

CHLORPYRIFOS AND PARATHION EFFECTS ON ENZYME ACTIVITIES IN FINGERLING  
CHANNEL CATFISH, Ictalurus punctatus: INTERACTIONS WITH DEF  
(S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE) AND AROCLOR 1254

By

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Candidate for Degree of Doctor of Philosophy

Channel catfish fingerlings were exposed to various xenobiotics in experiments to evaluate effects on enzyme activity.

In the first study, brain, gill, liver, muscle, and plasma were assayed for acetylcholinesterase (AChE) inhibition and gill, liver, and plasma were assayed for aliesterase (ALiE) inhibition in vitro by DEF and oxons of chlorpyrifos (Cpxn) and parathion (Pxn). DEF (1 mM) inhibited AChE activity <15% in all tissues. AChE  $I_{50}$  values for Cpxn were 28-33 nM, and for Pxn were 446-577 nM. ALiE  $I_{50}$  values for Cpxn were 0.1-0.2 nM, for DEF were 24-163 nM, and for Pxn were 6-46 nM.

In the second study, fish were exposed to chlorpyrifos (Cp), parathion (Pth), DEF, and combinations of the phosphorothionates and DEF for 4 h followed by a 388 h recovery period. AChE inhibition following Cp and Pth exposures was rapid. Cp led to more persistent inhibition than Pth. DEF treated fish had low inhibition in brain and muscle, and high inhibition in gill, liver, and plasma. In vitro and in vivo results suggest that DEF's disposition and mode of action are different than those of Cp or Pth. ALiE activities demonstrated that exposure to DEF or combination treatments resulted in persistent, high inhibition. Greater AChE inhibition in combination treatments was not evident suggesting that ALiEs do not serve to protect AChE, even though ALiEs are inherently more sensitive to inhibition.

In the third study, fish were exposed for 20 h to piperonyl butoxide (PBO) followed by a 4 h exposure to DEF. AChE activities were determined at 0 and 12 h after the exposure period. Inhibition of brain, plasma, and liver AChE activity by DEF was antagonized by PBO; muscle AChE was not inhibited by DEF. PBO did not antagonize the inhibition of liver or plasma AChE by DEF. These results suggest that PBO retards the formation of the metabolite(s) of DEF that inhibit AChE.

In the fourth study, fish were injected (IP) with 100 mg Aroclor 1254/kg body wt and sacrificed at 96 h. Microsomal cytochrome P450-mediated deethylation of 7-ethoxyresorufin was induced; however, effects on desulfuration or dearylation of Cp and Pth were not evident. Results indicate that Aroclor 1254 does not appreciably induce the isozymes of cytochrome P450 responsible for desulfuration and dearylation at the treatment rate tested.

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CHAPTER I  
GENERAL INTRODUCTION

Culture of channel catfish, Ictalurus punctatus, is a fast growing industry in the Mississippi Delta area. Flat terrain, an abundant, suitable and inexpensive water supply, and soil having a clay composition make this an ideal location for such an industry. Agriculture is a well established enterprise in this area because of these reasons and is often located in close proximity to catfish culture ponds.

Insect control on agricultural land is commonly accomplished by aerial application of insecticides throughout the growing season. While crop-dusting pilots are very conscious of all parameters affecting their activities, wind drift and over-spray of insecticides are very real possibilities for unintentional contamination. Defoliant (herbicides) are used at the end of the growing season to facilitate crop harvesting; these are also aerially applied and are therefore subject to wind drift and over-spray.

Banning of the use of DDT and other commonly used organochlorine (OC) insecticides in the early 1970's prompted the accelerated development and increased use of organophosphorus (OP) compounds. The OP insecticides are frequently more acutely toxic to mammals and more expensive, but are much less persistent in the environment. Steel (1991) reported that 70 percent of the restricted use pesticides listed by the EPA belong to the OP class of pesticides.

Two of the OP insecticides used presently or in the past in this region are chlorpyrifos (Lorsban<sup>®</sup>; O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) and parathion (O,O-diethyl O-4-nitrophenyl

phosphorothioate); the most frequently used OP herbicide is DEF<sup>o</sup> defoliant (S,S,S-tributyl phosphorotrithioate) (Figure 1.1).

The phosphorothionate insecticides (chlorpyrifos and parathion) are poor anticholinesterases; they must be activated to their oxon metabolites, chlorpyrifos-oxon (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphate) and paraoxon (O,O-diethyl O-4-nitrophenyl phosphate), to impair the function of the nervous system, their primary target; this activation is catalyzed by cytochromes P450. Murphy and DuBois (1959) suggested a possible cholinergic action by DEF because of symptoms of salivation, lacrimation, urination, and occasional tremors in treated rats which are also symptoms of OP poisoning; further experiments demonstrated that atropine was an ineffective antidote and provided further evidence that actions other than cholinesterase inhibition are important in poisoning by DEF. Casida et al. (1963) also found that actions other than cholinesterase inhibition are important in the toxicity of this phosphorotrithioate to mice, but did not identify these. Recent reports by Hur et al. (1992) implicate DEF sulfoxide and/or DEF sulfone as the anticholinesterase metabolites. Further research is still required to characterize the mode of toxicity of DEF.

Acute toxic effects are a result of binding to and inhibition of acetylcholinesterase (AChE), which is found at the synapses within the central and autonomic nervous systems and at nerve endings innervating striated muscles. Normally the neurotransmitter acetylcholine is hydrolyzed by AChE into acetate and choline promptly after it acts. In the presence of OP anticholinesterases, hydrolysis by AChE is decreased or prevented by the OP binding to a serine residue at the active site; acetylcholine accumulates resulting in excessive nerve excitation which causes muscle twitching and activation of other effectors (H. Chambers, 1992).

The acute toxicity of these compounds has been studied in many vertebrates. There is a great range in acute toxicity levels of OP's

(Table 1:1). This diversity could result from differences in inhibitory potency for the target and non-target enzymes, disposition, and/or metabolism.

Wallace (1992) cited differences in one or a combination of kinetic steps such as absorption, distribution, metabolic transformation, and elimination as causing species-related differences in sensitivity to OP compounds; species sensitivity may also be manifested at the receptor level (i.e., differences in the sensitivity of AChE to inhibition by OPs or differences in nicotinic or muscarinic acetylcholine receptor regulation and the effectors they control).

Metabolic contributions to differences in toxicity include activation (desulfuration) of the parent insecticides to their active metabolites or detoxication of the OP's and their metabolites; target enzyme (AChE) sensitivity to the metabolites is also important. Detoxication mechanisms include cytochrome P450-mediated dearylation, aliesterase phosphorylation, A-esterase mediated hydrolysis, and glutathione conjugation (Chambers and Chambers, 1991).

Phosphorothionate desulfuration and dearylation are metabolic routes of cytochrome P450-dependent activities of particular interest. Formation of the active oxon metabolite by desulfuration includes initial donation of a singlet oxygen atom to the sulfur atom of the parent compound. The chemical structure is believed to rearrange to form an unstable phosphooxythiiran (cyclic P-S-O) intermediate, which undergoes a cyclic electron shift with the loss of sulfur, forming the oxon; dearylation is possibly accomplished by hydrolysis of the P-O-aryl bond, and O rather than S is eliminated (H. Chambers, 1992; Neal and Halpert, 1982).

The liver is the major site of xenobiotic metabolizing enzymes and is typically assumed to be the major site of desulfuration in mammals, (J. Chambers, 1992; Nakatsugawa et al., 1969); this is also the case in fish (Melancon and Lech, 1983). The potential for

activation and/or detoxication may dictate the toxicity level of the compound. As an example, chlorpyrifos is expected to have the highest rate of activation since it is the most toxic. The structure of DEF prohibits both desulfuration and dearylation as forms of activation and detoxication, respectively; the major site of metabolism (to a sulfoxide or a sulfone) is also probably the liver, but this is yet to be identified.

Lipophilicity of a xenobiotic plays an important role in bioaccumulation and/or toxicity; lipophilic compounds tend to enter the body easily and to be excretable only when they have been rendered less lipophilic by metabolic action. A measure of lipophilicity is the log of the octanol:water partition coefficient ( $\log K_{ow}$ ); for chlorpyrifos, DEF, and parathion (@ 20°C) it is 5.11, 5.52 (Miles Agriculture Division, Personal Communication), and 3.81, respectively (Verschueren, 1983). A high partition coefficient indicates greater lipophilicity.

Barron et al. (1993) reported that hydrophobic chemicals such as chlorpyrifos ( $\log K_{ow}=5$ ) are generally more slowly eliminated across the gill because of binding to circulating blood proteins which reduces free chemical available for excretion; gill excretion can be rapid for chemicals with moderate hydrophobicity ( $\log K_{ow}=1-3$ ) or limited plasma protein binding.

Kopperman et al. (1974) noted that structure-toxicity correlations are only possible if the compounds examined have an identical mode of action; the acute toxicities of chlorpyrifos and parathion coincide with their  $\log K_{ow}$  in this respect. The  $\log K_{ow}$  of DEF is greater than that of chlorpyrifos, but the acute toxicity is less than chlorpyrifos; as mentioned previously, it has a different mode of toxicity. Lipophilicity is responsible for the accumulation of many organic xenobiotics by fish; metabolism can play an important role in determining both the extent of bioaccumulation of xenobiotics and their ultimate disposition and effects (Binder et al., 1984).

Toxicity potential can be observed in vitro by determining sensitivity of enzymes to an inhibitor. One index of sensitivity of an enzyme to an inhibitor is the  $I_{50}$  value. This is the concentration of the compound required to inhibit 50% of enzyme activity; it depends upon the affinity of the compound for the enzyme and the ability of the OP to phosphorylate the enzyme. A low  $I_{50}$  value indicates high potency of the inhibitor.  $I_{50}$  values are determined with the oxon of the phosphorothionate insecticide.

Another type of esterase that is responsible for detoxication of OP compounds in mammals are aliesterases (carboxylesterases). Aliesterases are assumed to provide protection against OP poisoning by their stoichiometric phosphorylation by the oxon with resultant destruction of the oxon (Chambers and Chambers, 1991). Phosphorylation occurs by binding the diethyl phosphate to the serine hydroxyl group while the leaving group, 3,5,6-trichloro-2-pyridinol or 4-nitrophenol, for chlorpyrifos-oxon and paraoxon, respectively, is released. ALiEs remain bound to the dialkyl phosphate and produce persistent inhibition (Chambers et al., 1990); saturation of this reaction can also occur and is similar to inhibition of AChE by the active metabolite. The  $I_{50}$  values indicate the sensitivity of the ALiEs to the active metabolites, and therefore, the potential of the ALiEs' to detoxify them. A low  $I_{50}$  value indicates a high sensitivity for the ALiEs and a high potential for protection.

In mammals, it is known that a variety of factors affect xenobiotic metabolism. In addition to species, strain, and individual variations in xenobiotic metabolizing enzyme systems, factors such as reproductive state, age, nutrition and exposure to environmental contaminants such as polycyclic aromatic hydrocarbons or polychlorinated biphenyls (PCBs) can also affect their activity (Hodgson, 1987). These factors also contribute to toxicity in fish (Binder et al., 1984).

PCBs are OC compounds which were used in the past in consumer goods requiring stability, and were exceptionally stable coolants for large transformers. Because of this stability, PCBs are extremely persistent in soil. The compounds are known to induce cytochrome P450 activities. Inducers of P450 can alter both the toxicity of the parent insecticide as well as the time course of esterase inhibition by the oxon by altering the rate of metabolism of the phosphorothionate. Depending on the nature of the induced cytochromes and the xenobiotics metabolized by the isozymes induced, induction can lead to either increases in the production of the activated metabolites and toxicity or increased detoxication and protection from toxicity (Binder et al., 1984).

Previous research has not determined the effects on enzyme activity of the above compounds on channel catfish, the possibility of interaction between the insecticides and the herbicide, or the effects of the cytochrome P450 inducer on chlorpyrifos and parathion toxicity in channel catfish. Multiple xenobiotic exposure is conceivable to fish in culture environments; therefore, interaction of these compounds should be investigated to determine if this is a possible environmental hazard to catfish.

The intent of this research is to assist in the understanding of exposure of channel catfish to three common agricultural pesticides (chlorpyrifos, DEF, and parathion) and to mixtures of these pesticides, to better define the metabolism of DEF, and to determine the effects of PCBs, a common soil contaminant, on chlorpyrifos and parathion metabolism.

This dissertation details a chronological series of studies culminating in an experiment dealing with the effects of PCB exposure on hepatic cytochrome P450 mediated activation and detoxication. Four specific objectives are addressed: 1) to determine the sensitivity of the target enzyme, AChE, and the protective enzyme, ALiE, in specific

tissues to chlorpyrifos-oxon, DEF, and paraoxon; 2) to determine inhibition and recovery of AChE and ALiEs in specific tissues by water column exposure to chlorpyrifos, DEF, and parathion, and possible interaction of chlorpyrifos and DEF or parathion and DEF; 3) to determine the effects of water-column exposure to piperonyl butoxide (a cytochrome P450 inhibitor) on the metabolism of DEF; and 4) to observe the effects of Aroclor 1254 (mixed PCBs) on desulfuration and dearylation of chlorpyrifos and parathion.

Table 1.1. Structures of chlorpyrifos, DEF, and parathion, and their respective metabolites.

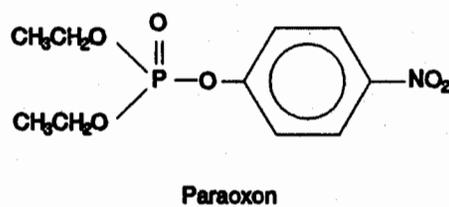
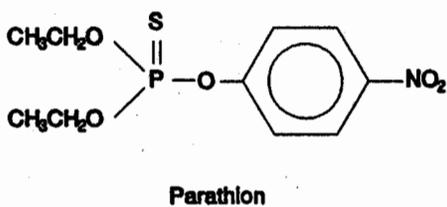
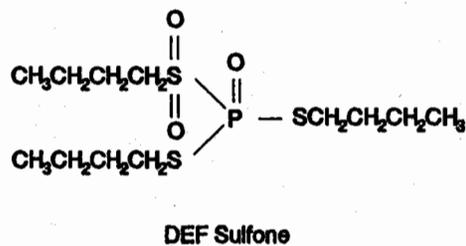
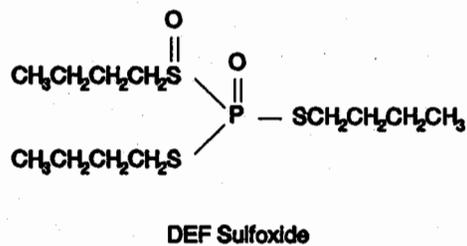
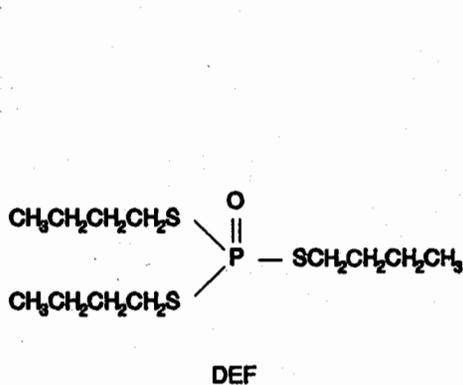
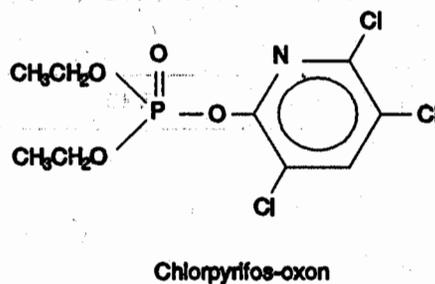
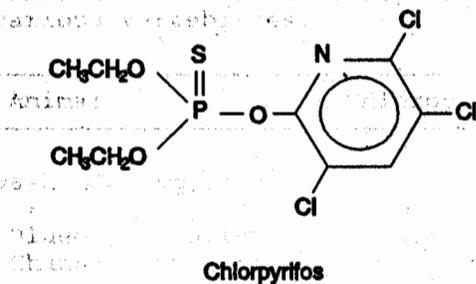


Figure 1.1. Structures of chlorpyrifos, DEF, parathion, and their respective metabolites.

Table 1.1. Acute toxicity of chlorpyrifos, DEF, and parathion in various vertebrates.

Animal	Chlorpyrifos	DEF	Parathion
96-h LC <sub>50</sub> (mg/l)			
Bluegill Sunfish	0.002 <sup>1</sup>	0.57 <sup>1</sup>	0.40 <sup>1</sup>
Channel Catfish	0.28 <sup>1</sup>	1.54 <sup>1</sup>	2.65 <sup>1</sup>
Mosquitofish	0.15 <sup>2</sup>	-----	0.32 <sup>1</sup>
Rainbow Trout	0.007 <sup>1</sup>	1.8 <sup>1</sup>	0.86 <sup>1</sup>
Oral LD <sub>50</sub> (mg/kg)			
Rat	151 <sup>3</sup>	325 <sup>4</sup>	13 <sup>5</sup>
Mallard Duck	75.6 <sup>3</sup>	2934 <sup>3</sup>	1.44 <sup>3</sup>
Pheasant	8.41 <sup>3</sup>	273 <sup>3</sup>	12.4 <sup>3</sup>

<sup>1</sup>Mayer and Ellersieck, 1986.

<sup>2</sup>Boone, 1991.

<sup>3</sup>Hudson et al., 1984.

<sup>4</sup>Murphy and DuBois, 1959.

<sup>5</sup>Gaines, 1969.

## CHAPTER II

### IN VITRO INHIBITION OF ACETYLCHOLINESTERASE AND ALIESTERASES OF FINGERLING CHANNEL CATFISH BY CHLORPYRIFOS-OXON, PARAOXON, AND DEF (S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE)

#### Introduction

Acute toxicity of a poison is governed by the inherent potency of the compound at the target site and the effective concentration reaching that target (Chambers and Chambers, 1991). One measure of this potency is the  $I_{50}$  value. As mentioned in the general introduction, the  $I_{50}$  value indicates the affinity of the active metabolite for the enzyme and its ability to phosphorylate the enzyme. For OPs, the oxon of the most acutely toxic pesticide (chlorpyrifos) is expected to have the highest potency for the target enzyme, AChE, and therefore the lowest  $I_{50}$  value. DEF should have a lower affinity or phosphorylation capacity followed by parathion.

ALiE  $I_{50}$  values should indicate the potential to detoxify the oxons in vivo. A low  $I_{50}$  value indicates a high potency and therefore a high potential for protection. If the  $I_{50}$  values are different, the oxon with the lowest value should offer the best protection.

Previous studies have looked at the in vitro toxicities of paraoxon (Boone, 1991; Johnson and Wallace, 1987) and chlorpyrifos-oxon (Boone, 1991) to teleosts, but there is a paucity of data on DEF or channel catfish. This experiment investigates the effects on AChE and ALiE activity by the oxons of the OP insecticides chlorpyrifos and parathion, and by DEF defoliant in fingerling channel catfish tissues.

between 1980 and 1984. The Materials and Methods

section of enzyme activity was analyzed in detail in the following section:  
**Chemicals**

Phosphorothionate oxons and nitrophenyl valerate were provided by Dr. Howard Chambers (Department of Entomology, Mississippi State University). DEF defoliant was a gift from Dr. Robert L. Graney (Miles Agriculture Division, Stilwell, KS). All biochemicals and reagents were from Sigma Chemical Company (St. Louis, MO).

#### Animals and Treatments

Channel catfish fingerlings, average wet weight 31 g (Appendix B) and of mixed sex were obtained for this research from the College of Veterinary Medicine, Mississippi State, MS. Fish were fed a commercial, 38% protein, floating catfish feed at the rate of 3% of body weight/day. Fish were not fed 24 h prior to the experiment to prevent organic matter from affecting exposure. Fish were held at  $30 \pm 1^\circ\text{C}$  and 12L/12D light cycle to minimize the reported seasonal variation in cytochrome P-450 monooxygenases, liver size, and other enzymes (Chambers and Yarbrough, 1979). Fish were acclimated for 2 weeks in a flow-through environment prior to each experiment in 76L glass aquaria containing 60L of aerated, dechlorinated water (pH = 7.5 to 7.7, total alkalinity = 60 to 90 mg/l as  $\text{CaCO}_3$ , chloride = 20 to 30 mg/l); flow rate was 60L/h. All animal procedures were done according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and under the supervision of the Laboratory Animal Veterinarian.

Analysis was carried out on three fish; individual assays were carried out in triplicate. For AChE and ALiE assays (see below), 10  $\mu\text{l}$  aliquots (in ethanol) of different concentrations of the phosphorothionate oxons or DEF were added to the enzyme sources prior to equilibration to allow for inhibition of the enzymes. Enzyme inhibition was accomplished using five or six concentrations and ranged

between 0 and 100%. The control contained 10  $\mu$ l of ethanol. The amount of enzyme activity was assayed as described below and the percent inhibition was calculated compared to the control.

#### Tissue Samples

Blood samples were taken, without the use of anesthesia (which could compromise the enzyme assays), via caudal vein puncture using a 22 gauge needle and a pediatric Vacutainer<sup>®</sup> containing EDTA. The fish were quickly decapitated and brain, gill, liver, and muscle were surgically removed.

All tissue samples were rinsed with 0.9% (w/v) sodium chloride solution and quickly chilled to decrease enzyme activity. Blood was centrifuged at 17,500  $\times$  g for 4 min to separate plasma. Tissue samples were stored at  $-70 \pm 2^\circ\text{C}$  until laboratory analysis could be performed.

#### Laboratory Analysis

##### Acetylcholinesterase Assay

Activity of AChE was quantified using a modification of Ellman et al. (1961) using acetylthiocholine as the substrate and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as the chromogen (which reacts with free -SH) and monitoring the production of nitrothiophenol as previously described (Chambers and Chambers, 1989). Brain, gill, liver, and muscle were homogenized in 0.05 M Tris-HCl buffer (pH 7.4), to give final assay concentrations of 0.2, 2.5, 1.8, and 1.0 mg/ml, respectively. Muscle samples (a source of peripheral AChE) were homogenized and filtered through glass wool. Plasma was diluted with 0.05 M Tris-HCl buffer (pH 7.4) to give a final assay concentration of 12.5  $\mu$ l/ml.

