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STUDY TITLE: Do Joint Exposures to Heavy Metals and Polynuclear Aromatic Hydrocarbons Elicit Nonadditive Responses in Benthic Invertebrates?

REPORT TITLE: Mixtures of Metals and Polynuclear Aromatic Hydrocarbons May Elicit Complex, Nonadditive Toxicological Interactions

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BACKGROUND: Many marine and estuarine organisms experience persistent exposure to mixtures of numerous individual compounds in different chemical classes at contaminated sites in nature. Sediments contaminated as mixtures of heavy metals and polynuclear aromatic hydrocarbons (PAH) are of particular concern in many marine environments, including those near oil production platforms. Biota may respond to contaminant mixtures in unexpected ways because the joint toxicity of combined contaminants may be expressed in a nonadditive fashion. Synergisms, in which the toxic effect of two or more combined contaminants is significantly greater than the additive effects of individual contaminants, and antagonisms, in which the toxic effect of two or more combined contaminants is significantly less than the additive effects of individual contaminants, are known to occur in some invertebrates, especially in binary exposures. Nevertheless, approximately 95% of all studies of sediment ecotoxicology investigate the toxicity of single contaminants and are unable to detect or predict nonadditive behavior in toxicant mixtures. Hazard assessment is therefore based on single-compound exposures, and compounds are assumed to have additive or independent effects in sediments with mixtures of contaminants. If toxicant interactions are common, hazard assessment based on predictions from minimum-effects criteria will be inaccurate.

OBJECTIVES: To contribute to an improved understanding of the role of chemical mixtures in structuring marine benthic systems at contaminated sites by investigating the joint toxicity of phenanthrene and fluoranthene (both commonly-used model PAH compounds) with the heavy metals cadmium, lead, and mercury. Few previous studies have tested for toxicological interactions between sediment-associated metals and PAH. The responses (acute and sublethal) of laboratory-cultured populations of harpacticoid copepods were studied in sediment and aqueous conditions, and contaminant uptake kinetics were examined through the use of a labeled contaminant.

DESCRIPTION: These studies employed factorial experiments and toxic unit methodology to test for nonadditive behavior in mixtures of sediment-associated PAH and metals. These methods expose organisms to a contaminant mixture in which each contaminant is present in equi-toxic proportions, and determine the nature of a mixture's acute toxicity. Results permit the estimation of the bulk-sediment concentrations of PAH and metals at which synergisms or antagonisms occur, making it possible to predict nonadditive effects at locations where sediment chemistry is known. In addition, the cause of an observed synergism in a copepod species was explored through the use of labeled contaminant uptake kinetics and by examination of interactive toxicology in sediment and aqueous exposures. Finally, sublethal mixture effects on feeding rate in a copepod species were examined to determine if feeding-rate effects contribute to nonadditive interactions causing lethality. This research examined the frequency of nonadditive interactions between PAH and metals in a group of animals (harpacticoid copepods) that is sensitive to contaminants, and facilitates predictions, through the use of both lethal and sublethal minimum-effects criteria, of synergisms or antagonisms at specific locations, such as in close proximity to oil production platforms.

SIGNIFICANT CONCLUSIONS: Synergisms between various combinations of metals (Cd, Hg, and Pb) and PAH (phenanthrene and fluoranthene) were found in two species of meiobenthic copepods suggesting that nonadditive toxicological interactions are common in this taxon known to be sensitive to sediment pollution. The cause of the interaction was probably not associated with factors related to uptake from sediment; strong synergisms were also found in aqueous exposures. In addition, Cd did not influence the uptake kinetics of phenanthrene. When additional metals (Hg and Pb) were combined with Cd, Cd-induced lethality was reduced, suggesting an antagonism among metals. This antagonism may have moderated the Cd-phenanthrene synergism in phenanthrene-Cd-Hg-Pb mixtures, suggesting that increasing toxicant diversity may reduce the strength of binary nonadditive interactions. Nevertheless, the strength of observed synergisms suggests that established sediment quality criteria may not be protective for joint exposures of PAH and metals, especially Cd-PAH mixtures.

STUDY RESULTS: Adults of the meiobenthic copepod, *Schizopera knabeni*, are highly tolerant of single-contaminant exposures to sediment-associated phenanthrene, Cd, Hg, and Pb, as well as a mixture of Cd, Hg, and Pb. However, when a mixture of Cd, Hg, and Pb is combined with phenanthrene, a greater than additive acute response occurs; the mixture is 1.5 x more lethal than predicted by separate exposures. Binary experiments reveal that although phenanthrene is individually synergistic with Cd, Hg, and Pb, a binary Cd-phenanthrene synergism is particularly strong (2.8 x more lethal than predicted by an additive model). A Cd-phenanthrene synergism in *S. knabeni* was also observed in aqueous exposures suggesting the synergism is related to a pharmacological insult rather than a sediment-related exposure effect. Cadmium does not influence phenanthrene uptake kinetics suggesting that Cd has no effect on phenanthrene biodynamics (however phenanthrene effects on Cd uptake kinetics were not studied due to the small mass of copepods). An antagonism between Cd, Hg, and Pb is also indicated, and this antagonism may moderate an observed Cd-phenanthrene synergism in Cd-Hg-Pb-phenanthrene mixtures. Grazing-rate bioassays suggest a response-additive sublethal toxicology between metals and phenanthrene. Experiments with a second meiobenthic copepod, *Amphiascoides atopus,* revealed that phenanthrene and fluoranthene are both synergistic with Cd. Overall, our research suggests that metal-PAH interactions may be common among benthic copepods (and perhaps other benthic taxa) and that strong nonadditive effects on lethality observed in binary mixtures may be moderated with increasing contaminant diversity in mixtures.

Hazard assessment of contaminated sediments is based on estimates such as ERM (effects range mid) that predict the likelihood of adverse effects to populations at specific levels of contamination. The ERM for Cd (9.6 mg kg^{-1} dry sediment) is well below our estimated LC_{50} value of 230 mg kg^{-1} dry sediment, suggesting this sediment quality criterion is protective of adult *S. knabeni*. Similarly, the ERM for phenanthrene (1.5 mg kg^{-1} dry sediment) is also well below the LC_{50} of 426 mg kg^{-1} dry sediment and is expected to be protective of adult *S. knabeni*. However, there are no guidelines for protection in Cd-phenanthrene mixtures when a synergism is indicated. In the present study, the TU_{50} of phenanthrene and Cd mixture was 162 mg kg^{-1} dry sediment; equi-toxic equivalents are 101 mg kg^{-1} dry sediment phenanthrene and 61 mg kg^{-1} dry sediment for Cd given the exposure ratio used. Assuming that 10% of an TU_{50} could serve as a protective standard, equi-toxic sediment concentrations as low as 10 mg kg^{-1} dry sediment for phenanthrene and 6 mg kg^{-1} dry sediment for Cd would be expected to cause lethal or sublethal effects in *S. knabeni*. Therefore, the established ERM for phenanthrene would be protective in an equi-toxic mixture with Cd, but the ERM for Cd may not be protective for adult *S. knabeni* in an equi-toxic mixture with phenanthrene. Thus, the strength of observed synergism suggests that established sediment quality criteria may not be protective for joint exposures of PAH and metals, especially Cd-PAH mixtures. The contaminants used in our studies (PAH, Cd, Pb and Hg) co-occur in sediments at oil production sites at concentrations that sometimes exceed those that may cause effects in our test species. Therefore, synergisms (if as strong as found in *S. knabeni*) could contribute to reductions in abundance of selected taxa observed near oil platforms.

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