SEDIMENT TOXICITY IN MID-CONTINENT GREAT RIVERS (USA)

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Abstract– As part of the Environmental Monitoring and Assessment Program for Great River Ecosystems (EMAP-GRE), sediment samples were collected from 447 randomly-selected littoral sites along the main channels of the Ohio, Missouri, and Upper Mississippi Rivers between 2004 and 2006. Toxicity of these sediment samples was measured using a 7-day *Hyalella azteca* survival and growth test. Sixty-five sites (14.5%) exhibited lethal toxicity and 130 sites (29.1%) exhibited reduced growth. In the EMAP-GRE probabilistic sampling design, each sampled site had a weight associated with it that determined the length (and proportion) of the river represented by that sample point in the population. Weighted whole-river estimates indicate that of the 4721 river km sampled, sediment from 15.9 ± 3.0% of the river (752 ± 50 km) were lethally toxic, 27.4 ± 3.5% (1289 ± 57 km) were toxic via growth inhibition, and 40.0 ± 3.7% (1887 ± 68 km) exhibited either lethal or growth toxicity. Selected toxic samples were analyzed for 21 pesticides, 20 polychlorinated biphenyl (PCB) congeners, and 6 polybrominated diphenyl ether (PBDE) congeners. For all samples tested, the concentration levels of these analytes were mostly below known toxicity thresholds, and neither un-ionized ammonia concentration, nor osmotic stress (as measured by conductivity) could account for the toxicity found in sediments. The spatial pattern of sediment toxicity cannot be readily explained by urbanization or agricultural land use at the subcatchment scale. We speculate that the distribution of toxic sediment is more likely due to a combination of localized sources, including polluted tributaries, and the redistribution of contaminated sediments from upriver. The sediment toxicity results from this study will be used, in combination with other sediment, biological, and habitat metrics and indicators collected in the EMAP-GRE study, to help interpret and assess the condition of the Ohio, Upper Mississippi, and Missouri Rivers.

INTRODUCTION

Agricultural and urban runoff are the two leading causes of surface water impairment in the United States (Tucker and Burton 1999). When assessing pollution sources and their effects on aquatic ecosystems, and prior to implementing source controls, it is necessary to define the stressors and receptors of exposure. Toxicity assays are a key component in integrated assessments, which may also include physical habitat, chemical, and biotic community characterization. Short-term growth and survival toxicity of urban and agricultural runoff have been measured both in the laboratory and in-situ. Urban runoff is often more toxic to organisms in the laboratory than demonstrated in in-situ studies; conversely, toxicity to organisms at agricultural sites is often greater during in-situ exposures, when compared to laboratory studies (Tucker and Burton 1999).

Subacute-duration sediment toxicity testing has become important in regulatory, monitoring, and scientific programs, serving as an indicator of pollution exposure for benthic organisms. Data compiled from Atlantic, Gulf of Mexico, and Pacific coastal (U.S.) studies by Hunt et al. (2001) showed that in 92% of the samples classified as toxic, at least one measure of benthic diversity or abundance indicated an impact (e.g., value < median of reference), and in 67% of the toxic samples, at least one measure of benthic abundance or diversity indicated a severe impact (value < 10^{th} percentile of reference). Hunt et al. (2001) concluded that impacts on the benthic fauna frequently corresponded to reduced amphipod survival in the laboratory toxicity tests. Other studies from marine systems have shown similar relationships between sediment toxicity and benthic macroinvertebrate impacts (Long et al. 2001, Preston 2002, Kuhn et al. 2002, Stronkhorst et al. 2003, Greenstein et al. 2008).

