

ENANTIOMERIC SULFOXIDATION OF THE ORGANOPHOSPHATE PESTICIDE FENTHION IN FISH



Rainbow Trout



Striped Bass



Tilapia

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RESULTS

ABSTRACT

The objective of this study is to examine the enantioselective sulfoxidation of fenthion, in liver microsomes of various fish species (rainbow trout, hybrid striped bass, tilapia). Microsomes from striped bass, trout, and tilapia primarily formed (-) sulfoxides in approximately 65% enantiomeric excess. Enzyme inhibitors lubrol (cytochrome P450) and methimazole (Flavin Monooxygenases) were used to determine sulfoxide relative contributions from each enzyme system. In striped bass microsomes, P450 was responsible for 74% of sulfoxide formation. Co-incubation with methimazole or lubrol in trout liver microsomes enhanced sulfoxide formation. Salt water treatments which typically induce FMOs, did not significantly alter enantioselectivity or rates of fenthion sulfoxidation, even though toxicity tests indicated that saline environments enhanced the toxicity of fenthion in trout. These results indicated either the formation of additional metabolites of fenthion or the contribution of additional oxygenases to S-oxidation.

INTRODUCTION

Fenthion is an organophosphate insecticide used primarily against fruit flies and mosquitoes. The EPA has classified Fenthion in toxicity class II, and as a Restricted Use Pesticide (RUP). While it is an effective insecticide, it is also moderately toxic to mammals, and highly toxic to birds. Due to its high toxicity to birds, fenthion is used in various parts of the world for bird control, mainly in the control of pigeons around public buildings. Little research has been done to study the effects of fenthion on aquatic organisms, such as fish. The sulfoxides of thioether pesticides are generally more toxic when compared to their parent compounds. Sulfoxidation may occur primarily through 2 enzyme systems, cytochrome P450 (P450) and Flavin-containing monooxygenases (FMO). The latter system (FMO) is unique in that expression in certain fish species appears to be directly related to salinity regimes in which they reside, as well as the stereo-selective sulfoxidation by specific FMO isoforms. Given the co-occurrence of pesticides with hyper-saline arid land agriculture, the ultimate objectives of this study are to evaluate the effects of salinity conditions during the biotransformation of the thioether pesticide fenthion.

MATERIALS & METHODS

Rainbow trout were donated by the U.S. Fish and Wildlife Mojave River Hatchery in Victorville, CA, and Tilapia and Hybrid Striped bass were donated by Kent Sea Tech Farms in Mecca, CA. Livers were taken from fish immediately after sacrificing, and were placed in -80°C until microsomes were made. Microsomes of each individual species were made by pooling livers from several fish and homogenizing them with buffer containing Tris-HCl, pH 7.4, KCl, and EDTA. This homogenate was then centrifuged at 20,000 x g at 4°C for 30 min eliminating the mitochondria and other cell debris into a pellet. The supernatant was taken out and centrifuged at 100,000 x g at 4°C for 90 min, separating the cytosol from the microsomal pellet. The microsomal pellet was re-suspended in potassium phosphate buffer, pH 7.4 containing 20% glycerol. Microsomes were also stored at -80°C until use. Assays were performed by mixing microsomal protein, NADPH, phosphate buffer pH 7.4, magnesium chloride, and 0.8mM (final concentration) fenthion to a total volume of 250 µL in a micro centrifuge tube. An internal standard, methyl (p) tolyl sulfoxide (R-Isomer) 0.04 mM (final concentration) was added to each sample in order to determine recovery rates. The average percent recovery is approximately 70%. The mixture was then incubated at 25°C for 30 min with intermittent mixing. Dichloromethane was added to stop the reaction and to extract the sulfoxides. The 2 phase mixture was then vortexed for 1 min and the organic layer was removed and vaporized under nitrogen gas to dryness then reconstituted in mobile phase. The samples were then analyzed by normal phase HPLC with UV detection at 254 nm using a Regis (R,R) Whelk 0-1 chiral column. Fenthion sulfoxide standards were also analyzed by HPLC to identify sample peaks and concentrations. For toxicity testing, 96 hour exposures were conducted in fresh and salt water at 12°C, with water being changed midway (48 hours). Fenthion was dissolved in methanol and the following concentrations were exposed to fish in duplicate with n=4 (fresh water), and n=8 (salt water): 0.1, 0.5, 1.0, and 10.0 mg/L, plus a control and solvent control.

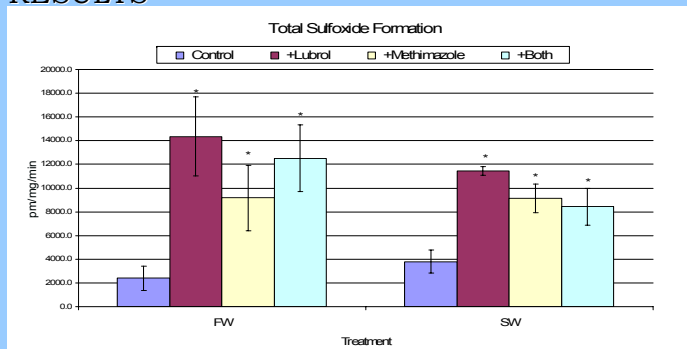


Figure 1: Effects of salt water and P450/FMO inhibitors on total fenthion sulfoxide formation from liver microsomes of rainbow trout. Each value represents the mean of 4 samples ± standard deviation. *Indicates a significant difference from the control at a p ≤ 0.05.

