

JOINT ACUTE TOXICITY OF ESFENVALERATE AND DIAZINON TO LARVAL FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)DEBRA L. DENTON,*† CRAIG E. WHEELOCK,‡ SHAUNA A. MURRAY,§ LINDA A. DEANOVIC,§
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Abstract—California (USA) agriculture employs pyrethroid and organophosphate insecticides to control insects in orchards and other crops. Diazinon and esfenvalerate were selected for this study because of their application overlaps. Toxicological and biochemical responses of larval fathead minnows (*Pimephales promelas*) exposed singly and in combinations to esfenvalerate and diazinon were determined. Exposures were 96-h static renewal tests that used standard U.S. Environmental Protection Agency acute toxicity test methods. After pesticide exposures, larvae were evaluated for carboxylesterase and acetylcholinesterase activity, and histopathological effects. Carboxylesterase activity was examined because of its potential influence on the toxicity of both organophosphates and pyrethroids. In vivo studies demonstrated that diazinon significantly inhibited carboxylesterase activity at nominal water concentrations as low as 50 µg/L. However, esfenvalerate did not affect carboxylesterase activity at any concentration tested. Liver glycogen depletion was the only histopathological effect observed; this effect was demonstrated with the individual pesticides and pesticide combinations (i.e., mixtures). The combinations of diazinon and esfenvalerate causing acute toxicity to fathead minnow larvae appeared to be greater than additive (i.e., synergistic) in all three tests.

Keywords—Diazinon Esfenvalerate Pesticide interactions Synergistic effect Carboxylesterase activity

INTRODUCTION

Central Valley Regional Water Control Board (Regional Water Board) staff have detected pesticides including organophosphates in storm and irrigation runoff from various crops into the San Joaquin River and Sacramento River (CA, USA) basins from the mid-1980s to the present [1–3]. Since 1986, the Regional Water Board has evaluated ambient water (i.e., receiving waters) toxicity using the U.S. Environmental Protection Agency (U.S. EPA) [4,5] three-species test methods (an alga, *Selenastrum*; an invertebrate, *Ceriodaphnia dubia*; and a larval fish, the fathead minnow [*Pimephales promelas*]). These tests have been performed in conjunction with toxicity identification evaluations to identify pesticides (e.g., diazinon, chlorpyrifos, carbofuran, and methyl parathion) that impair water quality.

This study examines the toxicological interaction of two pesticides (diazinon and esfenvalerate) applied to control overwintering pests such as peach twig borer (*Anarsia lineatella*), San Jose scale (*Quadraspidiotus perniciosus*), and aphids on stone fruit (peach, prune, nectarine, and plum) and almond orchards. Since the 1970s, organophosphate insecticides such as diazinon, naled, methidathion, and chlorpyrifos have been mixed with oils and applied during the dormant season for stone fruit and almonds throughout the Sacramento River and upper San Joaquin River basins. Dormant spray application has the following advantages over in-season application: less

exposure to field workers, no exposure to fruit (no residues), and fewer in-season applications (i.e., less pesticide usage and less disruption of beneficial insects [6]). However, dormant spray applications coincide with California's winter storm events, which allows pesticide movement from the application sites with runoff to aquatic environments [7–10]. California currently is developing diazinon and chlorpyrifos total maximum daily loads (TMDLs). The TMDLs will require inclusion of an implementation plan and may include discussion of alternative pesticides (e.g., pyrethroids and carbamates), environmentally lower-risk alternatives (e.g., *Bacillus thuringiensis*), or the development of best management practices to minimize off-site movement of pesticides. Epstein et al. [6] reviewed California Pesticide Use Reports and concluded that the percentage of the Sacramento–San Joaquin basin treated by organophosphates on stone fruit and almonds decreased from 1992 to 1998, as the applications of pyrethroids increased. With this increase in pyrethroid application levels, biota in aquatic systems may be exposed to mixtures of pyrethroid and organophosphate insecticides. Additionally, study of the interactions of pyrethroid and organophosphate insecticides is important because these two types of pesticides have different modes of toxic action. That is, multiple chemicals co-occur in aquatic systems at concentrations that may alter toxicity because of additive, antagonistic, or greater-than-additive (synergistic) effects.

