CHARACTERIZING THE CHEMICAL MIXTURE INTERACTIONS OF CHLORPYRIFOS, DIELDRIN, AND METHYL MERCURY

Jeffery A. Steevens and William H. Benson
Department of Pharmacology and Environmental and Community Health Research
Research Institute of Pharmaceutical Sciences
School of Pharmacy, University of Mississippi, University, Mississippi

INTRODUCTION

Chemicals in the environment rarely occur alone; however, 95 percent of all toxicological studies evaluate the effects of single chemicals (Yang 1994). Chemicals occurring as complex mixtures have the potential for interactions which include chemical-chemical, toxicokinetic, and toxicodynamic interactions (Shinn and Hogan 1988; Cassee et al. 1998).Chemical-chemical interactions result from chemical reactions between two or more chemicals in the mixture. Toxicokinetic interactions result from the alteration of chemical absorption, elimination, and distribution. Toxicodynamic interactions are those that develop at the site of action (i.e., receptor, enzyme).These types of interactions result in toxicological effects or responses that are difficult to predict based upon single chemical toxicological data. Toxicological effects and measured responses are typically utilized to characterize the interactions associated with chemical mixtures. These interactions include additivity, independence, synergism, or antagonism, and can be defined as:

Additivity: (dose additivity) the summation of toxic responses from multiple chemicals in proportion to the dose of each chemical in the mixture. The chemicals contribute to the resulting response or effect without modifying the mechanism or effect of other chemicals in the mixture.

Independence: (response additivity) the effect of a single chemical is not altered by the presence of another, indicating different mechanisms of toxicity. At low concentrations cumulative effects of multiple chemicals are not observed, however, at higher concentrations the cumulative effects of the mixture are additive.

Synergism: the interaction of multiple chemicals in which the toxic response is greater than would be predicted by additivity. The presence of a second chemical enhances or increases the toxicological effect of the first chemical.

Antagonism: the interaction in which the toxic response is less than would be predicted by additivity. The presence of a second chemical decreases the toxicological effect of the first chemical. (Calabrese 1991; Poch 1993; Yang 1994; Cassee et al. 1998).

Where chemical mixture interaction studies have been conducted, most have focused primarily on chemicals that are similar in either structure and/or mechanism. For these studies, the main focus was to evaluate similar chemicals for synergy and antagonism; deviations from additivity. There are numerous studies which have focused primarily on the effects of chemical mixtures containing metals (Lloyd 1961; Enserink et al. 1991; Hamilton and Buhl 1997).These studies concluded that metals frequently interact additively. Similar studies have focused on hydrocarbons (Ribo and Rodgers 1990), and pesticides (Kreitzer and Spann 1973; Keplinger and Deichman 1967) which also have reported additive interactions. Comprehensive reviews of the literature concluded that environmental toxicants having similar structure and mechanisms of toxicity act primarily through additive interactions (Yang 1994; Calabrese 1991; Broderius et al. 1995).

Currently, assessments of chemical mixtures in the environment are conducted based on the assumption that similar chemicals act additively (U.S. EPA 1990). For example, all divalent metals would be assumed to act additively in combination. The total quantity of divalent metals would be considered a single stressor as a component of a complex mixture. The predicted overall effects contributed by the components of the complex mixture are assessed utilizing the hazard index (HI) defined as:

$$HI = E_1/AL_1 + E_2/AL_2 + \ldots \ldots + E_x/AL_x$$

where: E = exposure concentration and AL = acceptable limit for toxicant HI values greater than 1 indicate a potential hazard

However, deviations from the hazard index exist where there are non-independent interactions between non-similar components and non-additive interactions between similar components of the complex mixture (Cassee et al. 1998). Interactions between two chemicals having different structure and mechanism often result in either greater than additive or less than additive interactions. Combinations of dissimilar

chemicals often interact by altering the uptake, distribution, metabolism, or elimination of the second compound (Shinn and Hogan 1988).These deviations from additivity, resulting from chemical mixture interactions, cannot be predicted based on traditional single chemical toxicological studies. In addition, there is a paucity of literature focusing on chemical mixture interactions of dissimilar chemicals (e.g., pesticides and metals).Therefore, chemical mixture interactions should be further studied so that the hazards and risks associated with multiple chemical exposure may be assessed (Cassee et al. 1998; Sexton et al. 1995).

The main objective of the current study was to assess the chemical mixture interactions of structurally dissimilar chemicals with similar toxicological mechanisms. Three model compounds chlorpyrifos, dieldrin, and methyl mercury, which represent environmentally relevant chemical contaminants were selected due to their persistence, mode of action, and occurrence at concentrations capable of producing adverse toxicological effects. All three bioaccumulative 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. Chlorpyrifos, an organophosphate insecticide, is widely used in the United States with more than 21 million pounds applied to crop land each year (USGS 1998). Chlorpyrifos exerts its toxicity by inhibiting acetylcholinesterase, an important enzyme which modulates the concentration of the neurotransmitter acetylcholine (Tomlin 1994). 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. Dieldrin is no longer used in the U.S.; however, dieldrin and similar organochlorines continue to persist in the environment due to their long half-life (2 to 10 years) (Mongomery 1993; Loganathan 1994). 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 1995). Methyl mercury was selected as a model organic metal toxicant primarily due to its ubiquitous occurrence and neurotoxic effects, as well as its ability to bioaccumulate. 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 (Lindquist et al. 1991). Methyl mercury is persistent in sediments and has been shown to bioaccumulate and biomagnify in fish and invertebrates (Suedel and Rodgers 1994). Methyl mercury can actively accumulate in an organism through an L-amino acid transporter and exerts its toxicity by depleting cellular stores of the antioxidant glutathione or by inducing oxidative stress (Mokrzan et al. 1995).