Final assay volume was 1.0 ml. Inhibition of AChE activity was accomplished with 10  $\mu$ l of  $10^{-3}$  M eserine sulfate ( $10^{-5}$  M final concentration); this served as a blank for non-AChE-mediated hydrolysis. Reaction mixtures were allowed to equilibrate for 15 min

at 30°C. A 10  $\mu$ l aliquot of 0.1 M acetylthiocholine iodide was then added and allowed to incubate for 15 min at 30°C. Reactions were terminated by the addition of 125  $\mu$ l of a 1:4 mixture of 0.95% DTNB and 5% sodium dodecyl sulfate, a detergent used to denature the enzyme and to make the reaction mixture clear. Absorbance was measured at 412 nm to determine the concentration of the product liberated.

#### Aliesterase Assay

Gill, liver, and plasma were assayed spectrophotometrically using 4-nitrophenyl valerate as the substrate and monitoring the production of 4-nitrophenol (a product of hydrolysis) for ALiE activity as previously described (Carr and Chambers, 1991). Preliminary assays on brain and muscle indicated that these tissues lacked measurable amounts of ALiEs. Gill and liver were homogenized in 0.05 M Tris-HCl buffer (pH 7.4), to give final concentrations of 2.5 and 0.5 mg/ml, respectively. Plasma was diluted with 0.05 M Tris-HCl buffer (pH 7.4) to give a final assay concentration of 2.8  $\mu$ l/ml.

Final assay volume was 1.0 ml. Inhibition of ALiE activity was accomplished with 10  $\mu$ l of  $10^{-3}$  M paraoxon ( $10^{-5}$  M final concentration); this served as a blank for non-enzymatic hydrolysis. Reaction mixtures were allowed to equilibrate for 15 min at 30°C. A 10  $\mu$ l aliquot of 50 mM 4-nitrophenyl valerate was then added and allowed to incubate for 15 min at 30°C. Reactions were terminated by the addition of 250  $\mu$ l of a mixture of 2% sodium dodecyl sulfate and 2% Tris base. Absorbance was measured at 400 nm to determine the concentration of the product liberated.

#### Protein Quantification

For all tissues, protein was quantified using the method of Lowry et al. (1951). Bovine serum albumin was used as the standard. Individual assays were carried out in triplicate.

### Statistical Analysis

$I_{50}$  values were calculated using log-probit analysis (SAS Institute, Cary, North Carolina) with 3 replications/data point;  $I_{50}$  value means were analyzed by analysis of variance followed by the Student-Neuman-Keuls means comparison test using SAS on a personal computer. A level of  $p < 0.05$  was used to conclude a significant difference among means.

### Results

DEF was a poor inhibitor of AChE;  $10^{-3}$  M inhibited AChE activity <15% in all tissues. Table 2.1 presents AChE and ALiE  $I_{50}$  values, standard error, and statistical mean comparison for chlorpyrifos-oxon, DEF, and paraoxon. Control specific activities for each tissue and enzyme is also displayed in Table 2.1.

All tissues exhibited similar sensitivities of AChE to chlorpyrifos-oxon; paraoxon also displayed similar sensitivities among the tissues. Chlorpyrifos-oxon was approximately 16-fold more potent than paraoxon to AChE in all tissues.

ALiEs were more sensitive to chlorpyrifos-oxon than to paraoxon by 287-, 105-, and 25-fold in gill, liver, and plasma, respectively. Liver ALiEs were most sensitive to chlorpyrifos-oxon, plasma least and gill intermediate, but the  $I_{50}$  values were all within the same order of magnitude. Paraoxon inhibited ALiEs to a greater extent than DEF but by the same ratio of approximately 3-fold in all three tissues. In contrast to chlorpyrifos-oxon, plasma ALiEs were the most sensitive to paraoxon and DEF, while gill ALiEs were the least sensitive to these compounds.

### Discussion

Chlorpyrifos-oxon was a much more potent anticholinesterase than paraoxon in all tissues, which correlates with the acute toxicity levels of the parent insecticides. The in vitro data do not explain

the relatively high acute toxicity of DEF since it is a poor anticholinesterase. Habig and DiGiulio (1988) reported that DEF did not affect brain AChE at concentrations of 10  $\mu$ M - 2.5 mM in in vitro experiments on channel catfish. The present study determined that 1 mM DEF (which is not physiologically relevant) inhibited <15% of AChE in all tissues examined. These results also agree with the findings of Casida et al. (1963), and suggests a different mode of toxicity. The metabolized form(s) of DEF may have produced different effects on  $I_{50}$  values, but these were not available for study.

Results of the present study for AChE inhibition are different than those found by Boone (1991) in mosquitofish (Gambusia affinis);  $I_{50}$  values for chlorpyrifos-oxon were 49.8 and 20.8 nM, and paraoxon were 270.8 and 1.2 nM, in brain and muscle, respectively. Refer to Table 1.1 for mosquitofish  $LC_{50}$  values. While the brain  $I_{50}$  values to both compounds and the muscle  $I_{50}$  value to chlorpyrifos-oxon were within the same order of magnitude, the mosquitofish muscle was over 400 times more sensitive to paraoxon than channel catfish. This difference in muscle AChE sensitivity between the two species may indicate that muscle AChE sensitivity plays an important role in acute toxicity as parathion is 8-fold more toxic to mosquitofish than to channel catfish. Wallace and Kemp (1991) suggest that such differences could be caused by the rate of phosphorylation of AChE and is influenced by the dimensions and the chemical properties of the active site. The potency of chlorpyrifos-oxon or paraoxon to tissues of the present study suggests that channel catfish may have a similar isozyme of AChE or analogous membrane composition in these tissues. Comparable binding capabilities or similar phosphorylation potential for the isozyme could also cause such an effect, while this does not seem to be true for mosquitofish.

Chlorpyrifos-oxon had similar potency for ALiEs in each tissue tested and suggests similar binding capabilities and comparable

phosphorylation potential for the isozymes. DEF or paraoxon, however, demonstrated differences in sensitivities to ALiEs of each tissue; the affinity and phosphorylation potential of these compounds for the isozymes appear to be different. While lipophilicity and membrane permeability of these compounds may be important in characterizing ALiEs sensitivity in these tissues, the ratio of the isozymes present in each tissue is probably the most important factor.

In all cases, the compounds were more potent ALiEs inhibitors than AChE inhibitors (noting that control specific activities of AChE were similar to control specific activities of tissues containing ALiEs), indicating that ALiEs could serve an important protective role by being stoichiometrically phosphorylated by the oxon to destroy the phosphate. According to  $I_{50}$  values of the present study, ALiEs would provide more protection from chlorpyrifos-oxon in the liver and from DEF and paraoxon in the plasma.

The structure of DEF incorporates a double bonded oxygen, similar to the phosphorothionate oxons, and was used as the inhibitor compound in these experiments. Hur et al. (1992) suggest that the active metabolite(s) of DEF may be the sulfone and/or the sulfoxide. Future experiments should determine the in vitro effects of these compounds, when they become available, as anticholinesterases. While DEF is not an anticholinesterase, its potency as an ALiE inhibitor suggests that it could interfere with some of the ALiE mediated protection occurring during phosphorothionate exposures. This conceivable potentiation was studied in the in vivo experiments described in Chapter III.

Table 2.1. Acetylcholinesterase (AChE) and aliesterase (ALiE)  $I_{50}$ <sup>1</sup> values for chlorpyrifos-oxon, DEF, and paraoxon on tissues of fingerling channel catfish.

Enzyme	Tissue	$I_{50}$ (nM)			Specific Activity <sup>2</sup>
		Chlorpyrifos-oxon	DEF	Paraoxon	
AChE	Brain	28.40 <sup>C</sup>	--- <sup>4</sup>	446.08 <sup>B</sup>	487.7
		2.1 <sup>3</sup>		34.4	1.3
	Gill	33.09 <sup>C</sup>	---	577.86 <sup>A</sup>	58.2
		0.7		15.6	4.2
	Liver	32.66 <sup>C</sup>	---	498.35 <sup>AB</sup>	45.4
2.5			20.0	5.2	
Muscle	31.59 <sup>C</sup>	---	528.85 <sup>AB</sup>	129.2	
	2.6		23.0	15.3	
Plasma	28.73 <sup>C</sup>	---	481.02 <sup>B</sup>	29.5	
	1.5		15.1	3.0	
ALiE	Gill	0.16 <sup>EF</sup>	163.36 <sup>A</sup>	45.99 <sup>B</sup>	30.6
		0.0	31.9	10.4	1.3
	Liver	0.13 <sup>F</sup>	34.32 <sup>B</sup>	13.66 <sup>C</sup>	44.4
0.0		3.1	0.5	3.7	
Plasma	0.23 <sup>F</sup>	24.21 <sup>B</sup>	5.73 <sup>D</sup>	71.5	
	0.0	1.6	0.4	2.5	

<sup>1</sup> $I_{50}$  values were determined by log [inhibitor] vs. probit % inhibition; means within an enzyme not followed by the same capital letter are significantly different ( $p < 0.05$ ) by the Student-Neuman-Keuls test.

<sup>2</sup>Specific activity expressed as  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ .

<sup>3</sup>Standard error based on 3 replications.

<sup>4</sup>DEF ( $10^{-3}$  M) inhibited AChE <15% in all tissues.

### CHAPTER III

## IN VIVO INHIBITION OF ACETYLCHOLINESTERASE AND ALIESTERASES OF FINGERLING CHANNEL CATFISH BY CHLORPYRIFOS, PARATHION, AND DEF (S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE)

### Introduction

Studying the time course of inhibition and recovery is necessary to determine the rate of enzyme inhibition and the speed of recovery from exposure to a sublethal dose of a particular xenobiotic(s). Rapid inhibition of target enzyme activity indicates less time is available for the fish to adapt to such an exposure. Uptake of these xenobiotics may occur across the membranes of the gills, through the intestine and/or by dermal absorption. Recovery rates of these enzymes are important to assess susceptibility to future exposures of OP toxicant(s).

OP insecticides are assumed to be toxic because they result in inhibition of AChE. The anticholinesterase metabolites of chlorpyrifos and parathion are their respective oxons; for DEF, these metabolites are the sulfone or the sulfoxide.

Cholinesterase effects of parathion (Benke et al., 1974; Benke and Murphy, 1974; Boone, 1991; Murphy et al., 1968) and chlorpyrifos (Boone, 1991; Jarvinen et al., 1983; Jarvinen et al., 1988; Van der Wel and Welling, 1989) have been studied in several teleosts, but there is a paucity of data on channel catfish. Habig and DiGiulio (1988) studied the cholinergic effects of DEF to channel catfish; however, only effects to brain and muscle AChE were quantified.

Chemical interaction has been previously studied; Fabacher et al. (1976) looked at mortality to study the apparent potentiation of DEF by

methyl parathion in mosquitofish, but there is little information on biochemical effects, especially to channel catfish.

This study was designed to investigate the effects of in vivo exposure to the pesticides chlorpyrifos, parathion, and DEF (S,S,S-tributyl phosphorotrithioate) on rates of inhibition and recovery of brain, gill, liver, muscle, and plasma AChE and gill, liver, and plasma ALiEs in fingerling channel catfish. Potential interaction of the pesticides were examined by treatments of chlorpyrifos and DEF or parathion and DEF.

### Materials and Methods

#### Chemicals

Phosphorothionates were provided by Dr. Howard Chambers (Department of Entomology, Mississippi State University). All other chemicals were obtained as in Chapter II.

#### Animals and Treatments

Channel catfish fingerlings, average wet weight 35 g (Appendix B) and of mixed sex, were obtained and held as noted in Chapter II; initial stocking rate was 20 fish/aquarium. There were three aquaria per treatment.

Fish were exposed to the pesticides suspended in 20 ml of reagent grade acetone (Table 3.1) for 4 hrs in a static environment. The high concentrations of parathion and chlorpyrifos were designed to be sublethal but of sufficient magnitude to yield a relatively high degree of AChE inhibition as well as overt signs of intoxication without significant mortality. A concentration of one half these concentrations was also tested. The concentration of DEF was selected to yield signs of poisoning without significant mortality. The system was then altered to allow for flow-through conditions; flow rate was 60L/h. Fish were sacrificed at 0, 4, 8, 12, 24, 48, 96, 144, 192, 240,

288, 336, or 384 h after the 4 h exposure period. A negative control (containing 20 ml acetone) was run for each replication.

#### Tissue Samples

Tissues were obtained as in Chapter II.

#### Laboratory Analysis

AChE and ALiE assays were carried out in duplicate and quantified as in Chapter II; protein was also quantified as in Chapter II.

#### Statistical Analysis

There were three replications per treatment, unless otherwise noted. Specific activities were analyzed by the General Linear Model procedure (due to missing data) followed by the Student-Neuman-Keuls means comparison test using SAS on a personal computer. A level of  $p < 0.05$  was used to conclude a significant difference among means.

#### Results

Mean AChE specific activity, standard error, percent inhibition, and statistical mean comparison are given in Tables 3.2-3.6 for brain, gill, liver, muscle, and plasma, respectively. Figures A.1-A.5 are presented in Appendix A to visualize the effects of the OPs. Mean control specific activities (expressed as  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ) for AChE in this study were 414.6, 36.4, 36.5, 122.0, and 22.1, for brain, gill, liver, muscle, and plasma, respectively. These specific activities are similar to those of the previous in vitro study.

Initial specific activities of AChE in each tissue were significantly different from controls in all treatments except for brain and muscle of DEF-only treatments; by 384 h, specific activities from fish of all treatments were significantly different from controls except for those of the DEF-only and the chlorpyrifos + DEF treatments in gill.

DEF was initially a poor inhibitor of brain AChE (Table 3.2, Figure A.1); specific activities were not significantly different from the control until 8 h and was greatest at 288 h (36%). Brain specific activities from fish of the DEF treatment did not differ significantly from 8 h through 384 h. Fish from all other treatments exhibited maximum inhibition greater than 81% by 8 h; enzyme activities in brains of each treatment recovered significantly.

Gill AChE yielded maximum inhibition of greater than 81% (Table 3.3, Figure A.2) by 8 h in all treatments. Enzyme activities recovered significantly in fish from each treatment by 384 h.

Liver showed the highest 'apparent' percentage of AChE inhibition of all tissues (Table 3.4, Figure A.3). Liver AChE was inhibited greater than 96% by 8 h in all treatments; specific activity recovered significantly by 384 h.

Toxicity of DEF for the target enzyme in muscle (Table 3.5, Figure A.4) showed similar results as the DEF treatment for brain AChE; maximum inhibition was 46% at 384 h. Results showed more variability than those in brain, but, in general, most of the activities throughout the treatment period did not differ from one another statistically, and, like brain, there was no evidence of recovery. Fish from all other treatments except for chlorpyrifos + DEF showed maximum inhibition greater than 93% by 4 h. Fish from the chlorpyrifos + DEF treatment yielded maximum muscle AChE inhibition (87%) at 240 h; however, specific activities within the treatment were not significantly different from 4 h through the end of the study. Fish from chlorpyrifos-only treatments demonstrated persistent AChE inhibition. Enzyme activities recovered significantly within treatments (other than DEF- and chlorpyrifos-only treatments) by 384 h.

Fish from all treatments yielded maximum AChE inhibition in plasma (Table 3.6, Figure A.5) by 8 h; this early inhibition was greater than 84% in treatments other than DEF. Fish from chlorpyrifos

treatments demonstrated statistically similar specific activities through 240 h; plasma AChE in chlorpyrifos-only treated fish recovered significantly by 384 h. AChE specific activities in fish from the parathion + DEF treatment recovered more rapidly than those of other parathion treatments, and were significantly different from them at 384 h. DEF treated fish yielded maximum inhibition of AChE by 8 h (56%) and recovered completely by 240 h; specific activities were statistically different from the controls through 96 h.

Figures A.1-A.5 demonstrate slight interaction between chlorpyrifos and DEF or parathion and DEF in AChE assays of all tissues, usually toward the end of the study. AChE specific activities of fish from the chlorpyrifos + DEF treatments were significantly different from fish of the 0.125 mg/l chlorpyrifos treatments for brain (336 and 384 h), gill (336 and 384 h), liver (240 h), muscle (336 h), and plasma (48 and 144 h); specific activities of fish from the parathion + DEF treatments were significantly different from fish of the 1.25 mg/l parathion treatments for brain (240 h), liver (240 h), and plasma (96, 144, 240, 288, 336, and 384 h). Chlorpyrifos exhibited potentiation with DEF in all tissues except plasma, where an antagonistic effect was evident. The greatest amount of potentiation between chlorpyrifos and DEF was evident in gill from 288 h through the duration of the study. Potentiation was evident in the parathion and DEF treatment of AChE in gill and liver, and an antagonistic effect in brain, muscle, and plasma; the greatest antagonistic effect was demonstrated in plasma after 48 h.

Mean ALiE specific activity, standard errors, percent inhibition, and statistical means comparison are given in Tables 3.7-3.9 for gill, liver, and plasma, respectively. Figures A.6-A.8 are presented in Appendix A to visualize the effects of the OPs. Mean control specific activities (expressed as  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ) for ALiEs in this study were 9.4, 63.5, and 71.3, for gill, liver, and plasma,

respectively. These specific activities are similar to those of the previous in vitro study.

Initial ALiE specific activities of each tissue were significantly different from the controls in all treatments; specific activities were significantly different from those of control fish through 384 h in all treatments except the 2.5 mg/l parathion treatment in gill, liver, and plasma, the 1.25 mg/l parathion treatment in liver and plasma. Liver ALiE specific activities of all treatments at 8, 12, 24, and 48 h (except DEF-only and parathion + DEF treatments at 24 h) were not significantly different from controls.

Gill ALiE activities (Table 3.7, Figure A.6) were somewhat erratic and reached maximum inhibition greater than 73% in all treatments by 12 h except for the chlorpyrifos + DEF and DEF-only treatments where maximum inhibition was reached by 144 and 336 h, respectively. Gill ALiEs were more persistently inhibited by treatment with chlorpyrifos-only than by parathion-only.

Treatments containing DEF inhibited liver ALiEs (Table 3.8, Figure A.7) better than chlorpyrifos- or parathion-only treatments; inhibition was persistent and was greater than 93% at 0 h. Liver ALiE activities of fish from chlorpyrifos-only treatments did not recover significantly; fish from the 1.25 mg/l parathion treatment yielded specific activities significantly different from activity at 0 h.

Plasma ALiEs (Table 3.9, Figure A.8) were inhibited persistently by DEF; inhibition was greater than 98% at all time points. Fish plasma ALiE from chlorpyrifos- and parathion-only treatments yielded over 94% inhibition through 48 h. Activities from chlorpyrifos-only treatments exhibited persistent inhibition; ALiE activities from the 0.125 mg/l treatment recovered slightly, but not significantly. Plasma ALiEs of fish from parathion-only treatments showed significant and substantial recovery.

Figures A.6-A.8 demonstrate an 'apparent' additive affect between chlorpyrifos and DEF or parathion and DEF in ALiE assays of all tissues. ALiE specific activities of fish from the chlorpyrifos + DEF treatments were significantly different from fish of the 0.125 mg/l chlorpyrifos treatments for gill (12, 144, 192, 288, 336 and 384 h) and liver (4, 240, 288, and 336 h); specific activities of fish from the parathion + DEF treatments were significantly different from fish of the 1.25 mg/l parathion treatments for gill (12, 48, 144, 192, 240, 288, 336, and 384 h), liver (4, 24, 96, 144, 240, 288, 336, and 384 h), and plasma (288, 336, and 384 h). The greatest effect was evident in plasma ALiEs after exposure to parathion and DEF.

#### Discussion

Fish from all treatments, except the control, displayed similar signs of stress during the static exposure period. Fish demonstrated signs of intoxication (i.e., torpor and lack of schooling behavior) in the DEF and parathion treatments by 1 h after exposure; piping at the surface was observed by 3 h. Chlorpyrifos-only treated fish displayed minor signs of stress during the static exposure period, but occasional mortalities occurred after the system was modified to flow-through conditions; infrequent mortality was also observed in parathion treatments. No mortality occurred in the DEF-only treatments.

Gill, liver, and plasma AChE exhibited high percentage inhibitions; low control specific activities indicate that fewer AChE molecules are probably present, so a lower amount of the cholinesterase inhibiting metabolite(s) would be necessary to cause such an inhibition as compared to muscle or brain.

Chlorpyrifos and parathion (alone or in combination with DEF) appear to have been rapidly metabolized to very potent and effective cholinesterase inhibitors according to inhibitions yielded by all tissues. A lower concentration of chlorpyrifos was required to inhibit a similar amount of AChE as in the parathion treatments. The active

metabolite of chlorpyrifos may be more toxic because of a higher rate of desulfuration or a higher affinity and phosphorylation potential for AChE; in vitro experiments of the present study (Chapter II) also demonstrate the greater toxicity of chlorpyrifos.

Chlorpyrifos-oxon led to more persistent cholinesterase inhibition than paraoxon in all tissues except plasma. In plasma, AChE activity recovered in a similar fashion after exposure to chlorpyrifos and parathion treatments. As mentioned in the introduction, lipophilic compounds such as chlorpyrifos are generally more slowly eliminated across the gill due to binding with blood proteins. This may help explain the persistent effects of the chlorpyrifos treatments; lipophilic compounds may also be sequestered. Such persistent AChE inhibition could jeopardize the fish in future exposures to OP compounds.

DEF yielded poor cholinesterase inhibition in brain and muscle; this coincides with results of previous in vitro assays. Defense mechanisms and/or the blood:brain barrier of the circulatory system may be preventing DEF from reaching these tissues as the active metabolite(s). An increase in inhibition near the end of the in vivo study suggests slow bioactivation. Habig et al. (1986) reported delayed inhibition in channel catfish brain (approximately 58%) and muscle (approximately 63%) after a 4 day exposure to DEF; however, they did not analyze gill, liver, and plasma.

