None of these are necessarily good or bad (false positives, false negatives), since neither type of test is an absolute barometer of toxicity. Both bulk sediment and elutriate tests are imperfect and have their strengths and weaknesses which result in limitations for each method. The fact that, for a few tests, the elutriate tests showed toxicity when the bulk sediment did not could indicate greater sensitivity for certain toxicants. -Whenever possible, the use of a ~~which would indicate that a~~ suite of toxicity test methods would provide the most complete measure of site condition. However, elutriate test methods do provide an efficient, cost-effective alternative to bulk sediment toxicity tests and can be used as a screening tool to monitor for sediment toxicity. A more intensive assessment of a site found to have consistently toxic sediment through elutriate testing may likely require the use of additional environmental measures, including bulk sediment testing, to determine the level and extent of toxicity.

For future research, two changes to the elutriate study described here could help to improve the overall utility of the test method. Work conducted with different sediment-to-water ratios used in preparing elutriates has shown potential to better reflect the toxicity of bulk sediments (Novelli et al. 2006). The addition of a chronic or sub-chronic endpoint would increase the

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

324 usefulness of the data, as well. For instance, adding a *D. magna* 4-day survival and growth
325 test (Lazorchak et al. 2009), or using it in place of the *P. promelas* acute test would provide a
326 sensitive sub-chronic endpoint. These improvements would increase the relevance of sediment
327 elutriate tests as a surrogate for bulk sediment testing.

328 **Acknowledgments**

329 The research reported in this document was funded by the U.S. Environmental Protection
330 Agency. This manuscript has been subjected to the Agency's peer and administrative review
331 and has been approved for publication. Approval does not signify that the contents reflect the
332 views of the Agency, nor does mention of trade names or commercial products constitute
333 endorsement or recommendation for use. The authors would like to acknowledge the statistical
334 technical support provided by Karen Blocksom, NERL USEPA, as well as the technical review
335 provided and editing by Justicia Rhodus, Dynamac. The comments of two anonymous reviewers
336 were most helpful.

337 **References**

338 Ahlf W, Wild-Metzko S (1992) Bioassay responses to sediment elutriates and multivariate data
339 analysis for hazard assessment of sediment-bound chemicals. *Hydrobiologia* 235-
340 236(1):415-418.

341 ASTM International (2000) Standard guide for collection, storage, characterization, and
342 manipulation of sediment for toxicological testing. In: ASTM Guide 1391-94, annual
343 book of ASTM standards. American Society for Testing and Materials, West

- 344 Conshohocken, Pennsylvania.
- 345 Burton GA Jr (1992) Sediment toxicity assessment. Lewis Publishers, Chelsea, Michigan.
- 346 Burton GA Jr, Norberg-King TJ, Ingersoll CG, Benoit DA, Ankley GT, Winger PV, Kubitz J,
347 Lazorchak JM, Smith ME, Greer E, Dwyer FJ, Call DJ, Day KE, Kennedy P, Stinson M
348 (1996) Interlaboratory study of precision: *Hyaella azteca* and *Chironomus tentans*
349 freshwater sediment toxicity assays. Environ Toxicol Chem 15(8):1335-1343.
- 350 Cairns MA, Nebeker AV, Gakstatter JH, Griffis WL (1984) Toxicity of copper-spiked sediments
351 to freshwater invertebrates. Environ Toxicol Chem 3(3):435-445.
- 352 Callier MD, Fletcher RL, Thorp CH, Fichet D (2009) Macrofaunal community responses to
353 marina-related pollution on the south coast of England and west coast of France. J Mar
354 Biol Assoc U.K. 89(1):19-29.
- 355 Chapman PM, Swartz RC, Roddie B, Phelps HL, van der Hurk P, Bulter P (1992) An
356 international comparison of sediment toxicity tests in the North Sea. Mar Ecol Prog Ser
357 91:253-264.
- 358 Finlayson B, Fujimura R, Huang Z-Z (2000) Toxicity of metal-contaminated sediment from
359 Keswick Reservoir, California, USA. Environ Toxicol Chem 19(2):485-494.
- 360 Hall RK, Wolinsky GA, Husby P, Harrington J, Spindler P, Vargas K, Smith, G (2000) Status of
361 aquatic bioassessment in U.S. EPA Region IX. Environ Monit Assess 64(1):17-30.
- 362 Karbe L (1992) Toxicity of surface microlayer, subsurface water and sediment-elutriates from
363 the German Bight: summary and conclusions. Mar Ecol Prog Se 91:197-201.
- 364 Lazorchak JM, Smith ME, Haring HJ (2009) Development and validation of a *Daphnia magna*
365 four-day survival and growth test method. Environ Toxicol Chem 28(5):1028-1034.