To characterize the chemical mixture interactions of the three model toxicants, survival of *Hyalella azteca* and accumulation and elimination of the model compounds were assessed. *H. 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 chemicals because it primarily feeds and lives in the upper layers of sediment where the concentration of contaminants is often the greatest. *H. azteca* is considered a sentinel testing species for benthic aquatic invertebrates, which are a major food source for commercially important fishes. Furthermore, *H. azteca* are cultured in large numbers (greater than 10,000 per 10 L aquarium) which are necessary to conduct a comprehensive evaluation of chemical mixture interactions. The number of organisms utilized would not be possible with traditional vertebrate organisms (e.g. fish, mammals, birds) based on economical, practical, as well as ethical considerations.

MATERIALS AND METHODS

Animal Model

Hyalella azteca culture was originally established at the University of Mississippi in 1994, with organisms obtained from the USGS Biological Resources Division, Environmental and Contaminants Research Center (Columbia, Missouri). Species identity has been verified by a genetic differentiation study (Duan et al. 1997). Organisms were cultured in flow-through dechlorinated tap water and fed flake food (aquatic ecosystems) and hard maple tree leaves. Dechlorinated tap water used for culturing and experiments is well water from the University of Mississippi.

Chemicals

Analytical grade chlorpyrifos (99.2 % pure) was obtained from Chem Service (Westchester, Pennsylvania). Methyl mercuric chloride (CH_3HgCl, 97 % pure) was obtained from Phaltz and Bauer, Inc (Waterbury, Connecticut). Technical grade dieldrin was obtained from Aldrich (Milwaukee, Wisconsin).

Chemical Analysis

Chlorpyrifos and dieldrin were analyzed utilizing a Hewlett-Packard 5890 gas chromatograph (GC) with dual electron capture detectors (ECD) as outlined by EPA Method 608 (CFR). The GC was equipped with a J&W DB-1 60 meter capillary column with 0.25 µm

diameter and 0.25 µm film. A Hewlett-Packard Vectra 25 GC data station with Hewlett-Packard Chemstation software was utilized for programmed autosampler operation. Integrations of eluted peaks were determined based on peak area and elution times based upon known standards and verified with internal calibration verification. Acceptable test conditions were verified by less than 10% variance of internal calibration verification. The detection limit for dieldrin and chlorpyrifos is 3 nM and 3 nmoles/Kg for water and tissue, respectively. Total mercury was determined following a method outlined by ASTM (1993). Total mercury was detected in the water and tissue samples utilizing a Varian Spectra AA-20 atomic absorption spectrophotometer and VGA-76 vapor generation system. The detection limit for mercury was 5 nM and 225 nM for water and tissue, respectively.

Survival Exposures

Juvenile and adult *H. azteca* were exposed to chemicals in water using a modification of methods outlined by U.S. EPA (1991). Saturated solutions of each toxicant were prepared as previously described and diluted to the appropriate concentration determined by geometric progression. Water quality and observations for surviving organisms were conducted every 24 hours throughout the four-day exposure. Exposure chambers, in replication of 20, consisted of a 12 ml test tube containing 10 ml of water/toxicant and one juvenile (age 13 to 14 days) or adult (sexually mature) organism. Conditions during the exposure period were maintained in a ventilated waterbath at 23°C ± 1°C and a light cycle of 16 light:8 dark. Surviving organisms were identified by gently blowing exposure water toward the organism with a glass dropper and making observations for movement. Toxicant concentrations were verified by conducting chemical analysis of stock solutions prior to initiation of the experiment. Single chemical experiments were designed utilizing a geometric progression based on LC_{50} values previously determined from range finding experiments. Chemical mixture experiments were designed utilizing a geometric progression of concentrations based on single chemical experiments. Binary combinations of the toxicants were selected based on a seven by seven factorial design. Concentrations for the factorial design were selected at 0.125, 0.25, 0.5, 1, 2, and 4 times the LC_{50} value.

Accumulation and Elimination Exposures

Accumulation and elimination of the three model toxicants was determined utilizing adult *H. azteca*. Organisms were exposed to chlorpyrifos (0.11 ± 0.05 nM), dieldrin (30.2 ± 6.6 nM), and methyl mercury (42.4 ± 3.0 nM) individually and in combination for 144 hours to evaluate accumulation. Concentrations, at which no mortality was observed, were selected from single chemical survival experiments. Elimination of the model toxicants was evaluated following the accumulation exposure by transferring organisms to toxicant free water for 144 hours. Exposure chambers consisted of 1000 ml glass beakers containing 100 adult organisms and 800 ml of toxicant and water. Water was renewed every twelve hours throughout the accumulation and elimination experiments. Organisms were fed 1 ml of 0.5 mg/ml YCT (yeast, Cerophyl®, trout chow) every 48 hours. Water quality parameters were measured throughout the exposures and included: dissolved oxygen, pH, ammonia, hardness, alkalinity, and conductivity. Water quality conditions were acceptable for the exposures. Initially, 30 exposure chambers were prepared for each treatment. Three chambers were removed and organisms and water sampled at 3, 12, 36, 72, and 144 hours for chemical analysis throughout the exposure period. Following transfer to fresh water, organisms and water from three chambers were sampled at 3, 12, 36, 72, and 144 hours to evaluate elimination of the model toxicants. Tissue samples were divided for total mercury analysis by atomic absorption, or dieldrin/chlorpyrifos by GC.

