CHEMICAL MIXTURES: EFFECTS OF CHLORPYRIFOS, DIELDRIN, AND METHYL MERCURY ON *HYALELLA AZTECA*

Jeffery A. Steevens and William H. Benson
Environmental Toxicology Research Program/ECHR and
Department of Pharmacology, School of Pharmacy
University of Mississippi

INTRODUCTION

Non-polar organic pesticides and organic metals are released into the aquatic environment and partition from surface water into sediment due to their hydrophobic nature. These non-polar chemicals have the potential to bioaccumulate in aquatic organisms exposed by direct contact with the sediment or indirectly through food. Recently, the U.S. EPA has proposed to establish guidelines for assessing sediments based on the concentrations of bioaccumulative chemicals in benthic invertebrates. The toxicity threshold values for bioaccumulative chemicals have typically been based upon single chemical studies. However, it is rare that a chemical occurs alone in the aquatic environment, particularly sediment. At the present time, there is limited knowledge regarding the bioaccumulative nature and effects of chemical mixtures. Furthermore, the mechanisms by which mixtures of bioaccumulative pesticides and metals elicit adverse effects are poorly understood.

The overall goal of the presented research is to evaluate the interactions of "real-world" bioaccumulative chemical mixtures having the potential for toxicological effects not predicted from single chemical toxicity experiments. The toxicological effects of the individual model chemicals, dieldrin, chlorpyrifos, and methyl mercury were evaluated using the amphipod, *Hyallea azteca*. Binary chemical interactions of the model chemicals will be characterized and the mechanism underlying the interactions determined. Additionally, bioconcentration data along with toxicological indices will be used to determine the critical body residue threshold concentrations at which toxicological effects occur. This research directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation.

Bioaccumulation

Contaminated sediments have become an increasingly important issue for human and ecological health. Presently, 15 percent of the nation's lakes, 4 percent of the nation's rivers, and 100 percent of the Great Lakes have fish consumption advisories associated with them (EPA 1996). Of the fish consumption advisories, greater than 95 percent are due to bioaccumulative chemicals including mercury, PCBs, organochlorine pesticides, and dioxin. Nationally, it is estimated that at least 29 percent of the benthic community in fresh and marine water is impacted by contaminated sediments (Veith 1996). Benthic aquatic organisms can bioaccumulate chemical contaminants from the sediment, by exposure either through food or direct contact with the sediment. Long term exposure to contaminants in the sediment can result in bioaccumulation of the chemical contaminant reaching concentrations capable of eliciting adverse toxicological effects (Borgmann 1991). Toxicity and bioconcentration data can be utilized to further characterize the dose-effect relationship of a chemical. The critical body residue is the whole body concentration in an organism associated with a measured adverse toxicological effect. It utilizes a one-compartment model to determine the total dose of a chemical that the organism receives following exposure from the water, food, or sediment. The critical body residue model accounts for variability in chemical bioavailability in the exposure media, metabolism, and uptake, and depuration kinetics. The use of critical body residues in aquatic organisms has been proposed as a method to assess sediment contamination and the potential toxicological effects in aquatic organisms. McCarty and Mackay (1993) suggested the use of critical body residues and corresponding biological responses be studied to validate laboratory and field-based assessments of sediments. Currently, the assessment of sediment contamination is based on measured sediment concentrations of individual chemicals and toxicity to laboratory organisms. Safe sediment concentrations of chemical contaminants in a sediment could be determined from the amount of that chemical accumulated and the corresponding measured toxicological effects. Due to site specific differences in chemical bioavailability and metabolism, the use of critical body residues may be a better predictor of the degree of ecological risk associated with contaminated sediments than sediment concentrations alone (Landrum et al. 1992).

Under the current U.S. EPA Contaminated Sediment Management Strategy (1994a), there are several main goals including the prevention of further contamination and the assessment of contaminated sediments. Prevention of sediment contamination is of particular interest for

regulation of new chemicals under the Toxic Substances Control Act (TSCA), National Pollution Discharge Elimination System (NPDES) permitting under the Clean Water Act (CWA), registration of new pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as well as permitting for ocean dumping under the Marine Protection, Resources and Sanctuary Act (MPRSA). Assessment of contaminated sediment is required under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Resources, Conservation, and Recovery Act (RCRA) to assess the necessary cleanup required and for disposal of dredged and contaminated sediments under the CWA (Southerland 1992). Presently, sediment quality criteria for five priority contaminants (dieldrin, endrin, acenaphthene, fluoranthrene, and phenanthrene) have been developed as guidance documents for the assessment of contaminated sediments (EPA 1993). Additionally, standardized methods have been developed to assess contaminated sediments using benthic invertebrates, and methods have been developed to measure bioaccumulation in aquatic invertebrates (EPA 1994b). Currently, the assessment of sediment contamination is based on measured sediment concentrations of individual chemicals and toxicity to laboratory organisms. However, bioaccumulation of contaminants by organisms exposed to the sediment may be a better indicator of the bioavailability and reduce the uncertainty when predicting the potential effects due to sediment contamination.