The use of synergists is not new to the pesticide industry because synergists have been used deliberately to enhance the effectiveness of some pesticides [11]. For example, piperonyl butoxide has long been used in pyrethroid tank mixtures to synergize pyrethroid toxicity by inhibiting monooxygenases and subsequently slowing pyrethroid metabolism and detoxi-

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fication [12]. However, synergistic interactions in the aquatic environment that can affect nontarget organisms are undesirable. To understand the potential for synergistic effects between chemicals, it is necessary to understand the toxic modes of action, sites of action, enzymes and rates necessary for both detoxification and activation, rates of uptake, and the sensitivity of enzymes to the chemical(s).

The pyrethroid insecticide esfenvalerate interferes with nervous system function by blocking the sodium and calcium channels in nerve membranes, thereby altering impulse conduction along nerve axons [13,14]. Initial symptoms of pyrethroid poisoning include decreased coordination and locomotor instability followed by hyperexcitation, tremors, and convulsions [15]. Fish exposed to sublethal levels of fenvalerate (a mixture of isomers) and esfenvalerate (the more toxic *S,S*-isomer) show behavioral changes such as rapid gill movement, erratic swimming, altered schooling activity, and swimming at the water surface [16].

Diazinon is an organophosphorus insecticide that also affects the nervous system. However, the mode of action of diazinon is different from that of pyrethroids. Larkin and Tjeerdema [17] provided an extensive discussion on the toxicology of diazinon to mammalian and aquatic organisms. Organophosphorus insecticides exert toxicity by inhibiting the neuronal enzyme acetylcholinesterase required for the metabolism of the neurotransmitter acetylcholine. An aquatic organism's sensitivity to diazinon will be determined by a combination of either sensitive acetylcholinesterase, a high rate of metabolic activation of diazinon to the toxic form (diazoxon; P450s are the metabolic activating enzymes for organophosphorus insecticides), a low activity of detoxifying enzymes such as carboxylesterases, or a combination of these. Diazinon-impaired fish exhibit muscular twitching, gyrating movements, and impaired optomotor behavior [18,19].

This study examined the acute toxicity of the two pesticides singly and in combinations to a standard freshwater fish model, the larval fathead minnow. After fish were exposed to the pesticides, we assessed histopathological condition and analyzed carboxylesterase and acetylcholinesterase activity to investigate the possible mechanisms responsible for the joint action of the two pesticides. Evaluation of the effects of possible chemical interactions that may occur in the aquatic environment and of the possible pesticide alternatives (i.e., pyrethroids) used to replace and supplement the usage of diazinon (and other organophosphates) is important. The results of this study should be considered in the development of diazinon TMDLs, in particular the elements of the numeric target selection and the margin of safety.

MATERIALS AND METHODS

Toxicity tests

The pesticide exposure treatments were conducted as 96-h daily static renewal tests according to standard procedures [20]. Seven-day-old fathead minnows were purchased from a certified test organism supplier (Aquatox, Hot Springs, AR, USA) for each test. Each pesticide exposure used 10 larvae per test chamber (i.e., 500-ml glass beakers), with three replicates for each of the five treatments. A control with methanol (i.e., pesticide-carrier solvent) used dilution water composed of synthetic moderately hard water. The methanol concentrations were always less than 0.5 mL/L. The fish were fed 200 to 500 *Artemia* nauplii per replicate, 2 h before the start of the test and at the time of water renewal. Water quality mea-

surements (temperature, pH, dissolved oxygen, and conductivity) and enumeration of larvae (alive or dead) were taken daily. All tests were conducted at 20°C. The pesticide exposures consisted of three trials of individual pesticide exposures of diazinon and esfenvalerate (singly) and concurrent equitoxic combinations of diazinon and esfenvalerate. Pesticide concentrations were chosen by using our laboratory's data and U.S. EPA Aquatic Toxicity Information Retrieval (Aquire) database search. The targeted esfenvalerate 96-h median lethal concentration (LC50) to 7-d-old fathead minnow larvae is 0.20 µg/L, and the targeted diazinon 96-h LC50 to 7-d-old fathead minnow larvae is 8,000 µg/L. The five nominal esfenvalerate concentrations we tested ranged from 0.10 to 0.30 µg/L. The nominal diazinon test concentrations tested ranged from 2,000 to 12,000 µg/L. Equitoxic concentrations of the two pesticides were prepared for testing by using 5, 10, 25, 50, and 100% of each pesticide's LC50.

