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Toxicity of Hexavalent Chromium from Aqueous and Sediment Sources to *Pimephales promelas* and *Ictalurus punctatus*

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Chromium toxicity to aquatic organisms is primarily from exposure to dissolved rather than particulate-sorbed toxicants (US EPA 1985). The range of sensitivities of benthic organisms and water-column organisms to chemicals are considered to be similar (US EPA 1989). This suggests that, although exposure routes are difficult to determine for benthic organisms, toxicant exposures to benthic and water-column organisms may not be substantially different. In fact, it is commonly assumed that pore waters serve as the primary route of exposure to sediment-dwelling organisms, and that biological effects concentrations are the same for both pore water and water-only exposures (US EPA 1989). This implies that sediment-sorbed toxicants play a minor role in sediment toxicity, and that sediment toxicity can be predicted primarily by aqueous (pore water and overlying water) toxicant concentrations.

Contaminated sediments may act as a toxicant source by releasing toxicants to pore waters (Salomons et al. 1987; Tessier and Campbell 1988), and the acute toxicity of metals in sediments has been correlated with pore water concentrations (Swartz et al. 1985, 1988; Di Toro et al. 1990). However, the additional toxicity contributed by particulate-sorbed toxicants might be important for benthic species that have substantial contact with contaminated sediments and pore waters. Therefore, it is hypothesized that the acute toxicity of metals to some aquatic species is different for water-only and sediment-source exposures. This hypothesis was tested by exposing both water column-dwelling fathead minnows (Pimephales promelas) and benthic channel catfish (Ictalurus punctatus) to aqueous and sediment-source hexavalent chromium. Hexavalent chromium was selected as a test chemical because of its ability to provide reproducible toxicity test results (Dorn et al. 1986) and because an extensive aquatic toxicity data base exists for this chemical (US EPA 1984).

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The objectives of this research were (1) to evaluate the relative importance of contaminated sediments as a source of chromium exposure to test fishes, and (2) to investigate species-specific differences in sensitivity to both aqueous and sediment-source chromium.

MATERIALS AND METHODS

Fathead minnows were cultured at the University of North Texas (UNT) for several generations, and were 3 to 14 days of age at the start of all toxicity tests. Two-week old channel catfish, obtained from D and B Fish Farms in Crockett, Texas were held in the laboratory for approximately two weeks prior to testing. Fish were not fed during 96-h tests and were fed once per day from days five through 30 in 30-d tests.

Sediment and aqueous trials occurred in polystyrene-covered glass beakers containing 500 or 1000 ml sediment/water or water-only, maintained at 23 to 26° C and a photoperiod of L:D 16:8. Aeration was not provided during 96-h tests, and slow (1 to 2 bubbles per second) aeration was provided during 30-d tests, beginning on day five.

Dechlorinated tap water (<2 ug chlorine/L) was used for culturing fathead minnows, holding channel catfish, and for definitive toxicity tests. Temperature (23-26°C.), pH (7.9-8.1), alkalinity (88-120 mEq/L), hardness (88-108 mEq/L), conductivity (340-420 umhos), and dissolved oxygen (7.9-8.5 mg/L) were measured initially and at the end of the 96-h tests. Dissolved oxygen was also monitored at 96-h intervals during 30-d exposures.

Test sediments were obtained from the UNT Water Research Field Station pond by grab sample, and were stored at 5 to 8° C. Physical characterization of test sediments included measurements of percent sand (48-72%), percent silt (13-25%), percent clay (14-27%), organic carbon content (0.1-2.4%), and cation exchange capacity (6.0-10.4 mEq/100g).

Stock solutions of Cr^{+6} were made in Mili-Q water from potassium dichromate ($K_2Cr_2O_7$), obtained from Fisher Scientific Company (Fair Lawn, NJ). The median pH of the 1000 mg/L Cr^{+6} standard, which was added directly to dilution water, was 3.98. At 100 mg Cr/L nominal, the pH of test waters was 6.74, which is within the 96-h pH tolerance limits of both fish species. Concentrations of chromium used in 30-d water-only tests ranged from 0 to 12 mg/L and were within the pH tolerance limits of both fish species. Lower pH tolerance limits (5d) were determined for both test fish species, and equalled 5.7 and 4.9 for P. promelas and I. punctatus, respectively.

