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Microsomal biotransformation of chlorpyrifos, parathion and fenthion in rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*): mechanistic insights into interspecific differences in toxicity

Ramon Lavado and Daniel Schlenk

Department of Environmental Sciences, University of California, Riverside, CA 92521

Abstract

Rainbow trout often serve as a surrogate species evaluating xenobiotic toxicity in cold-water species including other salmonids of the same genus, which are listed as threatened or endangered. Biotransformation tends to show species-specific patterns that influence susceptibility to xenobiotic toxicity, particularly organophosphate insecticides (OPs). To evaluate the contribution of biotransformation in the mechanism of toxicity of three organophosphate (phosphorothionate) insecticides, chlorpyrifos, parathion and fenthion, microsomal bioactivation and detoxification pathways were measured in gills, liver and olfactory tissues in juvenile rainbow trout (*Oncorhynchus mykiss*) and compared to juvenile coho salmon (*Oncorhynchus kisutch*). Consistent with species differences in acute toxicity, significantly higher chlorpyrifos bioactivation was found in liver microsomes of rainbow trout (up to 2-fold) when compared with coho salmon. Although bioactivation to the oxon was observed, the catalytic efficiency towards chlorpyrifos dearylation (detoxification) was significantly higher in liver for both species (1.82 and 0.79 for trout and salmon, respectively) when compared to desulfuration (bioactivation). Bioactivation of parathion to paraoxon was significantly higher (up to 2.2-fold) than detoxification to *p*-nitrophenol in all tissues of both species with rates of conversion in rainbow trout, again significantly higher than coho salmon. Production of fenoxon and fenthion sulfoxides from fenthion was detected only in liver and gills of both species with activities in rainbow trout significantly higher than coho salmon. NADPH-Dependent hydrolysis of fenthion was observed in all tissues, and was the only activity detected in olfactory tissues. These results indicate rainbow trout are more sensitive than coho salmon to the acute toxicity of OP pesticides because trout have higher catalytic rates of oxon formation. Thus, rainbow trout may serve as a conservative surrogate species for the evaluation of OP pesticides in coho salmon.

Keywords

organophosphate; cytochrome P450; biotransformation; rainbow trout; coho salmon

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Correspondence to: Daniel Schlenk.

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Introduction

Comparison of LD₅₀ or LC₅₀ values often indicates unique species-specific differences in xenobiotic toxicity among species of the same family or genus. Idiosyncratic differences in pharmacokinetic processes such as biotransformation can have significant contributions to these species differences. A prominent example of a class of compounds whose toxicity is significantly altered by biotransformation are the organophosphate insecticides (OPs). Phosphorothionate OPs possess a P=S group, which can be activated by cytochromes P450 (CYP) to the oxon (P=O) enhancing the inhibition of acetylcholinesterase (AChE) (Chambers and Carr, 1995). Thio-ether linkages several carbon units away from the phosphorothionate moiety provide an additional site for oxidation that further enhances potency in combination with oxon formation (Dauterman, 1971; Gadepalli et al., 2007).

The majority of studies evaluating the *in vitro* biotransformation of xenobiotics in vertebrates are carried out in liver microsomal systems (Miranda et al., 1998; Straus et al., 2000; Guengerich, 2001). However, extrahepatic biotransformation may also contribute to the overall biotransformation process. In fish, biotransformation has been observed in the gut, kidneys, gills and the olfactory system (Stegeman, 1989; Monod et al., 1994; Buhler and Wang-Buhler, 1998; Matsuo et al., 2008). One tissue that is of particular interest given its fundamental importance in migratory salmonids is the olfactory bulb (Tierney et al., 2010). Recent studies have indicated the presence of various Phase I enzymes (Matsuo et al., 2008), but their role in the activation or detoxification of xenobiotics is unknown. Consequently, the purpose of this study was designed to evaluate the contribution of biotransformation of 3 model OPs (fenthion, chlorpyrifos, and parathion) in three tissues of two salmonid species: rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*). The results of which will help determine if rainbow trout may be suited as a model for pesticide biotransformation in coho salmon, and whether species-specific differences in toxicity may be explained by bioactivation and detoxification pathways unique to each species.

Material and Methods

Choice of Pesticides

Fenthion (*O,O*-dimethyl-*O*-(4-methylmercapto)-3-methylphenylthio-phosphate), Chlorpyrifos (*O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl-phosphorothionate), and Parathion (*O,O*-diethyl-*O*-*p*-nitrophenyl-phosphorothionate) were selected for study due to the well-studied toxicity and biotransformation pathways previously reported in each species (Lavado et al., 2009; Schlenk 2005; Schlenk et al. 2008).