The hepatic MO system may be responsible for activating DEF to a toxic metabolite(s) which was not present in the brain or muscle to a great extent due to the route of the circulatory system. Several studies (Habig and DiGiulio, 1988; Wing et al., 1983; Wing et al., 1984) demonstrated that DEF was converted to a more potent inhibitor of AChE after incubation in the presence of liver microsomes; Hur et al. (1992) suggested that sulfoxidation catalyzed by liver microsomal

oxidases may be responsible for the active metabolite(s), possibly a sulfone and/or a sulfoxide.

An in vitro activation experiment in our labs (similar to the desulfuration assay described in Chapter V) with channel catfish hepatic microsomes demonstrated the ability of the metabolite(s) of DEF to inhibit channel catfish brain AChE by 12.6 and 21.8% in 15 min at 50 and 100  $\mu$ M final concentrations, respectively, in the presence of an NADPH-generating system. Habig and DiGiulio (1988) conducted a similar experiment with channel catfish hepatic microsomes and reported that channel catfish brain AChE was inhibited 35-40% in 15 min at 50  $\mu$ M DEF (final concentration); rat liver microsomes were found to more efficient at catalyzing DEF activation to inhibit channel catfish brain AChE (60%) at 15 min and 50  $\mu$ M.

DEF was not a good inhibitor in brain and muscle. The anticholinesterase metabolite(s) of DEF may not have been able to enter these tissues readily. Bioactivation, and resultant AChE inhibition, in brain and muscle would be expected to be slow.

Wallace and Herzberg (1988) suggest that de novo AChE synthesis is responsible for recovery in rainbow trout (Oncorhynchus mykiss) after exposure to malaoxon and paraoxon; they found that some reactivation occurs in fathead minnows (Pimephales promelas). Preliminary experiments in this lab (Russell Carr, personal communication) suggests that de novo synthesis is also responsible for recovery in channel catfish.

In the present study, the presumed metabolite(s) of DEF was/were very effective inhibitor(s) of AChE in gill and liver, demonstrating a similar pattern of inhibition and persistence as chlorpyrifos; this inhibition and persistence may also be attributed to the lipophilicity of DEF. Plasma AChE activity was inhibited moderately by DEF (56% at 8 h) but recovered completely by 240 h; this suggests that plasma can detoxify DEF more rapidly than the other tissues. Another

possibility could be that there is a more rapid turnover of AChE in plasma than in other tissues; however, if this were the case, chlorpyrifos and parathion treatments would also yield rapid recovery.

ALiEs are serine esterases and in mammals can serve as a protective mechanism against OPs by providing alternate phosphorylation sites for the active metabolite(s); thus resulting in destruction of the active metabolites (Chambers and Chambers, 1991). The concentration of ALiEs, the affinity of the metabolite(s) of chlorpyrifos, DEF, and parathion for the ALiEs, and their respective phosphorylation potential may also contribute to toxicity differences.

Fish from DEF treatments yielded the most effective and persistent inhibition of ALiEs in all tissues and treatments; fish from chlorpyrifos treatments yielded more persistent inhibition than parathion treatments in all tissues. Previous in vitro experiments (Chapter II) indicated that the order of potency to ALiEs was chlorpyrifos-oxon, paraoxon, and DEF. Results from the in vivo study suggest that a higher concentration of DEF may be reaching the ALiEs or that the affinity and phosphorylation potential of DEF for these ALiEs may be greater than the affinity and phosphorylation potential of chlorpyrifos-oxon or paraoxon. ALiE activity did not recover in the DEF treatments as it did in the chlorpyrifos and parathion treatments; this also suggests the higher affinity and phosphorylation potential of DEF for these ALiEs which could result in re-inhibition of ALiEs.

As mentioned earlier, liver ALiE specific activities of all treatments at 8, 12, 24, and 48 h (except DEF-only and parathion + DEF treatments at 24 h) were not significantly different from controls. The general trend may be of biological importance and further experiments with more replications may confirm this.

The 'apparent' additive affects between chlorpyrifos and DEF or parathion and DEF in ALiE assays of all tissues were probably due to the properties of DEF and its ability to effectively inhibit ALiEs in

vitro and in vivo; exposure to DEF could allow more of the metabolite(s) of chlorpyrifos or parathion to inhibit AChE in a real life scenario if ALiEs are present and if ALiEs serve a protective function. Potentiation was exhibited in gill (chlorpyrifos + DEF) and liver (chlorpyrifos and parathion + DEF) AChE assays by treatments utilizing the same concentration of the phosphorothionate as used in the interaction treatment; however, this potentiation was not as distinct as expected if ALiEs would have protected the AChE. Therefore, results suggest that ALiEs do not serve as a protective enzyme in channel catfish. Antagonism by DEF of plasma AChE inhibition following chlorpyrifos and parathion treatments was observed. Rapid de novo synthesis of plasma AChE inhibited by DEF may be responsible for recovery of specific activity.

The treatments containing 0.25 mg/l chlorpyrifos and 2.5 mg/l parathion demonstrated a similar inhibition to both enzymes as treatments containing half of their respective phosphorothionate. This suggests that saturation of the enzyme may have been reached with the lower dose and suggests that factors other than AChE inhibition in the tissues tested may also be involved in OP toxicity.

Future experiments should examine the role of ALiEs in fish since they are not protective enzymes as demonstrated in mammals.

Table 3.1. Nominal concentrations of parent compounds used in the channel catfish enzyme inhibition and recovery study.

Treatment <sup>1</sup>	Concentration
Chlorpyrifos	0.25 mg/l
0.5 Chlorpyrifos	0.125 mg/l
DEF	5.0 mg/l
Parathion	2.5 mg/l
0.5 Parathion	1.25 mg/l
Chlorpyrifos + DEF	0.125 mg/l + 2.0 mg/l
Parathion + DEF	1.25 mg/l + 2.0 mg/l

<sup>1</sup>Pesticides for each treatment were suspended in 20 ml reagent grade acetone.

Table 3.2. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling brain acetylcholinesterase after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	454.0 <sup>2,A,a</sup> 41.1 <sup>3</sup>	445.5 <sup>A,a</sup> 18.7	420.6 <sup>A,a</sup> 58.1	367.9 <sup>A,a</sup> 11.4	437.5 <sup>A,a</sup> 24.9	424.2 <sup>A,a</sup> 31.5	423.6 <sup>A,a</sup> 13.9	385.3 <sup>A,a</sup> 21.1	397.2 <sup>A,a</sup> 60.1	383.9 <sup>A,a</sup> 32.4	424.4 <sup>A,a</sup> 49.2	420.8 <sup>A,a</sup> 26.6	404.6 <sup>A,a</sup> 51.0
DEF	454.3 <sup>A,a</sup> 31.0 (0.0)	415.4 <sup>A,ab</sup> 27.1 (6.8)	386.8 <sup>A,abc</sup> 16.1 (8.0)	289.8 <sup>B,c</sup> 31.5 (21.2)	331.7 <sup>B,bc</sup> 29.7 (24.2)	313.5 <sup>B,bc</sup> 27.9 (26.1)	289.3 <sup>B,c</sup> 18.8 (31.7)	284.2 <sup>B,c</sup> 23.6 (26.2)	276.7 <sup>B,c</sup> 32.8 (30.3)	274.8 <sup>B,c</sup> 37.9 (28.4)	270.3 <sup>B,c</sup> 65.5 (36.3)	313.6 <sup>B,bc</sup> 27.7 (25.5)	328.2 <sup>B,bc</sup> 36.8 (18.9)
Chlorpyrifos	24.2 <sup>B,a</sup> 10.3 (94.7)	19.1 <sup>C,a</sup> 10.4 (95.7)	48.1 <sup>B,abc</sup> 12.0 (88.6)	43.6 <sup>C,ab</sup> 11.1 (88.1)	94.5 <sup>A,C,cd</sup> 3.2 (78.4)	78.2 <sup>C,bcd</sup> 15.8 (81.6)	93.1 <sup>C,cd</sup> 13.8 (78.0)	98.6 <sup>D,cd</sup> 5.7 (74.4)	119.0 <sup>C,de</sup> 26.6 (70.0)	125.5 <sup>D,de</sup> 12.2 (67.3)	160.4 <sup>B,ef</sup> 21.9 (62.2)	205.3 <sup>BC,9</sup> 36.3 (51.2)	196.3 <sup>D,fg</sup> 20.4 (51.5)
0.5 Chlorpyrifos	102.2 <sup>B,abc</sup> 25.8 (77.5)	40.3 <sup>C,a</sup> 13.0 (90.9)	52.4 <sup>B,ab</sup> 15.0 (87.6)	48.3 <sup>C,ab</sup> 2.3 (86.9)	91.2 <sup>C,ab</sup> 14.0 (79.2)	109.8 <sup>C,abc</sup> 26.4 (74.1)	104.5 <sup>C,abc</sup> 14.2 (75.3)	127.4 <sup>D,abc</sup> 42.8 (66.9)	133.4 <sup>C,bc</sup> 40.2 (66.4)	110.8 <sup>B,abc</sup> 32.1 (71.1)	177.9 <sup>B,cd</sup> 38.0 (58.1)	199.6 <sup>BC,d</sup> 52.0 (52.6)	257.0 <sup>BCD,e</sup> 52.8 (36.5)
Chlorpyrifos + DEF	105.6 <sup>B,ab</sup> 46.1 (76.7)	90.6 <sup>B,ab</sup> 12.0 (79.7)	78.6 <sup>B,a</sup> 21.5 (81.3)	88.4 <sup>C,ab</sup> 9.3 (76.0)	123.4 <sup>C,ab</sup> 6.2 (71.8)	123.0 <sup>C,ab</sup> 17.9 (71.0)	116.4 <sup>C,ab</sup> 16.2 (72.5)	125.1 <sup>D,ab</sup> 9.2 (67.5)	130.5 <sup>C,ab</sup> 7.4 (67.1)	147.6 <sup>D,abc</sup> 17.1 (61.6)	163.5 <sup>B,abc</sup> 28.8 (61.5)	168.5 <sup>C,bc</sup> 23.1 (60.0)	212.4 <sup>D,c</sup> 10.9 (47.5)
Parathion	27.2 <sup>B,a</sup> 25.7 (94.0)	34.4 <sup>C,a</sup> 8.1 (92.3)	33.6 <sup>B,a</sup> 2.7 (92.0)	49.5 <sup>C,a</sup> 3.4 (86.5)	105.2 <sup>C,b</sup> 11.5 (76.0)	123.6 <sup>C,b</sup> 8.9 (70.8)	140.7 <sup>C,bc</sup> 8.1 (66.8)	141.8 <sup>D,bc</sup> 10.8 (63.2)	185.7 <sup>BC,c</sup> 37.5 (53.2)	236.6 <sup>BC,d</sup> 16.6 (38.4)	269.8 <sup>B,de</sup> 33.8 (36.4)	267.1 <sup>BC,de</sup> 27.2 (36.5)	303.6 <sup>BC,e</sup> 18.3 (25.0)
0.5 Parathion	40.5 <sup>B,a</sup> 12.3 (91.1)	28.8 <sup>C,a</sup> 14.8 (93.5)	42.1 <sup>B,a</sup> 13.2 (90.0)	38.7 <sup>C,a</sup> 7.3 (89.5)	106.0 <sup>C,b</sup> 26.3 (75.8)	131.5 <sup>C,bc</sup> 10.3 (69.0)	141.0 <sup>C,bc</sup> 15.1 (66.7)	168.0 <sup>C,cd</sup> 20.5 (56.4)	163.9 <sup>BC,cd</sup> 35.5 (58.7)	198.4 <sup>BCD,de</sup> 27.8 (48.3)	216.4 <sup>B,e</sup> 20.4 (49.0)	217.4 <sup>BC,e</sup> 37.3 (48.3)	294.2 <sup>BC,f</sup> 33.7 (27.3)
Parathion + DEF	76.5 <sup>B,ab</sup> 29.6 (83.1)	50.9 <sup>C,a</sup> 14.5 (88.6)	60.1 <sup>B,a</sup> 18.6 (85.7)	67.4 <sup>C,a</sup> 16.9 (81.7)	101.5 <sup>C,ab</sup> 7.0 (76.8)	130.9 <sup>C,bc</sup> 17.9 (69.1)	129.5 <sup>C,bc</sup> 13.4 (69.4)	177.2 <sup>C,cd</sup> 4.0 (54.0)	198.5 <sup>BC,de</sup> 24.6 (50.0)	217.0 <sup>BC,def</sup> 10.6 (43.5)	244.6 <sup>B,ef</sup> 30.9 (42.4)	258.3 <sup>BC,fg</sup> 40.9 (38.6)	301.9 <sup>BC,g</sup> 23.4 (25.4)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as nmol·min<sup>-1</sup>·mg protein<sup>-1</sup>; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 99.5.

Table 3.3. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling gill acetylcholinesterase after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	34.5 <sup>A,a</sup> 4.3 <sup>3</sup>	33.0 <sup>A,a</sup> 6.7	53.5 <sup>A,a</sup> 18.6	42.9 <sup>A,a</sup> 11.1	38.0 <sup>A,a</sup> 2.4	46.7 <sup>A,a</sup> 14.6	53.3 <sup>A,a</sup> 16.0	27.5 <sup>A,a</sup> 1.9	33.3 <sup>A,a</sup> 4.1	24.0 <sup>A,a</sup> 4.5	30.3 <sup>A,a</sup> 4.9	25.4 <sup>A,a</sup> 0.8	30.8 <sup>A,a</sup> 4.1
DEF	23.7 <sup>B,ab</sup> 4.8 (31.4)	10.8 <sup>B,c</sup> 2.1 (67.3)	0.8 0.8 (81.1)	18.6 <sup>B,abc</sup> 3.8 (56.6)	16.2 <sup>B,abc</sup> 1.8 (57.3)	9.0 <sup>B,c</sup> 0.7 (80.7)	13.2 <sup>B,bc</sup> 1.2 (75.2)	12.1 <sup>B,c</sup> 1.6 (55.8)	13.4 <sup>B,bc</sup> 1.1 (59.9)	13.2 <sup>B,bc</sup> 1.5 (45.0)	17.2 <sup>B,abc</sup> 1.9 (43.2)	19.0 <sup>AB,abc</sup> 1.0 (25.0)	25.5 <sup>AB,a</sup> 2.0 (17.4)
Chlorpyrifos	1.5 <sup>C,a</sup> 0.4 (95.7)	1.8 <sup>B,a</sup> 0.5 (94.5)	5.5 <sup>B,ab</sup> 2.6 (89.6)	8.6 <sup>B,abc</sup> 1.8 (80.1)	9.6 <sup>A,C,abc</sup> 0.6 (74.8)	13.3 <sup>B,bc</sup> 4.7 (71.6)	13.4 <sup>B,bc</sup> 1.7 (74.8)	11.5 <sup>B,abc</sup> 0.2 (57.9)	11.0 <sup>B,abc</sup> 1.8 (66.9)	11.1 <sup>B,abc</sup> 0.7 (53.9)	13.5 <sup>B,bc</sup> 2.2 (55.6)	17.2 <sup>AB,c</sup> 2.5 (32.2)	14.0 <sup>B,bc</sup> 2.2 (54.7)
0.5 Chlorpyrifos	4.9 <sup>C,ab</sup> 1.1 (85.7)	3.7 <sup>B,a</sup> 1.0 (88.8)	4.5 <sup>A</sup> 0.9 (91.6)	7.4 <sup>B,abc</sup> 0.7 (82.9)	10.3 <sup>BC,abcd</sup> 0.6 (72.9)	10.7 <sup>B,abcd</sup> 4.3 (77.0)	13.8 <sup>C,de</sup> 0.1 (74.1)	10.8 <sup>B,abcd</sup> 2.2 (60.7)	14.7 <sup>B,de</sup> 2.5 (55.8)	11.8 <sup>B,bcd</sup> 1.1 (50.8)	21.9 <sup>B,f</sup> 2.5 (27.8)	19.1 <sup>AB,ef</sup> 0.5 (24.8)	23.4 <sup>AB,f</sup> 1.0 (24.0)
Chlorpyrifos + DEF	8.3 <sup>C,ab</sup> 2.5 (76.1)	4.2 <sup>B,b</sup> 0.6 (87.3)	8.6 <sup>B,ab</sup> 2.9 (83.9)	9.6 <sup>B,ab</sup> 1.9 (77.6)	11.5 <sup>BC,ab</sup> 0.9 (69.8)	12.6 <sup>B,ab</sup> 3.0 (73.0)	12.9 <sup>B,ab</sup> 1.5 (75.9)	12.9 <sup>B,ab</sup> 1.4 (53.0)	12.0 <sup>B,ab</sup> 0.4 (64.1)	12.4 <sup>B,ab</sup> 1.0 (48.2)	14.5 <sup>B,ab</sup> 1.0 (52.0)	15.4 <sup>B,a</sup> 3.1 (39.4)	18.4 <sup>B,a</sup> 4.4 (40.2)
Parathion	2.6 <sup>C,a</sup> 1.3 (92.5)	3.6 <sup>B,ab</sup> 1.9 (89.1)	6.2 <sup>B,abc</sup> 2.5 (88.4)	9.8 <sup>B,abcde</sup> 0.7 (77.2)	7.9 <sup>C,abcd</sup> 1.4 (79.2)	11.2 <sup>B,abcde</sup> 1.9 (75.9)	15.6 <sup>B,abcde</sup> 1.6 (70.7)	11.0 <sup>B,abcde</sup> 1.0 (60.1)	13.1 <sup>B,abcde</sup> 1.6 (60.7)	16.0 <sup>B,cd</sup> 1.6 (33.3)	19.5 <sup>B,ef</sup> 2.7 (35.8)	17.8 <sup>AB,ef</sup> 1.3 (30.0)	23.8 <sup>AB,f</sup> 5.1 (22.9)
0.5 Parathion	1.3 <sup>C,a</sup> 0.8 (96.1)	2.2 <sup>B,a</sup> 0.3 (93.2)	3.9 <sup>B,ab</sup> 1.0 (92.8)	5.9 <sup>B,ab</sup> 1.4 (86.3)	8.1 <sup>C,b</sup> 1.4 (78.8)	9.4 <sup>B,bc</sup> 2.6 (79.8)	14.3 <sup>B,cd</sup> 0.6 (73.2)	13.5 <sup>C,d</sup> 1.4 (50.6)	15.3 <sup>B,cd</sup> 2.1 (54.1)	16.5 <sup>B,de</sup> 0.7 (31.0)	20.3 <sup>B,ef</sup> 1.5 (33.0)	20.2 <sup>AB,ef</sup> 1.3 (20.6)	23.6 <sup>AB,f</sup> 2.4 (23.6)
Parathion + DEF	0.7 <sup>C,a</sup> 0.4 (97.9)	2.7 <sup>B,ab</sup> 1.0 (91.7)	3.3 <sup>B,ab</sup> 1.0 (93.8)	10.3 <sup>B,bc</sup> 3.0 (76.1)	10.6 <sup>BC,bc</sup> 1.1 (72.0)	15.6 <sup>B,cd</sup> 5.2 (66.5)	12.8 <sup>B,cd</sup> 1.6 (76.0)	17.6 <sup>B,cd</sup> 1.9 (35.7)	14.2 <sup>B,cd</sup> 1.0 (57.4)	15.7 <sup>B,cd</sup> 1.3 (34.7)	18.5 <sup>B,cd</sup> 0.7 (39.0)	17.5 <sup>AB,cd</sup> 2.0 (31.2)	22.4 <sup>AB,d</sup> 3.5 (27.4)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as mol·min<sup>-1</sup>·mg protein<sup>-1</sup>; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 9.3.