Toxicity testing of sediments collected from Great Rivers like the Mississippi, Ohio, and Missouri has been conducted on both a site-specific and stressor-specific basis. Three studies were performed in the Mississippi River in the late 1990s – two on the Upper Mississippi and one on the Lower Mississippi (Canfield et al. 1998, Brunson et al. 1998, Winger and Lasier 1998). In the Upper Mississippi River (UMR) studies, sample locations were chosen based on historical chemistry data measured in soft sediments from 13 navigational pools. In the first study, correlations between field-collected benthic measures, toxicity, sediment chemistry, and other abiotic parameters were weak (Canfield et al. 1998). This suggested that benthic invertebrate distribution and assemblage structure were most likely controlled by factors independent of contaminant concentrations in the sediment. The second UMR study examined bioaccumulation of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in the sediment from these same sites, using laboratory exposures of oligochaetes (Brunson et al. 1998). Correlations for concentrations of individual compounds between laboratory-exposed and field-collected oligochaetes were strongest for benzo(e)pyrene, perylene, benzo(b,k)fluoranthene, and pyrene. About 90% of the paired PAH concentrations in laboratory-exposed and field-collected oligochaetes were within a factor of three and the authors felt laboratory extrapolations to the field could be made with reasonable certainty (Brunson et al. 1998). The third study, conducted on the Lower Mississippi River, examined sediment samples collected at sites above and below five major cities (Winger and Lasier 1998). Toxicity of sediment and sediment pore water was used to assess the effects of cities on sediment quality along the Lower Mississippi River. Acute toxicity was determined by exposing *Hyalella azteca* to solid-phase sediment for 10 d and sediment pore water under static conditions for 96 h.

Sediments were analyzed for organics (organochlorine pesticides, PCBs, organophosphate insecticides, and PAHs) and metals (Cr, Cu, Pb, Mn, Ni, and Zn). Due to the heterogeneity of depositional sediments in the Mississippi River system, samples collected at the four sites upstream and four sites downstream of each city were averaged for comparative purposes (i.e., upstream vs. downstream comparisons by city; Winger and Lasier 1998). Although bottom sediments are perpetually being redistributed via erosion and deposition, Winger and Lasier (1998) felt that the variability found in individual site toxicities within each city may reflect the influence of point-source discharges, which are common in this system.

The U.S. Environmental Protection Agency's (U.S. EPA's) Environmental Monitoring and Assessment Program for Great River Ecosystems (EMAP-GRE) worked, in partnership with states, other federal agencies, and tribes, to develop bioassessment tools for the Nation's largest rivers (U.S. EPA 2004). A goal of EMAP-GRE was to develop methods that yielded unbiased data that could be used to inform management decisions for Great Rivers. The key questions that EMAP-GRE attempted to answer were: (1) What are the current conditions of our national aquatic ecosystems?, (2) What stressors are associated with biological conditions?, (3) Where are the conditions improving or declining?, and (4) Are management programs and policies working?. A variety of water quality, sediment, biological assemblage, and habitat metrics and indicators were collected at each of the sites selected in the EMAP-GRE study. During the summers of 2004 to 2006, EMAP-GRE crews collected sediment samples in the Upper Mississippi River (from Cairo, Illinois to St. Paul, Minnesota), in the Ohio River (from Pittsburgh, Pennsylvania to Cairo, Illinois), and in the Missouri River (from northeastern Montana to St. Louis, Missouri). These sediment samples were sent to the U.S. EPA laboratory

in Cincinnati, Ohio to test for toxicity via survival and growth endpoints. Sediment toxicity testing was conducted to help address questions (1) and (2) above, as they pertain to sediment quality and habitat condition. Unlike previous studies on Great Rivers (Canfield et al. 1998, Brunson et al. 1998, Winger and Lasier 1998, U.S. EPA 2004), EMAP-GRE samples were collected at randomly selected locations, rather than at or near known or suspected contaminated sites or contaminant sources. This paper reports the extent of sediment toxicity found in the Ohio, Missouri, and Upper Mississippi Rivers, based on survival and growth toxicity endpoints using a 7-day amphipod (*Hyalella azteca)* test method.

MATERIALS AND METHODS

Site selection

A probability survey algorithm with an explicit random element was used to select sample sites on each river in the site selection process (Stevens 1997), with spatial balance incorporated to disperse the sites longitudinally and increase the representativeness of the samples (see McDonald et al. 2004, Schweiger et al. 2004, and Angradi, Bolgrein et al. 2009 for further details of site selection). As part of the probabilistic sampling design, each site received a weight indicating the length (in km) and proportion of the river represented by that sampling site based on whole-river estimates (see www.epa.gov/nheerl/arm for additional design details).