Chemical analyses

Diazinon (99.4% pure), esfenvalerate (98.0% pure), and diazinon-*O*-analog (diazoxon; 90.5% pure) were obtained from Chem Service (West Chester, PA, USA). Analytical measurements were tested at three nominal pesticide concentrations. Diazinon was analyzed by gas chromatography-mass spectrometry following U.S. EPA standardized test methods (method 8141A [www.epa.gov/epaoswer/hazwaste/test/main/htm]). For one test, esfenvalerate was analyzed with gas chromatography-mass spectrometry and enzyme-linked immunosorbent assay (ELISA) to compare the recently developed ELISA test method [21] with U.S. EPA test method 8141A with a method detection limit of 0.10 µg/L. The esfenvalerate ELISA detection limit is 0.05 µg/L in water matrix.

Tissue preparations

At the end of the 96-h acute toxicity tests, surviving larvae were rinsed with U.S. EPA test water and flash frozen with liquid N₂ and stored at -80°C. Samples were homogenized in 100 mM sodium phosphate buffer (pH 7.4, 4°C) and centrifuged at 5,000 g for 15 min at 4°C. Aliquots of samples were prepared and immediately frozen at -80°C until used for enzyme assays.

Carboxylesterase analysis

Carboxylesterase activity was determined as the ability to hydrolyze *p*-nitrophenyl acetate according to Wheelock et al. [22]. Briefly, kinetic assays were run in 96-well Microtiter® plates (Dynatech Laboratories, Chantilly, VA, USA) in 100 mM sodium phosphate buffer (pH 7.4) at 30°C for 2 min. Absorbance was monitored at 405 nm for the production of the *p*-nitrophenolate anion after *p*-nitrophenyl acetate hydrolysis with a microplate reader (Molecular Devices, Palo Alto, CA, USA). All enzyme activities were corrected for background hydrolysis and normalized for protein concentration by the methods of Bradford [23] with bovine serum albumin as the standard. All enzyme assays were performed in triplicate.

Inhibition assays were conducted with unexposed fathead minnow larvae that were prepared as described above. Inhibitor stock solutions were prepared in ethanol and used at concentrations that never exceeded 1% of the total assay volume. Enzyme was incubated with the inhibitor for 5 min; enzyme activity analysis then was performed as described above. The concentration of inhibitor required to reduce enzyme activity

by 50% (IC₅₀) was determined by using a minimum of five datum points, with each point resulting from at least three individual analyses.

Acetylcholinesterase analysis

Acetylcholinesterase activity was assayed by the colorimetric method of Ellman et al. [24] modified for a 96-well microplate reader. The substrate for measuring fish acetylcholinesterase activity was 2 mM acetylthiocholine iodide.

Histopathological analysis

At the completion of the 96-h acute toxicity tests, individual surviving larvae were assigned a random identification code to assure that a blind study was performed. The fish were fixed in 10% buffered formalin, dehydrated, cleared, and embedded in paraffin. Two sections (5–7 μ m thickness) in series were mounted on two glass histology slides and stained with hematoxylin and eosin for survey. Severity of histopathologic alterations in each larval fish were semiquantitative; we ranked them on a scale from 0 (not present) to 3 (severe).