Statistical Analysis

Statistical analysis was conducted using Sigma Stat® version 2.03 statistical software (Jandel Scientific). Non-linear fitting and comparison of linear equations was conducted utilizing GraphPad Prism 2.00 (San Diego, California). Survival data from single chemical and mixture experiments were evaluated utilizing dose response curve analysis and data was fit to the four parameter logistic equation. Chemical interactions were evaluated by comparing binary chemical mixture interactions to predicted dose response curves indicative of the theoretical additive and independent interactions (Poch 1993; Poch and Pancheva 1995). Initially, single chemical toxicity experiments were conducted, and dose response curves developed. Theoretical additive and independent interaction dose response curves were determined from single chemical experiments and dose response curves. The four parameter logistic equation utilizes variables including the minimum effect (E_{min}), maximum effect (E_{max}), and the dose of the chemical to describe the measured response. Experimental dose response curves, including a shift of slope and effective dose (ED_{50}) of chemical A in the presence of a fixed dose of chemical B, were compared to theoretical dose response curves of additive and independent action. A shift of the combined dose response curve to the left would indicate synergism, while a shift to the right would indicate antagonism. Theoretical additive interaction

curves of chemical A in a constant concentration of chemical B was calculated by lowering the dose of A by the concentration of B (dose = conc. A - conc. B) and the increase of the minimum effect resulting from the effects of the constant concentration of B (E_{min} = fractional effect of B/1). The theoretical independent interaction curves were calculated by shifting the response (Y) due to the fractional increase of chemical B (response + fractional effect of B/1). Experimental interaction data were obtained by determining a dose response curve of one chemical in presence of constant concentration of the second chemical. Results of the dose response curves were evaluated by graphical analysis. Results from uptake-elimination experiments were evaluated by nonlinear regression of data utilizing Graphpad® statistical software. One phase exponential association and decay models were utilized. Uptake constants (K_U) and depuration constants (K_D) were compared by observing confidence intervals for overlap. Single time-point data for multiple treatments, from accumulation and elimination studies, were evaluated utilizing one-way analysis of variance (ANOVA) with Bonferroni pairwise comparisons. Where tests for normality ($p = 0.01$) and equal variance ($p = 0.01$) failed, Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's pairwise comparisons was utilized. Student's t-test was utilized to compare two treatments. When equal variance for Student's t-test failed, Mann-Whitney rank sum test was used.

RESULTS

Single Chemical Survival Experiments

Single chemical survival experiments were conducted for 96 hours with juvenile and adult *H. azteca* to determine the lethal concentration (LC_{50}) for chlorpyrifos, dieldrin and methyl mercury. LC_{50} values and 95% confidence intervals were determined for each chemical by non-linear dose-response curve fitting. Dieldrin LC_{50} (>200 nM) was highest followed by methyl mercury (109 nM) and chlorpyrifos (0.625 nM). Adult LC_{50} values were higher (2 - 6 fold) for all three chemicals compared to juvenile LC_{50} values (data not shown).

Chemical Mixture Survival Experiments

Chemical mixture interactions were assessed for juvenile (Fig. 1) and adult *H. azteca* (data not shown). Methyl mercury and chlorpyrifos (Fig. 1A) interacted additively, as apparent by the fit of the methyl mercury dose response curve in the presence of 0.42 nM chlorpyrifos to the theoretical additive interaction dose response curve. The LC_{50} value for methyl mercury of 17.8 nM (95% C.I. 14.3 - 22.1 nM) decreased to 9.3 nM (5.7 - 14.9 nM) in the presence of chlorpyrifos.

Chlorpyrifos and dieldrin mixtures (Figure 1B) and methyl mercury and dieldrin mixtures (Figure 1C) interacted independently. The chlorpyrifos LC_{50} value of 0.20 nM (95% C.I. 0.18 - 0.23 nM) did not change significantly in the presence of 20 nM dieldrin (0.23 nM; 95% C.I. 0.22 - 0.25). The chlorpyrifos dose response curve, in the presence of 20 nM dieldrin, fit the theoretical independent interaction dose response curve. Similarly, the methyl mercury dose response curve, in the presence of 20 nM dieldrin, fit the theoretical independent interaction dose response curve. The LC_{50} value for methyl mercury was 12.8 nM (95% C.I. 12.3 - 13.3 nM) and did not change significantly in the presence of dieldrin (12.5 nM; 95% C.I. 11.4 - 13.8 nM).

Accumulation/Elimination Experiments

Accumulation and elimination of the three toxicants, independently and in binary combination, were assessed for 288 hours. Uptake and elimination of methyl mercury by *H. azteca* exposed to 42.4 ± 3.0 nM methyl mercury are presented in Figure 2A and Figure 2B, respectively. In binary combination, organisms were exposed to methyl mercury and chlorpyrifos (0.11 ± 0.05 nM) or dieldrin (30.2 ± 6.6 nM). Rate of methyl mercury accumulation independently (K_u = 0.022 ± 0.016, R^2 = 0.93) was not significantly different from methyl mercury accumulation in the presence of dieldrin (K_u = 0.052 ± 0.012, R^2 = 0.96) or chlorpyrifos (K_u = 0.179 ± 0.085, R^2 = 0.76). However, tissue concentrations of methyl mercury in the presence of chlorpyrifos after 3 and 12 hours of exposure were statistically different from methyl mercury alone or in combination with dieldrin ($p < 0.05$).Throughout the remaining accumulation period, the rate of methyl mercury accumulation decreased in the presence of chlorpyrifos resulting in the same final tissue concentration (4180 ± 626 mmoles/g) as methyl mercury alone (6660 ± 1090 mmoles/g) or methyl mercury in the presence of dieldrin (4730 ± 643 moles/g). Elimination of methyl mercury (Figure 2B) was evaluated for methyl mercury independently and in combination with chlorpyrifos and dieldrin for 144 hours following the transfer to toxicant-free water. Methyl mercury concentrations decreased in organisms exposed to methyl mercury alone. Tissue concentrations after 3, 12, and 36 hours of transfer to toxicant free water were not significantly different ($p = 0.70$). However, after 144 hours of elimination, methyl mercury concentrations in organisms exposed to methyl mercury and chlorpyrifos were significantly higher than methyl mercury alone ($p = 0.014$). Elimination of methyl mercury in the presence of