Table 3.4. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling liver acetylcholinesterase after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	37.1 <sup>2, A, ab</sup> 5.3 <sup>3</sup>	47.2 <sup>4, a</sup> 2.7	38.2 <sup>4, ab</sup> 1.1	45.2 <sup>4, ab</sup> 10.0	43.9 <sup>4, ab</sup> 1.4	31.6 <sup>4, ab</sup> 4.4	39.4 <sup>4, ab</sup> 3.5	24.7 <sup>4, b</sup> 2.9	32.0 <sup>4, ab</sup> 4.6	30.2 <sup>4, ab</sup> 0.3	37.1 <sup>4, ab</sup> 3.9	27.4 <sup>4, ab</sup> 2.0	40.0 <sup>4, ab</sup> 2.7
DEF	1.6 <sup>8, a</sup> 0.6 (95.7)	0.4 <sup>8, a</sup> 0.4 (99.1)	1.1 <sup>8, a</sup> 0.4 (97.1)	2.1 <sup>8, a</sup> 1.3 (95.4)	1.1 <sup>8, a</sup> 0.3 (97.4)	1.1 <sup>8, a</sup> 0.8 (96.7)	2.9 <sup>8, a</sup> 0.6 (92.6)	4.0 <sup>8, a</sup> 0.7 (83.6)	4.5 <sup>8, a</sup> 1.0 (85.9)	7.5 <sup>8, a</sup> 1.5 (75.3)	10.1 <sup>8, ab</sup> 0.2 (72.9)	15.7 <sup>8, b</sup> 1.2 (42.9)	24.9 <sup>8, c</sup> 7.9 (37.7)
Chlorpyrifos	0.6 <sup>8, a</sup> 0.3 (98.4)	1.5 <sup>8, ab</sup> 0.4 (96.8)	2.7 <sup>8, ab</sup> 0.5 (92.9)	3.1 <sup>8, ab</sup> 1.5 (93.1)	4.4 <sup>4, 8, abc</sup> 0.9 (90.0)	3.8 <sup>8, ab</sup> 0.4 (88.0)	4.8 <sup>8, abc</sup> 0.2 (87.9)	6.5 <sup>8, bc</sup> 0.9 (73.8)	7.1 <sup>8, bc</sup> 0.7 (77.9)	5.9 <sup>8, abc</sup> 1.0 (80.6)	9.4 <sup>8, c</sup> 0.9 (74.7)	13.9 <sup>8, d</sup> 0.9 (49.2)	12.9 <sup>8, d</sup> 2.9 (67.8)
0.5 Chlorpyrifos	3.1 <sup>8, ab</sup> 0.3 (91.6)	1.7 <sup>8, a</sup> 0.2 (96.4)	2.4 <sup>8, ab</sup> 0.1 (93.8)	2.5 <sup>8, ab</sup> 0.2 (94.5)	5.6 <sup>8, ab</sup> 0.8 (87.3)	4.8 <sup>8, ab</sup> 0.9 (84.8)	6.6 <sup>8, ab</sup> 1.9 (83.2)	8.1 <sup>8, abc</sup> 1.4 (67.0)	9.7 <sup>8, abc</sup> 1.9 (69.7)	11.2 <sup>8, bcd</sup> 2.0 (63.0)	18.1 <sup>8, d</sup> 3.4 (51.2)	14.8 <sup>8, cd</sup> 1.9 (46.1)	18.2 <sup>8, d</sup> 3.4 (54.6)
Chlorpyrifos + DEF	1.8 <sup>8, a</sup> 0.2 (95.1)	0.6 <sup>8, a</sup> 0.6 (98.7)	0.5 <sup>8, a</sup> 0.3 (98.8)	2.6 <sup>8, a</sup> 0.7 (94.3)	4.1 <sup>8, a</sup> 0.3 (90.7)	2.7 <sup>8, a</sup> 0.4 (91.5)	3.4 <sup>8, a</sup> 0.2 (91.5)	4.5 <sup>8, a</sup> 0.2 (81.7)	6.0 <sup>8, ab</sup> 1.2 (81.4)	7.3 <sup>8, ab</sup> 1.0 (76.0)	8.0 <sup>8, ab</sup> 1.8 (78.4)	11.8 <sup>8, b</sup> 4.6 (57.0)	18.0 <sup>8, c</sup> 3.5 (54.9)
Parathion	0.2 <sup>8, a</sup> 0.1 (99.5)	0.5 <sup>8, a</sup> 0.5 (98.9)	1.0 <sup>8, a</sup> 0.2 (97.4)	2.4 <sup>8, a</sup> 0.7 (94.7)	3.8 <sup>8, a</sup> 0.4 (91.4)	5.3 <sup>8, a</sup> 0.9 (83.2)	4.7 <sup>8, a</sup> 0.6 (88.1)	6.7 <sup>8, a</sup> 0.6 (72.7)	7.5 <sup>8, a</sup> 2.2 (76.6)	16.5 <sup>8, b</sup> 3.5 (45.4)	16.4 <sup>8, b</sup> 4.1 (55.8)	20.1 <sup>8, b</sup> 0.9 (26.8)	23.3 <sup>8, b</sup> 6.4 (41.8)
0.5 Parathion	0.9 <sup>8, a</sup> 0.3 (97.6)	0.1 <sup>8, a</sup> 0.1 (99.7)	1.1 <sup>8, a</sup> 0.8 (97.2)	2.3 <sup>8, a</sup> 0.0 (95.0)	5.6 <sup>8, ab</sup> 0.5 (87.3)	6.3 <sup>8, ab</sup> 0.7 (80.1)	5.9 <sup>8, ab</sup> 1.4 (84.9)	9.1 <sup>8, bc</sup> 0.8 (63.1)	11.0 <sup>8, bc</sup> 1.2 (65.6)	13.0 <sup>8, cd</sup> 1.8 (56.9)	14.2 <sup>8, cd</sup> 1.6 (61.7)	18.0 <sup>8, d</sup> 2.0 (34.4)	26.8 <sup>8, e</sup> 3.7 (32.9)
Parathion + DEF	0.1 <sup>8, a</sup> 0.1 (99.7)	0.1 <sup>8, a</sup> 0.1 (99.7)	1.3 <sup>8, ab</sup> 0.4 (96.5)	1.5 <sup>8, ab</sup> 0.5 (96.8)	5.7 <sup>8, ab</sup> 1.9 (87.0)	4.9 <sup>8, ab</sup> 0.7 (84.5)	4.3 <sup>8, ab</sup> 0.6 (89.1)	9.1 <sup>8, abc</sup> 1.5 (63.0)	6.9 <sup>8, abc</sup> 0.6 (78.3)	8.6 <sup>8, abc</sup> 2.1 (71.5)	10.4 <sup>8, abc</sup> 3.5 (71.9)	13.2 <sup>8, bc</sup> 2.9 (51.8)	17.3 <sup>8, c</sup> 7.3 (56.7)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as nmol·min<sup>-1</sup>·mg protein<sup>-1</sup>; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 4.8.

Table 3.5. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling muscle acetylcholinesterase after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	133.3 <sup>2,3A,A</sup> 23.9 <sup>3</sup>	170.2 <sup>A</sup> 10.9	132.0 <sup>A</sup> 18.5	129.4 <sup>A</sup> 26.4	119.0 <sup>A</sup> 5.8	137.6 <sup>A</sup> 9.5	131.6 <sup>A</sup> 8.9	97.9 <sup>A</sup> 6.0	109.9 <sup>A</sup> 28.3	104.1 <sup>A</sup> 15.5	122.2 <sup>A</sup> 36.0	101.1 <sup>A</sup> 25.7	97.6 <sup>A</sup> 28.9
DEF	121.3 <sup>A,ab</sup> 3.7 (9.0)	118.1 <sup>B,ab</sup> 16.9 (30.6)	99.6 <sup>B,abc</sup> 9.6 (24.6)	131.7 <sup>A</sup> 4.2 (0.0)	129.8 <sup>A</sup> 11.9 (0.0)	86.4 <sup>B,abc</sup> 17.6 (37.2)	78.8 <sup>B,abc</sup> 11.7 (40.1)	77.7 <sup>A,abc</sup> 1.0 (20.7)	94.2 <sup>A,abc</sup> 26.8 (14.3)	72.8 <sup>B,bc</sup> 22.6 (30.1)	70.7 <sup>B,bc</sup> 16.1 (42.2)	83.6 <sup>B,abc</sup> 15.8 (17.2)	53.1 <sup>B,c</sup> 7.6 (45.5)
Chlorpyrifos	9.6 <sup>B,ab</sup> 2.4 (92.8)	9.1 <sup>C,ab</sup> 1.7 (94.7)	9.7 <sup>ab</sup> 1.9 (92.6)	8.0 <sup>B</sup> 0.6 (93.8)	20.5 <sup>A,B,ab</sup> 2.8 (82.7)	17.1 <sup>C,ab</sup> 3.0 (87.6)	20.9 <sup>C,ab</sup> 4.4 (84.1)	16.0 <sup>B,ab</sup> 2.0 (83.7)	17.2 <sup>B,ab</sup> 2.1 (84.3)	17.7 <sup>ab</sup> 3.6 (83.0)	17.2 <sup>B,ab</sup> 5.3 (85.9)	23.6 <sup>C,b</sup> 9.0 (76.6)	19.0 <sup>B,ab</sup> 4.8 (80.5)
0.5 Chlorpyrifos	33.5 <sup>B,A</sup> 7.3 (74.9)	12.8 <sup>C,ab</sup> 6.3 (92.5)	16.4 <sup>C,ab</sup> 8.2 (87.6)	10.9 <sup>B,b</sup> 1.9 (91.6)	29.0 <sup>ab</sup> 2.2 (75.7)	27.0 <sup>C,ab</sup> 6.3 (80.4)	19.2 <sup>C,ab</sup> 2.7 (85.4)	30.3 <sup>B,ab</sup> 12.3 (69.0)	18.5 <sup>B,ab</sup> 7.4 (83.2)	20.3 <sup>C,ab</sup> 5.8 (80.5)	25.8 <sup>B,ab</sup> 9.4 (78.9)	30.2 <sup>B,ab</sup> 13.3 (70.2)	26.6 <sup>B,ab</sup> 6.8 (72.7)
Chlorpyrifos + DEF	51.1 <sup>B,A</sup> 12.6 (61.7)	35.5 <sup>C,ab</sup> 5.9 (79.2)	29.3 <sup>C,ab</sup> 5.9 (77.8)	34.2 <sup>ab</sup> 0.6 (73.6)	28.6 <sup>ab</sup> 3.7 (76.0)	24.3 <sup>C,b</sup> 1.9 (82.3)	21.0 <sup>C,b</sup> 5.3 (84.0)	30.1 <sup>B,ab</sup> 12.6 (69.3)	17.9 <sup>B,b</sup> 2.3 (83.7)	14.0 <sup>C,b</sup> 3.7 (86.6)	18.6 <sup>B,b</sup> 5.3 (84.8)	20.1 <sup>C,b</sup> 5.1 (80.1)	26.1 <sup>B,b</sup> 7.8 (73.3)
Parathion	4.8 <sup>B,A</sup> 3.0 (96.4)	6.7 <sup>C,A</sup> 1.6 (96.1)	7.6 <sup>C,A</sup> 0.5 (94.2)	10.0 <sup>B,A</sup> 3.7 (92.3)	11.9 <sup>A</sup> 2.8 (90.0)	18.3 <sup>C,ab</sup> 1.0 (86.7)	28.9 <sup>C,bc</sup> 0.7 (78.1)	26.0 <sup>B,bc</sup> 5.7 (73.4)	28.9 <sup>B,bc</sup> 1.4 (73.7)	35.5 <sup>C,cd</sup> 3.9 (65.9)	47.0 <sup>B,d</sup> 2.7 (61.5)	48.5 <sup>B,c,d</sup> 6.9 (52.0)	43.7 <sup>B,d</sup> 6.7 (55.2)
0.5 Parathion	4.6 <sup>B,A</sup> 3.0 (96.6)	6.1 <sup>C,A</sup> 3.2 (96.4)	8.4 <sup>C,ab</sup> 2.4 (93.6)	9.7 <sup>B,abc</sup> 1.0 (92.5)	12.8 <sup>B,abcde</sup> 2.7 (89.2)	26.0 <sup>C,bcde</sup> 4.8 (81.1)	11.7 <sup>C,abcd</sup> 4.2 (91.1)	29.5 <sup>B,cde</sup> 1.5 (69.9)	28.0 <sup>B,bcde</sup> 8.3 (74.5)	30.9 <sup>C,cde</sup> 11.4 (70.3)	33.8 <sup>B,e</sup> 4.8 (72.4)	30.7 <sup>B,cde</sup> 7.3 (69.6)	32.6 <sup>B,de</sup> 8.7 (66.5)
Parathion + DEF	6.2 <sup>B,A</sup> 2.6 (95.3)	9.0 <sup>C,ab</sup> 2.2 (94.7)	6.3 <sup>C,A</sup> 1.6 (95.3)	12.3 <sup>B,ab</sup> 3.6 (90.5)	17.3 <sup>ab</sup> 2.6 (85.5)	24.8 <sup>C,abcd</sup> 0.3 (81.9)	21.0 <sup>C,abc</sup> 2.6 (84.1)	26.4 <sup>B,abcd</sup> 3.4 (73.1)	29.9 <sup>B,abcd</sup> 1.8 (72.8)	29.9 <sup>B,abcd</sup> 4.4 (71.2)	38.7 <sup>B,cde</sup> 2.4 (68.4)	51.9 <sup>B,c,e</sup> 14.5 (48.7)	42.0 <sup>B,de</sup> 5.1 (57.0)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as nmol·min<sup>-1</sup>·mg protein<sup>-1</sup>; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different (p ≤ 0.05) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 21.5.

Table 3.6. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling plasma acetylcholinesterase after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	20.2 <sup>2A,a</sup> 2.3 <sup>3</sup>	26.3 <sup>A,a</sup> 1.9	21.1 <sup>A,a</sup> 0.9	24.9 <sup>A,a</sup> 3.3	24.5 <sup>A,a</sup> 2.8	24.6 <sup>A,a</sup> 1.5	22.7 <sup>A,a</sup> 1.9	16.7 <sup>A,a</sup> 0.3	22.0 <sup>A,a</sup> 5.1	20.8 <sup>A,a</sup> 2.7	19.4 <sup>B,a</sup> 3.0	19.5 <sup>B,a</sup> 2.1	25.1 <sup>A,a,d</sup> 3.5
DEF	14.1 <sup>B,abc</sup> 1.2 (30.3)	12.2 <sup>B,ab</sup> 2.6 (53.7)	9.2 <sup>B,a</sup> 0.8 (56.3)	11.5 <sup>B,ab</sup> 1.8 (53.6)	11.8 <sup>B,ab</sup> 0.6 (51.8)	11.1 <sup>B,ab</sup> 0.8 (54.8)	12.8 <sup>B,ab</sup> 1.1 (43.9)	16.9 <sup>B,bc</sup> 0.0 (0.0)	19.1 <sup>A,c</sup> 2.5 (13.1)	23.9 <sup>A,d</sup> 1.2 (0.0)	26.4 <sup>A,d</sup> 1.2 (0.0)	25.4 <sup>A,d</sup> 3.0 (0.0)	26.8 <sup>A,d</sup> 0.2 (0.0)
Chlorpyrifos	1.2 <sup>D,a</sup> 0.8 (94.3)	1.7 <sup>C,ab</sup> 0.1 (93.5)	2.1 <sup>D,ab</sup> 0.5 (90.1)	3.1 <sup>C,ab</sup> 0.8 (87.6)	4.2 <sup>C,ab</sup> 0.4 (83.0)	4.2 <sup>CD,ab</sup> 1.0 (83.1)	2.7 <sup>D,ab</sup> 0.2 (88.0)	3.2 <sup>D,ab</sup> 0.6 (80.8)	3.9 <sup>B,ab</sup> 0.4 (82.2)	3.1 <sup>C,ab</sup> 0.6 (85.0)	4.7 <sup>C,ab</sup> 0.7 (75.7)	6.9 <sup>D,bc</sup> 1.3 (64.9)	9.6 <sup>C,c</sup> 2.9 (61.9)
0.5 Chlorpyrifos	6.0 <sup>C,ab</sup> 1.4 (70.1)	3.1 <sup>C,a</sup> 0.5 (88.1)	4.8 <sup>C,ab</sup> 0.3 (77.4)	4.7 <sup>C,ab</sup> 1.1 (81.0)	6.6 <sup>C,ab</sup> 1.1 (73.1)	5.4 <sup>CD,ab</sup> 0.9 (78.1)	3.7 <sup>D,a</sup> 0.5 (83.7)	5.4 <sup>CD,ab</sup> 1.7 (67.5)	5.2 <sup>B,ab</sup> 1.5 (76.6)	7.8 <sup>C,ab</sup> 0.8 (62.7)	9.1 <sup>C,bc</sup> 1.0 (53.3)	9.2 <sup>D,bc</sup> 0.9 (52.7)	11.7 <sup>C,c</sup> 1.3 (53.3)
Chlorpyrifos + DEF	7.0 <sup>C,abc</sup> 1.5 (65.4)	4.6 <sup>C,a</sup> 1.3 (82.5)	3.4 <sup>CD,a</sup> 0.2 (83.9)	6.1 <sup>C,abc</sup> 1.3 (75.4)	6.1 <sup>C,abc</sup> 1.1 (75.0)	6.8 <sup>C,abc</sup> 1.5 (72.5)	5.0 <sup>CD,ab</sup> 0.5 (78.1)	7.6 <sup>C,abc</sup> 0.8 (54.4)	8.0 <sup>B,abc</sup> 0.8 (63.8)	7.7 <sup>C,abc</sup> 1.0 (62.8)	10.2 <sup>C,bc</sup> 0.5 (47.3)	11.3 <sup>CD,c</sup> 2.2 (42.3)	10.0 <sup>C,bc</sup> 1.0 (60.3)
Parathion	0.7 <sup>D,a</sup> 0.7 (96.7)	0.6 <sup>C,a</sup> 0.1 (97.8)	1.8 <sup>D,a</sup> 0.4 (91.6)	1.5 <sup>C,a</sup> 0.1 (94.0)	2.2 <sup>C,a</sup> 0.3 (91.1)	3.0 <sup>D,ab</sup> 0.4 (87.7)	4.0 <sup>CD,abc</sup> 0.2 (82.5)	3.6 <sup>D,ab</sup> 0.4 (78.6)	5.7 <sup>B,bcd</sup> 0.9 (74.2)	6.6 <sup>C,cde</sup> 1.0 (68.3)	7.0 <sup>C,de</sup> 1.8 (63.8)	8.3 <sup>D,de</sup> 1.4 (57.5)	8.9 <sup>C,e</sup> 1.0 (64.6)
0.5 Parathion	0.8 <sup>D,a</sup> 0.2 (96.0)	0.5 <sup>C,a</sup> 0.3 (97.9)	1.4 <sup>D,a</sup> 0.2 (93.3)	2.0 <sup>C,a</sup> 0.3 (92.1)	2.8 <sup>C,ab</sup> 0.5 (88.5)	5.0 <sup>CD,bc</sup> 0.6 (79.9)	4.7 <sup>CD,bc</sup> 0.3 (79.4)	5.2 <sup>CD,bc</sup> 0.2 (68.8)	7.6 <sup>C,d</sup> 1.3 (65.6)	8.7 <sup>C,d</sup> 0.6 (58.2)	8.4 <sup>C,d</sup> 0.4 (56.5)	11.5 <sup>CD,e</sup> 1.9 (40.9)	8.8 <sup>C,d</sup> 0.8 (65.0)
Parathion + DEF	0.6 <sup>D,a</sup> 0.3 (96.8)	1.1 <sup>C,a</sup> 0.3 (95.9)	1.2 <sup>D,a</sup> 0.3 (94.4)	1.9 <sup>C,a</sup> 0.6 (92.4)	3.3 <sup>C,a</sup> 0.4 (86.5)	4.7 <sup>CD,a</sup> 0.6 (81.1)	6.9 <sup>C,ab</sup> 0.3 (69.8)	11.1 <sup>B,bc</sup> 0.6 (33.5)	11.9 <sup>B,bc</sup> 1.9 (45.9)	15.9 <sup>B,cd</sup> 3.3 (23.7)	20.4 <sup>B,d</sup> 2.6 (0.0)	18.0 <sup>B,c,d</sup> 1.9 (7.9)	18.3 <sup>B,d</sup> 2.3 (27.0)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 4.0.

Table 3.7. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling gill aliesterases after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	7.9 <sup>A,ab</sup> 1.9 <sup>b</sup>	7.9 <sup>A,ab</sup> 0.1	10.0 <sup>A,ab</sup> 2.8	6.5 <sup>A,b</sup> 3.3	8.8 <sup>A,ab</sup> 1.7	6.6 <sup>A,b</sup> 1.4	10.6 <sup>A,ab</sup> 2.7	9.4 <sup>A,ab</sup> 0.4	9.9 <sup>A,ab</sup> 1.5	14.3 <sup>A,a</sup> 0.5	10.3 <sup>A,ab</sup> 2.5	9.4 <sup>A,ab</sup> 1.4	10.0 <sup>A,ab</sup> 1.8
DEF	0.2 <sup>B,a</sup> 0.2 (97.5)	0.6 <sup>B,a</sup> 0.5 (93.4)	0.7 <sup>B,a</sup> 0.4 (93.4)	0.3 <sup>B,a</sup> 0.2 (94.9)	0.5 <sup>B,a</sup> 0.5 (94.5)	0.5 <sup>B,a</sup> 0.5 (92.8)	0.2 <sup>B,a</sup> 0.2 (98.3)	0.6 <sup>C,a</sup> 0.4 (93.6)	0.6 <sup>D,a</sup> 0.2 (93.7)	1.0 <sup>C,a</sup> 1.0 (93.1)	0.2 <sup>B,a</sup> 0.2 (97.9)	0.0 <sup>C,a</sup> 0.0 (100.0)	1.7 <sup>C,a</sup> 0.6 (83.3)
Chlorpyrifos	2.2 <sup>B,a</sup> 0.1 (72.0)	4.2 <sup>B,a</sup> 1.4 (47.1)	1.8 <sup>B,a</sup> 0.9 (82.3)	3.2 <sup>B,a</sup> 0.9 (50.9)	2.0 <sup>A,B,a</sup> 1.5 (77.3)	2.9 <sup>B,C,D,a</sup> 0.7 (56.1)	2.3 <sup>B,a</sup> 0.3 (78.8)	3.4 <sup>B,a</sup> 0.1 (63.9)	3.7 <sup>B,C,a</sup> 0.2 (62.4)	3.1 <sup>C,a</sup> 1.0 (78.4)	2.1 <sup>B,a</sup> 0.6 (79.5)	4.8 <sup>B,a</sup> 1.2 (49.6)	4.2 <sup>B,C,a</sup> 0.9 (57.6)
0.5 Chlorpyrifos	0.7 <sup>B,a</sup> 0.4 (90.9)	3.9 <sup>B,ab</sup> 0.4 (50.8)	2.5 <sup>B,ab</sup> 0.1 (74.5)	1.3 <sup>B,a</sup> 0.6 (79.9)	2.7 <sup>B,ab</sup> 0.4 (69.5)	2.4 <sup>B,C,D,ab</sup> 0.1 (64.2)	1.8 <sup>B,ab</sup> 1.2 (83.2)	3.7 <sup>B,ab</sup> 0.2 (60.6)	5.0 <sup>B,ab</sup> 0.6 (48.9)	2.7 <sup>C,ab</sup> 1.3 (81.0)	4.8 <sup>B,ab</sup> 2.2 (53.3)	6.4 <sup>A,B,b</sup> 0.6 (32.0)	5.3 <sup>B,C,ab</sup> 1.8 (47.4)
Chlorpyrifos + DEF	0.7 <sup>B,a</sup> 0.7 (90.6)	2.6 <sup>B,a</sup> 0.8 (67.2)	2.7 <sup>B,a</sup> 0.8 (73.1)	0.4 <sup>B,a</sup> 0.4 (94.2)	1.5 <sup>B,a</sup> 0.4 (82.7)	1.8 <sup>B,C,D,a</sup> 0.6 (72.2)	0.7 <sup>B,a</sup> 0.4 (93.4)	0.1 <sup>C,a</sup> 0.1 (98.7)	1.6 <sup>D,a</sup> 0.4 (83.9)	0.5 <sup>C,a</sup> 0.5 (96.7)	0.4 <sup>B,a</sup> 0.4 (96.0)	1.5 <sup>C,a</sup> 0.7 (84.3)	1.7 <sup>C,a</sup> 1.0 (83.1)
Parathion	2.2 <sup>B,a</sup> 0.2 (72.5)	2.6 <sup>B,a</sup> 1.0 (66.9)	4.0 <sup>B,a</sup> 0.7 (59.9)	2.2 <sup>B,a</sup> 1.0 (66.6)	3.8 <sup>B,a</sup> 0.3 (56.7)	4.0 <sup>B,C,a</sup> 1.1 (39.1)	5.2 <sup>B,a</sup> 1.1 (51.1)	4.0 <sup>B,a</sup> 0.7 (57.2)	4.7 <sup>B,a</sup> 1.0 (52.2)	2.9 <sup>C,a</sup> 1.1 (79.9)	7.0 <sup>B,ab</sup> 1.2 (32.4)	6.0 <sup>B,ab</sup> 0.8 (36.8)	9.3 <sup>A,B,b</sup> 1.6 (7.2)
0.5 Parathion	2.6 <sup>B,ab</sup> 0.8 (67.1)	2.2 <sup>B,ab</sup> 1.4 (71.9)	1.3 <sup>B,a</sup> 0.7 (86.6)	2.8 <sup>B,ab</sup> 0.7 (56.5)	2.8 <sup>B,ab</sup> 0.5 (68.1)	4.7 <sup>B,ab</sup> 0.7 (29.8)	4.1 <sup>B,ab</sup> 1.9 (61.1)	4.6 <sup>B,ab</sup> 1.2 (50.9)	6.3 <sup>B,ab</sup> 0.6 (35.8)	7.1 <sup>B,ab</sup> 1.3 (49.9)	7.5 <sup>B,ab</sup> 1.8 (27.7)	7.5 <sup>B,ab</sup> 0.8 (20.5)	12.2 <sup>A,C</sup> 2.4 (0.0)
Parathion + DEF	1.1 <sup>B,a</sup> 1.1 (85.8)	0.5 <sup>B,a</sup> 0.3 (93.8)	1.2 <sup>B,a</sup> 0.6 (88.0)	0.1 <sup>B,a</sup> 0.1 (98.1)	0.8 <sup>B,a</sup> 0.4 (90.8)	1.0 <sup>D,a</sup> 0.4 (85.2)	0.7 <sup>B,a</sup> 0.6 (93.1)	1.5 <sup>C,a</sup> 0.5 (84.3)	0.4 <sup>D,a</sup> 0.4 (95.8)	0.4 <sup>C,a</sup> 0.4 (97.4)	0.9 <sup>B,a</sup> 0.8 (91.1)	1.3 <sup>C,a</sup> 0.9 (86.4)	2.2 <sup>C,a</sup> 0.3 (77.9)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as nmol·min<sup>-1</sup>·mg protein<sup>-1</sup>; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 2.1.