Chemical Mixture Toxicity

Chemicals in the environment rarely occur alone; however, most toxicological studies are conducted using single chemical exposures. Therefore, it is necessary to characterize the toxicological hazards and risks associated with multiple chemical exposures (Feron et al. 1995). Chemicals occurring in complex mixtures have the potential for chemical to chemical interaction, toxicokinetic interactions, and toxicodynamic interactions affecting the resulting toxicological response (Shinn and Hogan 1988). Chemical mixtures are characterized as having additive, antagonistic, independent, or synergistic interactions and effects on the measured toxicological endpoint (Calabrese 1991). Additivity is the summation of toxic responses from multiple chemicals in a mixture. Antagonism is the interaction in which the toxic response is less than would be predicted by summation. Independence is the effect of a single chemical not altered by the presence of another, indicating different mechanisms of toxicity. Synergism is the interaction of multiple chemicals in which the toxic response is greater than would be predicted by simple summation. The deviation of chemical mixture toxicity from traditional individual toxicological testing makes it necessary to evaluate mixture interactions further so that the hazards and risks associated with multiple chemical exposure may be assessed (Sexton et al. 1995).

Aquatic toxicology has addressed concerns of environmental mixtures of chemical contaminants by either developing models or utilizing information and models from pharmacological drug interaction studies (Sprague 1970). Deterministic models used for evaluating interactions of chemicals include the isobolographic method for analyzing binary mixtures. Response surfaces of binary chemical mixtures can be plotted to determine synergistic, additive, or antagonistic interactions (Gessner 1995). Modifications of the isobole method for use in aquatic toxicology include the development of the toxic unit (TU) by Sprague and Ramsey (1965). The TU is the concentration of the toxicant exposure divided by the lethal threshold concentration. Potencies, based on the LC_{50} values of chemicals, can be compared and mixture interactions evaluated using the toxic unit. Other models for evaluating mixtures include the use of a mixture toxicity index (MTI), joint action ratio, and the combination index (Calabrese 1991; Yang 1993). The combination index, I_c, is a mathematical model developed by Berenbaum (1989). The model is the basis for the hazard index which the U.S. EPA presently utilizes to evaluate chemical mixtures (EPA 1990). The model uses the sum total toxic units of each chemical in the mixture to characterize the interaction effect. Values for the combination index indicate additivity if I_c is equal to one, synergy if I_c is less than one, and antagonism if I_c is greater than one. However, models such as the combination index and isobole may lead the investigator to draw misleading conclusions or they are difficult to interpret and analyze (Howard, personal communication; Pounds et al. 1998). Traditional dose response analysis utilizes the four parameter logistic equation (sigmoidal dose response curves, Equation 1) to characterize the toxicological effects of a single chemical. The same analysis can be utilized to determine the interaction of binary mixtures (Poch et al. 1993). Initially, single chemical toxicity experiments are conducted and dose response curves developed. Theoretical interaction dose response curves are determined from single chemical dose response curves. Experimental interaction data are obtained by conducting a dose response curve of one chemical in presence of constant concentration of the second chemical. The experimental dose response curves, including a shift of slope and ED_{50} of chemical A in the presence of a fixed dose of chemical B, are compared to theoretical dose response curves of additive (Figure 1) and independent action (Figure 2). Results of the dose response curves are evaluated by graphical analysis. The model has many advantages over other models including ease of interpretation and analysis as well as utilization of the entire dose response curve to more completely characterize mixture interactions.

$$Y = [(a - d)/(1 + x/c^b)] + d$$

a = Emax **b = slope**
c = ED50 **d = Emin**
Y = response **x = dose**

Equation 1. Four Parameter Logistic Equation

To date, aquatic toxicology studies have typically evaluated the interaction of chemicals having similar mechanisms of toxicity. Kraak et al. (1994) studied the effects of a mixture of cadmium, copper, and zinc in the Zebra Mussel (*Dreissena polymorpha*) and determined the mixtures to be additive. Similarly, zinc and copper were found to interact additively in the Rainbow Trout (Lloyd 1961). Therefore, reviews of the literature have concluded that most environmental toxicants act primarily through additive interactions (Parrott and Sprague 1993; Broderius et al. 1995). Mammalian toxicological studies have shown that at low concentrations of individual chemicals in a mixture, there is less than additive interaction and the assumption of additivity for assessing the risks associated with chemical mixtures are too conservative (Sexton et al. 1995). The interaction of chemicals is dependent on the mechanism by which they exert toxicity and the toxicological endpoints examined. Hoagland (1993) found that atrazine and bifenthrin, having dissimilar mechanisms of toxicity, were additive. Parrott and Sprague (1993) determined that copper, zinc, acetophenone, and diethylglycol acted either additively or less than additively when measuring inhibition of bacterial bioluminescence and survival of fathead minnows as toxicological endpoints. Spehar and Fiandt (1986) observed mixtures of metals at concentrations acceptable by the individual water quality criteria were not protective of daphnids and fish due to additivity interaction. Several studies in which chemicals having independent or dissimilar mechanisms of action have demonstrated non-additive interactions, and in some cases found synergistic and antagonistic effects (Marinovich et al. 1996). It is apparent that there have been a variety of conclusions drawn from chemical mixture interaction studies. Chemical interactions are more complex than the assumption of additivity presently utilized to assess the risks associated with multiple chemical contaminants in a sediment. Therefore, there is a need to more fully understand the underlying mechanisms of chemical mixtures responsible for deviations from additive interactions.

Project Background

Currently, experiments are being conducted to evaluate the interactions of bioaccumulative chemical mixtures having the potential for toxicological effects not predicted from single chemical toxicity experiments. Three model toxicants representing environmentally relevant chemical contaminants will be used to assess chemical mixture interactions. All three chemicals were selected due to their persistence, mode of action, and occurrence at concentrations capable of producing adverse toxicological effects. The model organism, *Hyalella azteca*, was selected due to its ecological importance, potential for exposure to the model pesticide and metals, and ease of culturing large numbers (thousands) necessary for comprehensive mixture toxicity experiments. In the first stage of the project, single-chemical, water-only experiments were conducted to characterize the effects of the individual model chemicals. Following the single chemical toxicity experiments, mixture experiments will be conducted to characterize the effects of binary chemical mixtures as additive, antagonistic, independent, or synergistic. Lastly, spiked sediment toxicity experiments will be conducted to validate the results observed with the water-only exposures.