dieldrin is not shown due to significant mortality and insufficient tissue for chemical analysis.

Accumulation and elimination of dieldrin, independently and in combination with chlorpyrifos and methyl mercury are shown in Figure 3. Rate of dieldrin accumulation (Figure 3A) alone (K_u = 0.022 ± 0.010, R^2 = 0.73) was not significantly different from organisms exposed to dieldrin and chlorpyrifos (K_u = 0.034 ± 0.005, R^2 = 0.96) or methyl mercury (K_u = 0.018 ± 0.007, R^2 = 0.84). Elimination of dieldrin (Figure 3B) alone (K_u = 0.056 ± 0.039, R^2 = 0.62) was not significantly different in slope, elevation, or y-intercept from organisms exposed to dieldrin and chlorpyrifos (K_u = 0.009 ± 0.012, R^2 = 0.72). Limited data were available for nonlinear regression analysis of dieldrin elimination in the presence of methyl mercury due to significant mortality and insufficient tissue for chemical analysis. However, 0 and 36 hour time point data were not significantly different from dieldrin alone.

Chlorpyrifos accumulation was not observed in adult *H. azteca* at 0.11 nM at the described exposure conditions. Exposure to chlorpyrifos in combination with methyl mercury did not result in accumulation above control organisms. Accumulation of chlorpyrifos in the presence of dieldrin was not detected by GC due to masking of the chlorpyrifos analyte by peaks associated with dieldrin.

DISCUSSION

Single Chemical Survival Experiments

Survival following exposure to the individual toxicants demonstrated different potencies between the three model compounds. Chlorpyrifos was the most potent toxicant to both juvenile and adult *H. azteca*, followed by methyl mercury and dieldrin. The LC_{50} value for chlorpyrifos obtained with adult *H. azteca* (0.625 nM) was similar to reported literature values for *H. azteca*; 3.7 nM for 1-day LC_{50} (Siefert 1984), 0.29 nM for 2-day LC_{50} (Moore et al. 1998), and 0.25 nM for 10-day LC_{50} (Phipps et al. 1995). Variations in the reported LC_{50} values may be due, in part, to the different age of the organisms and duration of exposure. The concentrations of chlorpyrifos at which effects were observed are within the range of measured environmental concentrations in surface water. Results of the National Water Quality Assessment Program demonstrated that 50 percent of the 1058 surface water sites sampled in the United States had chlorpyrifos concentrations of 0.037 nM (0.013 µg/L) or higher (U.S.G.S. 1998). The maximum concentration of chlorpyrifos in surface waters was 1.14 nM (0.4 µg/L). Higher concentrations in runoff waters have been observed adjacent to agricultural fields. Following a rainfall event, runoff from agricultural fields in northern Mississippi had concentrations greater than 2.0 µg/L chlorpyrifos 160 days after pesticide application (Smith et al. 1994).

The dieldrin LC_{50} value obtained (> 200 nM) was higher than reported literature values for *H. azteca* of 20 nM determined from flow through 10-day experiments (Hoke et al. 1995). The LC_{50} value reported in the current study may be greater due to the shorter duration of exposure and static conditions. Higher LC_{50} values were observed for other amphipods including *Gammarus fasciatus* (1580 nM) and *Gammarus lacustris* (1207 to 1837 nM) (U.S. EPA 1980).The higher LC_{50} values were greater than the water solubility and determined utilizing a solvent carrier potentially altering the uptake and bioavailability of dieldrin as well as the calculated LC_{50} values. The concentration of dieldrin at which effects were observed was greater than the measured dieldrin concentrations in surface waters. Results of the National Water Quality Assessment Program demonstrated that the maximum concentration of dieldrin in surface waters was 0.50 nM (0.19 µg/L) (USGS1998).

The methyl mercury LC_{50} value for adult *H. azteca* (109 nM) was lower than the reported value for the marine amphipod, *Gammarus duebeni*, at 748 nM (150 µg/L) (U.S. EPA 1985).The 96-hour LC_{50} value for the cladoceran, *Daphnia pulex*, was 7.2 nM (1.805 µg/L) (Tian-yi and McNaught 1992). These values are within a factor of 10 and the variation between them may be due to the species and age of the test organisms. In addition, freshwater organisms are more sensitive to methyl mercury (Eisler 1987). In fresh surface water, methyl mercury concentrations typically range from 0.16 to 2.9 nM (0.04 - 0.73 ng/L) (U.S. EPA 1997). Methyl mercury measured in water, represents only 1 to 14 percent of the total quantity of mercury. Additionally, environmental concentrations of methyl mercury are characteristically lower in surface water than in sediment. Sediment concentrations of total mercury are typically around 200 ng/g; however, in areas of heavy contamination the concentration of mercury may be as high as 1g/kg sediment (Eisler 1987). Similar to water, 0.5 to 5.3 percent of the total mercury present in sediment is methyl mercury. Although the environmental concentrations of methyl mercury are lower than inorganic mercury, methyl mercury is more toxic and has the potential to bioaccumulate to concentrations in the organism capable of eliciting adverse toxicological effects (McCarty and Mackay 1993).

