MECHANISMS OF FENTHION ACTIVATION IN RAINBOW TROUT (Oncorhynchus mykiss) ACCLIMATED TO HYPERSALINE ENVIRONMENTS
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BACKGROUND
• Fenthion [O,O-dimethyl-O-(4-methylmercapto)-3-methylphenylthio-phosphate] is an organophosphate pesticide (OP) widely used throughout the world as a broad-spectrum insecticide for numerous crops and also as an ectoparasiticide on farm animals (Roberts and Hulton, 1999).

• Upon uptake by organisms, fenthion undergoes oxidative metabolism, mediated by cytochrome P450 (CYP) and flavin-containing monooxygenases (FMO) forming primary and secondary metabolites, with enhanced or reduced potency to inhibit acetylcholinesterase (ACHE).

• In vitro and in vivo studies demonstrated that fenthion is biotransformed to fenoxon sulfoxide and fenoxon in liver microsomes of fish and rats (Kitamura et al., 2003) (Figure 1).

• Previous studies in rainbow trout have shown that acclimation to hypersaline environments enhances the toxicity of thioether organophosphate and carbamate pesticides (Wang et al., 2001; Bawardi et al., 2007).

To determine the role of biotransformation in this process, the metabolism of the thioether organophosphate biocide, fenthion was evaluated in microsomes from gills, liver and olfactory tissues in rainbow trout (Oncorhynchus mykiss) maintained in freshwater and 17% saline water.

MATERIALS & METHODS

Experimental procedures
1. Microsomal fractions obtained from liver (biotransformation), gills (osmoregulation) and olfactory tissues (behaviour).

2. In vitro incubations of microsomal proteins with 100 µM of substrate (fenoxon, fenoxon and fenthion sulfoxide) and 400 µM NADPH. After 1 h incubation, the reaction was stopped, extracted and analyzed by Chiral Normal Phase HPLC with UV detector (237 nm).

2.1. CYP and FMO inhibition studies: co-incubation with 500 µM methimazole (FMO inhibitor) or 500 µM ketocnazole (CYP inhibitor).

2.2. Carboxylesterase inhibition studies: co-incubation with TEPP (tetraethyl-pyrophosphate).

3. CYP1A, CYP2M1, CYP2K1 and CYP3A27 determined by Western blot.

RESULTS

Fenthion metabolism in different tissues

- Higher metabolism in liver.
- Only hydrolysis in olfactory tissue.

In liver and gills ratio S/R sulfoxidation is 65:35.

- NOVEL NADPH-dependent hydrolysis observed.

Effects of hypersaline conditions

- CYP may contribute more to fenthion sulfoxidation than FMO.
- Fenthion cleavage inhibited by TEPP: hydrolysis could be due to microsomal carboxylesterases.

CYP determinations in liver microsomes

- CYP3A27 (protein and testosterone hydroxylase associated activity) increased in hypersaline-acclimated fish.

SUMMARY AND CONCLUSIONS

- Hypersaline conditions increased the formation of fenoxon from fenthion and fenoxon sulfoxide from fenoxon, and reduced fenthion cleavage in liver microsomes from rainbow trout. In gills, hypersalinity reduced the formation of sulfoxides and increased fenthion esterase activity.

- Hypersalinity increased the content and catalytic activity of CYP3A27 in liver microsomes. Therefore CYP3A27 may contribute to enhanced fenthion oxidative metabolism and subsequent toxicity of fenthion of rainbow trout under hypersaline conditions.

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References


Figure 1. Metabolites produced from fenthion in microsomes isolated from different tissues in rainbow trout (Oncorhynchus mykiss) maintained in freshwater and 17% saline water.