Model Compounds

Chlorpyrifos. Chlorpyrifos, a model organophosphate, is widely used in the United States with more than 14.4 million pounds applied to crop land each year (USGS 1997). Chlorpyrifos can enter the environment by volatilization and run-off after application. Following a rainfall event, streams near agricultural fields in northern Mississippi have been shown to receive concentrations of greater than 2.0 ppb chlorpyrifos in runoff 160 days after pesticide application (Smith et al. 1994). Due to the low solubility (1.4 mg/L) and hydrophobic nature (Log Kow 3.31-5.27), chlorpyrifos rapidly partitions from the water and adsorbs to sediment particles (Montgomery 1993). In the sediment, chlorpyrifos has a long half-life (60-100 days) making exposure to aquatic benthic organisms possible (Tomlin 1994). Chlorpyrifos exerts its toxicity by inhibiting acetylcholinesterase, an important enzyme which modulates the concentration of the neurotransmitter acetylcholine.

Dieldrin. Dieldrin is an organochlorine insecticide used from the 1950s to the late 1980s to control agricultural pests and termites. In 1990, the U.S. EPA banned production, although its use on agricultural crops and buildings was already limited. Although organochlorines are no longer used in the U.S., they continue to persist in the environment due to their long half-life (Loganathan and Kannan 1994). The half-life of dieldrin in water and soil has been estimated to be from two to ten years (Montgomery 1993). In a toxicological assessment of wetlands in northern Mississippi, dieldrin concentrations in the water were measured as high as 2.0 part per billion (ppb) (Steevens et al. 1998). Additionally, dieldrin was the most abundant organochlorine pesticide measured in the Missouri River at water concentrations up to 3.1 part per trillion (ppt) (Petty et al. 1995). In the Louisianian province of the Gulf of Mexico, including the Mississippi Gulf Coast, 57% of the sediments sampled had levels of dieldrin above levels

expected to cause adverse biological effects (U.S. EPA 1995). Dieldrin exerts its toxicity by binding to the GABA-$_A$ receptor and blocking the flux of chloride ion which normally acts to inhibit neural transmission (Narahashi et al. 1995). Dieldrin is also a potent inhibitor of both Na, K ATPase and Ca, Mg ATPase.

Methyl Mercury. Approximately 4,500 metric tons of mercury are released into the environment each year by human activities such as combustion of fossil fuels and other industrial releases (Lindqvist et al. 1991). Anthropogenic sources account for nearly 30-60% of the total annual influx of mercury to the atmosphere (Benoit et al. 1994). Global mercury loading trends indicate that atmospheric concentrations are increasing annually by greater than 1 percent (Slemr and Langer 1992). In Mississippi, concern over mercury in the environment has increased as a result of increased mercury concentrations in fish tissue samples from the Sunflower and Yazoo River Basins and Enid Lake drainage (Bass, personal communication). Methyl mercury is persistent in sediments and has been shown to bioaccumulate and biomagnify in fish and invertebrates as reviewed by Suedel et al. (1994). Methyl mercury can accumulate by way of an L-amino acid transporter and exerts its toxicity by depleting cellular stores of the antioxidant glutathione or by inducing oxidative stress (Sorenson 1991).

Potential for Chemical Mixture Interactions

All three model chemicals are considered neurotoxicants, which elicit their effects through specific independent mechanisms. Although the toxicity of the individual toxicants is understood, very little is known regarding the binary interactions of these chemicals. Since all three bioaccumulative chemicals are persistent and have the potential to coexist in the environment, it is clearly necessary to understand the mechanisms by which they interact and result in toxicity.

It is likely that potential interactions between organophosphate insecticides and methyl mercury could exist through the inhibition of acetylcholinesterase and other enzymes including those involved in biotransformation and detoxification processes. Chlorpyrifos is known to inhibit acetylcholinesterase by irreversibly binding to the active site of the enzyme. Methyl mercury has also been shown to inhibit acetylcholinesterase in a dose dependent manner (Petruccioli 1991). Enzyme inhibition by mercury has been suggested to occur either by binding to sulfhydryl groups on proteins resulting in modifications of protein structure and activity, or through oxidative stress. Additionally, methyl mercury acts to increase the quantal release of acetylcholine from the presynaptic vesicles. Methyl mercury specifically induces a release of calcium from mitochondrial stores. The increase in calcium released at the terminal bouton of the nerve results in an increase in the quantal release of acetylcholine (Levesque 1988). Interaction at cholinergic neurons by both chlorpyrifos and methyl mercury through their specific independent mechanisms could potentially lead to a toxicological response not predicted by single chemical toxicological studies. Malathion, an organophosphate, when coadministered with methyl mercury has been shown to have a greater than additive effect on the inhibition of acetylcholinesterase activity in quail plasma and brain (Dieter and Ludke 1975). However, no further research has been conducted to evaluate the binary interaction of organophosphates and methyl mercury. Methyl mercury is known to cause oxidative stress and binds to sulfhydryl containing proteins. Methyl mercury has been shown to bind and deplete cellular stores of glutathione. Glutathione is important to protect the cell from oxidative stress by acting as a free radical scavenger as well as acting as a phase II conjugating molecule. One detoxification pathway for organophosphates is through a transfer of the alkyl side chains to glutathione by glutathione transferase (Ecobichon 1996). Organophosphates biotransformed by cytochrome P450 monooxygenases are also detoxified through conjugation reactions with glutathione. Depletion of glutathione following exposure to methyl mercury may modify the rate of detoxification of chlorpyrifos.