Standards and reagents

Fenthion (99.9%) (CAS 55-38-9), fenoxon, racemic fenthion sulfoxide, parathion (CAS 56-38-2) and chlorpyrifos (CAS 2921-88-2) were purchased from Chem Service (West Chester, PA). 3-Methyl-4-(methylthio)-phenol (MMTP), *p*-nitrophenol and paraoxon were purchased from Sigma (Milwaukee, WI). *R*- and *S*-fenthion sulfoxides were synthesized as described previously (Gadepalli et al., 2007). Methanol, ethanol, ethyl acetate, acetonitrile, *n*-hexane and isopropanol were of analytical grade (Fisher, Pittsburg, PA). Deionized water (DI water) was obtained using a Milli-Q water purification system (Millipore, Billerica, MA).

Organisms

Juvenile rainbow trout (*Oncorhynchus mykiss*) (age approximately 5 months, 16 ± 3 cm), without discernible gonadal morphology indicative of gender, were obtained from Jess

Ranch Fish Hatchery (Apple Valley, CA). Juvenile coho salmon (*Oncorhynchus kisutch*) were obtained from Nimbus Hatchery (Gold River, CA). Organisms were maintained in a flowthrough living-stream system with dechlorinated carbon-filtered municipal water at 13–15°C and acclimatized for 2 months before experimental use. Organisms were fed with commercial fish feed (Silver Cup, Murray, UT).

Subcellular fractionation

Livers, gills and olfactory tissues were selected due to their physiological roles in biotransformation (liver), osmoregulation (gill), and behavior (olfactory tissues). Subcellular fractionation was performed according to Lavado et al. (2009). Briefly, after weighing each tissue or tissue pool, it was rinsed in ice-cold 1.15% KCl and homogenized in 1:5 w/v of cold 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer pH 7.4, containing 100 mM KCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonylfluoride (PMSF) and 0.1 mM 1,10-phenanthroline. Olfactory tissues were pooled from 6–8 different individuals, but gills and liver were analyzed from individual organisms. Homogenates were centrifuged at 500g for 15 min, the fatty layer removed and the supernatant centrifuged at 12,000g for 20 min. The 12,000g supernatant was further centrifuged at 100,000g for 60 min to obtain microsomal fractions. Microsomal pellets were resuspended in a small volume of 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer pH 7.4, containing 100 mM KCl, 20% (w/v) glycerol, 1 mM EDTA, 0.1 mM PMSF and 0.1 mM 1,10-phenanthroline. Protein concentrations were determined by the Coomassie Blue method using a commercial kit (Pierce Inc., Rockford, IL) using bovine serum albumin as a standard. Biotransformation rates and measurements of protein concentration were collected at the same time using the same standard curve for both species.

Fenthion biotransformation

Fenthion biotransformation was evaluated as described in Lavado et al. (2009) which explored biotransformation in rainbow trout, but not coho salmon. Concentrations of fenthion ranged from 1 μM to 1 mM for kinetic evaluations in liver microsomes from rainbow trout and coho salmon. Fenthion metabolism was linear up to 1.2 mg protein and up to 60 min of incubation. Liver, gill and olfactory tissue microsomal fractions were incubated in 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer pH 7.6 with 100 μM of fenthion and 400 μM NADPH in a total volume of 500 μL . The reaction was initiated by the addition of substrate and samples were incubated for 60 min at 25°C. The reaction was stopped by placing the tube on ice and the addition of 1 mL ethyl acetate to extract the incubation mixture. An internal standard, *R*-methyl(*p*)tolyl sulfoxide (0.04 mM final concentration) was added to each sample after incubation was concluded in order to determine recovery rates. The extract was removed, transferred to glass HPLC vials and evaporated under nitrogen flow to dryness. Negative controls consisted of identical additives with the exception of NADPH or adding boiled proteins. Isopropanol (50 μL) was used to reconstitute the samples for analysis and 30 μL (injection loop was 50 μL) was injected into a chiral HPLC system (3 injection replicates for each sample).

[^{14}C]Chlorpyrifos biotransformation

[^{14}C]Chlorpyrifos metabolism was evaluated following the method of Choi et al. (2006) with minor modifications. Concentrations of [^{14}C]chlorpyrifos (15 Ci/mol; 96.4% purity) ranged from 1 μM to 1 mM for kinetic evaluations in liver microsomes. [^{14}C]Chlorpyrifos metabolism was linear up to 1.2 mg protein and up to 120 min of incubation. Liver, gill and olfactory tissue microsomal fractions were incubated in 10 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer pH 8.4 with 100 μM of chlorpyrifos and 500 μM NADPH. Samples were incubated for 90 min at 25°C. Incubations were stopped by adding 250 μL of acetonitrile and after centrifugation (10,000g; 10 min), 200 μL of supernatant was injected on to a reverse-phase HPLC column.