Table 3.8. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling liver aliesterases after a 4-hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	68.2 <sup>2A,ab</sup> 5.6 <sup>3</sup>	52.5 <sup>A,ab</sup> 8.7	38.8 <sup>A,b</sup> 2.8	44.8 <sup>A,ab</sup> 21.7	54.9 <sup>A,ab</sup> 29.2	51.9 <sup>A,ab</sup> 30.1	58.0 <sup>A,ab</sup> 17.0	65.1 <sup>A,ab</sup> 12.9	79.9 <sup>A,ab</sup> 25.7	64.8 <sup>A,ab</sup> 1.5	81.5 <sup>A,ab</sup> 7.9	64.6 <sup>A,ab</sup> 4.5	100.2 <sup>9A,a</sup> 22.2
DEF	2.2 <sup>B,a</sup> 0.2 (96.8)	1.9 <sup>C,a</sup> 1.2 (96.4)	7.2 <sup>A,a</sup> 4.6 (81.4)	6.8 <sup>A,a</sup> 6.1 (84.8)	2.0 <sup>B,a</sup> 1.2 (96.3)	5.6 <sup>A,a</sup> 3.9 (89.1)	7.1 <sup>B,a</sup> 3.2 (87.8)	4.6 <sup>C,a</sup> 0.7 (92.9)	8.4 <sup>B,a</sup> 4.9 (89.5)	5.2 <sup>D,a</sup> 1.0 (91.9)	6.7 <sup>C,a</sup> 0.6 (91.8)	5.5 <sup>D,a</sup> 0.9 (91.5)	5.0 <sup>10B,a</sup> 0.9 (95.1)
Chlorpyrifos	21.2 <sup>B,a</sup> 10.5 (68.9)	17.5 <sup>BC,a</sup> 10.4 (66.7)	20.5 <sup>A,a</sup> 9.1 (47.1)	22.0 <sup>A,a</sup> 6.6 (50.8)	14.7 <sup>5,AB,a</sup> 5.8 (73.2)	18.6 <sup>A,a</sup> 3.2 (64.3)	20.4 <sup>B,a</sup> 3.3 (64.9)	19.0 <sup>BC,a</sup> 3.2 (70.8)	26.6 <sup>B,a</sup> 1.6 (66.7)	24.9 <sup>C,a</sup> 8.4 (61.5)	29.1 <sup>B,a</sup> 3.1 (64.3)	23.5 <sup>DB,a</sup> 2.4 (63.6)	22.4 <sup>11B,a</sup> 2.5 (77.7)
0.5 Chlorpyrifos	23.7 <sup>B,a</sup> 12.5 (65.2)	33.5 <sup>B,a</sup> 2.7 (36.1)	21.0 <sup>A,a</sup> 3.8 (45.8)	15.8 <sup>A,a</sup> 8.1 (64.8)	22.7 <sup>AB,a</sup> 4.7 (58.6)	21.0 <sup>A,a</sup> 2.4 (59.6)	19.0 <sup>B,a</sup> 1.7 (67.2)	22.5 <sup>BC,a</sup> 1.0 (65.4)	23.1 <sup>B,a</sup> 1.0 (71.1)	26.7 <sup>C,a</sup> 2.0 (58.8)	40.7 <sup>D,a</sup> 2.0 (50.1)	27.8 <sup>BC,a</sup> 8.3 (57.0)	22.6 <sup>12B,a</sup> 4.6 (77.4)
Chlorpyrifos + DEF	4.6 <sup>B,a</sup> 1.5 (93.3)	3.5 <sup>C,a</sup> 2.5 (93.4)	12.3 <sup>A,a</sup> 7.3 (68.4)	1.5 <sup>A,a</sup> 1.5 (96.7)	3.1 <sup>6,AB,a</sup> 1.3 (94.3)	5.6 <sup>A,a</sup> 2.9 (89.2)	20.5 <sup>B,a</sup> 16.5 (64.7)	11.1 <sup>BC,a</sup> 7.8 (83.0)	7.2 <sup>B,a</sup> 1.2 (91.0)	6.6 <sup>B,a</sup> 2.4 (89.8)	6.4 <sup>C,a</sup> 1.5 (92.1)	10.4 <sup>D,a</sup> 2.4 (83.9)	16.9 <sup>13B,a</sup> 4.6 (83.1)
Parathion	28.6 <sup>B,abc</sup> 5.6 (58.1)	21.0 <sup>BC,ab</sup> 4.7 (60.1)	27.3 <sup>A,abc</sup> 10.0 (29.6)	25.5 <sup>A,abc</sup> 5.1 (42.9)	9.8 <sup>7,AB,a</sup> 1.1 (82.2)	20.6 <sup>A,ab</sup> 2.1 (60.4)	33.1 <sup>AB,abc</sup> 4.9 (43.0)	25.2 <sup>BC,abc</sup> 5.7 (61.3)	41.3 <sup>B,bc</sup> 4.5 (48.3)	43.9 <sup>B,bc</sup> 2.1 (32.3)	40.9 <sup>B,bc</sup> 4.8 (49.9)	42.8 <sup>B,bc</sup> 7.0 (33.7)	51.3 <sup>14,AB,c</sup> 5.4 (48.8)
0.5 Parathion	21.9 <sup>B,a</sup> 2.7 (67.8)	22.7 <sup>BC,a</sup> 2.2 (56.8)	21.6 <sup>A,a</sup> 4.6 (44.3)	18.6 <sup>A,a</sup> 2.4 (58.4)	24.4 <sup>AB,a</sup> 5.7 (55.6)	25.4 <sup>A,a</sup> 6.2 (51.0)	29.5 <sup>AB,a</sup> 2.3 (49.1)	33.6 <sup>B,ab</sup> 5.6 (48.5)	35.4 <sup>B,ab</sup> 9.7 (55.7)	38.3 <sup>B,ab</sup> 2.9 (41.0)	45.1 <sup>B,ab</sup> 6.6 (44.7)	43.5 <sup>B,ab</sup> 2.3 (32.7)	50.3 <sup>15,AB,b</sup> 3.5 (49.8)
Parathion + DEF	2.8 <sup>B,a</sup> 0.9 (95.9)	7.5 <sup>C,a</sup> 3.4 (85.6)	11.7 <sup>A,a</sup> 11.5 (69.8)	9.7 <sup>A,a</sup> 5.7 (78.4)	0.0 <sup>8,B,a</sup> 0.0 (100.0)	2.0 <sup>A,a</sup> 1.3 (96.2)	7.6 <sup>B,a</sup> 3.8 (86.9)	11.9 <sup>BC,a</sup> 4.4 (81.8)	10.2 <sup>B,a</sup> 2.6 (87.3)	7.8 <sup>B,a</sup> 1.3 (88.0)	6.7 <sup>C,a</sup> 1.1 (91.8)	6.6 <sup>D,a</sup> 0.4 (89.7)	10.8 <sup>16,AB,a</sup> 3.5 (89.3)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as nmol·min<sup>-1</sup>·mg protein<sup>-1</sup>; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 58.0.

<sup>5</sup>n = 1; value = 21.7; no standard error.

<sup>6</sup>n = 2; arithmetic mean = 6.2.

<sup>7</sup>n = 2; arithmetic mean = 12.9.

<sup>8</sup>n = 2; arithmetic mean = 0.0.

<sup>9</sup>n = 2; arithmetic mean = 100.2.

<sup>10</sup>n = 1; value = 10.2; no standard error.

<sup>11</sup>n = 2; arithmetic mean = 22.4.

<sup>12</sup>n = 1; value = 27.8; no standard error.

<sup>13</sup>n = 2; arithmetic mean = 16.9.

<sup>14</sup>n = 2; arithmetic mean = 51.3.

<sup>15</sup>n = 1; value = 55.6; no standard error.

<sup>16</sup>n = 2; arithmetic mean = 10.8.

Table 3.9. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling plasma aliesterases after a 4 hour static exposure to each treatment. Refer to Table 3.1 for treatment concentrations.

Treatment	Time (h)												
	0	4	8	12	24	48	96	144	192	240	288	336	384
Control	62.5 <sup>2A,a</sup>	52.8 <sup>A,a</sup>	74.1 <sup>A,a</sup>	73.0 <sup>A,a</sup>	48.5 <sup>A,a</sup>	65.7 <sup>A,a</sup>	60.4 <sup>A,a</sup>	63.7 <sup>A,a</sup>	72.2 <sup>A,a</sup>	85.2 <sup>A,a</sup>	86.4 <sup>A,a</sup>	85.5 <sup>A,a</sup>	96.6 <sup>A,a</sup>
	25.1 <sup>3</sup>	20.4	7.9	20.8	12.0	27.0	16.7	19.3	12.4	28.1	32.0	19.0	34.8
DEF	0.0 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.6 <sup>B,a</sup>	0.1 <sup>B,a</sup>	1.1 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.6 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.3 <sup>B,a</sup>	1.1 <sup>B,a</sup>	2.0 <sup>B,a</sup>	0.2 <sup>B,a</sup>
	0.0	0.0	0.5	0.1	1.1	0.0	0.0	0.6	0.0	0.2	0.8	0.5	0.2
	(100.0)	(99.9)	(99.2)	(99.9)	(97.7)	(100.0)	(100.0)	(99.1)	(100.0)	(99.7)	(98.7)	(97.7)	(99.8)
Chlorpyrifos	0.4 <sup>B,a</sup>	0.3 <sup>B,a</sup>	1.5 <sup>B,a</sup>	1.6 <sup>B,a</sup>	1.1 <sup>B,a</sup>	1.1 <sup>B,a</sup>	1.7 <sup>B,a</sup>	2.5 <sup>B,a</sup>	2.0 <sup>B,a</sup>	1.5 <sup>B,a</sup>	3.9 <sup>B,a</sup>	4.9 <sup>B,a</sup>	4.6 <sup>B,a</sup>
	0.4	0.3	0.5	1.1	0.8	0.6	1.0	0.9	1.3	0.4	1.2	2.4	1.7
	(99.5)	(97.9)	(97.9)	(97.8)	(97.8)	(98.3)	(97.2)	(96.1)	(97.3)	(98.2)	(95.5)	(94.3)	(95.2)
0.5 Chlorpyrifos	1.7 <sup>B,a</sup>	1.5 <sup>B,a</sup>	1.6 <sup>B,a</sup>	1.4 <sup>B,a</sup>	2.8 <sup>B,a</sup>	4.8 <sup>B,a</sup>	3.4 <sup>B,a</sup>	1.7 <sup>B,a</sup>	3.4 <sup>B,a</sup>	3.1 <sup>B,a</sup>	14.2 <sup>B,a</sup>	6.9 <sup>B,a</sup>	27.9 <sup>B,a</sup>
	1.7	0.8	0.9	0.9	0.5	0.3	1.1	1.1	2.7	2.0	9.2	3.1	18.6
	(97.7)	(97.2)	(97.8)	(98.0)	(94.2)	(92.6)	(94.4)	(97.4)	(95.3)	(96.4)	(83.5)	(91.9)	(71.2)
Chlorpyrifos + DEF	1.4 <sup>B,a</sup>	0.6 <sup>B,a</sup>	1.1 <sup>B,a</sup>	0.3 <sup>B,a</sup>	0.8 <sup>B,a</sup>	0.3 <sup>B,a</sup>	1.5 <sup>B,a</sup>	1.4 <sup>B,a</sup>	0.2 <sup>B,a</sup>	0.1 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.1 <sup>B,a</sup>	1.1 <sup>B,a</sup>
	1.4	0.5	0.8	0.2	0.5	0.3	0.8	1.0	0.2	0.1	0.0	0.1	0.6
	(97.7)	(98.9)	(98.5)	(99.6)	(98.4)	(99.5)	(97.5)	(97.8)	(99.7)	(99.9)	(99.9)	(99.8)	(98.9)
Parathion	0.1 <sup>B,a</sup>	0.8 <sup>B,a</sup>	1.8 <sup>B,a</sup>	3.2 <sup>B,a</sup>	2.9 <sup>B,a</sup>	1.7 <sup>B,a</sup>	3.0 <sup>B,a</sup>	6.7 <sup>B,a</sup>	9.8 <sup>B,a</sup>	20.5 <sup>B,a</sup>	45.2 <sup>AB,b</sup>	55.5 <sup>AB,b</sup>	60.4 <sup>AB,b</sup>
	0.1	0.8	1.0	1.2	0.2	0.8	1.7	0.9	2.2	4.3	15.2	7.8	7.4
	(99.9)	(98.5)	(97.6)	(95.6)	(94.0)	(97.5)	(95.1)	(89.6)	(86.4)	(76.0)	(47.6)	(35.1)	(37.5)
0.5 Parathion	2.7 <sup>B,a</sup>	1.5 <sup>B,a</sup>	1.4 <sup>B,a</sup>	1.3 <sup>B,a</sup>	3.7 <sup>B,a</sup>	1.8 <sup>B,a</sup>	5.1 <sup>B,a</sup>	8.9 <sup>B,a</sup>	21.0 <sup>B,a</sup>	45.8 <sup>B,b</sup>	46.4 <sup>AB,b</sup>	62.8 <sup>B,b</sup>	84.5 <sup>A,c</sup>
	1.9	0.9	0.8	1.2	0.3	1.0	1.5	2.3	11.7	7.6	3.8	12.3	14.6
	(95.7)	(97.2)	(98.1)	(98.2)	(92.4)	(97.3)	(91.6)	(86.0)	(71.0)	(46.3)	(46.2)	(26.6)	(12.6)
Parathion + DEF	0.6 <sup>B,a</sup>	0.4 <sup>B,a</sup>	0.0 <sup>B,a</sup>	1.5 <sup>B,a</sup>	1.3 <sup>B,a</sup>	1.0 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.0 <sup>B,a</sup>	0.9 <sup>B,a</sup>	0.4 <sup>B,a</sup>	0.9 <sup>B,a</sup>	0.0 <sup>B,a</sup>
	0.6	0.4	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.8	0.2	0.9	0.0
	(99.1)	(99.3)	(100.0)	(97.9)	(97.2)	(98.5)	(100.0)	(100.0)	(100.0)	(98.9)	(99.5)	(98.9)	(100.0)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ; means within a time not followed by the same capital letter or within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>n = 2; arithmetic mean = 2.8.

## CHAPTER IV

### EFFECTS OF PIPERONYL BUTOXIDE ON THE METABOLISM OF DEF IN FINGERLING CHANNEL CATFISH

#### Introduction

Organophosphorus (OP) insecticides, such as chlorpyrifos and parathion, or their metabolites are assumed to poison by inhibiting AChE in the central and peripheral nervous systems. The mode of action of DEF, also an OP, is unclear; however, Hur et al. (1992) suggested that the sulfone and/or the sulfoxide is responsible for the toxic effects.

Earlier experiments (Chapter II) have shown that DEF is a poor anticholinesterase in vitro in brain, gill, liver, muscle, and plasma; DEF is a potent inhibitor of ALiEs. In vivo results from previous experiments (Chapter III) demonstrated that DEF is a poor anticholinesterase in channel catfish brain and muscle (maximum inhibition at 288 h (36%) and 384 h (46%), respectively); in addition, rapid and substantial inhibition of AChE was displayed in gill and liver, while plasma demonstrated intermediate inhibition with rapid enzyme recovery. Chapter III also discusses an in vitro activation experiment (with channel catfish hepatic microsomes) demonstrating the ability of the metabolite(s) of DEF to inhibit channel catfish brain AChE in the presence of a NADPH-generating system.

Piperonyl butoxide (PBO) is a methylenedioxyphenyl (MDP) compound that has routinely been used as a synergist in pesticide formulations; it exerts this effect through competitive and noncompetitive inhibition of cytochromes P450 by acting as an alternative substrate (Franklin, 1977; Hodgson and Philpot, 1974; Wilkinson et al., 1984). This

compound has also been demonstrated to be an inducer of P450s in fish (Erickson et al., 1988) and mice (Fennell et al., 1980; Skrinjaric-Spoljar et al., 1971).

In a recent study, Hur et al. (1992) demonstrated that PBO blocks the microsomal oxidative activation of DEF completely (in the presence of mouse liver microsomes plus NADPH) as an electric eel AChE inhibitor in vitro, but increased its rate of rat erythrocyte AChE inhibition in vivo. They suggested that sulfoxidation catalyzed by liver microsomal oxidases may be responsible for activation.

The objectives of this study were to determine if PBO affects the metabolism of DEF and influences the rates of inhibition of brain, liver, muscle, and plasma AChE following in vivo exposures; effects on liver and plasma ALiEs were also measured.

### Materials and Methods

#### Chemicals

Dr. Howard Chambers (Department of Entomology, Mississippi State University) provided the PBO. All other chemicals were obtained as in Chapter II.

#### Animals and Treatments

Channel catfish fingerlings, average wet weight 32 g (Appendix B) and of mixed sex, were obtained and held as in Chapter II; initial stocking rate was 10 fish/aquaria. There was one aquarium per treatment.

Fish were exposed to 1 mg/l PBO for 20 h followed by a 4 h exposure to 5 mg/l DEF in a static environment at 30°C; compounds for each treatment were suspended in 10 ml of reagent grade acetone. A negative control (containing 10 ml acetone), a DEF control, and a PBO control were included in the study. The system was then converted to flow-through conditions; flow rate was 60L/h. Fish were sacrificed at 0 or 12 h after the 24 h PBO exposure period.

### Tissue Samples

Tissue samples were obtained as in Chapter II. Gill samples were not assayed.

### Laboratory Analysis

AChE and ALiE assays were carried out in duplicate and quantified as in Chapter II; protein was also quantified as in Chapter II.

### Statistical Analysis

There were three replications per treatment. Specific activities were analyzed by the General Linear Model procedure (due to missing data) followed by the Student-Neuman-Keuls means comparison test using SAS on a personal computer. A level of  $p < 0.05$  was used to conclude a significant difference among means.

### Results

Table 4.1 displays mean, standard error, percent inhibition, and statistical mean comparison for each treatment of AChE specific activity in brain, liver, muscle, and plasma, and for ALiE specific activity in liver and plasma. Control specific activities were similar to previous in vivo experiments.

Brain AChE specific activity in the DEF only treatment was inhibited slightly but not significantly by 12 h after the exposure period; PBO prevented apparent DEF induced inhibition. Muscle AChE was not inhibited by exposure to the DEF treatment; specific activity in the DEF + PBO treatment at 0 h was significantly different from the 12 h specific activity.

Inhibition of fish liver AChE by treatment with DEF was 4.5-fold greater at 0 h than inhibition by the DEF + PBO treatment; specific activity of the DEF + PBO treatment was not significantly different from the control. Specific activities in the DEF treatment and the DEF + PBO treatment at 12 h were significantly different from the control;

the DEF treatment resulted in 1.3-fold more inhibition than the DEF + PBO treatment.

Inhibition of plasma AChE in the DEF treatment was 3.1- and 3.6-fold greater than inhibition by the DEF + PBO treatment at 0 and 12 h, respectively. Specific activity of the DEF treatment at 12 h was significantly different from the control.

AChE of brain, liver, muscle, and plasma in the PBO treatment appeared to be lower than controls at 12 h, but specific activities were not significantly different.