The toxic effects of organophosphates have been suggested to be antagonized by concurrent exposure to organochlorine insecticides. Classical studies by Triolo and Coon (1966) demonstrated that aldrin antagonized the effects of parathion, paraoxon, as well as several other organophosphates. Keplinger and Deichmann (1967) demonstrated DDT and aldrin antagonized the effects of acute organophosphate toxicity. The protective effects of aldrin has been suggested to be through induction of beta-esterases (aliesterases). Beta-esterases are carboxylesterases that bind organophosphates with greater affinity than acetylcholinesterases (Chambers and Chambers 1990). Therefore, an increase or induction of beta-esterase may be protective against the effects of organophosphate exposure. Additionally, dieldrin induces cytochrome P450 which is important for the bioactivation and detoxification pathways of organophosphates (Wright et al. 1972). Several organophosphates, such as chlorpyrifos, require bioactivation by cytochrome P450 mediated oxidative desulfuration to the chlorpyrifos oxon prior to binding to acetylcholinesterase. Induction of cytochrome P450 would increase the rate of chlorpyrifos bioactivation, potentially increasing the toxicity. Conversely, the main mechanism of detoxification of organophosphates is through cytochrome P450 mediated dealkylation and dearylation of the side groups (Ecobichon 1996). An increase in cytochrome P450 would result in an increase in the rate of detoxification and lowered toxicity following exposure to organophosphates.

Organochlorine pesticides and methyl mercury act on the CNS through two primary mechanisms, the inhibitory $GABA_A$ receptor, and through inhibition of Na, K ATPase. The primary action of dieldrin, is through blockage of the $GABA_A$ receptor and associated chloride channels on the terminal bouton of neurons (Narahashi et al. 1995). The $GABA_A$ receptor is responsible for inhibition of neural transmission through hyperpolarization of the neuron. Blockage of the chloride channels disables the inhibitory mechanism, resulting in hypopolarization and uncontrolled excitability. Methyl mercury also binds to the $GABA_A$ receptor, acting synergistically with receptor agonists resulting in enhanced GABAergic responses (Komulainen et al. 1995). Benzodiazipine, a GABA receptor agonist, binds to the GABA receptor and is enhanced by binding of methyl mercury to the receptor (Corda et al. 1981). However, methyl mercury has been shown to block chloride ion flux by binding to the chloride ion channel (Arakawa et al. 1991). The overall physiological effects of methyl mercury on the GABAergic system are still unclear; however, the potential for chemical and drug interaction exists. Additionally, both dieldrin and methyl mercury are inhibitors of Na, K ATPase (Rajanna and Hobson 1985; Ballatori et al. 1988). Na, K ATPase is responsible for the repolarization of membranes and as a modulator of synaptosomal uptake of dopamine and norepinephrine. Inhibition of Na, K ATPase can lead to hyperexcitability of neurons due to the inability to repolarize as well as sequester neurotransmitters (Ecobichon 1996).

Model Organism.

Hyalella azteca (class crustacea, order amphipoda) is a benthic amphipod found in fresh and estuarine waters of North and South America. *H. azteca* is exposed to environmental xenobiotics because it primarily feeds and lives in the upper layers of sediment where the concentration of contaminants is often the greatest. Physiologically, amphipods are similar to crustaceans such as crabs, crawfish, and shrimp (Pennak 1989). *H. azteca* is a sentinel testing species for benthic aquatic invertebrates, which are a major food source for commercially important fishes. *H. azteca* has been used to assess bioaccumulation of metals and toxicity of sediments (Ingersoll et al. 1994). Furthermore, *H. azteca* has been endorsed as an aquatic invertebrate testing organism for evaluating toxicity as demonstrated by the U.S. EPA standard guidelines for sediment testing (U.S. EPA 1994b). *H. azteca* obtained from the U.S. Geological Service, National Biological Service are presently cultured in a flow-through aquarium system in the aquatic toxicology research facility of the School of Pharmacy at the University of Mississippi.

MATERIALS AND METHODS

Chemical Exposures

Range Finding Exposures. *H. azteca* were exposed to chemicals in water using a modification of methods outlined by U.S. EPA (1991). Organisms were exposed to model toxicants using concentrations following a geometric progression. Replication of 20 organisms, using greater than 150 organisms for each experiment, was necessary to adequately meet the requirements of the statistical model. Exposure chambers contained 10 ml of exposure water with the toxicant and one organism. Experiments were monitored daily, including water quality analysis, feeding, and observations of mortality. Water quality parameters monitored include: dissolved oxygen, pH, ammonia, hardness, alkalinity, and salinity.

Adult Exposures. Ten-day exposures at selected concentrations were conducted using adult test organisms to obtain adequate tissue for analytical and biochemical measurements. Water quality and feeding were conducted daily. Exposure chambers consist of a 1000 mL glass beaker containing 100 adult organisms, nitex substrate, and 800 mL of test water. At termination of the exposures, surviving organisms were counted and placed in vials to be stored in a -80°C freezer until chemical and biochemical analysis. Growth of *H. azteca* was determined by measuring length using a Videometric 150 Image Analyzer by American Innovision® as described by Steevens and Benson (1998).