Accumulation and Elimination of Single Chemicals

The bioaccumulation of chlorpyrifos, dieldrin, and methyl mercury have been previously reported in aquatic organisms (Suedel and Rodgers 1992; Tsuda et al. 1992; Serrano et al. 1997). In the current study, the uptake and evaluation of the individual model toxicants was determined in order to assess the toxicokinetic interactions following exposure to a chemical mixture.

Accumulation of chlorpyrifos was not observed in *H. azteca* exposed to 0.11 nM of the chemical. Method detection limits of 3 nmoles/kg would have been sufficient to detect a bioconcentration of 30 times the water concentration (0.11 nM). Based on the chemical properties of chlorpyrifos (log K_{ow} 4.82), a high degree of accumulation would be expected. Bioconcentration factors for fish typically range from 2.7 to 5100 depending on the species and experimental conditions (Barron and Woodburn 1995). However, bioconcentration factors for fish have been reported as high as 28000 (Deneer 1993). Only one study has reported the accumulation of chlorpyrifos in invertebrates. A bioconcentration factor of 262 was reported for the isopod *Asellus aquaticus* exposed to 2.0 nM chlorpyrifos (0.7 µg/L) for 48 hours (Cid Montanes et al. 1995). These data suggest that the balance of accumulation and elimination of chlorpyrifos is highly species and age dependent.

Accumulation of methyl mercury by *H. azteca* was rapid and continued throughout the 6 day exposure period without reaching a maximum concentration. The accumulation of methyl mercury in aquatic organisms including fish and invertebrates is well documented (Olsen 1975; Eisler 1987; U.S. EPA 1996, 1997).The uptake of methyl mercury was rapid, primarily because accumulation of methyl mercury is an active process facilitated by an amino acid transporter (Clarkson 1994). Once reaching the systemic circulation, methyl mercury binds to sulfhydryl containing peptides/proteins resulting in decreased elimination. Elimination of mercury in invertebrates primarily occurs through demethylation of the mercury to inorganic mercury, or potentially through the loss of protein bound mercury during molt (Dallinger and Rainbow 1992).The large differences between uptake and elimination may, in fact, be responsible for the ability of methyl mercury to biomagnify and reach high concentrations in tissue (Suedel and Rodgers 1994). The potential for dieldrin to accumulate led to its eventual banning in the 1980s (Loganathan and Kannan 1994). In the current study, the rate of dieldrin accumulation in *H. azteca* exposed to 42.4 nM was rapid. Dieldrin accumulation appears to be a passive process, primarily due to its hydrophobicity and affinity for lipophilic substances, as indicated by a high octanol/water partition coefficient (log K_{ow} = 4.55). Dieldrin accumulation is known to occur directly through exposure in the water, with very little occurring through the ingestion of food or sediment.

Chlorpyrifos-Methyl Mercury Interactions.

Currently, the binary interactions of chlorpyrifos, dieldrin, and methyl mercury would not be predicted to interact additively, but rather independently based on the known mechanisms of the individual chemicals. Methyl mercury and chlorpyrifos elicit their toxicity through different mechanisms. Chlorpyrifos is known to inhibit acetylcholinesterase by irreversibly binding to the active site of the enzyme. Methyl mercury is known to cause oxidative stress and binds to sulfhydryl containing proteins. In addition, 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. Based upon known mechanisms, the prediction utilizing the current models would be that chlorpyrifos and methyl mercury interact independently. However, results of the binary interaction survival experiments demonstrate that methyl mercury and chlorpyrifos interact additively. An additive interaction would imply that the two chemicals are acting by the same mechanism and at the same target. Synergy of mercury and the organophosphates, malathion and parathion, has been reported in Coturnix quail (*Coturnix japonica*) (Dieter 1974; Dieter and Ludke 1975). Quail, age 4 weeks, were administered 2, 4, 6, 8, and 10 mg/kg orally in combination with 4 mg/kg methyl mercury (morsodren). LD_{50} values for parathion treated birds were 5.86 mg/kg and 4.24 mg/kg in the presence of 4 mg/kg methyl mercury. Based on these findings, the authors reported that methyl mercury synergized the effects of parathion. In addition, they concluded that methyl mercury increased the inhibition of acethlcholinesterase by acting at the enzyme or by increasing the bioavailability of parathion to the enzyme. However, the study did not include dose response curves of methyl mercury alone, and the synergy was determined based upon the increased toxicity due to exposure to both compounds. Therefore, the actual interaction may have been additive. To date, no further studies have been conducted evaluating methyl mercury and organophosphate insecticides.

