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Exposure to Cadmium-Phenanthrene Mixtures Elicits Complex Toxic Responses in the Freshwater Tubificid Oligochaete, *Ilyodrilus templetoni*

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Abstract. The joint toxicity of metal–hydrocarbon mixtures in sediments was investigated using cadmium (Cd) and phenanthrene (Phen) as model contaminants. Sediment bioassays were utilized to quantify effects of individual and combined contaminants in the bulk-deposit feeding oligochaete *Ilyodrilus templetoni*. Combined contaminants elicited antagonistic lethal effects and independent responses for feeding rate (measured as sediment ingestion). The 10-d LC_{50} for Cd alone was 1375 mg kg^{-1} (95% C.I. 1340–1412), whereas Phen elicited no mortality even when loaded to sediment saturation. The presence of Phen decreased Cd lethality, increasing the LC_{50} of Cd by as much as 40%. Regression analyses indicated that Phen was nearly 10 times more potent than Cd in eliciting feeding rate reductions. Exposure to Cd–Phen mixtures resulted in feeding rate reductions equivalent to those caused by Phen alone. The marked reduction in sediment ingestion induced by the co-pollutant Phen reduced exposure to Cd via ingestion. We suggest that this Phen-induced reduction in Cd exposure decreased Cd bioaccumulation and subsequent lethality. More generally, we suggest that even if the toxicological effects among dissimilarly acting chemicals (including metals and hydrocarbons) are independent, contaminant mixtures may elicit unexpected interactive effects facilitated by modifying exposure.

Environmental pollution often occurs as a mixture of various classes of chemical constituents (Kennicutt *et al*. 1996). The toxicity of contaminant mixtures to organisms in natural environments is complex and is related to environment-specific bioavailability, organismal exposure, biodynamics (bioaccumulation versus elimination rates), and mixture toxicology. Regulators often use data from single-contaminant toxicity tests to estimate maximum allowable concentrations of individual chemicals within a given environment. In areas where contaminant mixtures are present, environmental risk is typically determined assuming concentration-additive toxicity (USEPA 2000b), which is defined as toxicity proportional to the summed toxicities of each individual contaminant concentration (Cassee *et al*. 1998). Concentration-additive toxicity has been demonstrated to be an effective predictor of the overall toxicity of combined chemicals that have similar toxicological effects and/or those having related quantitative structure–activity relationships (Altenberger *et al*. 2004; Van Leeuwen *et al*. 1996). Many organic contaminants fit this relationship well (Faust *et al*. 2000; Broderius *et al*. 1995). Contaminants with dissimilar modes of toxic action, on the other hand, are generally hypothesized to elicit independent joint toxicity, which occurs when the contaminants in a mixture have no influence on one other's toxic effects, thus resulting in less than concentration-additive toxicity (Altenberger *et al*. 2004; Faust *et al*. 2000; Cassee *et al*. 1998).

Because 95% of all toxicity studies investigate single compounds (Yang 1994), the assumptions that concentration-additive or independent toxicity are representative of contaminant mixtures (especially independence among dissimilar classes of contaminants) remain largely untested. Because metals and hydrocarbons are generally dissimilar in terms of their molecular structure and modes of toxic action, they are hypothesized to elicit independent toxic effects. However, a literature review of studies investigating metal–hydrocarbon mixtures (Gust 2005a) suggests that effects can be additive, independent, or interactive (synergistic or antagonistic). Twenty-six of 31 studies suggest that toxicity is interactive, with a compelling trend toward synergism (22 of 31; Gust 2005a). This is of concern because mixtures of heavy metals and polynuclear aromatic-hydrocarbons (PAHs) may be prevalent in benthic and wetland sediments as a result of urbanization and industrial contamination (Callender and Rice 2000, Van Metre *et al*. 2000, Sanger *et al*. 1999a, 1999b) which may lead to complex and deleterious environmental effects.