Chemical Analysis

Water and tissue samples were held at 4°C in amber glass jars and analyzed within seven days. Chlorpyrifos and dieldrin were analyzed using a Hewlett-Packard 5890 gas chromatograph with dual electron capture detectors as outlined by EPA method 608 (CFR). Inorganic mercury and methyl mercury is determined by solvent extraction and detected using a Varian Spectra AA-20 atomic absorption spectrophotometer and VGA-76 vapor generation system (Filippell 1987). Detection limits for all analytes were less than 1.0 ug/L.

Statistical Analysis

Data from whole organism responses such as survival and growth was evaluated using dose-response curve analysis as previously described. In the case of single point interaction data, one-way analysis of variance (ANOVA) was used.

SigmaStat/Plot statistical analysis and graphing software will be utilized (Jandel Scientific).

RESULTS AND DISCUSSION

Presently, single chemical experiments for the three model compounds, chlorpyrifos, dieldrin, and methyl mercury have been conducted. Three main areas of experimentation have been conducted, including the evaluation of mortality, accumulation, and growth following exposure to the three model compounds. Initial experiments included four-day single chemical exposures to determine mortality endpoints (*e.g.*, LC_{50}) in both juvenile and adult *H. azteca* for all three model compounds (Table 1). These experiments were necessary to determine the level at which effects will be observed, as well as to determine the concentrations for subsequent experiments.

Variation observed in LC_{50} values demonstrates the potential differences between juveniles and adult *H. azteca* at these conditions when conducting toxicological experiments. Additionally, the toxicological data are presented on a molar basis to allow for comparison of potencies. Molar comparisons in toxicity demonstrate that chlorpyrifos is more potent than both methyl mercury and dieldrin, respectively. Additional data interpreted from non-linear dose-response curve fitting included slope and no-effect level threshold concentrations (not shown). Age related differences were observed for the LC50 values as determined from single chemical exposures. These differences ranged from 1.8-fold for chlorpyrifos, to more than 6-fold for dieldrin. Differences in susceptibility to the toxicants may be attributed to body size, developmental differences in biotransformation enzymes (modifying either elimination or bioactivation), or differences related to the specific mechanisms of action of the toxicant.

Accumulation of the three model compounds was evaluated over an exposure period of 96 hours. *H. azteca* exposed to 2.8 nM dieldrin for 96 hours accumulated levels reaching 1950 nM (Figure 3). The calculated bioconcentration factor (BCF) was 742. Similarly, methyl mercury was observed to accumulate linearly in *H. azteca* to 10,300 nM following exposure to 30 nM methyl mercury (Figure 4). Significant lethality was observed at a body residue concentration of 10,000 nM or greater. Accumulation of chlorpyrifos was below method detection limits (5.7 nM) during an exposure to 0.57 nM for 96 hours. The low chlorpyrifos concentration in tissue suggests that little or no accumulation occurs, the opposite of what would be expect- ed from the chemical properties (log Kow: 4.82) and literature values for BCFs (10 - 1000). The high potency (effects at less than 1 nM) and the low chemical concentration in the tissue suggest that *H. azteca* have a high capacity to metabolize chlorpyrifos. These results correspond to the high rate of metabolism and elimination of DDT observed in *H. azteca* by other investigators (P. Landrum, Great Lakes Environ. Res. Lab, Personal Communication).

Growth of juvenile *H. azteca* was evaluated during 10-day exposures to the individual chemicals. There were no statistically significant differences observed between control groups and treated groups. Therefore, life stage experiments will be conducted for 28 days starting with 2 to 3 day old organisms. The longer exposure duration will allow for an assessment of growth during the first 9 instars of development to sexual maturity.

CONCLUSION

To date, the toxicological effects of the single chemicals has been determined. Additional experiments are being conducted to determine the effects of the single chemicals during the sensitive developmental life stage (days 2 through 28). Following these experiments, the binary chemical mixture experiments will be conducted. These experiments will characterize the chemical mixture interactions of the model chemicals. Following the characterization, the biochemical mechanism of interaction will be determined. These endpoints include acetylcholinesterase, lipid peroxidation, protein oxidation, Na,K ATPase, total lipid content, glutathione-S-transferase activity, and total protein thiol content. Lastly, sediment toxicity experiments will be conducted. In summary, the research project outlined here, integrates traditional pharmacology and ecotoxicology to develop a novel approach to address chemical mixture toxicity. Results will contribute to our presently limited knowledge of multi-chemical exposure in the environment and, therefore, make a significant contribution to our fundamental understanding of the "real-world" toxicological effects of chemicals. Accordingly, this project is directly applicable to Mississippi and the South Atlantic-Gulf because of the importance of accurately assessing ecological risk.

ACKNOWLEDGEMENTS

Support for the research activities was provided, in part, by USGS Award 1434-HQ-96-GR-02679 (U.S. Department of Interior, U.S. Geological Survey, Cooperative State Water Resources Program) and Environmental and Community Health Research, The University of Mississippi.

LITERATURE CITED

Arakawa, O., M. Nakahiro, and T. Narahashi. 1991. Mercury modulation of GABA-activated chloride channels and non-specific cation channels in rat dorsal root ganglion neurons. Brain Res. 551: 58-63.

Ballatori, N., S. Chenyang, and J. L. Boyer. 1988. Altered plasma ion permeability in mercury-induced cell injury: studies in hepatocytes of easmobranch Raja erinacea. Toxicol. Appl. Phamacol. 95: 279-291.