Liver ALiEs were inhibited to approximately the same extent by treatment with DEF or DEF + PBO at both time periods; this inhibition of specific activities were significant for both treatments at 0 h, but not at 12 h. PBO treatment yielded 1.7- and 2.5-fold higher specific activities than the controls at 0 and 12 h, respectively, and were significantly different from the controls.

Inhibition of plasma ALiE specific activities were similar in DEF or DEF + PBO treatments at both time periods and these inhibitions were significant. Plasma ALiE specific activity was significantly lower in the treatment with PBO at 0 h, but not at 12 h; in contrast, liver ALiEs displayed significantly higher specific activities as compared to the controls. Although there were no statistical differences, the 0 and 12 h specific activity means of control ALiEs in both tissues were quite different from one another.

#### Discussion

Fish did not display signs of stress during the 20 h exposure to PBO. Signs of stress were apparent by 1 h after exposure to DEF; piping at the surface was noted by 3 h.

The concentration of DEF selected was the same concentration used in the previous experiment of the present study (Chapter III); the concentration of PBO was used in accordance with studies by Melancon et al. (1977) and Erickson et al. (1988). The previous experiment of the

present study demonstrates the time course of inhibition and recovery (after a 4 h static exposure) of DEF through 384 h; therefore, only 0 and 12 h time periods were employed to demonstrate the effects of PBO on DEF in this experiment. Inhibition of AChE and ALiEs were similar for each time period in both studies.

Fish treated with DEF yielded poor cholinesterase inhibition in brain and muscle, which is similar to the results of previous in vivo assays. In the present study, exposure of fish to DEF led to appreciable AChE inhibition in liver and moderate inhibition in plasma; as reported in the previous chapter, close proximity to hepatic metabolic enzymes and therefore a reactive metabolite may be responsible for greater inhibition in these tissues. Additionally, the higher specific activities of AChE in brain and muscle implies more AChE molecules, so minor amounts of active metabolite would yield only a small proportion of inhibition.

AChE inhibitions yielded from DEF + PBO treatments suggest that DEF's effects are antagonized by PBO in brain (12 h), liver (0 and 12 h), and plasma (0 and 12 h), and the antagonism among tissues decreases with time. This antagonism (demonstrated by lower specific activity in the DEF + PBO treatment) was only significantly different from DEF treatments in liver (0 h) and plasma (12 h) but suggests the trend that the antagonistic effects of PBO may be time dependent. Since PBO exerts its effects by acting as an alternative substrate, oxidative metabolism may transform this compound to a non-inhibitory product over time; hence, PBO would be less of an antagonist to DEF as time increases.

Initial inhibition of hepatic monooxygenase (MO) activity followed by induction occurs over time in mammalian species following treatment with MDP compounds (Hodgson and Philpot, 1974; Skrinjaric-Spoljar et al., 1971). Hur et al. (1992) demonstrated that PBO enhanced the rate of DEF induced rat erythrocyte AChE inhibition during

a 24 h period and increased the extent of inhibition; rats were administered an intraperitoneal (IP) injection of PBO 1 h prior to IP treatment with DEF. In contrast, results of the present study indicate that PBO has antagonistic properties on DEF inhibition of AChE in channel catfish and suggests that the MO system of rats and fish metabolize these compounds differently or that there may be differences in time course of inhibition/induction.

Specific activities of brain AChE were higher in the DEF + PBO treatment than in the controls; although these specific activity differences were not statistically significant because of variability among replications, they may suggest possible induction of AChE by this treatment; however, this seems unlikely since AChE is not a MO. Erickson et al. (1988) observed induction of ECOD (ethoxycoumarin-O-deethylase) and EROD (ethoxyresorufin-O-deethylase) activity in rainbow trout by PBO during in vivo studies; in contrast, they demonstrated that PBO was an inhibitor of hepatic MO activity during in vitro studies.

While 12 h liver ALiE specific activities did not show a statistically significant difference between the control and DEF treatments, the general trend may be of biological importance and further experiments with more replications may confirm this.

Mean specific activities of liver ALiEs at both times in the PBO treatment were significantly higher than control values. Similar protein measurements of individual assays for all treatments of this tissue suggests that possible induction of ALiEs may have occurred. As mentioned above, previous studies have implicated PBO in inducing microsomal enzymes, and this may well have occurred here.

In summary, results suggest that PBO retards the formation of the metabolite(s) of DEF that inhibit brain, liver, and plasma AChE, and can significantly induce liver ALiEs of channel catfish. Future research should determine the monooxygenase responsible for activation

of DEF and investigate the induction of liver AChEs and their possible function in channel catfish fingerling acetylcholinesterase and its function after a 20 day exposure to 1 mg/L of dieldrin (DDT) followed by a 4 h exposure to 5 mg/L DEF

Group and Sample	Time (h)	Control	DEF	DDT	DEF + DDT
Group 1: Acetylcholinesterase	0	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1
	4	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1
Group 2: Acetylcholinesterase	0	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1
	4	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1	142.7 ± 1.1

The results of the present study indicate that the induction of liver AChE in channel catfish fingerling is significantly higher in the DEF treated group compared to the control group. This suggests that DEF is a potent inducer of AChE in channel catfish fingerling. The induction of AChE by DEF is likely due to the inhibition of AChE by DEF, which leads to an increase in the activity of AChE. The induction of AChE by DEF is also likely due to the inhibition of AChE by DEF, which leads to an increase in the activity of AChE.

Table 4.1. Least squares mean<sup>1</sup> specific activity and percent inhibition (in parentheses) of channel catfish fingerling acetylcholinesterase and aliesterases after a 20 h exposure to 1 mg/l piperonyl butoxide (PBO) followed by a 4 h exposure to 5 mg/l DEF.

Tissue and Enzyme	Time (h)	Treatment			
		Control	DEF	PBO	DEF + PBO
Brain Acetylcholinesterase	0	442.9 <sup>2,A,a</sup> 21.0 <sup>3</sup>	458.8 <sup>A,a</sup> 28.6 (0.0)	421.1 <sup>A,a</sup> 23.2 (4.9)	547.0 <sup>A,a</sup> 76.6 (0.0)
	12	432.3 <sup>A,a</sup> 34.5	337.8 <sup>A,a</sup> 17.4 (21.9)	370.2 <sup>A,a</sup> 4.4 (14.4)	473.5 <sup>A,a</sup> 42.9 (0.0)
Liver Acetylcholinesterase	0	33.1 <sup>A,a</sup> 3.0	2.6 <sup>C,a</sup> 0.1 (92.1)	42.4 <sup>B,a</sup> 3.7 (0.0)	26.4 <sup>A,a</sup> 2.7 (20.3)
	12	40.3 <sup>A,a</sup> 6.8	0.9 <sup>B,a</sup> 0.5 (97.8)	33.7 <sup>A,a</sup> 3.2 (16.4)	10.0 <sup>B,a</sup> 2.6 (75.2)
Muscle Acetylcholinesterase	0	103.1 <sup>A,a</sup> 0.8	132.0 <sup>A,a</sup> 8.9 (0.0)	125.9 <sup>A,a</sup> 29.8 (0.0)	110.2 <sup>A,a</sup> 6.0 (0.0)
	12	137.0 <sup>4,A,a</sup> 5.1	138.8 <sup>A,a</sup> 21.8 (0.0)	99.8 <sup>A,a</sup> 17.4 (27.1)	126.8 <sup>A,b</sup> 5.8 (7.5)
Plasma Acetylcholinesterase	0	17.5 <sup>A,a</sup> 1.2	9.5 <sup>A,a</sup> 1.4 (45.6)	16.6 <sup>A,a</sup> 1.0 (5.1)	14.9 <sup>A,a</sup> 3.5 (14.5)
	12	15.5 <sup>A,a</sup> 0.7	6.5 <sup>B,a</sup> 0.8 (57.9)	14.3 <sup>A,a</sup> 0.7 (7.7)	13.1 <sup>A,a</sup> 0.8 (15.9)
Liver Aliesterases	0	79.4 <sup>5,A,a</sup> 9.2	9.6 <sup>C,a</sup> 3.5 (91.0)	136.9 <sup>6,B,a</sup> 1.0 (0.0)	13.5 <sup>C,a</sup> 1.4 (87.2)
	12	51.8 <sup>A,a</sup> 8.8	5.9 <sup>A,a</sup> 3.2 (88.6)	130.8 <sup>B,a</sup> 20.4 (0.0)	10.3 <sup>A,a</sup> 5.1 (80.1)
Plasma Aliesterases	0	127.3 <sup>A,a</sup> 3.7	0.9 <sup>C,a</sup> 0.5 (99.3)	72.6 <sup>B,a</sup> 8.0 (42.9)	0.7 <sup>C,a</sup> 0.3 (99.4)
	12	84.4 <sup>A,b</sup> 11.9	0.4 <sup>B,a</sup> 0.4 (99.5)	67.3 <sup>A,a</sup> 2.9 (20.3)	1.1 <sup>B,a</sup> 0.6 (98.7)

<sup>1</sup>Means adjusted for varying sample sizes by the General Linear Model procedure.

<sup>2</sup>Specific activity expressed as  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ . Within each tissue/enzyme combination, means within a time not followed by the same capital letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test. Within each tissue/enzyme combination, means within a treatment not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the analysis of variance test.

<sup>3</sup>Standard error based on 3 replications.

<sup>4</sup> $n = 2$ ; arithmetic mean = 135.8.

<sup>5</sup> $n = 2$ ; arithmetic mean = 79.9.

<sup>6</sup> $n = 2$ ; arithmetic mean = 137.3.

## CHAPTER V

### EFFECTS OF AROCLOR 1254 ON HEPATIC DESULFURATION AND DEARYLATION OF CHLORPYRIFOS AND PARATHION IN FINGERLING CHANNEL CATFISH

#### Introduction

Monooxygenase (MO) systems of fishes are inducible by xenobiotics, as are mammalian systems, but on a more limited scale (Chambers and Yarbrough, 1976). Depending on the type of induced cytochrome, and the xenobiotic metabolized, induction may lead to either an increase in the production of activated metabolites and toxicity or increased detoxication and protection from toxicity.

Studies have shown that hepatic MO activities in fish can be increased greatly by exposure to polychlorinated biphenyls (PCBs) which are commonly present in the aquatic environment (Lidman et al., 1976; Addison et al., 1978; Elcombe and Lech, 1979). Levels as low as 0.2 mg/kg of the PCB mixture, Aroclor 1254, have been demonstrated to induce ECOD and EROD (7-ethoxycoumarin- and 7-ethoxyresorufin-O-deethylase) activities in hepatic microsomes (Melancon and Lech, 1983). Ankley et al. (1986) examined the effects of Aroclor 1254, on hepatic MO activity toward ECOD, EROD, and benzo[a]pyrene, as well as hepatic cytosolic glutathione S-transferase activity and hepatic microsomal UDP-glucuronosyltransferase activity; the increase of these activities suggested that Aroclor 1254 could alter patterns of xenobiotic metabolism and toxicity.

Several studies on cytochrome P450-mediated desulfuration and dearylation have been carried out on mammals (Chambers et al., 1994; Forsyth and Chambers, 1989; Levi et al., 1988); however, studies on

fish are limited to mosquitofish (Boone, 1991) and sunfish (Benke et al., 1974). Activation of OP insecticides in liver slices has been examined in brook trout, brown trout, black bullhead, winter flounder, and shorthorn sculpin (Potter and O'Brien, 1964; Murphy, 1966). Desulfuration and dearylation in channel catfish hepatic microsomes has not been reported.

The present study was designed to observe the effects of Aroclor 1254 on hepatic cytochrome P450-mediated desulfuration and dearylation of chlorpyrifos and parathion in fingerling channel catfish.

### Materials and Methods

#### Chemicals

Aroclor 1254 was obtained from Chem Service, West Chester, PA. Phosphorothionates were provided by Dr. Howard Chambers (Department of Entomology, Mississippi State University). All biochemicals and reagents were from Sigma Chemical Company (St. Louis, MO).

#### Animals and Treatments

Channel catfish fingerlings, average wet weight 58 g (Appendix B) and of mixed sex, were obtained and held at 30°C as in Chapter II; initial stocking rate was 26 fish/aquarium. There was one aquarium per treatment.

Fish were treated with 100 mg Aroclor 1254 in Sigma corn oil/kg body weight by intraperitoneal (IP) injection and sacrificed after 96 h. Untreated and vehicle controls were also included. Desulfuration, dearylation, and EROD activity assays were carried out on hepatic microsomes; chlorpyrifos and parathion were used as substrates for desulfuration and dearylation assays.

#### Tissue Samples

Fish were quickly decapitated and the liver was surgically removed and rinsed with 0.9% (w/v) sodium chloride solution; tissue samples

were quickly chilled to decrease enzyme activity. Due to the small size of the fish, 8 livers were combined for each replication.

Tissues were homogenized in 0.05 M Tris-HCl + 0.15 M KCl (pH 7.4) buffer, using a glass-Teflon Potter-Elvehjem tissue grinder driven by a Wheaton overhead mixer. Microsomal fractions were isolated by differential centrifugation. The microsomal fraction was a 110,000 x g, 60 min, pellet of a 17,000 x g, 15 min supernatant. Microsomes were covered in 0.05 M Tris-HCl + 0.15 M KCl (pH 7.4) buffer and stored at  $-70 \pm 2^{\circ}\text{C}$  until laboratory analysis could be performed.

#### Laboratory Analysis

Protein was quantified as in Chapter II.

#### Desulfuration Assay

The amount of desulfuration of the substrate was quantified by a modification of the method of Forsyth and Chambers (1989). Individual assays were carried out in duplicate.

Liver microsomal pellets were resuspended to 15% wet weight equivalent (0.15 g/ml) in 0.1 M Tris-HCl + 0.005 M MgCl<sub>2</sub> (pH 7.4) buffer. The microsomal suspension (100  $\mu\text{l}$ ; 15 mg wet weight equivalent) was incubated in the presence or absence of 50  $\mu\text{l}$  of a NADPH-generating system solution consisting of 0.01 M glucose-6-phosphate and 0.001 M NADP<sup>+</sup> dissolved in buffer, and 1 U glucose-6-phosphate dehydrogenase; final reaction volume was 250  $\mu\text{l}$ . Reaction mixtures not containing the NADPH-generating system were used as blanks. A 100  $\mu\text{l}$  aliquot of an exogenous source of AChE was used to trap the oxon as it was formed (supernate of 8.5 mg/ml channel catfish brain homogenized in 0.1 M Tris-HCl + 0.005 M MgCl<sub>2</sub>, (pH 7.4) and centrifuged at 17,500 x g for 1 min). Inhibition of AChE activity was accomplished by adding 50  $\mu\text{l}$  of  $10^{-3}$  M eserine sulfate ( $10^{-5}$  M final concentration) to one tube of each sample; this tube served as a blank for non-AChE-mediated hydrolysis.

Final substrate concentrations were achieved by addition of 10  $\mu$ l of chlorpyrifos or parathion in ethanol to give 10 and 50  $\mu$ M. Assays were incubated for 5 min (chlorpyrifos) or 15 min (parathion) at 30°C. Reactions were diluted with 1.75 ml of 1  $\mu$ M acetylthiocholine (ATCh) in 0.05 M Tris-HCl buffer (pH 7.4) to retard desulfuration of the compounds. The ATCh served as the substrate for the AChE. The assay was incubated 5 min at 30°C to allow time for the inhibition of the exogenous AChE by the oxon produced. Final volume was 2.0 ml. Reactions were terminated by the addition of 250  $\mu$ l of a 1:4 mixture of 0.95% 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), the chromogen (which reacts with free -SH), and 5% sodium dodecyl sulfate, a detergent used to denature the enzyme and to make the reaction mixture clear. Absorbance was measured at 412 nm.

Standard curves of inhibition of channel catfish brain AChE versus concentration of chlorpyrifos-oxon (0.03  $\mu$ M - 0.4  $\mu$ M) or paraoxon (0.1  $\mu$ M - 2.0  $\mu$ M) were established for microsomes of each replication. Results of each assay were compared to its respective curve to quantify the amount of oxon produced during desulfuration.

#### Dearylation Assay

The amount of dearylation was quantified by a modification of the method described by Boone (1991). Individual assays were carried out in duplicate.

Liver microsomal pellets were resuspended to 100% wet weight equivalent (1 g/ml) in 0.1 M Tris-HCl + 0.005 M MgCl<sub>2</sub> (pH 7.4) buffer. The microsomal suspension (100  $\mu$ l; 100 mg wet weight equivalent) was incubated in the presence or absence of 100  $\mu$ l of a NADPH-generating system solution consisting of 0.1 M glucose-6-phosphate and 0.01 M NADP<sup>+</sup> dissolved in buffer; 1.6 U of glucose-6-phosphate dehydrogenase was added to each tube containing the generating system. Final reaction volume was 1000  $\mu$ l for chlorpyrifos and 500  $\mu$ l for parathion. Reaction mixtures not containing the NADPH-generating system were used

as blanks. Mixtures were warmed for 2 min at 30°C and initiated with the addition of 5  $\mu$ l of chlorpyrifos or parathion in ethanol to give a final concentration of 50  $\mu$ M; reactions were incubated at 30°C for 15 min. Reactions were terminated by the addition of 50  $\mu$ l of 50% trichloroacetic acid (TCA) and chilled for 15 min to precipitate proteins.

For parathion, the reaction mixture was centrifuged for 2 min at 17,500 x g at room temperature to precipitate proteins. Tris base (300  $\mu$ l of 48%) was added to 400  $\mu$ l of the supernate. Absorbance was measured at 400 nm and 30°C; production of 4-nitrophenol was quantified using a standard curve ranging from 0.4 to 5  $\mu$ M. For chlorpyrifos, the reaction mixture was centrifuged for 2 min at 17,500 x g to precipitate proteins; absorbance was measured at 340 nm to detect 3,5,6-trichloro-2-pyridinol and compared to a standard curve ranging from 10 - 60  $\mu$ M.

#### EROD Activity

The amount of EROD activity was quantified by the procedure of Lake and Paine (1983), a modification of the method of Burke and Mayer (1974). Individual assays were carried out in duplicate.

Liver microsomal pellets were resuspended to 100% wet weight equivalent (1 g/ml) in 0.1 M Tris-HCl + 0.005 M MgCl<sub>2</sub> (pH 7.4) buffer. The microsomal suspension (100  $\mu$ l; 100 mg wet weight equivalent) was warmed at 30°C for 5 min in the presence of: 200  $\mu$ l of a NADPH-generating system solution consisting of 0.01 M glucose-6-phosphate and 0.001 M NADP<sup>+</sup> dissolved in buffer, 10 U of glucose-6-phosphate dehydrogenase, 100 mM MgSO<sub>4</sub>, and 0.05 M Tris-HCl. The reaction was initiated by the addition of 10 nM of 7-ethoxyresorufin (substrate) and incubated at 30°C for 5 min; final volume was 1 ml. Reaction mixtures not containing the substrate were used as blanks. The reaction was terminated by the addition of 500  $\mu$ l of 5% ZnSO<sub>4</sub> and 500  $\mu$ l of saturated Ba(OH)<sub>2</sub>, and centrifuged for 15 min at 17,500 x g and 0-4°C to precipitate proteins. Resorufin content was determined by mixing 1

ml supernate with 2 ml of 0.5 M glycine-NaOH buffer (pH 8.5) and measuring the fluorescence at 582 nm with excitation at 535 nm in a Perkin-Elmer LS 50B luminescence spectrometer.

### Statistical Analysis

There were <sup>3</sup> replications per treatment, unless otherwise noted. Specific activities were analyzed by the General Linear Model procedure followed by the Student-Neuman-Keuls test using SAS on a personal computer. A level of  $p < 0.05$  was used to conclude a significant difference among means.

### Results

Table 5.1 displays the mean, standard error, and statistical mean comparison for desulfuration and dearylation of chlorpyrifos and parathion; mean, standard error, and statistical mean separation for the EROD assay are also included. No statistically significant differences were observed between vehicle and untreated controls of each parameter.

There was no statistical significance between hepatic microsomal desulfuration activities of the control sample for each substrate and the sample from Aroclor 1254-treated fish. No statistically significant differences were evident between chlorpyrifos or parathion desulfuration activities at 10  $\mu\text{M}$  final concentration. At 50  $\mu\text{M}$  final concentration, chlorpyrifos showed significantly higher desulfuration activities than parathion. Chlorpyrifos desulfuration activities at 50  $\mu\text{M}$  substrate concentrations were significantly different from 10  $\mu\text{M}$  substrate concentrations for each treatment; parathion activities at 50  $\mu\text{M}$  in the control and Aroclor 1254 treatments were significantly different from the other parathion desulfuration activities.

Channel catfish hepatic microsomes were found to dearylate chlorpyrifos to a greater extent than parathion at the same substrate concentration (50  $\mu\text{M}$ ). There were no statistically significant

differences in activities between the control microsomes for each substrate and the microsomes from Aroclor 1254-treated fish.

EROD activities yielded statistically significant induction between the control microsomes (naive and vehicle) and the microsomes from Aroclor 1254 treated fish.

### Discussion

In a recent review of the cytochrome P450 system in fish, Goksoyr and Forlin (1992) identified CYP1A1 as the only gene yet to be described (Heilmann et al., 1988); they reported that 154 mammalian P450 genes have been sequenced. P450 protein purification and characterization has progressed faster than gene characterization, and the predominate isozyme in fish is 1A1. Organic xenobiotics such as petroleum hydrocarbons, PCBs, chlorinated dioxins, and chlorinated dibenzofurans induce this isozyme, and the prototypical activities to quantify this induction include EROD, ECOD, and AHH (aryl hydrocarbon hydroxylase) (Goksoyr and Forlin, 1992).

Treatment dose and time-course of inhibition of Aroclor 1254 were selected according to a study on channel catfish by Ankley et al. (1986) which optimized these parameters. The EROD assay was used as a positive control to verify induction of cytochrome P450 in the present study.