Of additional concern, recent studies indicate that even if dissimilar chemicals elicit independent toxicological effects, contaminant mixtures may produce unexpected interactive effects facilitated via changes in environmental exposure. This may be considered an ''apparent'' interactive effect (Norwood *et al*. 2003). For example, Gust (2006) observed that Cd–Phen mixtures elicited synergistic lethal effects in the amphipod *Hyalella azteca* in sediment exposures, but not in

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aqueous exposures. Further investigation has suggested that the toxicology between Cd and Phen is likely independent in *H. azteca*, but sediment-mediated effects of this mixture alter bioaccumulation rates, thereby increasing lethality (Gust and Fleeger 2005b).

We investigated the toxicity of metal–hydrocarbon mixtures in sediments using the PAH phenanthrene (Phen) and the toxic heavy metal Cd as model contaminants. Freshwater sediment-toxicity bioassays were conducted with the bulk-deposit feeding tubificid oligochaete *Ilyodrilus templetoni* (Southern) where lethal and sublethal (feeding rate) endpoints were observed. The null hypothesis for the present study was as follows: The joint-toxic effects of Cd–Phen mixtures in sediments are independent. The objective was to test the joint-toxic effects of metals and PAHs in deposit-feeding organisms in environmentally relevant exposures (*i.e.*, sediments).

Materials and Methods

Sediment Preparation and Test Organisms

Sediment used in all bioassays was collected from Bayou Manchac, a relatively uncontaminated freshwater bayou near Baton Rouge, Louisiana. Sediment characteristics and methods for amending Cd and phenanthrene to sediments are described in Gust (2006). Amended sediments were stored at 4°C for no more than 7 d prior to bioassay initiation, and concentrations of Cd and Phen were measured in sediments collected at experiment initiation. Cadmium concentrations were measured by inductively coupled argon plasma spectrophotometry and Phen concentrations by high-performance liquid chromatography. Sediment extraction methods and analytical procedures for contaminant analyses are described in Gust (2006).

I. templetoni cultures were maintained at 23 ± 1°C in multiple 2-L round aquaria containing sediment and overlying water. Culture sediments consisted of Better Homes and Gardens potting soil that passed through a 2-mm sieve. Culture chambers were individually aerated and experienced a natural light cycle of indirect light. Worms were fed mixed-grain baby cereal twice a week with supplemental spinach amendments once a month. Culture water and sediment were replaced every 3 weeks and 6 months, respectively. Prior to initiation of toxicity bioassays, 10 or 20 (depending on experiment) adult *I. templetoni* were transferred to Petri dishes that were randomly assigned to experimental treatments.

Feeding Rate Bioassays

Tubificid feeding rate is a sensitive indicator of sediment-hydrocarbon contamination (Lotufo and Fleeger 1996). *I. templetoni* feeds on organic carbon adsorbed to sediment particles. Individuals ingest several times their body weight of sediment per day, indicating that egestion rate is a close approximation of feeding rate.

The effect of Phen on *I. templetoni* feeding rate was tested in an experiment with a randomized design including seven treatments, each including six replicates. Measured Phen concentrations in experimental treatments were: 0, 7, 51, 109, 112, 131, and 193 mg kg^{-1} dry weight sediment. Treatment sediments in amounts that approximated a 50:1 total organic carbon/worm dry weight ratio (approximately 17 mL $replicate^{-1}$) were inserted into 50-mL glass centrifuge tubes (USEPA 2000a). Twenty-five milliliters of artificial pond water (APW, 0.5 mM NaCl, 0.05 m*M* KCl, 0.2 m*M* $NaHCO_3$, 0.2 m*M* $MgCl_2$, 0.4 m*M* $CaCl_2$) was added and allowed to equilibrate with sediment in the dark for 24 h. Polyester filter floss topped with two layers of gauze was held in place at the sediment–water interface with a polyvinyl chloride split ring to facilitate feces collection (Lotufo and Fleeger 1996). Artificial pond water was replaced 1 h prior to experiment initiation and was replaced every 2 d thereafter. Twenty randomly selected adult worms were transferred into each replicate, and were observed to pass through the feces collector and burrow into the sediment shortly after insertion. Experimental treatments were incubated at 25 ± 1°C in the dark.