Bass, P., Chief of Field Services Division, Mississippi Department of Environmental Quality. Personal communication.

Berenbaum, M. C. 1989. What is synergy? Pharmacol. Rev. 41: 93-141.

Borgmann, U., W. P. Norwood, and I. M. Babirad. 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 48: 1055-1060.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Broderius, S. J., M. D. Kahl,, and M. D. Hoglund. 1995. Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. Environ. Toxicol. Chem. 14: 1591-1605.

Calabrese, E. J. 1991.Multiple Chemical Interactions. Chelsea, MI: Lewis Publishers, Inc.

Chambers, J. E. and H. W. Chambers. 1990. Time course of inhibition of acetylcholinesterase and aliesterases following parathion and paraoxon exposures in rats. Toxicol. Appl. Pharmacol. 103: 420-429.

Code of Federal Regulations. 40 Part 136, Appendix A. Methods for organic chemical analysis of municipal and industrial wastewater: Method 608-organochlorine pesticides and PCBs.

Corda, M. G., A. Concas, S. Rossetti, P. Guarneri, F.P. Corongiu, and G. Biggio. 1981. Methyl mercury enhances [^{3}H]diazepam binding in different areas of the brain. Brain Res. 229: 264-269.

Dieter, M. P. and J. L. Ludke. 1975. Studies on combined effects of organophosphates and heavy metals in birds. I. Plasma and brain cholinesterase in coturnix quail fed methyl mercury and orally dosed with parathion. Bull. Environ. Contam. Toxicol. 13: 257-262.

Ecobichon, D. J. 1996. Toxic effects of pesticides. In: Casarett & Doull's toxicology: the basic science of poisons, Fifth Edition. Curtis D. Klassen, Ed. McGraw-Hill, New York, NY.

Feron, V. J., J. P. Groten, D. Jonker, F. R. Cassee, and P. J. van Bladeren. 1995. Toxicology of chemical mixtures: challenges for today and the future. Toxicology. 105: 415-427.

Filippelli, M. 1987. Deermination of trace amounts of organic and inorganic mercury in biological materials by graphite furnace atomic absorption spectrometry and organic mercury speciation by gas chromatography. Anal. Chem. 59: 116-118.

Gessner, P. K. 1995. Isobolographic analysis of interactions: an update on applications and utility. Toxicology. 105: 161-179.

Hoagland, K. D., R. W. Drenner, J. D. Smith, and D. R. Cross. 1993. Freshwater community responses to mixtures of agricultural pesticides: effects of atrazine and bifenthrin. Environ. Toxicol. Chem. 12: 627-637.

Howard, C. L. Professor of Environmental Toxicology and Ecology. University of Houston, Clear Lake, Houston, TX. Personal Communication.

Ingersoll, C. G., W. G. Brumbaugh, F. J. Dwyer, and N. E. Kemble. 1994. Bioaccumulation of metals by *Hyalella azteca* exposed to contaminated sediments from the upper Clark Fork River, Montana. Environ. Toxicol. Chem. 13: 2013-2020.

Keplinger, M. L. and W. B. Deichmann. 1967. Acute toxicity of combinations of pesticides. Toxicol. Appl. Pharmacol. 10: 586-595.

Komulainen, H., A. Keranen, and V. Saano. 1995. Methylmercury modulates $GABA_A$ receptor complex differentially in rat cortical and cerebellar membranes in vitro. Neurochem. Res. 20: 659-662.

Kraak, H. S., L. Daphna, H. Schoon, M. Toussaint W. H. M. Peeters, and N. M. van Straalen. 1994. Ecotoxicity of mixtures of metals to the zebra mussel Dreissena polymorpha. Environ. Toxicol. Chem. 13: 109-114.

Landrum, P. F., H. Lee, II, and M. J. Lydy. 1992. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ. Toxic. Chem. 11: 1709-1725.

Levesque, P. C. and W. D. Atchison. 1988. Effect of alteration of nerve terminal Ca^{2+} regulation on increased spontaneous quantal release of acetylcholine by methyl mercury. Toxicol. Appl. Pharm. 94: 55-65.

Lindquist, O. K., M. A. Johansson, A. Anderson, L. Bringmark, G. Hovsenius, A. Iverfeld, M. Meili, and B. Timm. 1991. Mercury in the Swedish environment. Water Air Soil Pollut. 55:193-216.

Lloyd, R. 1961. The toxicity of mixtures of zinc and copper sulphates to rainbow trout. Ann. Appl. Biol. 49: 535-538.

Loganathan, B. G., and K. Kannan. 1994. Global organochlorine contamination trends: an overview. Ambio. 23: 187-191.

Marinovich, M., F. Ghilardi, and C. L. Galli. 1996. Effect of pesticide mixtures on in vitro nervous cells: comparison with single pesticides. Toxicology. 108: 201-206.

McCarty, L. S. and D. Mackay. 1993. Enhancing ecotoxicological modeling and assessment. Environ. Sci. Technol. 27: 1719-1727.

Montgomery, J. H. 1993. Agrochemicals desk reference, environmental data. Chelsea, MI: Lewis Publishers.

Narahashi, T., D. B. Carter, J. Frey, K. Ginsburg, B. J. Hamilton, K. Nagata, M. L. Roy, J. H. Song, and H. Tatebayashi. 1995. Sodium channels and $GABA_A$ receptor-channel complex as targets of environmental toxicants. Toxicology Letters. 82: 239-245.