For water-only tests, mean LC50s were determined for each fish species based on measurements of chromium in water at 96 hours (96-h tests). For 30-d tests, total chromium concentration was determined initially and at the end of the 30-d trials to evaluate differences in chromium concentration over time. Only minimal differences were observed; therefore, the means of initial and 30-d measurements were used for computing 30-d LC50s.

Sediment tests were conducted utilizing a sediment to water ratio of 1:4. Chromium was added directly to wet sediments in varying concentrations as 1000 mg/L standard. Sediments were stirred completely with a glass rod, and dilution water was added slowly down the side of the test beaker. Fish were added 24 h after preparation of sediment-water test systems. Chromium concentrations in test sediments were measured at 96h (96-h tests) and both initially and at 30d (30-d tests). The mean of the initial and 30-d measurements were determined for each exposure concentration, and these means were used to calculate distribution coefficients and LC50s. Distribution coefficients (DCs) are defined as (mg Cr/kg sediment) / (mg Cr/L overlying water).

Water samples (2 ml) for chromium determinations were withdrawn from test beakers prior to the addition of test fish and again at the end of each trial. These samples were acidified with concentrated nitric acid (pH 1.5 to 2.0) and stored under refrigeration for later analyses. Chromium was extracted from sediment samples (1.0 g) following the procedure of Plumb (1981). An aliquot of the filtrate was removed for analysis by atomic absorption with graphite furnace. The detection limit for total chromium in water was 0.001 mg/L, while the lowest concentration of chromium measured in control sediments equalled 0.3 mg/L. The degree to which control sediments were contaminated with chromium was not assessed.

Mortality, defined as the lack of response of a test fish to gentle prodding, was the endpoint selected for all tests. Dead fish were removed each morning. Two replicates of 10 fish each were run per concentration, and the number of survivors was determined. Results were not adjusted for control mortality, which remained acceptable (<10%, 96-h tests; <20%, 30-d tests). Using probit analysis, the LC50 and 95% confidence intervals were determined for each replicate. Since the 95% confidence intervals around the mean LC50s overlapped substantially between replicates, survival data were pooled for each set of replicates and percent mortality was determined on pooled data. Differences between replicates were not evaluated for statistical significance. Independent t-tests assessed for possible interspecific differences in chromium survivability.

RESULTS AND DISCUSSION

Measured values of Cr^{+6} agreed well with nominal values (measured = 96-113% of nominal), and measured chromium concentrations did not change substantially during test durations. Sediments had a buffering effect on chromium solutions, with the pH of overlying waters in the presence of chromium-spiked sediments remaining above 7.4 during all trials involving sediments. Maximum observed mortality of control organisms for both water-only and sediment/water tests was nil in 96-h tests and 15 percent in 30-d tests.

Distribution coefficients were calculated for both 96-h and 30-d exposures. Excluding controls, distribution coefficients ranged from approximately three to 600 in 96-h test systems, indicating generally unstable aqueous exposures in those test systems containing lower chromium concentrations (Table 1). Apparently, at lower chromium concentrations, 96h is insufficient time for equilibrium to be achieved in test systems.

Table 1. Chromium concentrations in sediments (SED) and overlying waters (OW) presented to *Pimephales promelas* and *Ictalurus punctatus* for 96h. Cr^{+6} concentrations are based on 96-h SED and OW measurements.

Mean Cr+6 concentration in SED (mg/kg) and OW (mg/L)					
Pimephales promelas			*Ictalurus punctatus*		
SED	OW	DC 1/	SED	OW	DC
0.5	<0.001	>500	0.6	<0.001	>500
127.3	4.2	30	61.5	0.1	615
169.8	17.1	10	77.5	8.3	9
274.6	52.5	5	94.1	28.1	3
375.8	87.6	4	155.6	44.5	3
			254.2	49.9	5

1/ DC (Distribution coefficient) = (mg Cr+6/kg SED) / (mg Cr+6/L OW)

Because chromium concentrations were not appreciably different between initial and 30-d measurements, distribution coefficients (and 30-d LC50s) were based on the mean of initial (day 1) and final (day 30) measurements. In 30-d test systems, excluding controls, distribution coefficients ranged from 5 to 25 (Table 2). The results presented in Table 2 suggest that conditions approaching equilibrium were reached between sediment and overlying water chromium concentrations within 30d for all test systems except the controls.