Feces were collected every 2 d, dried at 70°C, and dry weights were recorded. On d 10, treatment sediments were washed onto a 125-μm sieve. Surviving *I. templetoni* were enumerated, transferred to dishes containing APW, and allowed to clear their guts for 12 h. Then, worms were rinsed, transferred to preweighed 8-mL glass vials, frozen, and freeze dried. The average dry weight $worm^{-1}$ was calculated for each replicate.

At the end of the 10-d feeding bioassay, mortality in each treatment was low (<10%), and no treatment varied significantly from the control [analysis of variance (ANOVA), $p > 0.05$] for mortality. Day-10 egestion rate data were unreliable because of deterioration of the feces collectors and were excluded from subsequent analyses. Dry weight measurements for egested material were summed over the 8-d period, and the average egestion rate day^{-1} was calculated. Because feeding rate is approximately equal to egestion rate for *I. templetoni*, egestion rate will be hereafter referred to as feeding rate. To account for the variability in body size between replicates, feeding rate was expressed as worm dry weight for each replicate (g dry wt. egested material g dry wt. $worm^{-1}$ day^{-1}). The affect of Phen on feeding rate was tested using ANOVA and the concentration–response relationship between sediment-Phen concentration and feeding rate was developed using linear regression, both calculated using SigmaStat Software (Jandel Scientific).

A separate experiment was conducted to test the effect of sediment-amended Cd on *I. templetoni* feeding rate. The experimental design and methods used to conduct this bioassay were equivalent to those described above. Treatments included 11 Cd-spiked sediments and an unamended control, each having 6 replicates. Measured Cd concentrations in treatments included: 0, 10, 40, 88, 124, 165, 520, 803, 833, 1183, 1346, and 1396 mg kg^{-1} dry weight sediment. At the end of the 10-d bioassay, mortality in each treatment was low (<10%), and no treatment varied significantly from the control (ANOVA, $p > 0.05$) for mortality. For consistency, feeding-rate data for d-10 were excluded and the average dry weight of egested material d^{-1} was quantified. The affect of Cd on feeding rate was tested using ANOVA, and the concentration–response relationship between sediment Cd concentration and feeding rate was developed using linear regression.

An experiment with randomized design and 4 × 3 factorial treatment arrangement was conducted testing the effects of Cd-Phen mixtures on *I. templetoni* feeding rate. The interaction term was used to test for interactive effects among contaminants. Four Phen treatments 0, 50, 250, and 500 mg kg^{-1} dry weight sediment (nominal, see Table 1 for measured concentrations) were combined with 3 Cd treatments: 0, 497, and 887 mg kg^{-1} dry weight sediment (measured concentrations). No significant mortality was detected compared to controls (ANOVA, $p > 0.05$). Treatment effects on average feeding rate d^{-1} for the 8-d period were analyzed by analysis of covariance (ANCOVA) on natural-log-transformed data (due to nonhomogeneity of variance) using SAS software 8.1 (SAS Institute Inc.).