- Liß W, Ahlf W (1997) Evidence from whole-sediment, porewater, and elutriate testing in toxicity assessment of contaminated sediments. *Ecotoxicol Environ Saf* 36(2):140-147.
- Marin MG, Da Ros L, Moschino V, Campesan G (2001) Sediment elutriate toxicity testing with embryos of sea urchin (*Paracentrotus lividus*). *Aquat Ecosys Health Manage* 4(2):215-221.
- Mucha AP, Bordalo AA, Vasconcelos MTSD (2004) Sediment quality in the Douro river estuary based on trace metal contents, macrobenthic community and elutriate sediment toxicity test (ESTT). *J Environ Monit* 6(7):585-592.
- Novelli AA, Losso C, Libralato G, Tagliapietra D, Pantani C, Ghirardini AV (2006) Is the 1:4 elutriation ratio reliable? Ecotoxicological comparison of four different sediment:water proportions. *Ecotoxicol Environ Saf* 65(3):306-313.
- OSPAR (1997) JAMP guidelines for general biological effects monitoring, OSPAR Agreement 1997-7. Technical annexes revised 2007. OSPAR Commission, London.
- Phipps GL, Mattson VR, Ankley GT (1995) Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. *Arch Environ Contam Toxicol* 28(3):281-286.
- Sousa ECPM, Abessa DMS, Rachid BRF, Gasparro MR, Zaroni LP (2007) Ecotoxicological assessment of sediments from the Port of Santos and the disposal sites of dredged materials. *Braz J Oceanogr* 55(2):75-81.
- Stewart AJ, Kszos LA, Harvey BC, Wicker LF, Haynes GJ, Bailey RD (1990) Ambient toxicity dynamics: assessments using *Ceriodaphnia dubia* and fathead minnow (*Pimephales promelas*) larvae in short-term tests. *Environ Toxicol Chem* 9(3):367-379.
- Suedel BC, Rodgers JH Jr, Clifford PA (1993) Bioavailability of flouranthene in freshwater

sediment toxicity tests. Environ Toxicol Chem 12(1):155-165.

Tabak HH, Lazorchak JM, Smith ME, Ferretti J. A toxicity assessment approach for
evaluation of in-situ bioremediation of PAH contaminated sediments. 2005. In
Techniques in Aquatic Toxicology, Vol. 2. G. K. Ostrander, Editor

USEPA (1988) Bioassay protocols for assessing acute and chronic toxicity at hazardous waste
sites. U.S. Environmental Protection Agency, Corvallis Environmental Research
Laboratory, Corvallis, Oregon.

USEPA (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated
contaminants with freshwater invertebrates, 2nd ed. EPA-600-R-99-064. U.S.
Environmental Protection Agency, Office of Water, Washington, DC.

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to
freshwater and marine organisms, 5th ed. EPA-821-R-02-012. U.S. Environmental
Protection Agency, Office of Water, Washington, DC.

USEPA – USACOE (1998) Evaluation of dredged material proposed for discharge in waters of
the U.S.- Inland testing manual. EPA-823-B-98-004. U.S. Environmental Protection
Agency, Office of Water, and U.S. Army Corps of Engineers, Washington, DC.

West CW, Mattson VR, Leonard EN, Phipps GL, Ankley GT(1993) Comparison of the relative
sensitivity of three benthic invertebrates to copper-contaminated sediments from the
Keweenaw Waterway. Hydrobiologia 262(1):57-63.

