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Joint toxicity of fluoranthene and pentachlorobenzene to *Hyalella azteca* and *Chironomus dilutus*

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ABSTRACT

Nonpolar organic chemicals such as polycyclic aromatic hydrocarbons and chlorobenzenes are expected to act additively when exposed as a mixture. The present study examined the toxicity of fluoranthene (FLU) and pentachlorobenzene (PCBz) individually and in a binary mixture using the whole-body residue as the dose metric. Body residues were based on the toxic equivalent body residue, which included the parent compound plus the organically extractable metabolites for FLU and the parent compound only for PCBz. Using a toxic unit (TU) approach, the binary mixtures of FLU and PCBz following 4- and 10-d water-only exposures acted additively. The lethal residue (LR50) values for mixtures of the compounds for *Hyalella azteca* were 1.26 (1.19–1.33) TU and 1.27 (1.20–1.34) TU for 4- and 10-d exposures, respectively. For *Chironomus dilutus*, the 4-d and 10-d values were 0.93 (0.90–0.97) TU and 1.01 (0.96–1.06) TU. Additionally, the total molar sum of PCBz and FLU whole-body residues in a mixture were compared to residues from single compound exposures. For both species tested, the LR50 values based on the total molar sum fell within the range of those determined from the single compound tests; providing additional support for molar additivity for nonpolar narcotic compounds. Assuming that residue-effects data among narcotic compounds (e.g., LR50) are similar, applying the molar sum methodology to narcotic compounds in tissues determined from routine biomonitoring programs and risk specific sampling may be a valuable tool to assess potential effects to biota in the field.

1. Introduction

Although some potential environmental hazards involve significant exposure to only a single compound, most instances of environmental contamination involve a simultaneous exposure to a mixture of compounds. The number of pollutants and their concentrations in the aquatic environment are extremely variable and, as such, a virtually unlimited number of different mixtures can be found. Consequently, an experimentally based hazard assessment of all conceivable mixtures is simply impossible. Given this, regulatory agencies including US Environmental Protection Agency (US EPA), US Department of Agriculture, and others have been attempting to develop guidelines for determining effects from chemical mixtures.

In response to the need to predict effects to biota from mixtures, various models including concentration addition (Loewe additivity; Altenburger et al., 2000) and independent action (Bliss independence; Altenburger et al., 2000) have been evaluated for mixture toxicity. In terms of model selection, concentration addition assumes that all the components of the mixture have the same mode of action, whereas the independent action model assumes that the chemicals act by separate modes of action. Mode of action is defined as a common set of physiological and behavioral signs that characterize a specific type of response (Rand et al., 1995). These models have been widely used in aquatic toxicity with reliable predictions of mixture toxicity (Swartz et al., 1995; Altenburger et al., 2000; Faust et al., 2000, 2001; Belden and Lydy, 2006). One of the most common methods of assessing concentration addition is to use toxic units (TU) (Lydy et al., 2004; Belden and Lydy, 2006). The TU approach has been used to predict the toxicity of chemical mixtures from the toxicity of individual compounds. A toxic unit (TU) is defined as the ratio of the concentration in the environmental media of each component to an effect concentration in that media (i.e., LC50) (Sprague and Ramsay, 1965; Pape-Lindstrom and Lydy, 1997). The TU approach has been successfully used as a means to predict mixture toxicity and as a tool for assessing combination effects (i.e., additivity, synergism, and antagonism) (Altenburger et al., 2000). However, because the variables are linked to environmental concentrations, the ability of a selected model to accurately predict effects suffers from the same shortcomings as those identified for assessing the risk of individual chemicals

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including bioavailability, route of uptake, and chemical and species sensitivities.

An alternative approach uses our knowledge of the hazard of the individual components to predict the overall toxicity of a mixture. For a mixture of narcotic chemicals, the total molar concentration in the biological membranes (i.e., target site) is proportional to the overall effect. For example, PAH toxicity showed approximately molar additivity in *Diporeia*, an organism with minimal biotransformation capability (Landrum et al., 2003). Simply put, nonpolar chemicals can be considered as dose additive if each component can be thought of as a concentration of the other chemicals in the mixture. The mixture toxicity of similar compounds acting with the same mode of action is hypothesized to be additive and as such; a residue-based approach can be applied to the TU model, where the previously mentioned confounding factors are minimized, thereby, allowing a better understanding of biological effects based on chemical concentrations determined in tissue residues.

The objective of the present study was to evaluate the toxicity of binary mixtures of fluoranthene and pentachlorobenzene using the toxic unit approach applied to whole-body residues. Additionally, the total molar concentration was compared to the concentrations predicted for the individual components to determine its potential for use in predicting effects for use in the field.