Lethality Bioassay

Phenanthrene caused no detectible mortality compared to controls (ANOVA, $p > 0.05$) at concentrations approaching sediment saturation (~450 mg kg^{-1}) based on sediment-organic-carbon and equilibrium partitioning theory. An experiment with randomized design and 4 × 8 factorial treatment arrangement was used to test the

Table 1. Measured sediment phenanthrene concentrations from experiment testing Cd-phenanthrene mixture effects on *Ilyodrilus templetoni* feeding rate

Nominal phenanthrene concentration	0 Cd conc. (0)[a]	500 Cd conc. (497)[a]	1000 Cd conc. (887)[a]
0	0	0	0
50	37	47	36
250	188	161	191
500	318	314	370

Notes: All contaminant concentrations in mg kg^{-1}. Values represent mean (n = 2).
[a] Measured Cd concentrations.

effect of sublethal concentrations of Phen on Cd lethality. Phenanthrene treatments included: 0, 81, 189, 378 mg kg^{-1} dry weight sediment (measured concentrations) and 8 Cd concentrations 1000, 1300, 1600, 1900, 2200, 2500, 2800, 3100 mg kg^{-1} dry weight sediment (see Table 2 for measured concentrations). Each treatment included 4 replicates containing 10 randomly selected worms $replicate^{-1}$. LC_{50} values including 95% confidence intervals (C.I.) were calculated using probit analysis with SAS Software. Nonoverlapping 95% C.I.s were used as the criterion for identifying significant differences in mortality among Phen treatments.

Results

Individual Contaminant Effects

Phenanthrene significantly reduced *I. templetoni* feeding rate (Figure 1A, $p < 0.001$). Dunnett multiple comparisons test ($p = 0.05$) indicated significant reductions in feeding rate compared to the control at Phen concentrations at and above 112 mg kg^{-1}. Data yielded the regression [(feeding rate) = 7.977 – (0.0264 * Phen concentration), $p < 0.001$, $R^2 = 0.366$], which was used to compare the individual effects of Phen and Cd on *I. templetoni* feeding rate (next two paragraphs).

Cadmium significantly reduced *I. templetoni* feeding rate (Figure 1B, $p < 0.001$). With the exception of the 124 mg kg^{-1} Cd treatment, none of the "low" Cd treatments (10, 40, 88, 165, 520 mg kg^{-1}) caused significant reductions in feeding rate compared to the control as indicated by Dunnett multiple comparisons test ($p = 0.05$). In all other Cd treatments, worms were observed to have significantly lower feeding rates than the control. As the concentration of Cd increased, feeding rate decreased [(feeding rate) = 8.209 – (0.00331 * Cd concentration), $p < 0.001$, $R^2 = 0.473$; Figure 1B].

The y-intercept values for both the Cd alone and Phen alone regression equations were similar; however, the slope for the Phen alone exposure is nearly an order of magnitude steeper than the Cd alone exposure. These results indicate that control animals behaved similarly among feeding-rate bioassays, and Phen was nearly 10 times more potent than Cd in eliciting feeding rate reductions.

Cd-Phenanthrene Mixture Effects

The concentration-responses for Cd-Phen mixture effects on feeding rate did not differ significantly from the concentration-response generated for Phen alone (Figure 2). Results of ANCOVA indicated that Phen significantly reduced *I. templetoni* feeding rate ($p < 0.0001$) and Cd did not ($p = 0.5561$). No significant interaction occurred among contaminant treatments ($p = 0.1625$) suggesting the joint effects of Cd and Phen on feeding were independent in *I. templetoni.*

Range-finding experiments indicated that Phen caused no detectable lethality even when loaded to sediment saturation (~450 mg kg^{-1}). Cadmium elicited mortality in *I. templetoni* at relatively high concentrations (>1300 mg kg^{-1}). *I. templetoni* consistently demonstrated a threshold lethal response when exposed to Cd alone or in combination with Phen in 10-d bioassays (Figure 3). Within relatively small increments of increasing Cd concentrations (<200 mg kg^{-1}), mortality shifted from equivalence with uncontaminated controls to complete mortality.