Yegane O, Parlack H, Arslan OC, Boyacioglu M (2008) Sediment toxicity of streams flow
through the inner part of Izmir Bay with *Daphnia magna* Straus, 1820. EU J Fish Aquat
Sci 25(1):47-51.

Formatted: Line spacing: Double

Formatted: Font: Not Bold

410 Zumwalt DC, Dwyer FJ, Greer IE, Ingersoll CG (1994) A water-renewal system that
411 accurately delivers small volumes of water to exposure chambers. Environ Toxicol Chem
412 13(8):1311-1314.
413

Table 1 Index of sampling sites used in elutriate and bulk sediment testing. Numbers in parenthesis indicate number of samples collected at each site.

Site name (# of visits)	Sample IDs	State	Location
Willow Creek (2)	NM1-A	New Mexico	Lat 35°45'27"
	NM1-B		Long 105°40'17"
Middle Fork Gila River (1)	NM2	New Mexico	Lat 33°13'35"
			Long 108°14'30"
Elephant Butte Reservoir (2)	NM3-A	New Mexico	Lat 33°09'01"
	NM3-B		Long 107°10'56"
Black Bear Creek (2)	OK1-A	Oklahoma	Lat 36°17'58"
	OK1-B		Long 96°43'12"
Ellison Creek (4)	TX1-A	Texas	Lat 39°55'12"
	TX1-B		Long 94°43'48"
	TX1-C		
	TX1-D		
Medina River (3)	TX2-A	Texas	Lat 29°13'45"
	TX2-B		Long 98°27'30"
	TX2-C		
Finfeather Lake (3)	TX3-A	Texas	Lat 30°38'56"
	TX3-B		Long 96°22'16"
	TX3-C		

Site name (# of visits)	Sample IDs	State	Location
Bryan Municipal Lake (2)	TX4-A	Texas	Lat 30°38'27"
	TX4-B		Long 96°21'37"
Lake Palestine (3)	TX5-A	Texas	Lat 32°12'01"
	TX5-B		Long 95°27'41"
	TX5-C		
Dixon Creek (1)	TX7	Texas	Lat 35°44'32"
			Long 101°20'30"
Alligator Bayou (2)	TX8-A	Texas	Lat 29°52'39"
	TX8-B		Long 93°58'44"

416

417

Table 2 Summary of sediment elutriate samples and bulk sediment samples found to be toxic.

An X indicates a test where the sample was determined to be toxic with that species; a blank cell indicates no toxicity effect.

Sample ID	Sample Collection Date	96-hour acute sediment elutriate tests		10-day bulk sediment tests	
		<i>Ceriodaphnia dubia</i>	<i>Pimephales promelas</i>	<i>Hyaella azteca</i>	<i>Chironomus tentans</i>
TX1-A	2/11/02			X	
TX2-A	2/20/02				X
NM1-A	3/13/02	X	X	X	X
TX1-B	7/25/02	X	X		
TX2-B	8/20/02				
TX3-A	8/26/02		X		X
TX4-A	8/26/02				X
TX1-C	9/23/02		X	X	X
TX3-B	11/4/02				
TX4-B	11/4/02	X			X
NM2	11/13/02				
TX2-C	2/11/03			X	X
NM1-B	3/17/03	X	X	X	X
TX5-A	4/7/03				
NM3-A	4/21/03				X

Sample	Sample	96-hour acute sediment elutriate tests		10-day bulk sediment tests	
ID	Collection	<i>Ceriodaphnia</i>	<i>Pimephales</i>	<i>Hyaella</i>	<i>Chironomus</i>
	Date	<i>dubia</i>	<i>promelas</i>	<i>azteca</i>	<i>tentans</i>
TX5-B	5/5/03				
TX-7	5/13/03				X
OK1-A	5/27/03				
TX5-C	6/9/03				
OK1-B	6/23/03				
TX1-D	7/7/03	X			
TX8-A	11/17/03	X	X	X	X
NM3-B	11/17/03				
TX8-B	12/8/03	X	X	X	X
TX3-C	12/8/03		X		