The accumulation and elimination of methyl mercury was influenced by coexposure to chlorpyrifos. Initially, organisms exposed to methyl mercury and chlorpyrifos accumulated methyl mercury more rapidly, within the first three hours, than methyl mercury alone. Following

the initial increase of methyl mercury accumulation, the rate of accumulation was decreased significantly. The concentration at the end of the 144 hour exposure period, was the same for both treatments. Following the 144 hour exposure period, the organisms were placed in toxicant free water and allowed to eliminate methyl mercury. It is likely that chlorpyrifos decreases the elimination of methyl mercury resulting in an apparent increased rate of accumulation of methyl mercury. Methyl mercury is normally eliminated by conjugation to glutathione utilizing the enzyme glutathione-S-transferase. Chlorpyrifos is also eliminated through the conjugation to glutathione. A competition for glutathione or a subsequent decreased concentration of available glutathione would decrease the elimination. Few studies have evaluated the toxicokinetics of methyl mercury in the presence of pesticides. The increased accumulation of methyl mercury in liver of rats was demonstrated in the presence of atrazine (Meydani and Hathcock 1984). An increase in neurotoxicity also was observed, however, the mechanism of the interaction was never elucidated. A similar study focused on the accumulation and distribution of mercury and methyl mercury compounds in brown trout (*Salmo trutta*) in combination with sulphur containing ligands (Gottofrey and Tjalve 1991). Included in the ligands that were tested, was a phosphorothionate compound (sodium diisopropyldithiophosphate) with a similar structure to chlorpyrifos. The presence of the sulphur ligand significantly increased the accumulation of methyl mercury in the tissues of brown trout. Potential interactions exist through the metabolic pathways of methyl mercury. Methyl mercury is demethylated by the cytochrome P450 enzymes and eliminated by conjugation to glutathione (Clarkson 1994). Altered activity of specific cytochrome P450 isoforms may result in decreased demethylation of methyl mercury and subsequent decreased elimination.

Dieldrin-Methyl Mercury Interactions

To date, there are no other studies which have evaluated the interactions between metals and organochlorine pesticides. In the current study, dieldrin and methyl mercury interacted independently on the survival of *H. azteca*. In addition, the binary combination did not alter the toxicokinetics of either dieldrin or methyl mercury. Dieldrin and methyl mercury elicit their toxicity through similar biochemical pathways. 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. In addition to the oxidative stress and depletion of glutathione, methyl mercury also binds to the $GABA_A$ receptor. Methyl mercury has been demonstrated to act synergistically with receptor agonists resulting in enhanced GABAergic responses (Komulainen et al. 1995). Benzodiazipine, a GABA receptor agonist, can bind 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). 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). Although the known mechanisms of dieldrin and methyl mercury have the potential to interact in a way to alter the toxicity or toxicokinetics of the other chemical, no dose additive, synergistic, or antagonistic interactions were observed.

Chlorpyrifos-Dieldrin Interactions

Chlorpyrifos and dieldrin interacted independently with survival as the endpoint. These results suggest that the mechanism of toxicity associated with the two chemicals in combination is dissimilar and the presence of the second chemical does not alter or contribute to the toxicity of the other. Similar studies on survival of birds and mammals have evaluated the interactions of organochlorine and organophosphate insecticides. Kreitzer and Spann (1973), determined that dieldrin and diazinon, an organophosphate, interacted additively in pheasants and quail. These results do not distinguish between additivity and independence; however they do not indicate the presence of synergy or antagonism between dieldrin and an organophosphate. Keplinger and Deichmann (1967) evaluated the interactions of eight organochlorine and five organophosphate insecticides on rats and mice. The investigators concluded that the toxicity of dieldrin was synergistic with all five organophosphates, delnave, diazinon, V-C 13, malathion, and parathion. In addition, it was concluded that aldrin, DDT, and toxaphene were antagonistic with organophosphates in rats, but not mice. The Keplinger and Deichmann study did not evaluate the dose-response of the individual chemicals and is, therefore, limited in its ability to distinguish between additivity and

deviations such as synergy and antagonism. The mechanism of interaction between organochlorine and organophosphate insecticides may exist through the metabolic capacity of an organism to eliminate the highly toxic organophosphates. Pretreatment of mice with DDT or aldrin decreases the toxicity associated with parathion (Bass et al. 1972). The mechanism of the antagonistic interaction was determined to be through the increased activity of A-esterase and B-esterase which play a role in the detoxification and toxicity, respectively (Triolo and Coon 1966).

Accumulation of dieldrin, in the current study, was not altered by the presence of chlorpyrifos. Mechanisms of interaction for organochlorine and organophosphates typically occur through the modification of metabolic pathways for organophosphates. Organochlorines have the potential to alter the metabolizing enzymes for other pesticides. Hexachlorobenzene and DDT have been demonstrated to increase the elimination rate of dieldrin in feces and urine as well as decreasing the concentration of dieldrin in adipose (Clark et al. 1981). However, no data exists suggesting that the organophosphates alter the accumulation or elimination of organochlorine pesticides. In addition, the accumulation of chlorpyrifos was not observed in *H. azteca* under the described exposure conditions.

CONCLUSIONS

The current study demonstrates the potential for chemical interactions to occur beyond what would be expected or predicted based on single chemical experiments. While previous studies have reported synergism for methyl mercury and chlorpyrifos, these results demonstrate an additive interaction as well as alterations in accumulation and elimination of methyl mercury by chlorpyrifos. Methyl mercury and dieldrin did not interact to alter the toxicity or toxicokinetics of either chemical. Previous studies have reported both synergy and antagonism for mixtures of organochlorine and organophosphate insecticides. However, these results demonstrate that dieldrin and chlorpyrifos interact independently.

Current models utilized to evaluate chemical mixtures would have predicted the effects of organochlorines with organophosphates and methyl mercury. However, these models would not have predicted the additive effects of methyl mercury and chlorpyrifos. In addition, there is previous knowledge of synergistic and antagonistic interactions with all three chemicals. The species of chemical and animal model as well as conditions of exposure must be considered to meaningfully assess the toxicological effects of chemical mixtures. In addition to that, it is imperative that further knowledge on the mechanisms of interactions be acquired to understand and accurately predict the toxicological effects of chemical mixtures.