The combination of Cd and Phen at all Phen concentrations (81, 189, and 378 mg kg^{-1}) significantly increased survivorship compared to worms exposed to Cd alone (Figure 3). The LC_{50} values for Cd alone and Cd in combination with 81, 189, and 378 mg kg^{-1} Phen were 1375 (1340–1412), 1929 (1852–2005), 1770 (1701–1840), 1779 (1682–1883) mg kg^{-1} Cd, respectively (95% C.I.s in parentheses). Phenanthrene alone caused no lethal effects, and based on the assumptions of either independent or concentration-additive toxicity, should have had no effect on the concentration-response relationship of Cd. The greatest enhancement in survivorship, a 40.1% increase in Cd LC_{50}, occurred at the lowest Phen concentration, suggesting that optimal survivorship in the presence of lethal concentrations of Cd may occur at low Phen concentrations.

Discussion

The observed tolerance of *I. templetoni* to high concentrations of sediment-associated Cd (in excess of 1300 mg kg^{-1}) is likely the result of a relatively high species-specific metal tolerance (Gust unpublished) and limited bioavailability of Cd associated with the muddy sediment used in our bioassays. *I. templetoni* exhibits lethality in aqueous exposures at Cd concentrations comparable to related freshwater oligochaetes including *Lumbriculus variegatus* and *Tubifex tubifex* (Rathore and Khangarot 2003; USEPA 2000a), which are relatively metal-tolerant (Milani *et al*. 2003). The 24-h LC_{50} for *I. templetoni* exposed to aqueous Cd was determined to be 2.4 mg L^{-1} (95% C.I. 2.1–2.7) in "reconstituted water" (hardness 90–100 mg L^{-1} as $CaCO_3$ at pH 7.8–8.2) (Gust unpublished observations). Extreme tolerance to Cd (low mortality at 34,000 mg kg^{-1} total Cd) has also been observed in natural populations of tubificid oligochaetes living in heavily Cd-polluted sediments (Klerks and Bartholomew 1991). In the present study, the presence of Phen reduced the lethal toxicity of Cd in *I. templetoni*, increasing the LC_{50} of Cd by as much as 40.1% (95% C.I. 38.2–42.0%; Figure 3). Phenanthrene alone caused no detectible lethality even when loaded to sediment saturation. Therefore, the lethal joint-toxic effect of the Cd–Phen mixture in the present sediment bioassay is characterized as antagonistic for *I. templetoni*. There are several possible mechanisms that may have contributed to the observed antagonism including mixture-mediated effects on the following: behavior that influences

Table 2. Measured sediment Cd concentrations from Cd-phenanthrene mixture toxicity bioassay

	Measured Cd concentration ($mg\ kg^{-1}$) within each treatment							
Measured phenanthrene conc. ($mg\ kg^{-1}$)	1	2	3	4	5	6	7	8
0	914	1273	1548	1528	1859	2310	2675	2667
81	853	1323	1651	1103	2100	2108	2392	2524
189	951	1357	1547	1545	1866	2328	2576	2885
378	959	1307	1569	1562	2011	2257	2462	2741

Notes: All contaminant concentrations measured as $mg\ kg^{-1}$. Values represent means ($n = 2$).