Sediment collection

Sediment samples were collected, using a hand scoop or petite ponar, at 10 evenly-spaced locations along a 500-m shoreline transect at each site. The top 2 cm of fine substrate within a 225-cm^2 area (15 x 15 cm) was obtained at each sampling location, at a depth of up to 0.3 m (U.S. EPA 2006). Four liters of sediment were collected and composited for each site. The

composite sample was stored in a polyethylene bag and held on ice prior to shipment to the laboratory. All sediment samples were refrigerated at 4°C until tested; testing was conducted within eight weeks of sample collection (U.S.EPA 2006).

During the summers of 2004 through 2006, sediment samples were collected at 447 sites, representing 4721 river km. Toxicity assays were conducted within the allowed sample holding period (i.e., within eight weeks of collection) and run in manageable batches of up to 20 samples, including control (U.S. EPA 2002a).

Sediment test procedure

Reformulated moderately-hard reconstituted water (RMHRW; hardness of 100 mg/L as $CaCO_3$) was used for reference toxicity testing (U.S. EPA 2002a) and as the overlying water in the sediment toxicity tests. RMHRW was prepared using Super-Q® (Millipore Corporation, Billerica, MA) ultrapure water and reagent-grade chemicals at least three days prior to the start of the test to allow time for stabilization.

EMAP-GRE sediment testing generally followed the standard 10-day *H. azteca* sediment toxicity procedure (U.S. EPA 2002a), with minor modifications, including the use of a 7-day exposure at 25°C (U.S. EPA 2002b, U.S. EPA 1994, Smith et al. 1997, Tabak et al. 2003). Samples were homogenized using a stainless-steel paint mixing paddle attached to a power drill. Forty (40)-mL subsamples of sediment from each site were dispensed into four 250-mL test beakers, as replicates; control samples of washed sand were prepared in a similar fashion. One hundred and sixty (160) mL of RMHRW was gently added to each beaker to minimize any disturbance of the sediment. Samples were held overnight at 25 ± 1ºC. The next day, twenty 7- to 10-d old *H. azteca* were added to each test beaker to initiate testing.

Routine chemical parameters (dissolved oxygen, pH, and conductivity) were measured at day 0, before organisms were added, and on day 7, prior to test takedown; temperature was measured on a daily basis. One-hundred percent (100%) of the water was exchanged daily, then each beaker was fed 2 mL of *H. azteca* test food, a 1:1 ratio of algae *Pseudokirchneriella subcapitata*:alfalfa (Tabak et al. 2003).

Beaker contents were sieved with a #30-mesh (500-micron) sieve to retrieve surviving organisms at the conclusion of exposures. Live amphipods were washed from the sieve into a glass dish and counted. They were dried for 24 h at 60°C and cooled in a desiccator for 1 h. All animals from each replicate beaker were weighed as a group to the nearest 0.01 mg on tared pans.

Statistical analysis

The sample survival and growth data were analyzed against the control survival and growth data for each test batch using *t*-tests (Excel v 2003, Microsoft Corporation, Redmond, WA). The data were tested for basic parametric assumptions and it was determine that mortality and growth toxicity would be determined by comparison of respective replicate control and sample data via a one-tailed, type 3 (two-sample unequal variance) t-test ($\alpha = 0.05$).

A sample was classified as lethally toxic if the *p*-value of the *t*- test was < 0.05 and survival was $< 80\%$ in the sample. A sample significantly different from the control, with survival $\geq 80\%$ was considered non-toxic. Test-acceptability criteria used in this study was a minimum control survival of $\geq 80\%$.