Statistical analyses

Lethal concentrations were calculated for each pesticide and pesticide combination by the Probit Model of ToxCalc® (Ver 4.0, Tidepool Software, McKinleyville, CA, USA). Based on the three independent tests, SAS® (Ver 8.1, SAS Institute, Cary, NC, USA) was used to obtain a probit model [25] to estimate the probability of survival at the 24-h, 48-h, 72-h, and 96-h LC₅₀ for each pesticide and each pesticide combination. To calculate the interactive ratio (IR), the LC₅₀ estimates associated with the pesticide mixtures were first converted to toxic units (TU = 100/LC₅₀) based on LC₅₀ estimates from the single pesticide LC₅₀ (μ g/L) [26,27]. The predicted TU for esfenvalerate was calculated as the measured concentration in the mixture divided by the test esfenvalerate LC₅₀. The same method was used to calculate the predicted diazinon TU. The TU measurement of the mixture then was 100 divided by the LC₅₀ of the mixture. The IR was calculated as the measured TU of the combination divided by the predicted TU of diazinon plus the predicted TU of esfenvalerate. If IR = 1, the interaction is directly additive; if IR > 1, the interaction is greater than additive (synergistic); and if IR < 1, the interaction is antagonistic. If the toxicity of the mixture is strictly additive, then 50% LC₅₀ of each pesticide would contribute 0.5 TU diazinon and 0.5 TU esfenvalerate, respectively, to total 1.0 TU.

RESULTS

Toxicity test results and interaction calculations

All of the toxicity tests met the minimum control survival of 90% or more, and had water quality parameters that were within the guidelines specified by the U.S. EPA [20]. The measured pesticide concentrations ranged from 57 to 100% recovery for diazinon, and from 50 to 133% recovery for esfenvalerate. The recovery ranged from 70 to 90% for esfenvalerate with ELISA.

Table 1 summarizes the LC₅₀ and IR values for each pesticide and pesticide combination, for all three tests. For test 1, the IR was 1.7 TU, meaning that a mixture of the two pesticides at concentrations of 24.8% of their respective LC₅₀ contributed to the total toxicity (Table 1). The IR results were 1.4 TU and 1.4 TU for the two repeated tests.

We used the probit model to estimate the probability of

Table 1. The 96-h median lethal concentration (LC₅₀) and interactivity ratio (IR) results

Parameter	Test 1	Test 2	Test 3
Diazinon LC ₅₀ (μ g/L)	6,393	5,048	7,969
Predicted Diazinon toxic units (TU)	1.3	1.6	1.0
Esfenvalerate LC ₅₀ (μ g/L)	0.18	0.22	0.22
Predicted esfenvalerate TU	1.11	0.91	0.91
Predicted esfenvalerate + predicted Diazinon TU	2.4	2.5	1.9
Esfenvalerate + Diazinon mixture LC ₅₀ (%)	24.8	28.8	37.9
Measured TU mixture	4.0	3.5	2.6
IR	1.7	1.4	1.4

survival for the combined three tests (Table 2). This model indicated that the three tests were different, and that the pesticides had significant ($p = 0.01$) effects. Confidence intervals were generated around the probability of survival for each 24-h time exposure. At the 48-h LC₅₀, lethal effects were evident at the highest individual pesticide concentrations tested, and at a combination of 80% for the mixture. From 48 to 72 h of exposure, the amount of pesticide affected the survival and larvae become more susceptible to further addition of pesticides. At 72 h, the LC₅₀ for the mixture was 35% (~ 50% reduction compared to the 48-h curve).

Examination of the median time to lethality (LT₅₀) (Fig. 1) revealed a similar pattern for each pesticide and pesticide mixture, and demonstrated a good concentration–response relationship. The LT₅₀ data for the three tests are given in Table 3. Examination of the results shows that the higher concentration caused fathead minnows to die sooner, compared to the lower concentrations. Each increase in pesticide concentration required a shorter time to cause mortality. The actual effect of the two pesticides in mixture was more pronounced (i.e., mortality was evident sooner) than predicted if the effect was merely additive.

Results of histopathological, carboxylesterase, and acetylcholinesterase analyses

The pesticides, singly or in combination, did not cause major histopathological damage to gill or liver tissues of the fathead minnow larvae within the 96-h exposure period (data not shown). However, within each of the pesticide tests, liver glycogen levels were depleted, relative to controls.