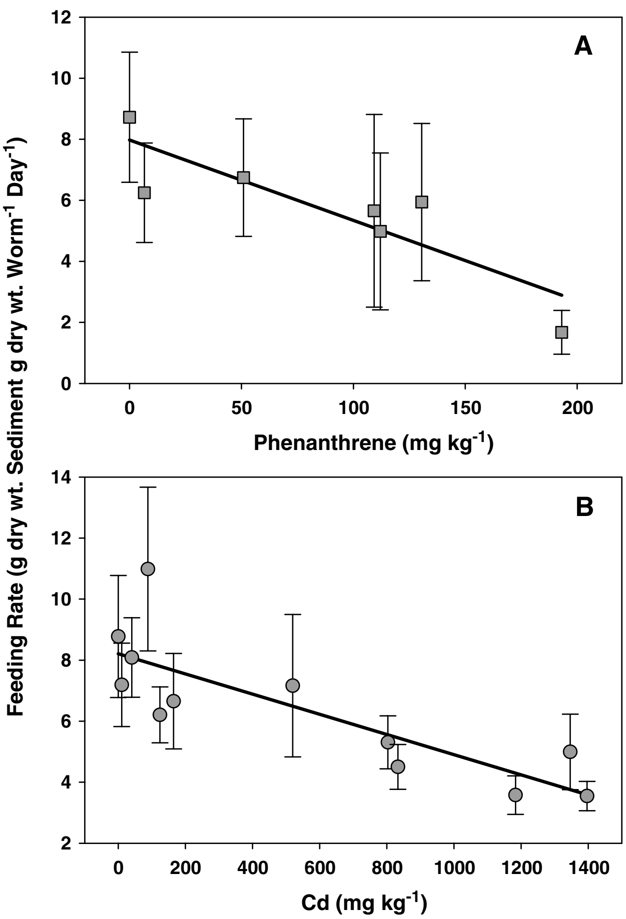

Fig. 1. Effect of phenanthrene (**A**) and Cd (**B**) on *Ilyodrilus templetoni* sediment feeding rate. Symbols represent means ±1 S.D. ($n = 6$). Dose–response relationships are characterized by linear regression. Phenanthrene and Cd concentrations represent measured values. Concentrations are displayed as $mg\ kg^{-1}$ dry wt. sediment

contaminant exposure, contaminant biodynamics, contaminant bioavailability, and toxicological interactions.

Effects of Behavior on Contaminant Exposure

Bioaccumulation of metals by deposit feeders occurs predominantly via uptake from feeding on contaminated sediments (Wang and Fisher 1999). Therefore, the rate of sediment ingestion likely influenced Cd bioaccumulation in our experiments. Cadmium and Phen each reduced feeding rates in *I. templetoni* with increasing concentration (Figure 1). Feeding rate proved to be a sensitive indicator of Phen contamination, with significant reductions compared to controls detected at concentrations as low as 112 $mg\ kg^{-1}$ Phen (Figure 1A). *Limnodrilus hoffmeisteri* also demonstrated feeding-rate sensitivity to sediment containing Phen (Lotufo and Fleeger 1996). Feeding rate was a less sensitive indicator of sediment-Cd contamination (Figure 1B). Similarly, *Capitella* sp. I feeding rate was unaffected when exposed to sediment-associated Cd even though their Cd body burden increased dramatically during feeding (Selck *et al*. 1998). In the present study, the combination of Cd and Phen elicited an independent joint-toxic response for feeding rate in *I. templetoni*. Reduced feeding rates appeared to be driven primarily by the concentration of Phen in sediment (Figure 2), likely causing reduced bioaccumulation/toxicity of the co-contaminant, Cd.

Sediment-burrowing avoidance was a predominant behavioral response in *I. templetoni* exposed to the highest Phen concentrations regardless of the presence of Cd (Gust 2005a). Although avoidance provides a potential means for reducing exposure to sediment-bound contaminants, it did not enhance survivorship compared to *I. templetoni* exposed to low to moderate Phen concentrations (Figure 3).

Biodynamics

Biodynamic models, such as the bioenergetic-based kinetic model proposed by Wang and Fisher (1999) and Luoma and Rainbow (2005), utilize physiological parameters including metal uptake from the dissolved phase (k_u), assimilation efficiency (AE) of metal from ingested material, ingestion rate (IR), and metal efflux rates (k_e) to predict animal body burdens. We estimated these model parameters for *I. templetoni* under our experimental conditions (Gust 2005a). Assuming that each parameter value, except for IR, remains constant in the presence of Phen and assuming that a high percentage of the Cd body burden accumulates through the ingestion route of uptake (Wang and Fisher 1999), Phen-induced reductions in IR are predicted by model calculations to reduce Cd body burdens in *I. templetoni* by as much as 72%. This large impact suggests that Phen may confer increased survivorship by lowering Cd bioaccumulation rates.