Parott, J. L., and J. B. Sprague. 1993. Patterns in toxicity of sublethal mixtures of metals and organic chemicals by Microtox and by DNA, RNA, and Protein Content of Fathead Minnows. Can. J. Fish. Aquat. Sci. 50: 2245-2253.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States. Third Ed. New York, NY: John Wiley & Sons.

Petruccioli, L., and P. Turillazzi. 1991. Effect of methylmercury on acetylcholinesterase and serum cholinesterase activity in monkeys, *Macaca fascicularis*. Bull. Environ. Contam. Toxicol. 46: 769-773.

Petty, J. D., J. N. Huckins, C. E. Orazio, J. A. Lebo, B. C. Poulton, R. W. Gale, C. S. Charbonneau, and E. M. Kaiser. 1995. Determination of waterborne bioavailable organochlorine pesticide residues in the lower Missouri River. Environ. Sci. Technol. 29: 2561-2566.

Poch, G. 1993. Combined effects of drugs and toxic agents: modern evaluation in theory and practice. Wien-New York: Springer-Verlag.

Pounds, J. G., D. G. Chen, and M. Mumtaz. 1998. Importance of model fitting in assessment of chemical mixtures. Toxicological Sciences. 42 (1-S): 343.

Sexton, K., B. D. Beck, E. Bingham, J. D. Brain, D. M. Demarini, R. C. Hertzberg, E. J. O'Flaherty, and J. G. Pounds. 1995. Chemical mixtures from a public health perspective: the importance of research for informed decision making. Toxicology. 105: 429-441.

Shinn, A. F., and M. J. Hogan. 1988. Evaluations of Drug Interactions, Fourth Edition. The C.V. Mosby Company. St. Louis, MO.

Slemr, F., and E. Langer. 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the atlantic ocean. Nature. 355: 434-437.

Smith, S. Jr., R. F. Cullum, and J. D. Schreiber. 1994. Pesticides in runoff and shallow ground water from upland corn production in north Mississippi, USA. Proceedings of the second international conference on ground water ecology. 249-258.

Sorensen, E. M.. 1991. Metal Poisoning in Fish. 312-323. Boca Raton, FL: CRC Press.

Southerland, E., M. Kravitz, and T. Wall. 1992. Management framework for contaminated sediments (the U.S. EPA sediment mangement strategy). Sediment toxicity assessment, edited by G. A. Burton, Jr. Chelsea, MI: Lewis, 1992. 341-370.

Spehar, R. L., and J. T. Fiandt. 1986. Acute and chronic effects of water quality criteria based metal mixtures on three aquatic species. Environ. Toxicol. Chem. 5: 917-931.

Sprague, J. B., and B. A. Ramsey. 1965. Lethal levels of mixed copper-zinc solutions for juvenile salmon. J. Fish. Res. Bd. Can. 22: 425-432.

Steevens, J. A., S. S. Vansal, K. W. Kallies, S. S. Knight, C.M. Cooper and W.H. Benson. 1998. Toxicological evaluation of constructed wetland habitat sediments utilizing *Hyalella azteca* 10-day sediment toxicity test and bacterial bioluminescence. Chemosphere. In press.

Steevens, J.A . and W. H. Benson. 1998. *Hyalella azteca* 10-day sediment toxicity test: Comparison of growth measurement endpoints. Environ. Toxicol. Water Qual. 13 (3).

Tomlin, C. 1994. The Pesticide Manual, 10th Edition. British Crop Protection Council and The Royal Society of Chemistry, U.K.

Triolo, A.J .and J. M. Coon. 1966. The protective action of aldrin against the toxicity of organophosphate anticholinesterases. J. Pharmacol. Exp. Therap. 154: 613.

U.S. Environmental Protection Agency. 1990. Technical support document on health assessment of chemical mixtures. EPA/600/8-90/064. Washington, D.C: U.S. Government Printing Office.

U.S. Environmental Protection Agency. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, Fourth Ed. EPA/600/4-90/027. Washington, D.C.: Office of Research and Development, EPA.

U.S. Environmental Protection Agency. 1993. Sediment quality criteria for the protection of benthic organisms: dieldrin (additional documents: endrin, acenaphthene, fluoranthrene, and phenanthrene). EPA-822-R-93-015. Washington, D.C.: Office of Science and Technology.

U.S. Environmental Protection Agency. 1994a. EPA's contaminated sediment management strategy. EPA 823-R-94-001. Washington, D.C.: Office of Water.

U.S. Environmental Protection Agency. 1994b. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-94/024. Washington, D.C.: Office of Research and Development.

U.S. Environmental Protection Agency. 1995. EMAP-Estuaries Louisianian Province-1993, Statistical Summary. EPA/620/R-96/003. Washington, D.C.: Office of Research and Development.

U.S. Environmental Protection agency. 1996. National listing of fish and wildlife consumption advisories. EPA-823-F-96-006. Washington, D.C.: Office of Water.

U.S. Geological Survey. 1997. Pesticides in surface and groundwater of the United States: Preliminary results of the national water quality assessment program (NAWQA).

Veith, G. D. 1996. Presentation at the National sediment bioaccumulation conference. Bethesda, Maryland. September, 11-13, 1996. Office of Research and Development, Washington, D.C.

Wright, A. S., D. Potter, and M. F. Wooder. 1972. The effects of dieldrin on the subcellular structure and function of mammalian liver cells. Food Cosmet. Toxicol. 10: 311-322.