A sample was classified as growth inhibitory if the mean dry weight of test organisms was significantly less than the control ($p < 0.05$). No minimally-acceptable growth criteria have

been determined yet for controls. The extent of river km in each river exhibiting lethal toxicity, growth inhibition, or general toxicity (lethal or growth-inhibitory toxicity, or both) were determined based on the individual weights of sites from the probability design. Both number and percent of river km in each condition were calculated using R statistical software (v. 2.4.0, R Foundation for Statistical Computing, Vienna, Austria) and an R contributed library, psurvey.analysis (v. 1.4, USEPA, http://www.epa.gov/nheerl/arm), developed specifically for the statistical analysis of probability survey design data. The details of using this statistical package are described by Stoddard et al. (2005).

In addition, two types of regressions were performed on the data. First, two stepwise logistic regressions were performed for each river to predict the occurrence of lethal and growth-inhibitory toxicity; a Hosmer-Lemeshow test (Agresti 1996) was used to evaluate the fit of each final model. Then, a best subsets regression was conducted, with mean percent survival corrected for control survival (i.e., divided by control survival) as the response variable, and Mallow's C_p (Myers 1990) used to identify the best models for best subsets regression for each river separately. Thirty (30) abiotic variables were used as predictors in each model (Table 1), and each variable was transformed according to its use in a stressor gradient developed in Angradi, Pearson et al. (2009).

Land use - toxicity relationship analysis

The 2001 National Land Cover Dataset (Homer et al. 2004) was used to determine percent developed (NLCD classes 21–24) and percent agriculture (NLCD classes 81–82) land use within a subcatchment at each sample site. Any National Hydrography Dataset Plus (NHDPlus; http://www.horizon-systems.com/nhdplus) catchment that intersected the Great

River or one of its tributaries within a 50-km flow distance upstream of the sample site was used in analysis. Angradi et al. (2010) found subcatchments of this size to be useful for identifying major urban areas along mid-continent Great Rivers. The estimated land use for each sample location was calculated by summing the total land use area within the selected NHDPlus catchments.

Sediment chemistry analysis

Sediment samples that were toxic to amphipods were prioritized for chemical analysis. Due to limited analytical capacity, analyses were performed only on those samples exhibiting the highest toxicity. Samples expressing toxicity in both survival and growth were given the highest priority, followed by those expressing only toxicity based on survival, and finally, only toxicity based on amphipod growth. Samples were analyzed for 21 organic pesticides [aldrin (309-00-2); chlordane-cis (5103-71-9); chlordane-trans (5103-74-2); 2,4'-DDD (53-19-0); 4,4'-DDD (72-54-8); 2,4'-DDE (3424-82-6); 4,4'-DDE (72-55-9); 2,4'-DDT (789-02-6); 4,4'-DDT (50-29-3); dieldrin (60-57-1); endosulfan I (959-98-8); endosulfan II (33213-65-9); endrin (72-20-8); heptachlor (76-44-8); heptachlor epoxide (1024-57-3); hexachlorobenzene (118-74-1); hexacholorocyclohexane (Gamma-BHC/Lindane, 58-89-9); mirex (2385-85-5); trans-nonachlor (3765-80-5); cis-nonachlor (5103-73-1); oxychlordane (27304-13-8)], 6 polybrominated diphenyl ethers (PBDEs; 47, 99, 100, 153, 154, and 183), and 20 PCB congeners [8, 18, 28, 52, 44, 66, 101, 77 (coplanar), 118, 153, 105, 138, 187, 128, 180, 170, 195, 206, 209, 1and 26 (coplanar)]. The detection limit for all analytes was 0.001 ppm. These analytes were chosen because they represent legacy contaminants most often measured in sediments and were also measured and reported in an associated EMAP-GRE study examining fish tissue (Blocksom et

al. 2010).