Aside from feeding-rate effects, it is possible that Phen may alter other parameters in the biodynamic model that contribute to Cd bioaccumulation. Moreau *et al*. (1999) indicated that Phen has the capacity to reduce metal bioaccumulation from the dissolved phase (k_u). In that study, Phen reduced Zn

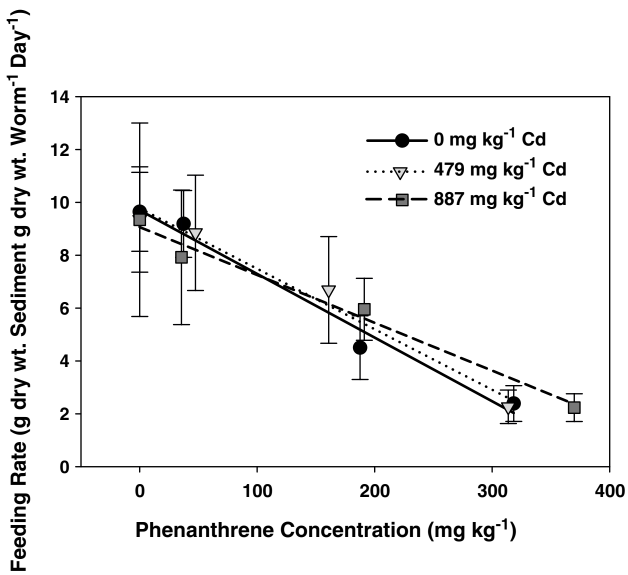

Fig. 2. Effect of Cd-phenanthrene mixtures on *Ilyodrilus templetoni* sediment feeding rate. Symbols represent means ±1 S.D. (n = 6). Dose–response relationships characterized by linear regression. Phenanthrene and Cd concentrations represent measured values. Concentrations are displayed as mg kg^{-1} dry wt. sediment

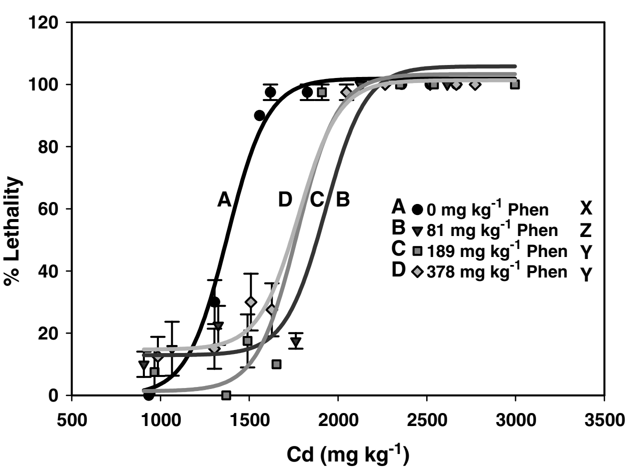

Fig. 3. Effect of Cd–phenanthrene mixtures on *Ilyodrilus templetoni* lethality in a 10-d sediment bioassay. Symbols represent treatment means and error bars represent standard error (n = 4). Dose–response curves were generated using probit analysis. Letters (A–D) are used to match concentration–response curves to corresponding treatments, and letters (X–Z) denote nonoverlapping 95% confidence intervals among dose–response curves. Phenanthrene and Cd concentrations represent measured values. Concentrations are displayed as mg kg^{-1} dry wt. sediment. Phen = phenanthrene

bioaccumulation in sheepshead minnow (*Cyprinodon variegatus*), which was associated with decreased lethality. Phenanthrene-induced modifications in Cd AE, k_u, and k_e could also contribute to reductions in Cd bioaccumulation. Results from kinetic-uptake bioassays with *I. templetoni* suggest that tissue-Cd concentrations are reduced in the presence of Phen (Gust 2005a).