The inhibition of carboxylesterase activity was pesticide-dependent. Esfenvalerate did not significantly alter carboxylesterase activity from that of the controls at any of the tested concentrations (data not shown). However, carboxylesterase activity was inhibited by 40% by diazinon (reflected by p -

Table 2. Median lethal concentration (LC₅₀) results

LC ₅₀ end-point (h)	Esfenvalerate (μ g/L)	Diazinon (μ g/L)	Esfenvalerate and Diazinon (%)
24	NC ^a	NC	NC
48	0.30	11,200	84.2
72	0.26	8,400	32.0
96	0.20	6,600	25.2

^a NC = not calculable because the median time to lethality was greater than 96 h.

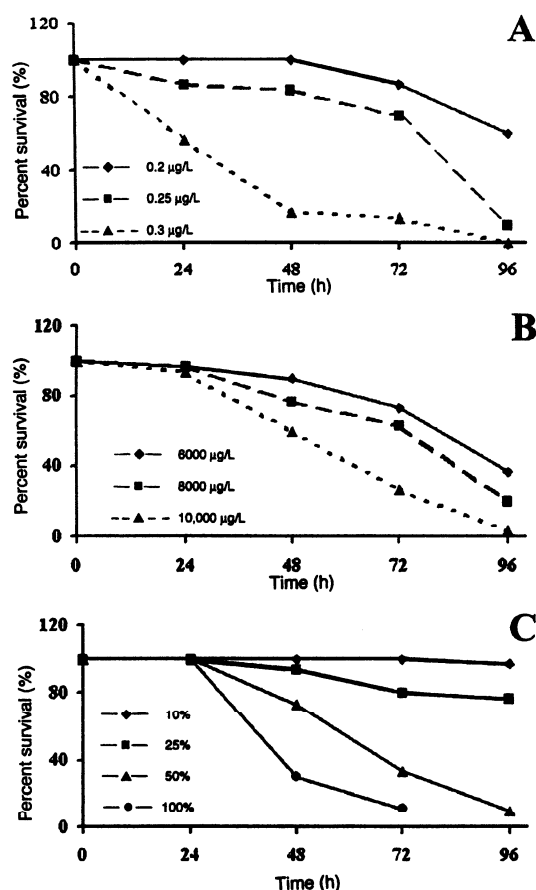


Fig. 1. (a) Esfenvalerate 96-h median lethal concentration (LC50) curves, (b) Diazinon 96-h LC50 curves, and (c) esfenvalerate plus Diazinon 96-h LC50 curves.

nitrophenyl acetate hydrolysis), at a nominal concentration of 50 µg/L (Fig. 2). The maximum inhibition observed was 50% of total specific activity at diazinon at 500 µg/L. We observed essentially no difference in inhibition potency above 500 µg/L (Fig. 2). Differences in inhibition potency were insignificant, for carboxylesterase activity of fish exposed to mixtures versus diazinon alone (data not shown).

Carboxylesterase inhibition studies were performed in vitro with diazinon and its oxon analog, by using unexposed fathead

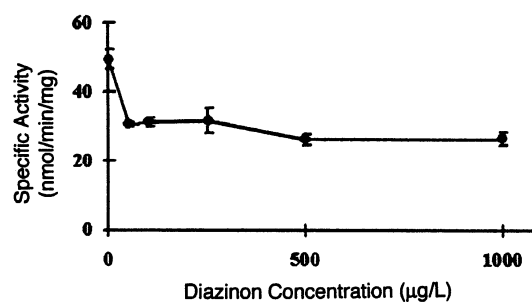


Fig. 2. Carboxylesterase specific activity after in vivo exposure to diazinon in fathead minnows.

minnows to determine the sensitivity of carboxylesterase activity to diazinon inhibition. The IC50 was determined for each chemical individually. Examination of the results showed that the parent compound, diazinon, is not a potent carboxylesterase inhibitor (it had an IC50 of >500 µM). However, the oxon form was a very potent inhibitor (it had an IC50 of 1.51 ± 0.09 µM).