Parathion biotransformation

Parathion biotransformation in microsomes was evaluated following the method of Sultatos and Murphy (1983) with minor modifications. Concentrations of parathion also ranged from 1 μ M to 1 mM for kinetic evaluations in liver microsomes. Parathion metabolism was linear up to 0.8 mg protein and up to 120 min of incubation. Liver, gill and olfactory tissue microsomal fractions were incubated in 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer pH 7.6 with 50 μ M of parathion and 300 μ M NADPH. Samples were incubated for 60 min at 25°C. Incubations were stopped by adding 250 μ L of acetonitrile and after centrifugation (10,000g; 10 min), 200 μ L of supernatant was injected into a reverse-phase HPLC system.

HPLC methods

For fenthion metabolites, HPLC analyses were performed on a SCL-10AVP Shimadzu HPLC system equipped with a 250 mm $\times$ 4.6 mm Chiralcel OJ-H chiral column (Daicel Chemical Industries, Fort Lee, NJ). Separation of fenthion and fenthion metabolites (3-methyl-4-(methyltio)-phenol (MMTP), fenoxon, *R*- and *S*-fenthion sulfoxides) was performed using an HPLC gradient system elution at a flow rate of 1 mL/min with a mobile phase composed of (A) 24% isopropanol and 76% hexane and (B) 100% isopropanol. The run consisted of a 3 min linear gradient from 100% A to 92% A, and 3–35 min linear gradient to 80% A. Peaks were monitored with a UV-detector SPD-10AVP Shimadzu at 237 nm, quantified by integrating the area under the peaks and identified with co-elution of authentic standards (the retention times observed were 7.2 for MMTP, 10.1 for fenoxon, 12.9 min for fenthion, 14.1 for *S*-fenthion sulfoxide and 16.2 for *R*-fenthion sulfoxide; and the recovery of each varied from 95.1 to 98.7%). The detection limit of each metabolite was 0.3 pmol/min/mg protein.

For [^{14}C]chlorpyrifos incubation extracts, HPLC analyses were performed on the same Shimadzu HPLC system using a 250 $\times$ 4.6 mm Atlantis C18 (5 μ m) reverse-phase column (Waters, Milford, MA). Separation of [^{14}C]chlorpyrifos metabolites employed an HPLC gradient system elution at a flow rate of 1 mL/min with a mobile phase composed of (A) 89% water, 10% acetonitrile with 1% phosphoric acid and (B) 99% acetonitrile with 1% phosphoric acid. The run consisted of a 5 min linear gradient from 90% A to 100% B, 3 min 100% B, 2 min linear gradient from 100% B to 40% A and 15 min linear gradient to 90% A. Chromatographic peaks were monitored by on-line radioactivity detection with a radioflow detector β -ram Model 3 (INUS Systems Inc., Tampa, FL) using In-Flow 2:1 (INUS Systems Inc.) as scintillation cocktail. Chlorpyrifos (13.7 min) and its metabolites, chlorpyrifos-oxon1 (12.3 min) and 3,5,6-trichloropyridinol (11 min) were identified by co-elution with authentic standard compounds and quantified by integrating the area under the radioactive peaks (recovery was from 96.1% to 99.4% for each metabolite and the detection limit was 0.1 pmol/min/mg protein).

For parathion biotransformation, HPLC analyses were performed on the same Shimadzu HPLC system but in this case equipped with a 250 $\times$ 4.6 mm Hypersil ODS C18 (3 μ m) reverse-phase column (ThermoFisher Scientific Inc., MA). Separation of parathion metabolites employed an HPLC isocratic system elution at a flow rate of 1 mL/min with a mobile phase composed of 40% water and 60% acetonitrile. Chromatographic peaks were monitored with a UV-detector SPD-10AVP Shimadzu at 290 nm, quantified by integrating the area under the peaks and identified with co-elution of authentic standards (the retention times observed were 3.9 for *p*-nitrophenol, 5.0 min for paraoxon and 12.6 min for parathion; and the recovery for each metabolite varied from 97.6 to 99.0%). The detection limit of each metabolite was 0.3 pmol/min/mg protein.