Bioavailability

Phenanthrene may have altered Cd toxicity by modifying the bioavailability of Cd in sediments. For example, if Phen modified the physicochemistry of sediments in a way that enhanced Cd partitioning to recalcitrant environmental ligands (such as Cd binding to acid volatile sulfides), Cd bioaccumulation would likely be reduced. The potential for hydrocarbon contamination to alter metal bioavailability remains to be tested, and is of critical importance in natural environments where microbial communities control sediment biogeochemistry.

Mixture Toxicology

Phenanthrene may be involved in a true antagonistic toxicological interaction with Cd. For example, Phen-mediated interference with mechanisms that confer Cd toxicity and/or enhancement of Cd detoxification mechanisms could theoretically mitigate Cd toxicity. In the European eel *Anguilla anguilla*, Cd-PAH mixtures elicited greater production of both metal- and PAH-detoxification enzymes than expected by summing responses elicited by the individual chemicals (Lemaire-Gony and Lemaire 1992).

General Considerations

The 10-d bioassays conducted in the present study tested acute effects of Cd–Phen mixtures. Effects of chronic exposure to Cd–Phen mixtures have not been examined, but should be considered. In the present study, Phen- and Cd-mediated reductions in *I. templetoni* feeding rate were associated with reductions in growth rates (data not presented). Growth rates were negative (–2.59% growth day^{-1}) at high Phen concentrations compared to 0.35% growth d^{-1} in control animals and –0.75% growth d^{-1} in worms exposed to low Phen concentrations. Reduced feeding as the result of chronic exposure to high Phen and Cd concentrations may impart additional lethal effects not detected in acute bioassays. The antagonistic effect of Phen was not linearly related to reductions in feeding rate (*i.e.,* the lowest concentration of Phen reduced feeding rate slightly but conferred the maximum enhancement of survivorship when exposed to Cd). Effects of low Phen concentrations on feeding rate and growth were relatively minor, suggesting that chronic effects of Phen at low concentrations might be negligible. If so, the antagonistic effect of Phen on Cd lethality may be long-lived.

Results of the current study suggest that Phen-induced reductions in feeding rate can reduce exposure to sediment-bound Cd in bulk deposit feeders. Several toxicants have been shown to reduce feeding rate in a broad range of benthic taxa (Forrow and Maltby 2000; Blockwell *et al*. 1998; Lotufo and Fleeger 1996; Weston 1990). Given that sediments are frequently contaminated with multiple chemicals, it is plausible that feeding rate changes driven by one contaminant that alter a deposit-feeding organism's exposure to co-contaminants might be a common occurrence. This type of relationship should be considered when attempting to predict the toxicity of chemical mixtures in natural environments.

Implications and Importance

The concepts of additive and independent mixture toxicology are founded on the fundamental toxicological unit, the dose. In environmental exposures, especially in sediments, determination of expected dose is complex. In the present study, a PAH reduced exposure to a heavy metal, thereby likely reducing the heavy metal dose. Conversely, the same exposure regime elicited synergistic lethal effects in *H. azteca* (Gust 2006) in which PAH increased the heavy metal dose (Gust and Fleeger 2005b). These experiments indicate that exposure source may be more important than toxicological interactions in determining contaminant mixture effects in sediment environments. There are two fundamental challenges to expanding the concepts of dose-additive and independent toxicity to ecological risk assessment: (1) Although many ''similar'' chemicals are well characterized by dose-addition, the assumption of independent joint toxicology for dissimilar chemicals should be used with caution; most combinations have never been tested. (2) Observed interactions may be manifested via mechanisms unrelated to mixture toxicology. Our studies indicate that contaminant mixtures in complex exposure sources (*i.e.*, sediments) may elicit unexpected effects on exposure and subsequent toxicity. If this trend is widespread, understanding how species are exposed, determining route of uptake, and understanding how environmental characteristics affect exposure may be more important in determining mixture effects than knowing mixture toxicology.

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