Table 2. Chromium concentrations in sediments (SED) and overlying waters (OW) presented to *Pimephales promelas* and *Ictalurus punctatus* for 30d. Cr+6 concentrations are based on means of initial and 30-d SED and OW measurements.

Mean Cr+6 concentration in SED (mg/kg) and OW (mg/L)					
Pimephales promelas			*Ictalurus punctatus*		
SED	OW	DC 1/	SED	OW	DC
0.9	<0.001	>900	1.3	<0.001	>1300
2.4	0.15	16	3.0	0.12	25
4.1	0.26	16	4.9	0.28	18
8.6	1.73	5	9.4	1.95	5
21.1	2.08	10	20.0	2.48	8

1/ DC (Distribution coefficient) = (mg Cr+6/kg SED) / (mg Cr+6/L OW)

Table 3 presents the results of the 96-h and 30-d water-only Cr exposures for both fathead minnows and channel catfish. Included in this table are PP/IP ratios, defined as the mean LC50 calculated for *P. promelas* divided by the mean LC50 calculated for *I. punctatus*. PP/IP ratios are presented for each set of exposure scenarios, and these ratios indicate relative differences in species-specific sensitivities, with ratios at unity indicating equal sensitivity to chromium.

Table 3. *Pimephales promelas* and *Ictalurus punctatus* exposed to hexavalent chromium in water for 96 h and 30 d. Two trials per species per concentration. Ten fish per trial.

Organism	Exposure		Mean LC50 (mg/L)	LC50 95% C.I. (mg/L)	PP/IP Ratio 1/
P. promelas	Water-only	96 h	23.9	(19.0-28.7)	
I. punctatus	Water-only	96 h	14.8	(12.2-17.2)	1.6
P. promelas	Water-only	30 d	0.9	(0.1-1.6)	
I. punctatus	Water-only	30 d	1.5	(0.5-2.2)	0.6

1/ Mean LC50 *P. promelas* / Mean LC50 *I. punctatus*

Hexavalent chromium tests with sediments resulted in appreciably higher mortalities to both fish species compared to similar aqueous exposures in water-only tests. Channel catfish were more susceptible to sediment-source hexavalent chromium than fathead minnows in 96-h tests, yet the results of 30-d tests revealed no important differences in species-specific sensitivities. The mean 96-h LC50 of fathead minnows was more than six times greater than that of channel catfish in sediment/overlying water tests, while a difference in mean LC50s of only two times was noted in water-only tests. The results of both 96-h and 30-d sediment tests are presented in Table 4. In addition, PP/IP ratios similar to those presented for water-only exposures are presented in Table 4.

Table 4. <u>Pimephales promelas</u> and <u>Ictalurus punctatus</u> exposed to hexavalent chromium in sediment/water for 96 h and 30 d. Two trials per species per concentration. Ten fish per trial.

Organism	Exposure		Mean LC50 (mg/L)	LC50 95% C.I. (mg/L)	PP/IP Ratio 1/
<u>P.promelas</u>	Sed/OW	96h	12.3	(8.0-18.0)	
<u>I.punctatus</u>	Sed/OW	96h	1.9	(0.4-4.9)	6.5
<u>P.promelas</u>	Sed/OW	30d	0.041	(0.016-0.082)	
<u>I.punctatus</u>	Sed/OW	30d	0.054	(0.021-0.109)	0.8

1/ Mean LC50s based on Cr+6 measurements in overlying water (OW)
2/ Mean LC50 <u>P. promelas</u> / Mean LC50 <u>I. punctatus</u>

Of the four PP/IP ratios presented in Tables 3 and 4, only the ratio associated with the 96-h sediment tests (6.5) was substantially different from one.

Results of independent t-tests indicated that a significant (α = 0.05) difference in mean LC50s existed between test species in 96-h tests (p = 0.0023, OW; p = 0.0210, water-only). In contrast, no significant difference was found in mean LC50s between test species in 30-d tests.