Inhibition of acetylcholinesterase activity was dose-dependent for diazinon, with about a 30% reduction in activity from control values at 50 µg/L and an additional approximately 30% reduction at 100 µg/L (nominal water concentration). The majority of the activity was inhibited at 250 µg/L, but activity did continue to decrease in a dose-dependent manner (Fig. 3). Acetylcholinesterase activity assays were not performed for the esfenvalerate exposures because pyrethroids do not interact with this enzyme.

DISCUSSION

This study used an interactivity ratio approach to show that the effects of esfenvalerate and diazinon in combinations were greater than additive (i.e., synergistic). Recently, several researchers [28–32] have demonstrated that various pesticides and metal-pesticide combinations result in synergistic toxicity responses. Belden and Lydy [31] found that the herbicide atrazine might affect midge larvae (*Chironomus tentans*) and increase the toxicity of diazinon. Compounds with the same toxic mode of action (such as the two organophosphates diazinon and chlorpyrifos) have been found to be strictly additive [27].

These results did not include a chronic follow-up, but this outcome does not exclude the possibility of histopathological effects from longer exposures. Histopathology results of the present study reflect early response of the liver to the compounds. The loss of glycogen (a secondary stress response)

Table 3. Median lethal time (LT50) results

Pesticide	Concentration (µg/L)	LT50 (h)		
		Test 1	Test 2	Test 3
Esfenvalerate	0.20	77	NC ^a	NC
	0.25	60.8	70.5	65.2
	0.30	48.7	26.9	62.0
Diazinon	6,000	NT ^b	89.9	NT
	8,000	50.2	72.1	nc
	10,000	54.2	51.2	72.8
	12,000	33.9	NT	NT
Esfenvalerate and Diazinon	25%	93.4	NC	NC
	50%	66.2	61.0	62.6
	75%	64.8	NT	NT
	100%	42.3	41.7	46.5

^a NC = not calculable because LT50 was greater than 96 h.

^b NT = concentration not tested.

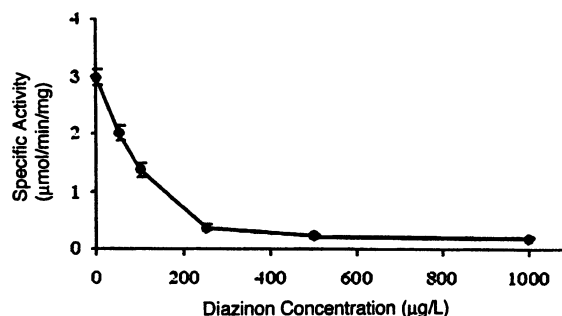


Fig. 3. Acetylcholinesterase specific activity after in vivo exposure to diazinon in fathead minnows.

should be regarded as a nonspecific response signifying stress and has been linked to changes in cortisol during exposure to various stressors [33].

Carboxylesterase activities were examined in this study as a possible mechanistic rationale for the observed greater-than-additive effects of diazinon plus esfenvalerate. Carboxylesterases are an important class of serine hydrolases that cleave esters into the corresponding alcohol and acid. Numerous examples exist of ester-containing compounds that have environmental importance (e.g., herbicides, phthalate esters, and pyrethroids). Carboxylesterases are inhibited by organophosphates and carbamates and detoxify pyrethroids [34]. Therefore, the potential for greater-than-additive toxicity exists after diazinon inhibition of carboxylesterases, because the latter enzymes are then prevented from hydrolyzing esfenvalerate. This toxicity has been observed in mammalian studies [35], but has not yet been verified in studies with fish.

Carboxylesterases also are important in the detoxification of organophosphates. Barron et al. [36] concluded that carboxylesterase activity in fish might influence the accumulation and toxicity of pesticides and other toxic esters. Furthermore, carboxylesterases may act as a sink for organophosphates through irreversible binding, thus preventing the compounds from inhibiting acetylcholinesterase [37]. Wogram et al. [38] showed that carboxylesterase was 13 to 17 times more sensitive to paraoxon than either brain or axial muscle acetylcholinesterase in vitro in three-spined sticklebacks (*Gasterosteus aculeatus*). In the present study, we examined toxicity (i.e., fathead minnow survival) and carboxylesterase activity after exposure to diazinon and esfenvalerate. The observed toxicity was greater than additive and could be explained by inhibition of carboxylesterase activity. Our results showed that the constitutive level of carboxylesterase activity in larval fathead minnows was 50.1 ± 2.9 nmol/min/mg protein. These results compare well to those reported by Barron et al. [36], who listed juvenile fathead minnow carboxylesterase activity as approximately 80 nmol/min/mg protein. Differences in activity could be due to the greater age of the fish in the study of Barron et al. [36], because carboxylesterase activity has been shown to increase with developmental stage in rainbow trout [39].