When samples were being prepared for sediment toxicity, a subsample was collected and frozen in a glass jar at -20ºC for chemical analysis. When samples were selected for chemical analysis, the samples were thawed to room temperature and mixed with a spatula prior to extraction. For samples consisting of ≤ 50% water, a 15-g sample was dried using sodium sulfate and loaded into an ASE-200 accelerated solvent extractor (Dionex, Sunnyvale, CA). For samples with > 50% water, the sample mass was first adjusted so there was no more than 7 g of water in a sample. An extraction solvent of acetone:hexane (50:50 v/v) was used and each sample extracted at 100°C and 1000 psi for two, 7-min cycles. The extract was concentrated to 10 mL and dried through a 25-g sodium sulfate column. A 2-mL aliquot was then loaded onto an alumina column (3 g) and the column eluted with 14 mL of hexane:methylene chloride (80:20, v/v). The sample extract was re-concentrated under nitrogen at 50°C to a final volume of 1 mL. This extraction procedure was based on U.S. EPA method # 508.1 (U.S. EPA 1995). For samples that contained sulfur, the 1-mL alumina-cleaned extract was transferred to a centrifuge tube with 2 g of oxide-cleaned copper and mixed for 1 min. The sample was separated from the copper by centrifugation at 3000 rpm for 5 min, and the solvent layer decanted as described by U.S. EPA method # 3660B (U.S. EPA 2008). Samples were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a micro electron capture detector (μECD) that used a dual-column system for analyte confirmation.

All 447 sediment samples were analyzed for total ammonia. Levels of un-ionized ammonia (NH_3), the ammonia species responsible for lethality toxicity in aquatic organisms, were calculated from total ammonia concentration, pH, and temperature within the water

column. Ammonia concentration was measured on day 0 using an Orion Expandable Ion Analyzer, EA 940 (Orion Research Inc., Boston, MA).

Nonparametric Spearman rank correlation analysis (SYSTAT v 11, Systat Software Inc., San Jose, CA) was used to determine the relationship of un-ionized ammonia concentrations to survival. The effect of un-ionized ammonia concentration on survival and growth endpoints was examined using logistic regression (PROC LOGISTIC, SAS v9.1, SAS Institute Inc., Cary, NC). From these logistic regressions, the log likelihood (L), a measure of model fit with lower numbers indicating better fit, and the likelihood ratio (LR), a measure of the significance of the model, were used to determine the importance of relationships between $\log_{10}$-transformed un-ionized ammonia and toxicity.

RESULTS

Sediment toxicity test results

Sediment from 65 of the 447 sites visited in this study (14.5%) was found to be acutely toxic to *H. azteca* (Fig. 1), and sediment from 130 sites (29.1%) was found to inhibit growth in *H. azteca*. One-hundred eighty-two (182) sites (40.7%) were characterized as lethally toxic, growth inhibitory, or both. Table 2 presents the sediment toxicity results for each of the Great Rivers.

Of the 447 sites assessed, 144 were on the Mississippi River, 183 on the Missouri River, and 120 on the Ohio River. Twenty-six (26) of the 144 Mississippi River sites (18.1%) were characterized acutely toxic, while 37 (25.7%) were found to inhibit growth within 7 d. Fifty-six (56) sites (38.9%) expressed toxicity for at least one endpoint, with 19 (33.9%) revealing acute toxicity only, 30 (53.6%) exhibiting only inhibition of growth within 7 d, and 7 (12.5%)

displaying toxicity both for survival and growth.

On the Missouri River, twenty-five (25) of the 183 sites (13.7%) were characterized acutely toxic, while 58 (31.7%) were found to inhibit growth within 7 d. Seventy-nine (79) sites (43.2%) expressed toxicity for at least one endpoint, with 21 (26.6%) revealing acute toxicity only, 54 (68.4%) exhibiting only inhibition of growth within 7 d, and 4 (5.1%) displaying toxicity both for survival and growth.

Fourteen (14) of the 120 Ohio River sites assessed (11.7%) were characterized acutely toxic, while 35 (29.2%) were found to inhibit growth within 7 d. Forty-seven (47) sites (39.2%) expressed toxicity for at least one endpoint, with 12 (25.5%) revealing acute toxicity only, 33 (70.2%) exhibiting only inhibition of growth within 7 d, and 2 (4.3%) displaying toxicity both for survival and growth.