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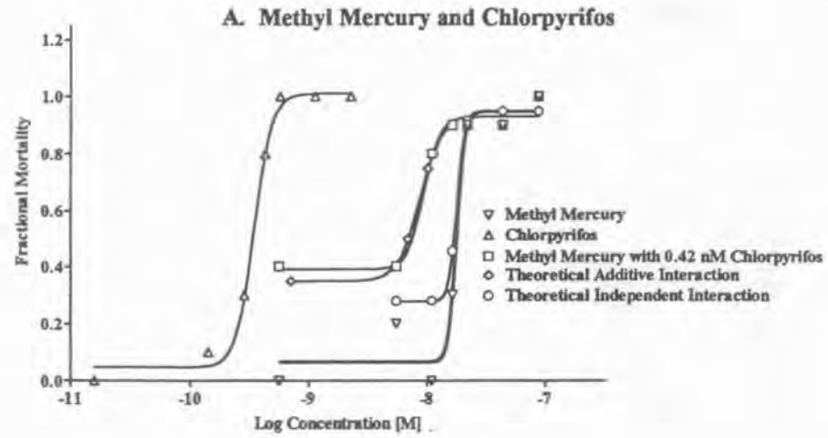
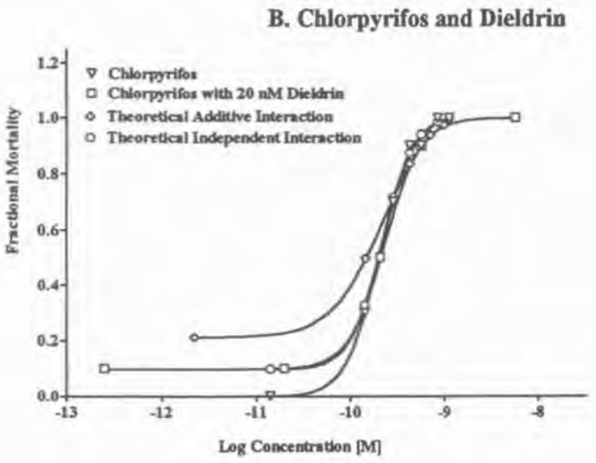




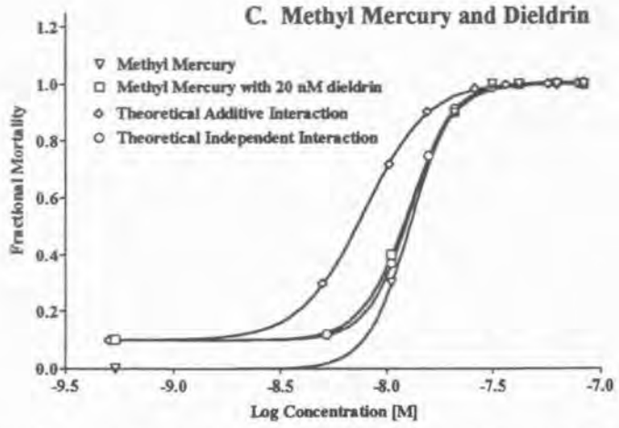


Figure 1. Survival of juvenile *Hyalella azteca* following exposure to binary chemical mixtures of chlorpyrifos, dieldrin, and methyl mercury for 96 hours. Interactions were characterized as additive or independent by graphical analysis of dose response curves. Dose response curves of one chemical were evaluated in the presence of a constant concentration of a second chemical.