Sediments contaminated with hexavalent chromium may add to the toxicity associated with dissolved chromium in the water column by releasing additional chromium to overlying waters, via pore water. This release appears to be a function of initial sediment chromium concentration and duration of sediment/water contact, and is expected to vary, depending on sediment-specific properties (e.g., grain size and chemical composition). Distribution coefficients were less variable at higher chromium concentrations, and exposure concentrations within the test system approached equilibrium within 96h and 30d at all but the lowest concentrations tested. These results suggest that chromium concentrations in overlying waters depend primarily on initial sediment chromium concentration and, to a lesser degree, exposure duration.

In this study, the sensitivity of both fishes to hexavalent chromium was increased in the presence of spiked sediments compared to water-only exposures. Increased toxicant exposures probably resulted from contact with pore waters, which are expected to contain higher concentrations of chromium than overlying waters, and contact with particulate-sorbed chromium. Until equilibrium is achieved within the test system, which appears to be concentration and time dependent, the sediment-water interface is expected to be associated with higher chromium concentrations than those of the upper water column. Channel catfish were observed to perturb sediments throughout the test duration, and most often were observed directly on or partially buried within test sediments. This behavior is expected to contribute to increased contact with pore waters and particulate-sorbed chromium compared to fathead minnows.

Significant differences between sensitivities of test species to hexavalent chromium were detected in all 96-h tests, while 30-d tests failed to reveal significant differences. These differences were most pronounced in sediment tests, with channel catfish exhibiting increased sensitivity to sediment-source chromium relative to fathead minnows. These results indicate that (1) chromium-spiked test sediments contributed additional toxicity to both test species compared to water-only exposures and, (2) the additional toxicity associated with contaminated sediments was most pronounced in channel catfish. The latter finding indicates that channel catfish were more sensitive to sediment-source hexavalent chromium than fathead minnows, even though generally similar toxicities were observed between test species in water-only tests. These findings do not support the assumptions that (1) toxicant exposures are equal for both benthic and water column-dwelling organisms and, (2) aquatic exposure assessments should be based solely on dissolved chemical concentrations. The results of this study suggest that the biology of exposed animals and the variability in routes of toxicant exposure should be considered in the development of sediment quality criteria.

REFERENCES

Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mays SM, Redmond MS (1990) Toxicity of cadmium in sediments: The role of acid volatile sulfide. Environ Toxicol Chem 9: 1489-1504

Dorn PB, Rodgers JH Jr, Jop KM, Raia JC, Dickson KL (1986) Hexavalent chromium as a reference toxicant in effluent toxicity tests. Environ Toxicol Chem 6: 435-444

Plumb, RH Jr. (1981) Procedures for handling and chemical analysis of sediment and water samples. Environmental Laboratory. U.S. Army Engineers Waterway Experiment Station. Vicksburg, MS

Salomons W, de Rooij NM, Kerdijk H, Bril J (1987) Sediments as a source for contaminants? Hydrobiology 149: 13-30

Swartz RC, Ditsworth GR, Schults DW, Lamberson JO (1985) Sediment toxicity to a marine infaunal amphipod: Cadmium and its interaction with sewage sludge. Mar Environ Res 18: 133-153

Swartz RC, Kemp PF, Schults DW, Lamberson JO (1988) Effects of mixtures of sediment contaminants on the marine infaunal amphipod, *Rhepoxynius abronius*. Environ Toxicol Chem 7: 1013-1020

Tessier A, Campbell PGC (1988) Partitioning of trace metals in sediments. In: Kramer JR and Allen HE (eds), Metal Speciation: Theory, Analysis and Application. Lewis Publishers, Inc., Chelsea, MI, pp. 183-199.

US EPA (1985) Ambient Water Quality Criteria for Chromium - 1984. EPA-440/5-84-029. Office of Water Regulations and Standards. Washington, DC.

US EPA (1989) Briefing Report to the EPA Science Advisory Board on the Equilibrium Partitioning Approach to Generating Sediment Quality Criteria. EPA-440/5-89-002. Office of Water Regulations and Standards. Washington, DC.

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