From the EMAP-GRE probabilistic sampling design, each sampled site reported above had a weight associated with it that determined the length (in km) and proportion of the river represented by that sample point in the population. Using those weights, of the 4721 river km included in the sample design, sediment from 15.9 ± 3.0% (752 ± 50 km) of the river, by length, were acutely toxic, 27.4 ± 3.5% (1289 ± 57 km) were growth inhibitory, and 40.0 ± 3.7% (1887 ± 68 km) exhibited either acute toxicity or growth inhibition. Probability estimates of river lengths with toxic sediments in each of the Great Rivers are presented in Table 3.

Based on an in-house calculated reference toxicant, a no observable effects concentration (NOEC) of 1.4 mg/L as un-ionized ammonia had been established. Five (5) out of 447 samples had un-ionized ammonia values above 1.4 mg/L, therefore, the toxicity found in those samples may be due to un-ionized ammonia. Conductivity measured in the sediment samples ranged from

316–531µmhos, well below 843 µmhos, which was the calculated NOEC from in-house reference toxicant tests.

Statistical analysis results

Although at least one predictor variable was selected in each regression, all models explained low proportions of the total variance. Associations with land use or other in-stream chemistry variables were weak at best (Tables 4 and 5). The strongest regression was for lethal toxicity in the Ohio River ($R^2 = 0.35$), exhibiting an association with local network agriculture and total nitrogen. These same two variables were associated with mean survival in the Ohio River, as well. There were no clear patterns across rivers otherwise.

Land use - toxicity relationship analysis results

Sites that were acutely toxic or that showed toxicity via growth inhibition were distributed throughout each Great River (Fig. 1). There was no consistent longitudinal pattern or relationship between land use and sediment toxicity on any river. Some major urban reaches, including the Quad Cities (Davenport, IA; Rock Island, IL; Moline, IL; and Bettendorf, IA) on the Upper Mississippi (ca. 700–800 km from the confluence with the Ohio River); Kansas City, MO, on the Missouri (ca. 500–650 km from the mouth); and Louisville, KY, on the Ohio (ca. 550–650 km from the mouth) had very few toxic sites. Mean percent developed or agricultural land use was not different between sites with and without acutely toxic sediments, except on the Missouri River where sites with toxic sediments had a slightly higher percentage of agricultural land use (Fig. 2). Results for sites that were toxic via growth inhibition were similar.

Chemical analysis results

Ninety-four (94) of the samples found to be toxic during sediment toxicity testing were

analyzed for pesticides, PCBs, and PBDEs. Twenty (20) of the samples (21.3%), 19 of which were from the Ohio River, had measureable concentrations of pesticides; nineteen (19) samples (20.2%), 17 of which were from the Ohio River, had measurable concentrations of PCB congeners; and only eleven (11) of the 94 samples (11.7%), 10 from the Ohio River, had measurable PBDE concentrations (Table 6).

Nonparametric Spearman rank correlation for lethally toxic samples revealed a weak correlation between ammonia concentration and survival (r = -0.09, n = 65 , p = 0.46). Un-ionized ammonia was predictive for survival toxicity (L = 300.7, LR = 67.4, p <0.0001, n = 439), but not growth toxicity (L = 536.7, LR = 0.34, p = 0.56, n = 437) .

DISCUSSION

The 7-day *Hyalella azteca* sediment toxicity test detected varying levels of sediment toxicity in all three Great Rivers sampled as part of EMAP-GRE during 2004–2006. Little toxicity could be attributed to un-ionized ammonia, and none to osmotic stress (as indicated by conductivity). However, we acknowledge we may have oversimplified major ion effects by only measuring conductivity (Mount et al. 1997). Based on sediment testing of EMAP wadeable streams, 3-5% of the samples exhibited toxicity for at least one endpoint using the 7-day *Hyalella azteca* method (Unpublished results of EMAP-Mid Atlantic Highlands and Integrated Assessment sediment toxicity results). In contrast, approximately 40% of the Great River samples were toxic for at least one toxicity endpoint (based on similar field and laboratory methods).