Desulfuration was similar for both phosphorothionates at 10  $\mu\text{M}$  final concentration, but at 50  $\mu\text{M}$ , desulfuration of chlorpyrifos was approximately 2-fold greater than parathion. While this indicates that chlorpyrifos may be more susceptible to desulfuration at higher concentrations, the high toxicity of chlorpyrifos might prevent this concentration from being achieved in vivo; 50  $\mu\text{M}$  chlorpyrifos is equal to 17.5 mg/l and this would be lethal to channel catfish. The dearylation activity of chlorpyrifos was approximately 60-fold greater than that of parathion at 50  $\mu\text{M}$  final concentration according to the control specific activities for each substrate, implying a far greater

detoxication potential for chlorpyrifos than for parathion. For chlorpyrifos, dearylation activity was 15-fold greater than desulfuration activity at 50  $\mu\text{M}$  final concentration; desulfuration activity of parathion at 50  $\mu\text{M}$  final concentration was 2.5-fold greater than dearylation activity. These results do not correlate with the acute toxicities of the two compounds.

Boone (1991) demonstrated that desulfuration and dearylation of chlorpyrifos was approximately 2-fold greater than parathion using 50  $\mu\text{M}$  final concentrations of each substrate in mosquitofish (Gambusia affinis) hepatic microsomes; no inducers were used in his study. Comparison of his work with the controls of the present study demonstrates that mosquitofish desulfuration was greater (6- and 4-fold, respectively) for chlorpyrifos and parathion, than channel catfish; dearylation of chlorpyrifos was greater (4-fold) in channel catfish than in mosquitofish, while dearylation of parathion was greater (7-fold) in mosquitofish than in catfish. The great disparity in dearylation activities of the two compounds in the present study does not help explain the acute toxicity values of chlorpyrifos and parathion to channel catfish.

Desulfuration and dearylation activities of chlorpyrifos or parathion (50  $\mu\text{M}$ ) in rats was higher than found here in channel catfish (Chambers et al., 1994). Assay incubation temperatures for the rat and the channel catfish were 37°C and 30°C, respectively. The higher rates of desulfuration and dearylation in the rat may be due in part to the adaptation to a higher physiological operating temperature.

In the present study, hepatic microsomal MO activity toward 7-ethoxyresorufin was induced while microsomal protein was not; induction of desulfuration and dearylation of chlorpyrifos and parathion were not evident. These results indicate that treatment with 100 mg Aroclor 1254/kg does not induce the isozymes of the cytochrome P450's most

responsible for desulfuration and dearylation, and that the isozymes responsible for these activities are not primarily CYP1A1.

The control EROD specific activity from the study of Ankley et al. (1986) was  $6.7 \text{ pmol resorufin} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ; a study by Ankley and Blazer (1988) showed that EROD specific activities varied from 10.6-19.5 according to diet. Another study by Ankley et al. (1989) reported EROD specific activities four-fold higher than previous studies in their lab and indicated that this was probably due to use of a new batch, with possible higher purity, of resorufin standard for calibration of activity. Other factors such as channel catfish strain differences or laboratory technique may affect results of the EROD assay.

Other studies have exposed channel catfish to Aroclor 1254 in the water column (Hill et al., 1976) and orally (Klaunig et al., 1979); they noted significant increases in hepatic aminopyrine demethylase (APDM) activity. The studies of Ankley et al. (1986) and Lipsky et al. (1978) showed that IP injections of Aroclor 1254 did not affect ADPM activity. Ankley et al. (1986) suggests that the bioavailability of Aroclor 1254 may be influenced by different routes of exposure.

Lipsky et al. (1978) reported that treatment of channel catfish with 350 mg Aroclor 1254/kg body weight produced significant increases in hepatic microsomal cytochrome b5 concentration and NADPH-cytochrome c reductase activity; Ankley et al. (1986) saw no evidence of this at 100 mg Aroclor 1254/kg, and suggested that higher levels are required to increase these enzymes. A higher dose may also be necessary to induce dearylation and desulfuration in the present study.

Ankley et al. (1986) also reported that they saw little if any increase in the total concentration of cytochrome P450, which indicated that the levels of cytochrome P450 induced in channel catfish by Aroclor 1254 may not be sufficient to alter the levels of the total cytochrome P450 pool.

Current methods for monitoring the dearylation of phosphorothionates are inadequate for fish (due to low activities) and must be modified to give more accurate results. In vitro monitoring of dearylation is accomplished by a colorimetric biochemical assay; using channel catfish hepatic microsomes, the very low absorbance readings yielded great variability among replications.

Future studies should evaluate known cytochrome P450 inducers and inhibitors as potential inducers or inhibitors of phosphorothionate desulfuration or dearylation activities; such evaluations could possibly lead us to a better understanding of OP toxicity.

Table 5.1. Mean specific activity of channel catfish fingerling hepatic microsomal dearylation, desulfuration, and 7-ethoxyresorufin-O-deethylase (EROD) after a 96 hour IP injection of 100 mg/kg Aroclor 1254 *in vivo*.

Parameter	Insecticide	Substrate Concentration ( $\mu\text{M}$ )	Treatment		
			Control	Vehicle Control <sup>1</sup>	Aroclor 1254
Desulfuration	Chlorpyrifos	10	44.9 <sup>2,A,a</sup> 17.7 <sup>3</sup>	46.6 <sup>A,a</sup> 7.9	53.5 <sup>A,a</sup> 13.0
			Chlorpyrifos	50	112.7 <sup>A,c</sup> 16.3
	Parathion	10			49.1 <sup>A,a</sup> 8.1
			Parathion	50	69.4 <sup>A,ab</sup> 8.3
Dearylation	Chlorpyrifos	50			1684.2 <sup>4,A</sup> 377.2
			Parathion	50	28.1 <sup>A</sup> 12.4
EROD	-----	--			65.8 <sup>6,A</sup> 9.1

<sup>1</sup>Dosage = 2 ml/kg Sigma corn oil.

<sup>2</sup>Specific activity expressed as pmol oxon produced  $\cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ; means within a row not followed by the same capital letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test. Desulfuration means not followed by the same lower case letter are significantly different ( $p \leq 0.05$ ) by the Student-Neuman-Keuls test.

<sup>3</sup>Standard error based on 3 replications, unless indicated.

<sup>4</sup>Specific activity expressed as pmol product formed  $\cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ; product formed: chlorpyrifos = 3,5,6-trichloro-2-pyridinol; parathion = p-nitrophenol.

<sup>5</sup>n = 2.

<sup>6</sup>Specific activity expressed as pmol resorufin formed  $\cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ .

## CHAPTER VI

### CONCLUSION

Control specific activities of individual tissues were similar in each experiment of the present study. Random variation between replications could be due to experimental error, but a more likely cause would be attributed to individual animal differences since fish do not demonstrate the inbreeding associated with pure strains of laboratory animals such as rats and mice.

Differences in acute toxicity of one species to various pesticides does not always correlate to the inhibition found in vitro. Such a poor relationship can be explained by the observation that the toxicity of compounds can be controlled by different rate-limiting reactions and processes such as uptake kinetics, internal distribution, rates of biotransformation into active or detoxified metabolites, or the probability that the active metabolite will reach the target site at a concentration that is sufficient to exert toxic effects.

Acute toxicity of chlorpyrifos and parathion to channel catfish is reflected by  $I_{50}$  values for their respective oxons; the acute toxicity of DEF suggests that the mode of action is yet to be determined for this compound. In all cases, the compounds (i.e., the two oxons and DEF) were more potent ALiEs inhibitors than AChE inhibitors.

The 384 h in vivo study demonstrated the persistence of chlorpyrifos and the rate of enzyme recovery after exposure to chlorpyrifos, DEF, and parathion. Persistence of chlorpyrifos presumably results from its lipophilic nature (i.e., sequestering of the parent compound); enzyme recovery is reported to be from de novo synthesis. DEF appeared to be bioactivated to inhibit AChE rapidly in

gill, liver, and plasma; inhibition in brain and muscle was delayed. Multiple xenobiotic exposure is conceivable to fish in culture environments and these experiments demonstrated that some interaction between chemicals took place. Greater AChE inhibition in combination treatments (i.e. chlorpyrifos + DEF or parathion + DEF) was not evident suggesting that ALiEs do not serve to protect AChE, even though ALiEs are inherently more sensitive to inhibition. Further research should evaluate the role of ALiEs in fish. Inhibition and recovery of AChE and ALiEs after water column exposure to the parent compounds provides insight into the extent of damage that can be caused by over-spray. The enzyme recovery period is useful in determining how long channel catfish remain more susceptible to future exposures.

The PBO-DEF study demonstrated the antagonistic effect of PBO on DEF induced AChE inhibition in brain, liver, and plasma of channel catfish as opposed to the enhanced effect that PBO exhibited on DEF induced inhibition of rat erythrocyte AChE (Hur et al., 1992). Future research should determine the monooxygenase responsible for activation of DEF and investigate the induction of liver ALiEs.

Results of the hepatic desulfuration and dearylation study demonstrated that cytochrome P450-mediated activation and detoxication were not significantly induced by treatment of channel catfish with 100 mg Aroclor 1254/kg body wt. The rate of dearylation was much higher for chlorpyrifos than for parathion, which does not correspond to the acute toxicity of the compounds.

Future studies should be designed to observe the in vivo effects of organophosphorus compounds on cytochrome P450-mediated induction. Further research should be done to identify the mode of action of DEF and to clarify the differential tissue effects of AChE inhibition.

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APPENDIX A  
IN VIVO EFFECTS OF CHLORPYRIFOS, DEF, AND PARATHION  
TO FINGERLING CHANNEL CATFISH

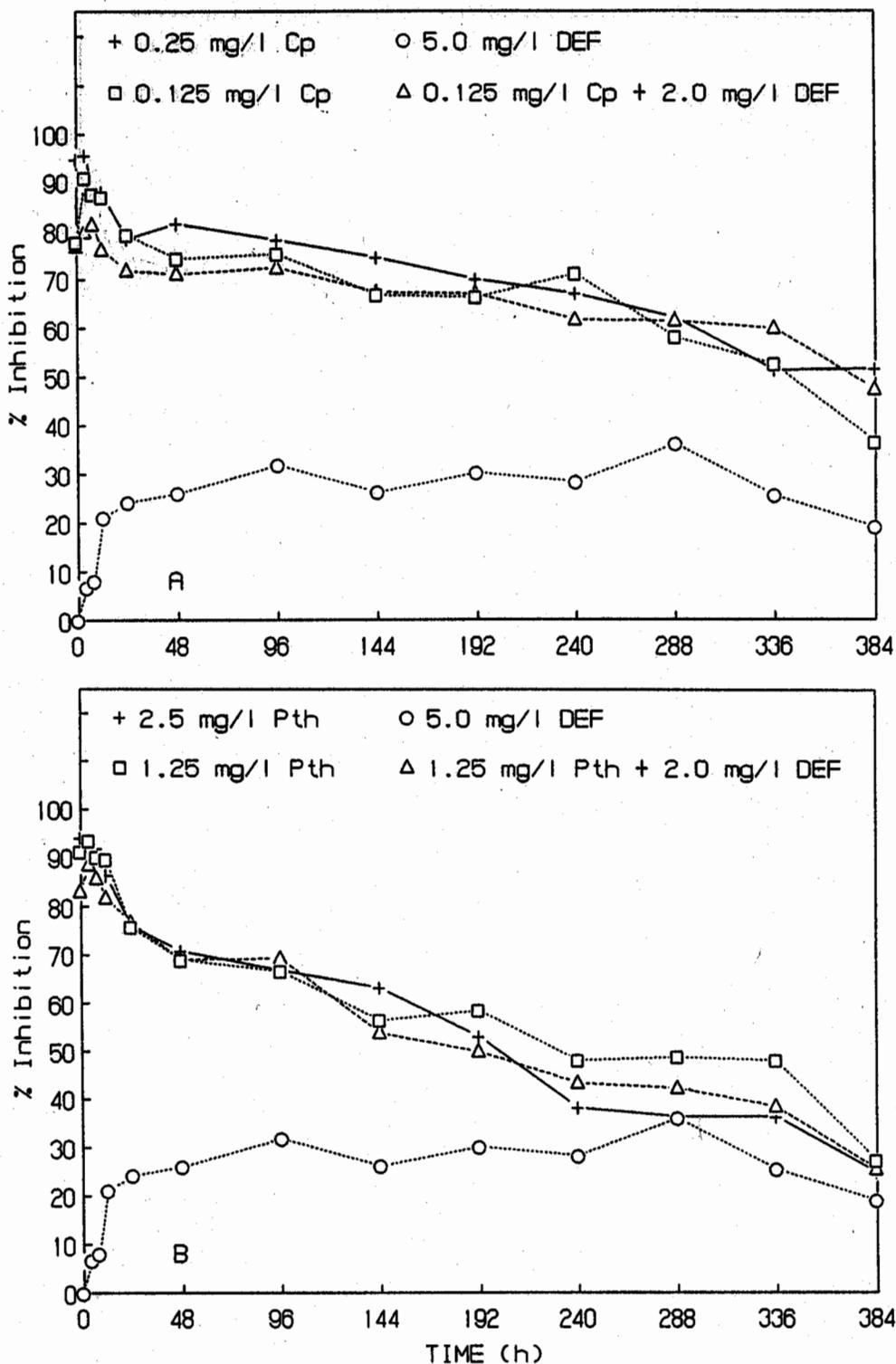


Figure A.1. Brain AChE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

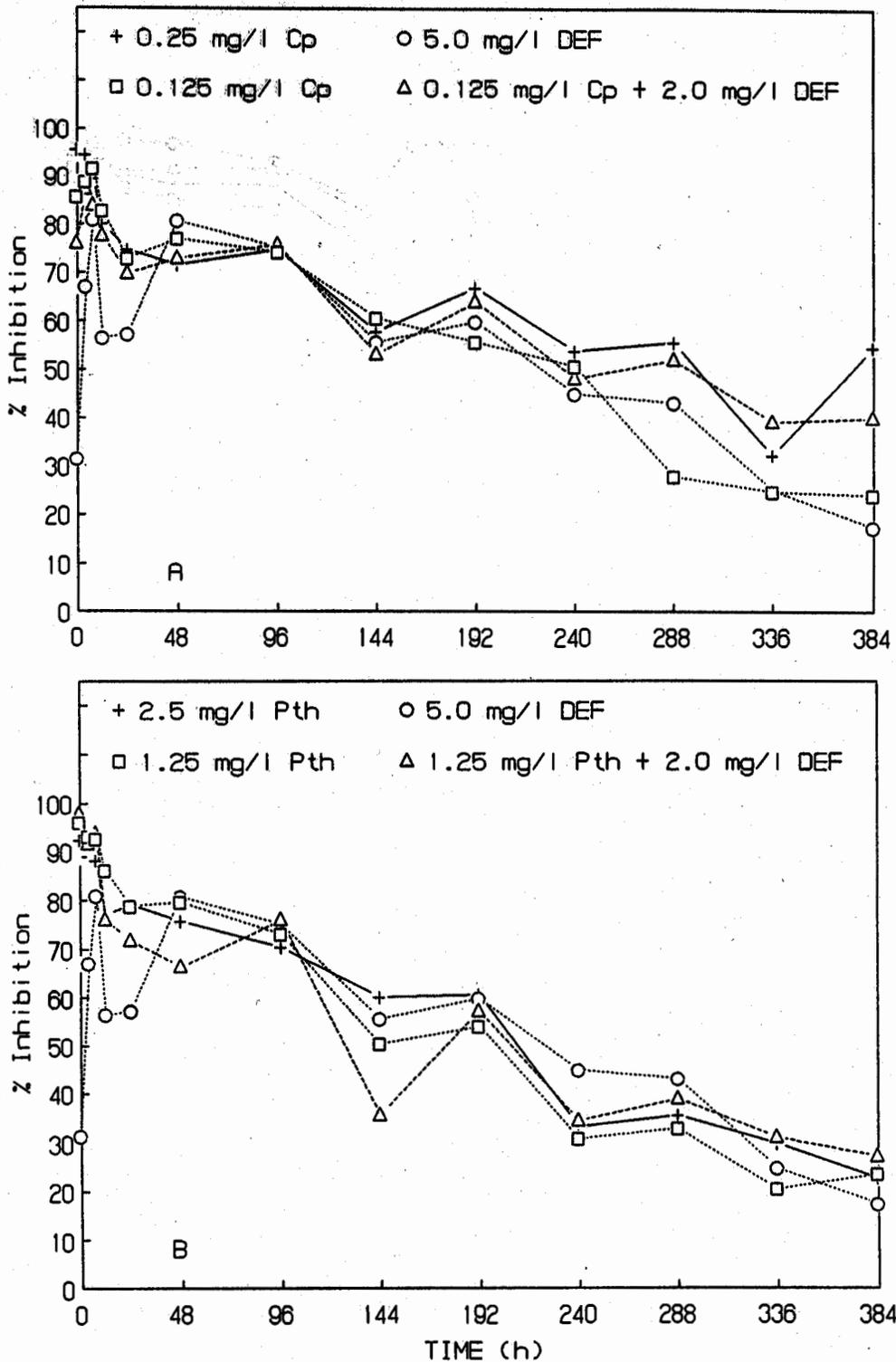


Figure A.2. Gill AChE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

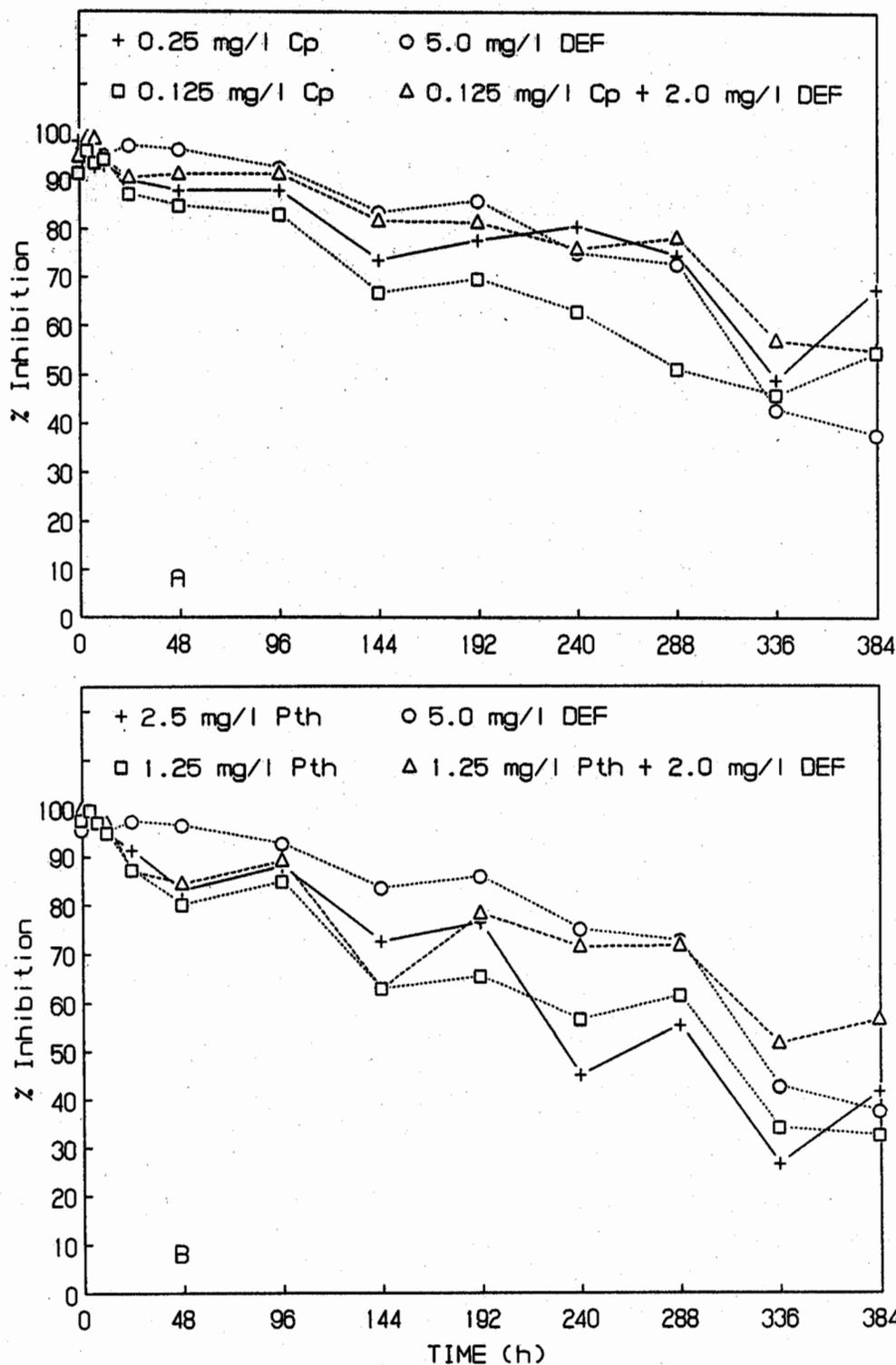


Figure A.3. Liver AChE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated, in A and B. Cp = chlorpyrifos; Pth = parathion.