2. Materials and methods

2.1. Organisms

Two invertebrates, *Hyalella azteca* (juvenile, approximately 14-d old) and *Chironomus dilutus* (3rd instar) were selected for use in the present study. These two species were chosen because they have been recommended by the US Environmental Protection Agency (US EPA) for sediment toxicity testing (US EPA, 2000), because of their ecological importance and geographical distribution, and their use in previous bioassays. All organisms were cultured at Southern Illinois University in accordance with US EPA methods (US EPA, 2000).

2.2. Chemicals

Radiolabeled ^{14}C-pentachlorobenzene (PCBz) and ^{3}H-fluoranthene (FLU) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Unlabeled compounds were obtained from Chemservice (West Chester, PA, USA). Radiolabeled compounds were tested for purity using an Agilent Model 1100 high-pressure liquid chromatograph (HPLC) and a Packard Model 2900TR liquid scintillation counter (LSC). Stock solutions were prepared by adding known quantities of radiolabeled compound to known amounts of unlabeled compound using acetone as a carrier. The specific activities were recalculated by adjusting for the isotopic dilution.

2.3. Exposure media

All experiments employed an aqueous exposure route. The actual route of exposure was not important, since all measurements were linked to body residue levels. Moderately hard exposure water (MHW) was prepared by adding the necessary salts to deionized water and then allowing the water to mix overnight to ensure the required water quality (US EPA, 1991). The spiking procedure consisted of adding the predetermined amount of contaminant(s) (radiolabeled and unlabeled) using acetone as a carrier solvent to a bulk aliquot of water. The spiked waters were mixed for 30 min to ensure the chemicals were equally distributed. The volume of carrier was equal across all exposures and was <100 μL L^{-1}.

2.4. Bioassays

To test the hypothesis that narcotic compounds exert additive toxicity with respect to lethal body residues, the toxicity of *H. azteca* and *C. dilutus* were examined using 4- and 10-d exposures. All experiments were conducted in Precision Scientific Environmental Chambers (Chicago, IL, USA) maintained at 23 °C with a 16 light: eight dark photoperiod using ultra-violet filtered fluorescent light to minimize photoinduced FLU toxicity. Organisms were exposed to a series of five contaminant concentrations, predetermined from previous experiments (Schuler et al., 2004). Exposures for *H. azteca* and *C. dilutus* were conducted using 250-mL beakers containing 200 mL of dosed water. A total of three replicates and 10 organisms per replicate were used in each experiment. The toxicity of the chemicals was determined individually and in mixtures.

The toxicity of the binary mixtures was examined by applying a toxic unit (TU) approach (Pape-Lindstrom and Lydy, 1997) to lethal body residues. In this approach, the LR50 value for each compound corresponds to 1 TU. In the TU exposures, the organisms were exposed to a mixture of ^{14}C–PCBz and ^{3}H–FLU where concentrations of each compound were added at proportions of their respective LC50s so that the summation of lethal body residues were expected to yield body residues equivalent to 0.25, 0.50, 1.00, 1.50, and 2.00 TU.

Organisms were fed 1 mL of a 6 mg mL^{-1} suspension of Tetramin® (Spectrum Brands, Atlanta, GA, USA) daily. Mortality was assessed at the end of the 4- and 10-d exposures and the live organisms remaining were removed and body residues measured using LSC. Briefly, the organisms were removed from the water, rinsed, blotted dry, and weighed to the nearest 0.01 mg using a Mettler analytical balance (Toledo, OH, USA). The radioactivity analysis was performed by placing the organisms directly into scintillation cocktail (ScintiSafe 50%, Fisher Scientific, Hampton, NH, USA), sonicating for 30 s (Tekmar Corp., Solon, OH, USA) and then counting via LSC using a dual-label program. Prior to counting via LSC, samples were stored in darkness for at least 24 h to aid in the final extraction of the radiolabeled compounds and to minimize chemiluminescence. Sample blanks were included in each analysis to track any potential background contamination and samples were corrected for background and quench using the external standards ratio method.

Quantification of biotransformation allowed for the quantification of the toxic fraction (parent compound and organically extractable metabolites) of body residues. Biotransformation was assessed from three additional replicates following a 4-d exposure to FLU as previously described (Schuler et al., 2004, 2006). Pentachlorobenzene has been shown to not be biotransformed to any significant extent by *C. dilutus* or *H. azteca* (Sijm et al., 1993; Schuler et al., 2006) and was not reevaluated in the present study.

2.5. Analysis of lethal body residues in mixture exposures

Lethal residues determined from individual organisms were converted to TU using the following equation:

$$\mathrm{TU}_{Ri} = \frac{R_i}{\mathrm{LR50}_i} \quad (1)$$

where R was the residue measured in live organisms, LR50 was the lethal residue causing 50% mortality, and i was the individual chemical (PCBz or FLU). The LR50 values and 95% confidence intervals were determined using Trimmed Spearman Karber analysis. When live organisms were not available at the end of the exposure period, the whole-body residues of dead organisms were used. Previous research has demonstrated that there are no significant differences in residues between live and dead organisms (Steevens and Landrum,