The spatial pattern of sediment toxicity cannot be readily explained by land use at the subcatchment scale. We speculate that the distribution of toxic sediment is more likely due to a

combination of localized sources, including polluted tributaries, and the redistribution of contaminated sediments from upriver. A source of uncontrolled variation in this study is benthic conditions at sediment collection sites. Some locations were dominated by coarse artificial substrates, such as riprap or other revetments. At these typically erosional locations, it was often difficult to obtain a sample of fine substrate that was not dominated by sand or fine gravel, which are generally less toxic than silt substrate. Because artificial revetments are more common in urban than in non-urban reaches (authors' personal observation), sediment toxicity may have been underestimated for urban reaches due to littoral substrate conditions.

Samples indicated to be toxic by the amphipod tests were prioritized for chemical analysis based on the bioassay results. Standard chemical screenings for various organic contaminants, including chlorinated pesticides, PCBs, and PBDEs, revealed very low concentrations of these analytes. Of 4418 results from chemical screening (94 samples * 47 analytes), contaminants were detected in only 184 cases (4.2%). The sediment toxicity detected in the samples during testing could not be attributed to these contaminants, however, because the overwhelming majority of contaminants were present in the samples at levels well below thresholds known to cause toxicity. ~~(Table 6).~~ MacDonald et al. (2000) determined that the threshold effect concentration (TEC) and probable effect concentration (PEC) for total PCBs in sediment is 60 µg/kg and 680 µg/kg, respectively. The highest concentration of total PCBs in any of the EMAP-GRE sediment samples was ≅ 42 µg/kg; PCB contamination in the majority of these samples was at concentrations less than 10% of TEC and an order of magnitude below the levels expected to cause harmful effects. Similarly, the highest concentrations of 4,4'-DDE (1.98 µg/kg), 4,4'-DDT (3.18 µg/kg), 4,4'-DDD (1.40 µg/kg), 2,4'-DDD (1.11 µg/kg),

Comment [c1]: I had a table in the older version of the document from which is referenced here. This reference to Table 6 is vestigial and is no longer applicable to what we are now calling table 6.

hexachlorobenzene (11.99 ug/kg) and chlordane (3.86 µg/kg) found in the EMAP-GRE sediment chemistry samples approached threshold effect levels, but were mostly below the sediment quality guidelines for organics [Burton 2002, U. S. EPA 2000]. However, not all compounds detected in our sediment chemistry analysis had an associated sediment quality criterion, including endosulfan, nonachlor, or any of the PCB congeners. Moreover, no sediment quality guidelines exist for PBDEs [MacDonald et al 2000]. Based on median concentrations of PCBs, PBDEs, and total chlordane, higher levels of contaminants were found in samples from the Ohio River versus those from the Missouri and Upper Mississippi Rivers. However, since the concentrations of chemicals did not exceed published sediment quality guidelines, it appears samples should not to be considered deleterious via the specific contaminants analyzed (McDonald et al. 2004, Burton 2002, U.S. EPA 2000, Johannessen et al. 2008).

The results of this study demonstrate the potential utility of an amphipod-based toxicity test as an indicator of stress (e.g., from polluted runoff and contaminated river sediments) that might otherwise not be detected from sediment chemical analysis or other methods. More analyses are needed to detect the potential sources of sediment toxicity found in the Great Rivers assessed in this study (e.g., screening for heavy metals, PAHs and pyrethroid pesticides, etc.), along with additional research into the synergistic, additive, or subtractive effects of chemical mixtures potentially influencing toxicity. Toxicity identification evaluation (TIE), for instance, may be a consideration for future studies to aid in identification of potential toxic fractions that should be analyzed chemically. The toxicity results from this study will be used, in combination with other sediment, biological, and habitat metrics and indicators collected in the EMAP-GRE study, to help interpret and assess the condition of the Ohio, Upper Mississippi, and Missouri

Rivers. In addition to other parameters, the absence of sediment toxicity may also be used as a criterion for selecting reference condition.

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Fig. 1. Distribution of acute and toxic via growth inhibition and non-toxic sediment samples by river (Triangles are individual samples). Lines show the percent developed and agricultural land use for the 50-km network subcatchment of the sample site (see Methods). Gaps for the Missouri River are unsampled mainstem reservoirs.

Fig. 2. Mean percent land use by river for sites with acutely toxic and non-toxic sediments.