

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

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

## 42 **Introduction**

43 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 CaCO<sub>3</sub>, 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

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

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#### 111 Sediment Elutriate Preparation

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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.

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#### 127 Toxicity Tests with Sediment Elutriate Samples

128

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

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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.

## 152 Bulk Sediment Toxicity Tests

153 The 10-day static-renewal bulk sediment toxicity tests with *H. azteca* and *C. tentans* were  
154 conducted using standard USEPA sediment testing methods (USEPA 2002). The tests for both  
155 species were conducted at  $23 \pm 1^\circ\text{C}$ , using 100 ml of sediment and 175 ml of MHRW as the  
156 overlying water. The control sediment was a commercially available washed-grade 40 white  
157 silica sand that is typically used as a landscape material. The sand was acid washed then rinsed  
158 with deionized water prior to use. It was, supplemented by the addition of 1% liquid alfalfa for  
159 use in testing. This control sediment has been extensively used in testing conducted for the  
160 Environmental Monitoring and Assessment Program-Great Rivers Ecosystems project (EMAP-  
161 GRE) as well as in the assessment and remediation of contaminated sediments (Tabak et al.  
162 2005). Each control or sediment treatment used six replicate 400-ml beakers as test chambers,  
163 with 10 animals in each replicate (60 organisms total per concentration). The photoperiod  
164 during testing was 16 h light:8 h dark and a water change of two volume additions (350 ml) was  
165 performed daily, using a modified Zumwalt renewal system (Zumwalt et al. 1994). Temperature  
166 was measured on a daily basis and routine physical/chemical parameters (pH, dissolved oxygen,  
167 conductivity, and temperature) were measured on initial setup (day 0) and final takedown (day  
168 10) for each test concentration or control sample. The sediment testing conducted under the  
169 EPA Region 6 TOXNET program provides a screening level assessment. Therefore the majority  
170 of the toxicity results do not have accompanying chemical analysis data. Such data would be  
171 generated as part of an intensive special study to evaluate spatial and temporal characteristics of  
172 toxicity at a designated location.

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

174 USEPA-Cincinnati facility. Prior to testing, both species were held at  $25\pm 1^\circ\text{C}$  and fed daily.  
175 At the start of testing, the *C. tentans* were third instar larvae (10-day old) and the *H. azteca* were  
176 7- to 10-days old. The feeding regimes for both species followed standard USEPA guidance  
177 (USEPA 2000). Each species was fed 1.0 ml YCT (yeast, trout chow, cerophyll mixture) daily  
178 throughout the duration of the test.

#### 179 Data Analysis

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

186 Statistical analyses were performed on the results of these toxicity bioassays to establish  
187 associations between the results from elutriate test methods and bulk sediment methods to  
188 determine if the use of elutriate testing is just as applicable in determining toxicity as bulk  
189 sediment methods. Comparisons between the elutriate versus bulk sediment methods were made  
190 by McNemar's test of symmetry ( $p=0.05$ ) in Systat 11 (Systat Software, San Jose, CA) to derive  
191 whether the probability of rating a sample as toxic is similar between the two methods being  
192 compared. Cohen's Kappa measure of agreement (values between 0 and 1) in Systat 11 was also  
193 performed on elutriate versus bulk sediment methods to determine the strength of association  
194 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%.

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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

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

#### 221 Bulk Sediment Tests

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

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

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

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

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

## 240 Discussion

241 The purpose of this study was to assess the utility of using sediment elutriate tests as a tool for  
242 monitoring sediment condition as part of a long-term ambient toxicity monitoring program.

243 Results from this study indicate that the 10-day bulk sediment toxicity tests conducted with *C.*  
244 *tentans* were the most sensitive, with 12 of the 25 sediment samples exhibiting toxicity to this  
245 species (Table 2, Fig. 2). The *H. azteca* bulk sediment tests (Table 2, Fig. 2) and *C. dubia*  
246 elutriate tests (Table 2, Fig. 1) were the least sensitive, exhibiting toxicity in only 7 of the 25  
247 sediments, while the *P. promelas* elutriate tests found 8 of the 25 sediments to be toxic (Table 2,  
248 Fig. 1). Two of the sites that were sampled and tested multiple times (OK1 and TX5) did not  
249 exhibit toxicity with either the bulk sediment or elutriate tests.

250 The results from the statistical comparison of elutriate testing data and bulk sediment  
251 testing data reveal interesting trends. McNemar's test of symmetry indicated there was no  
252 significant difference between the ability of an elutriate test or bulk sediment test to predict the  
253 toxicity of a sample. Cohen's Kappa measure of agreement suggested that both elutriate tests  
254 and bulk sediment tests alone lacked the qualitative ability to predict toxicity in a given sample.  
255 This could be due to a variety of factors, including the sensitivity of the species tested and the  
256 toxic components found in the samples. Based on the results of both data analysis methods, this  
257 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

280 community (Callier et al. 2009). Based on the ability of elutriate tests with *Daphnia magna* to  
281 determine toxic sites in Izmir Harbor in western Turkey, the elutriate test with *D. magna* has  
282 been proposed as a low-cost, efficient method to screen for sediment toxicity (Yegane et al.  
283 2008). Others have made this same proposal, based on the ease of conducting elutriate tests and  
284 the associated resource savings (Marin et al. 2001).

285 The original purpose behind the design of the sediment elutriate test is another factor to  
286 consider when determining which method to use. This test method was originally designed to  
287 assess the impact of re-suspension of sediment contaminants due to dredging and the release of  
288 the dredged material back into an aquatic environment (ASTM International 2000, USEPA  
289 1988). The re-suspension of sediments is not limited to dredging. Flood events can cause  
290 significant quantities of bottom sediment to be re-suspended (Mucha et al. 2004), as can boat and  
291 ship traffic in harbors, rivers, and recreational lakes and reservoirs (Sousa et al. 2007). The  
292 water bodies tested in this study are all large enough to support recreational use, including boat  
293 traffic. They are also subject to high flow levels and flood events. These factors indicate the  
294 Elutriate tests would be an appropriate screening tool to use to monitor for the effects of this  
295 type of activity, as well in these systems.

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

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

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

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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.

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414 **Table 1** Index of sampling sites used in elutriate and bulk sediment testing. Numbers in  
 415 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		

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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"

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

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

421

Sample ID	Sample Collection Date	96-hour acute sediment elutriate tests		10-day bulk sediment tests	
		<i>Ceriodaphnia</i>	<i>Pimephales</i>	<i>Hyaella</i>	<i>Chironomus</i>
		<i>dubia</i>	<i>promelas</i>	<i>azteca</i>	<i>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 ID	Sample Collection Date	96-hour acute sediment elutriate tests		10-day bulk sediment tests	
		<i>Ceriodaphnia</i>	<i>Pimephales</i>	<i>Hyaella</i>	<i>Chironomus</i>
		<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		