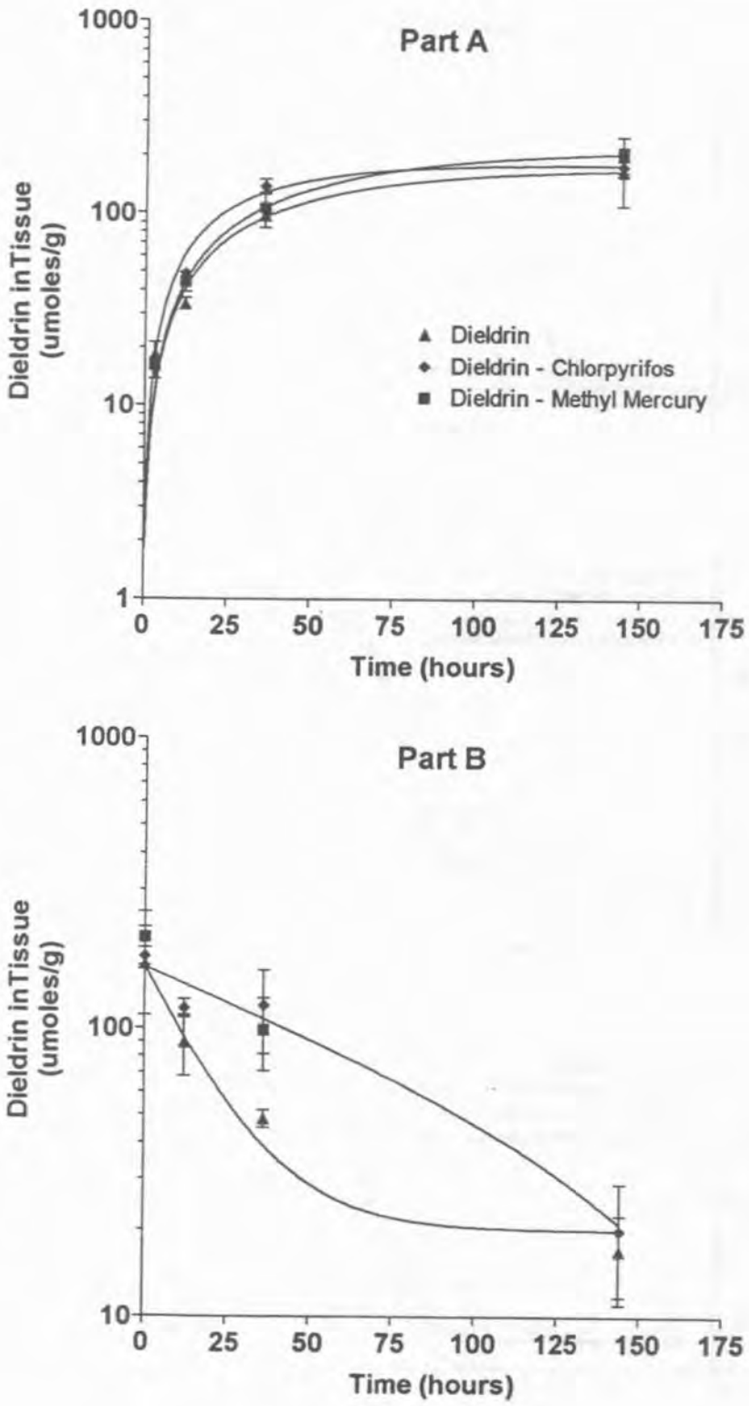
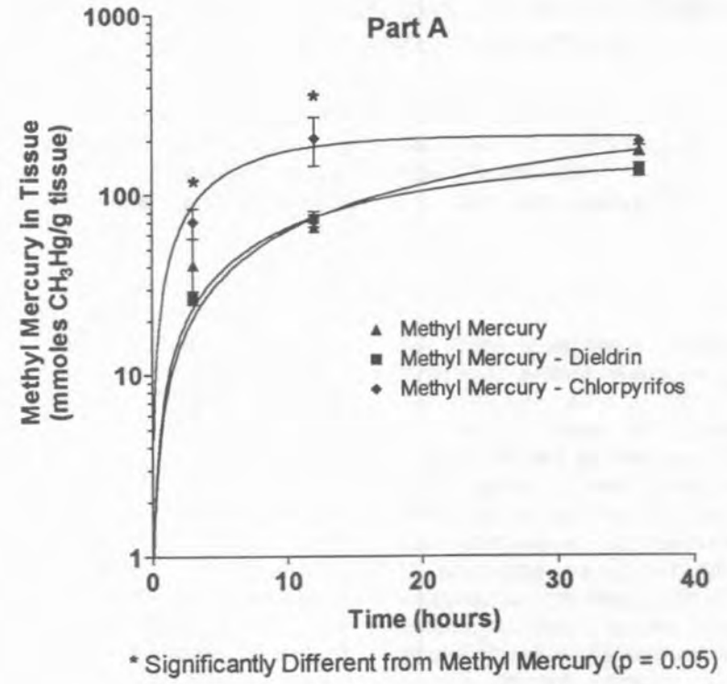


Figure 2. Accumulation (A) and elimination (B) of dieldrin in *Hyalella azteca* following exposure to methyl mercury (42.4 ± 3.0 nM), chlorpyrifos (0.11 ± 0.05 nM) and dieldrin (30.2 ± 6.6 nM).



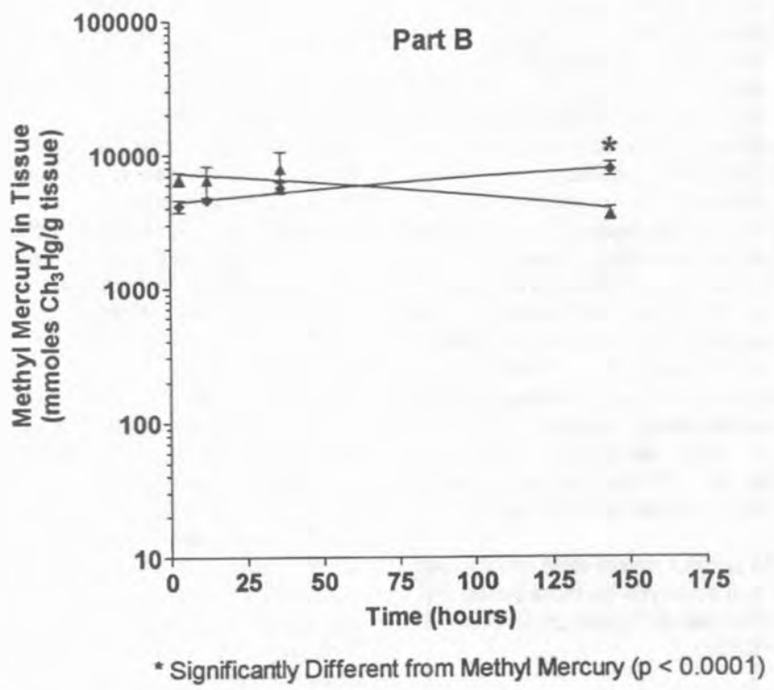


Figure 3. Accumulation (A) and elimination (B) of methyl mercury in *Hyalella azteca* following exposure to methyl mercury (42.4 ± 3.0 nM), chlorpyrifos (0.11 ± 0.05 nM) and dieldrin (30.2 ± 6.6 nM).