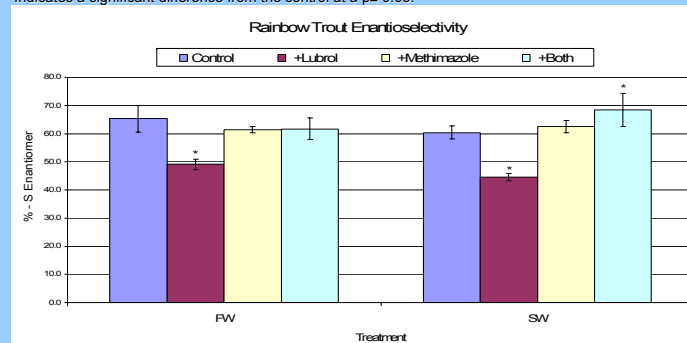


Figure 2: Effects of salt water and P450/FMO inhibitors on the enantioselectivity of sulfoxidation (-S enantiomer) by rainbow trout microsomes. Each value represents the mean of 4 samples ± standard deviation. *Indicates a significant difference from the control at a p ≤ 0.05.

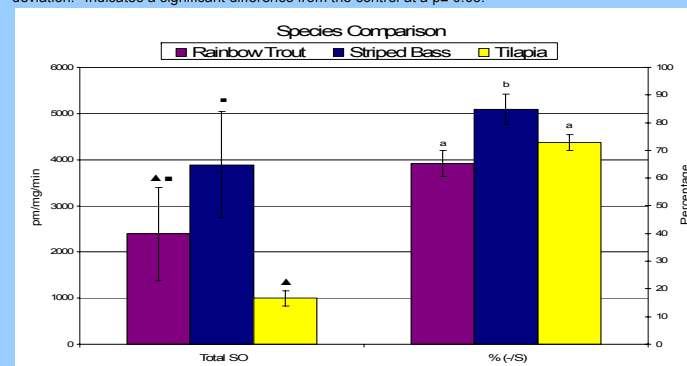


Figure 3: Comparison of total sulfoxide formation and the % (-S) of the enantiomer formation by each fish species in fresh water without the use of inhibitors. A difference in symbols or letters indicates a significant difference of p ≤ 0.05.

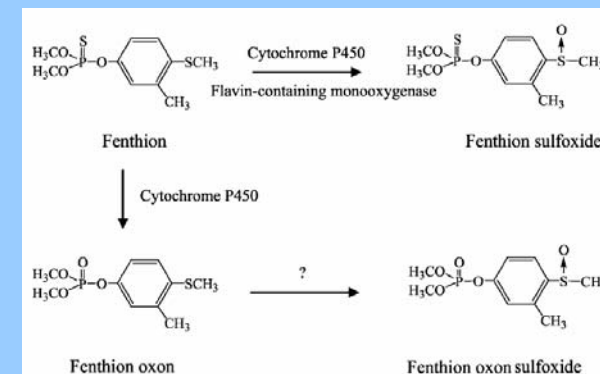


Figure 4: The mechanism of fenthion oxygenation by cytochrome P450 and FMO.

CONCLUSIONS

- Fenthion is more toxic to rainbow trout when exposed in a hyper-saline environment (LC50 0.18 mg/L) compared to freshwater exposures (LC50 1.12 mg/L).
- Microsomes from saltwater treated rainbow trout had slightly higher rates of fenthion sulfoxidation than fish maintained in fresh water.
- Saltwater treatment did not significantly alter enantioselectivity of fenthion sulfoxidation.
- Lubrol co-incubation significantly increased fenthion sulfoxidation in trout, indicating P450 may be responsible for fenthion sulfoxidation and formation of additional unknown metabolites.
- Lubrol treatment also enhanced the formation of (R) enantiomers in salt water treated trout, suggesting the contribution of FMO1-like proteins, likely induced by salt water in trout.
- Co-incubation with lubrol and/or methimazole in trout liver microsomes led to significant increases in fenthion sulfoxide formation indicating other enzyme systems may be responsible for the formation of sulfoxide metabolites or a shift in oxonic metabolite formation.
- Species comparisons:
 - Total SO in fresh water: striped bass > rainbow trout > tilapia
- Enantioselectivity:
 - % -S Isomer in fresh water: striped bass > tilapia > rainbow trout

ACKNOWLEDGEMENTS

This work was supported by the UC Riverside USDA/ Agricultural Experiment Station. We would also like to thank Mary Ann Irwin and Lingtian Xie for their help with the fish, and Yelena Sapozhnikova for her instrumental guidance. We are grateful to The U.S. Department of Fish and Wildlife (Mojave River Hatchery) and Kent Sea Tech Farms for their generous contribution of fish samples.