Table 1. 96-Hour LC_{50} values for Adult and Juvenile *Hyalella azteca*

	Chemical	LC_{50} (nM) [A]	95% C.I.
Adult	Chlorpyrifos	0.625	N/A [B]
	Dieldrin	> 200	N/A
	Methyl Mercury	109	44-270
Juvenile	Chlorpyrifos	0.340	0.294-0.393
	Dieldrin	44.7	25.1-79.8
	Methyl Mercury	17.8	15.7-20.1

[A] Values calculated by non-linear sigmoidal dose-response curve fitting.
[B] Confidence interval not calculated, correlation coefficient squared is 1.0 for non-linear fit.

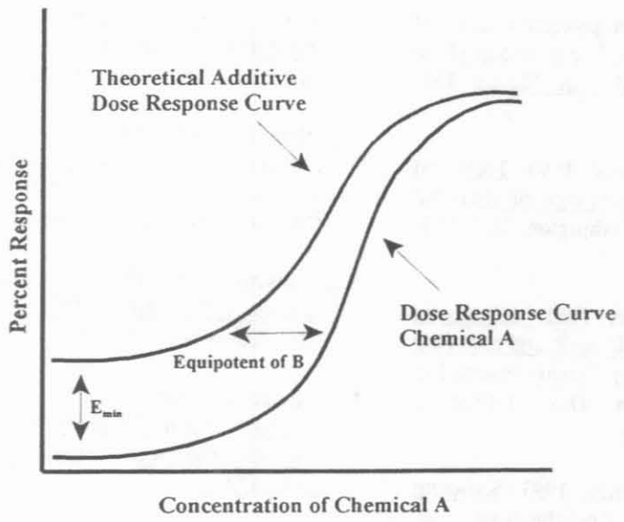

Figure 1. Construct Theoretical Additive Interaction Curve.

- Response (Y) shifted by change in equipotent concentration (x) of chemical B.
- Minimum effect (E_{min}) modified by a fraction of response associated with the constant concentration of chemical B.

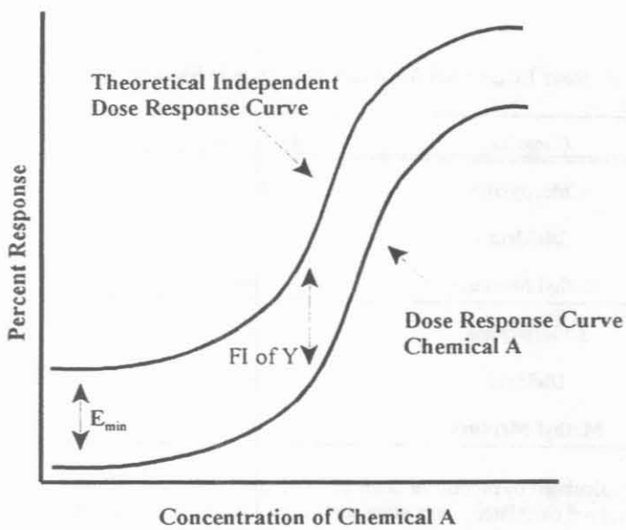

Figure 2. Construct Theoretical Independent Interaction Curve

- Response (Y) shifted upwards by a fractional increase due to the presence of chemical B
- Minimum effect (E_{min}) modified by a fraction of response associated with the constant concentration of chemical B.

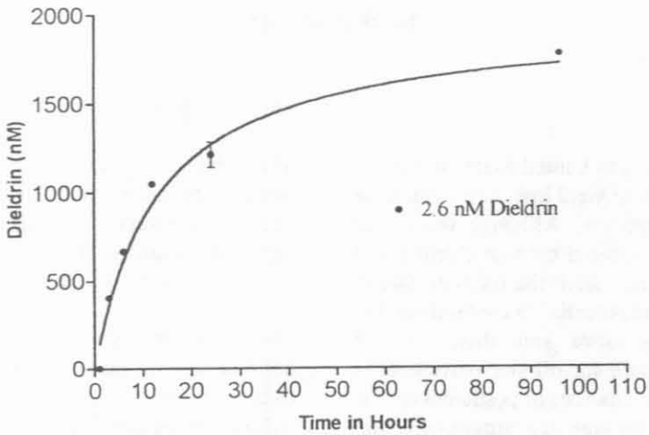

Figure 3. Uptake of Dieldrin by *Hyalella azteca*

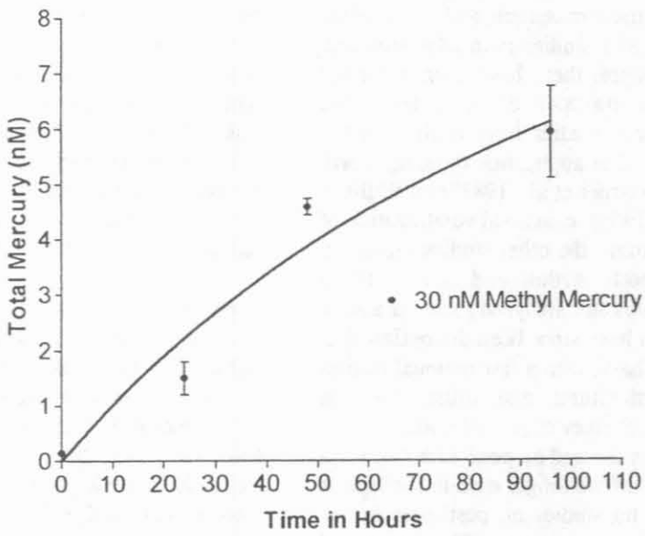

Figure 4. Uptake of Methyl Mercury by *Hyalella azteca*