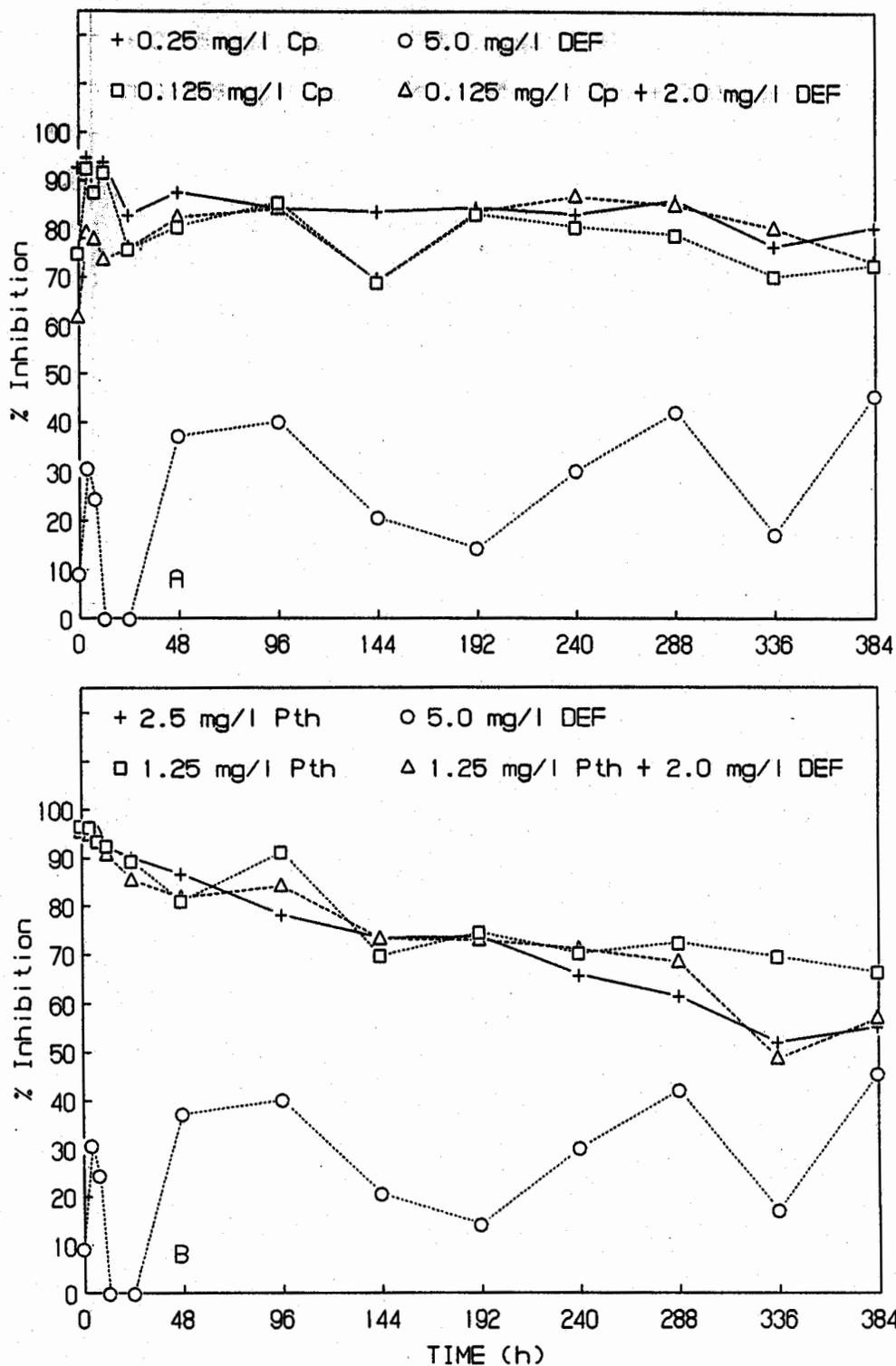


Figure A.4. Muscle AChE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

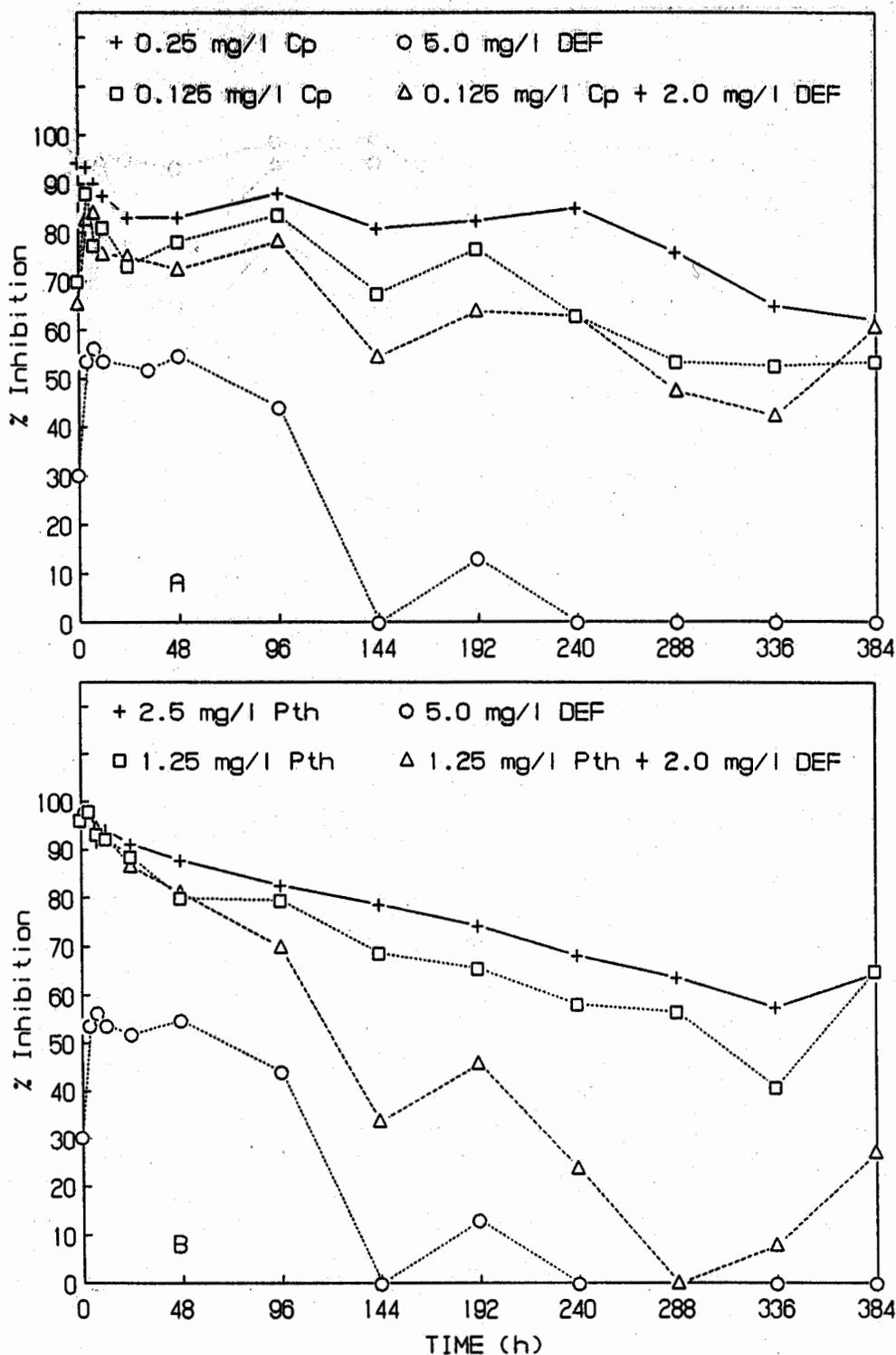


Figure A.5. Plasma AChE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

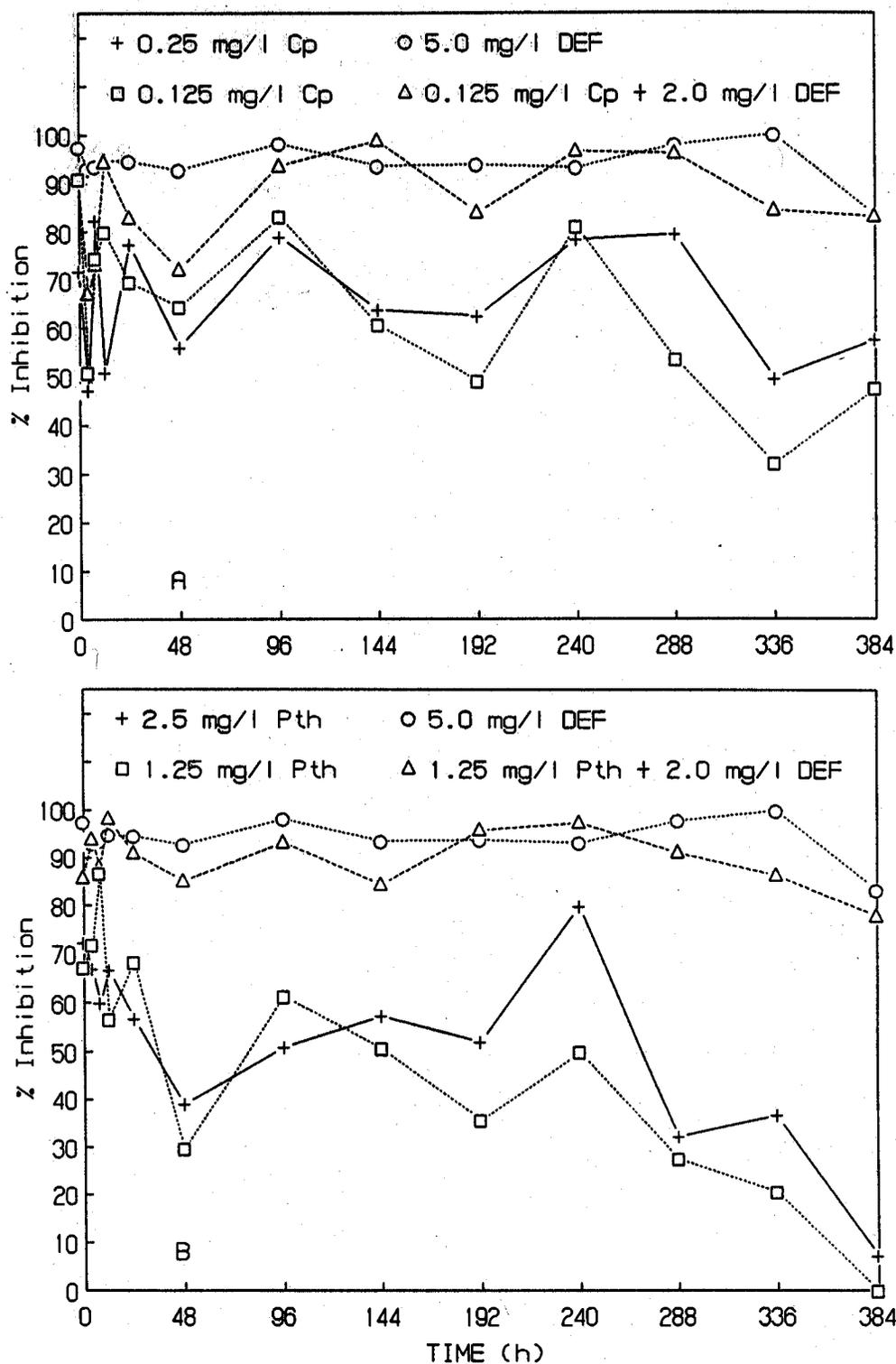


Figure A.6. Gill ALiE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

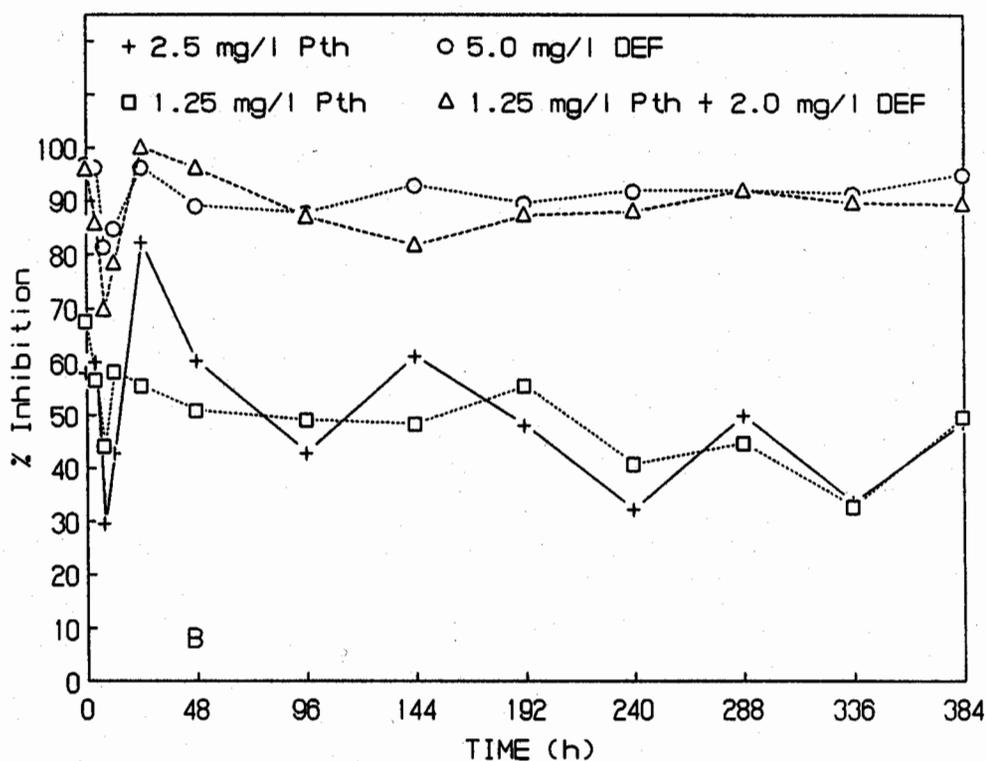
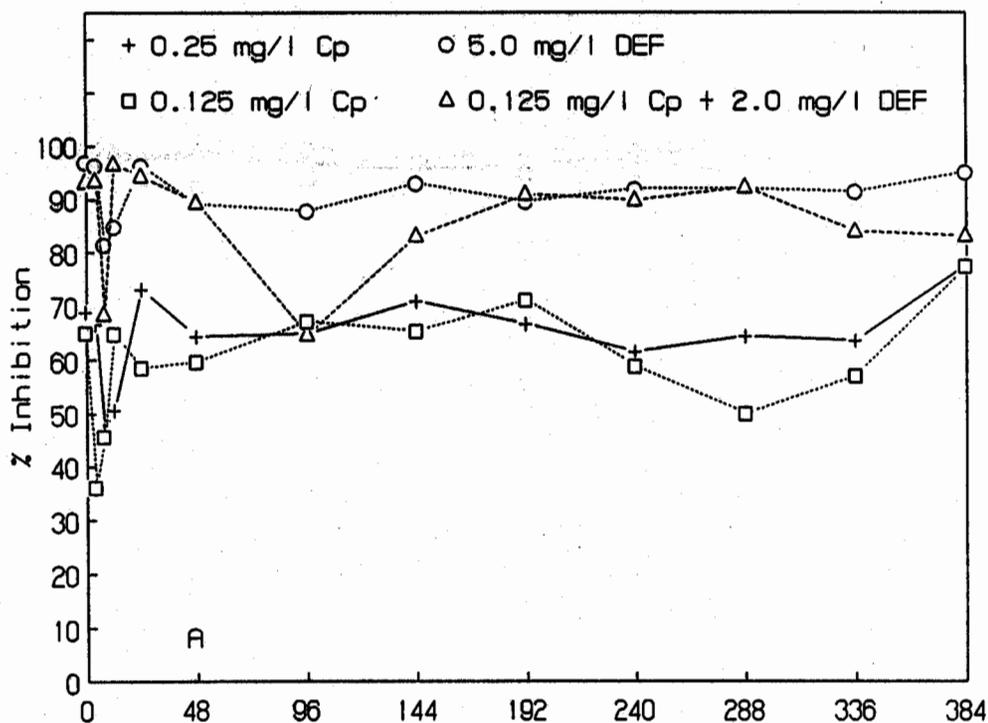


Figure A.7. Liver ALiE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

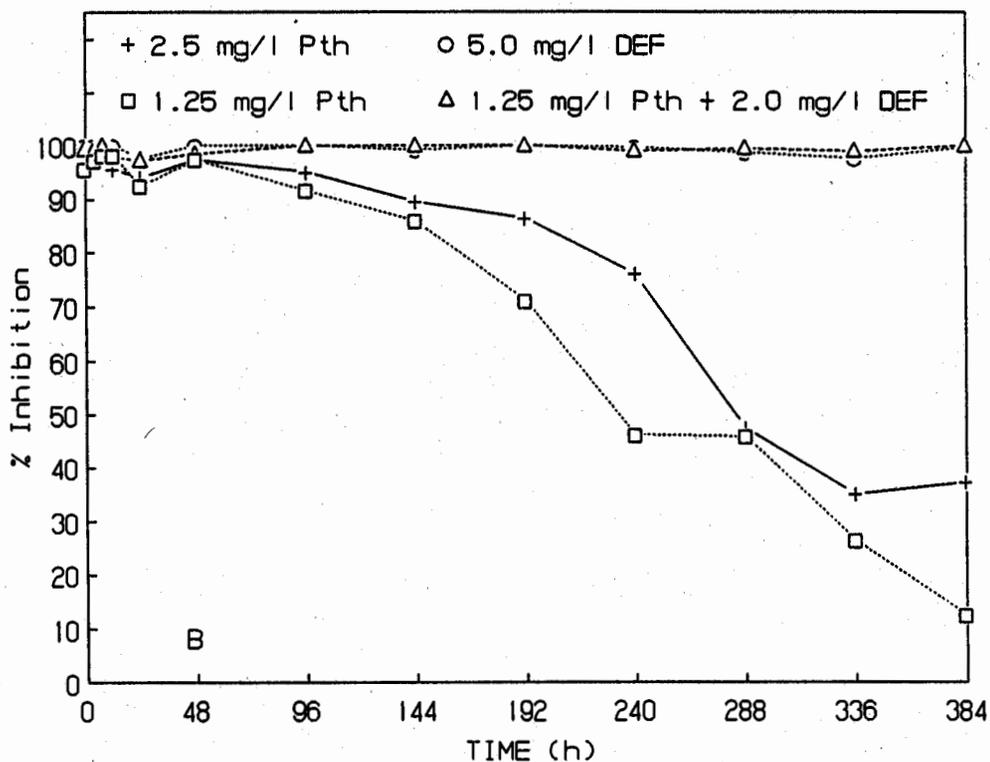
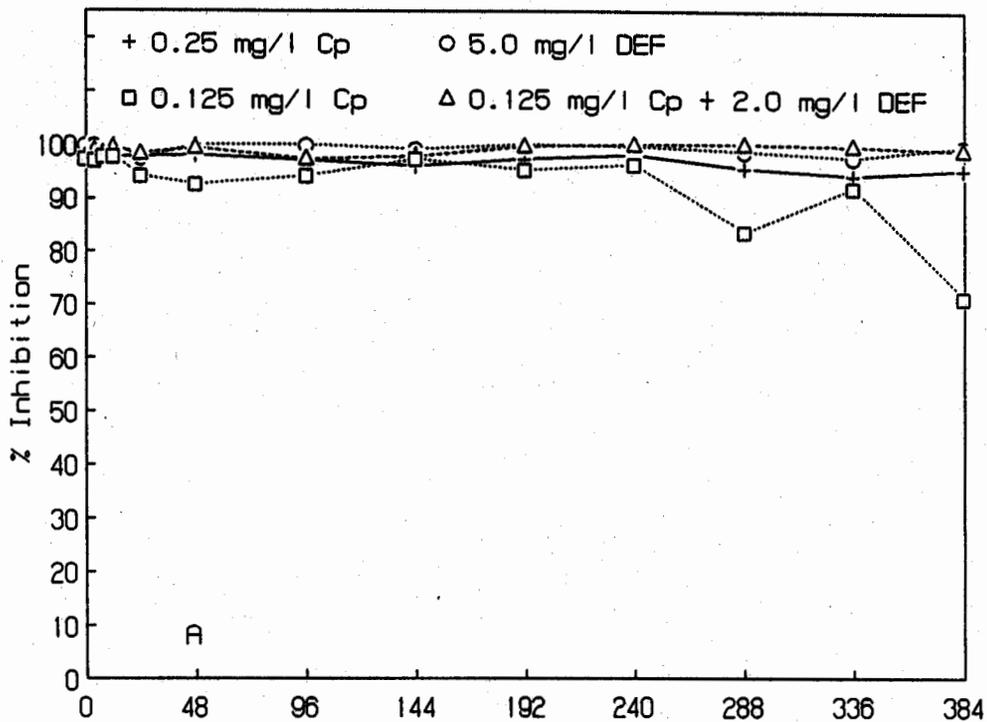


Figure A.8. Plasma ALiE inhibition in fingerling channel catfish after a 4 h static exposure to chlorpyrifos (A) and parathion (B) alone and in combination with DEF; flow-through conditions were initiated at 0 h. The DEF treatment is duplicated in A and B. Cp = chlorpyrifos; Pth = parathion.

Table 3.1. Wet weight of fingerling rainbow trout for each of the treatments in the experiment.

[Faint table content, likely containing experimental data on fish weights]

**APPENDIX B**

**INDIVIDUAL FISH WET WEIGHTS FOR EACH EXPERIMENT**

[Faint table content, likely containing individual fish wet weights for each experiment]

Table B.1. Wet weight of fingerling channel catfish for in vitro study of the effects of organophosphate treatment on enzyme activity.

Replication	Weight (g) <sup>a</sup>
1	34.03
2	27.64
3	32.50

<sup>a</sup>Mean = 31.4, standard error = 1.9

Table B.2. Mean wet weight of channel catfish fingerlings for each replication of the enzyme inhibition and recovery study.

Replication	Weight (g)
1	30.48 1.0 <sup>a</sup>
2	41.38 2.6
3	44.98 1.2

<sup>a</sup>Standard error based on 20 fish.

Table B.3. Mean wet weight<sup>a</sup> of fingerling channel catfish used to study the effect of piperonyl butoxide (PBO) on the metabolism of DEF in fingerling channel catfish.

	Treatment		
	Control	DEF	PBO DEF + PBO
	30.68	32.21	33.91 32.04
	2.83 <sup>b</sup>	2.35	1.53 1.77

<sup>a</sup>Overall mean = 32.2 g.

<sup>b</sup>Standard error based on 8 fish.

Table B.4. Mean wet weight<sup>a</sup> of fingerling channel catfish in the Aroclor 1254 study on microsomal activity effects.

	Treatment	
	Control	Aroclor 1254 Corn Oil
	55.40	48.41 48.48
	1.60 <sup>a</sup>	2.05 2.23

<sup>a</sup>Overall mean = 50.76 g.

<sup>b</sup>Standard error based on 26 fish.