The inhibition observed in the in vivo exposures varied from approximately 40 to 50% inhibition of carboxylesterase activity. This inhibition was observed at all concentrations examined in this study, and did not exhibit a strong dose-response relationship. Thus, additional isoforms of carboxylesterase may exist that are capable of hydrolyzing *p*-nitrophenyl acetate, but that are insensitive to inhibition by diazinon. This possibility could be explored further by the use of other esterase inhibitors to examine the inhibition of the remaining activity, and suggests the importance of studying the diversity of isozyme abundance and physical characteristics of fathead minnow carboxylesterase. Inhibition studies with diazinon and the oxon analog showed that the parent compound does not significantly inhibit carboxylesterase activity, whereas the oxon is a potent carboxylesterase inhibitor. These results agree well with other studies on carboxylesterase inhibition by organophosphates [38]. Numerous carboxylesterase inhibitors reported in the literature have IC₅₀ values in the nanomolar range. Evaluation of their ability to inhibit fathead minnow carboxylesterase would be interesting. These more potent inhibitors could potentially affect the remaining 50% of carboxylesterase activity that is insensitive to diazinon

inhibition (Fig. 3). In addition, further studies are needed to determine the exact role of carboxylesterase activity and inhibition in relation to fish susceptibility to these pesticides. Work could productively focus on determining the ability of fish carboxylesterases to hydrolyze pyrethroids (i.e., kinetic studies to measure the Michaelis constant [K_m] and maximal velocity [V_{max}]), as well as the effect of complete carboxylesterase inhibition upon organophosphate and pyrethroid toxicity with other potent carboxylesterase inhibitors, such as trifluoromethyl ketones. We did not observe an effect of esfenvalerate exposure on carboxylesterase activity. Given that esfenvalerate is a substrate for carboxylesterases, we did not expect to observe enzyme inhibition. However, no dose-response induction in activity was observed either. Specific activity levels were statistically equivalent at all exposure concentrations. Fish were exposed for 96 h, this length of time possibly is insufficient to induce carboxylesterase. Alternatively, pyrethroids possibly are incapable of inducing these enzymes in this species. Knowing which of these possibilities is correct is not possible without further study. This study demonstrated that diazinon is a potent in vivo inhibitor of carboxylesterase at nominal water concentrations as low as 50 µg/L, which could explain the observed enhanced toxicity of the combined pesticide exposure.

CONCLUSIONS

Given that several classes of pesticides may coexist in aquatic systems, it is paramount that pesticide interactions be examined. Concern exists about initiating regulatory action against one pesticide (i.e., diazinon) when replacement pesticides (i.e., esfenvalerate) may be more toxic or persistent in the environment. The consideration of chemical mixtures is important because although regulatory TMDLs are developed for single chemicals in a water body, the more usual condition is that a mixture of chemicals is present in the listed water body. The potential effects of chemical mixtures in water bodies should be considered during the selection of a numeric target and the development of a margin of safety section of a TMDL. This is critical because the development of TMDLs currently considers only single-chemical interactions and single-chemical target values.

The utility of understanding the biochemical mode of action for pyrethroids is that it may lead to the development of a forensic fingerprint that can be used to identify the presence of pyrethroid compounds in water. The next step would be to identify a carboxylesterase inhibitor that is nontoxic to the target organism, is water soluble at its effective dose, is chemically available, and can generate a response that indicates the presence of pyrethroid insecticides. A carboxylesterase inhibitor could serve as a new tool to account for the presence of pyrethroid-induced toxicity, thereby allowing for the elucidation of toxicity caused by these pesticide mixtures.

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