Statistical procedures

Statistical differences between two groups was assessed using Student's *t* test, and to evaluate differences between more than two groups, one-way ANOVA analysis was applied, with the use of a SPSS v15.0 software package (SPSS Inc., Chicago, IL). A *p*-value of less than 0.05 was considered statistically significant unless otherwise indicated. If an overall significance was detected, Tukey's multiple range tests were performed. Samples showing levels below the detection limits were considered as having 50% of the minimal values detectable only for statistical comparisons. All data were analyzed prior to statistical analysis to meet the homocedasticity and normality assumptions of parametric tests. Kinetic parameters (Lineweaver-Burk plots) were calculated using Graphpad Prism v5.0 software package using non-linear regression (Graphpad Software Inc., La Jolla, CA).

Results

Kinetic Parameters for Hepatic Transformation

The kinetics of the three OPs (fenthion, FTH; chlorpyrifos, CPF; and parathion, PTH) in liver microsomes from rainbow trout and coho salmon are shown in Table 1. Principal metabolites of FTH included fenthion sulfoxide, fenoxon and the cleavage product 3-methyl-4-(methyltio)-phenol (MMTP). *S*-Oxygenation of FTH creates a chiral center with *S*- and *R*-oxides (Gadepalli et al., 2007). In this study, fenoxon sulfoxide was not detected in any of the incubations with FTH. MMTP was the main metabolite, accounting for 43.0 to 46.8% of total fenthion biotransformation in both fish species (Table 2). Fenthion sulfoxide production ranged from 32.1 to 42.9% of total metabolites formation. Fenoxon formation was 14.1% and 18.9% in trout and salmon, respectively.

In both species, NADPH-independent MMTP production in liver had the lowest catalytic efficiency, whereas the NADPH-dependent MMTP formation had the highest efficiency (Table 1). However, salmon were less efficient than trout in hydrolyzing FTH. The efficiency for fenoxon production was similar in both species (0.61–0.72). However, the efficiency of conversion to the remaining metabolites was up to 2-fold higher in rainbow trout compared with salmon. Significant differences were observed in sulfoxide formation, although stereoselectivity was unchanged.

CPF was converted to chlorpyrifos-oxon (CPO) (27.8–35.2% of metabolism) and 3,4,5-trichloropyridinol (TCP) (64.8–72.2% of total biotransformation) in liver microsomes from both species. A higher efficiency (>2-fold) was observed towards TCP production than CPO production for both species. However, overall efficiency was nearly 50% less in salmon, which had a significantly lower $V_{\max}$ compared to rainbow trout.

Paraoxon (POX) and *p*-nitrophenol (PNP), were the principal metabolites from PTH hepatic incubations. Higher efficiencies toward POX relative to PNP production was observed in both species with efficiency differences driven by significantly lower $V_{\max}$ in salmon (1.6-fold). Apparent K_m 's were generally lower and catalytic efficiencies higher for PTH relative to CPF or FTH in both species.

Tissue comparisons of organophosphate biotransformation in each species

Biotransformation of FTH, CPF and PTH showed tissue-specific differences in both fish species. For FTH, MMTP was the predominant metabolite in all three tissues evaluated (liver, gills and olfactory tissues) for both species (Figure 1). NADPH-independent hydrolysis of FTH was significantly higher in gills compared to liver (up to 17-fold in trout and up to 4-fold in salmon) and olfactory tissues (up to 1.8-fold in both species). Rates of NADPH-dependent hydrolytic cleavage of FTH were highest in olfactory tissues of the

rainbow trout, but relatively equivalent between the three tissues of salmon. Oxidation was not detected in olfactory tissues in either species. Oxidation products (fenoxon and fenthion sulfoxides) were highest in liver for both species. In salmon, the tissue-specific activities showed the same trend as rainbow trout, with the exception that fenoxon production in gills was not detected.

CPO and TCP were detected in microsomal incubations of liver and gill, but not olfactory tissues of both species (Figure 2). TCP formation was higher in both tissues of each species (Figure 2). Significantly higher CPF biotransformation was observed in liver when compared with gills ($p < 0.05$). As observed in liver, salmon had 1.5-fold lower transformation activities in the gill compared to rainbow trout.

Parathion biotransformation in liver was higher than activities in gills and olfactory tissues of both species (Figure 3). Respective POX and PNP production was up to 120-fold and 17-fold higher in liver than in gills ($p < 0.05$). Oxidative desulfuration was higher in liver (up to 1.4-fold), whereas dearylation to PNP was higher (up to 3.5-fold) in gill. In olfactory tissues, desulfuration of parathion was not observed in either species. As described for the previous two pesticides, parathion biotransformation in liver, gills and olfactory tissues was significantly higher in rainbow trout compared to coho salmon (Figure 3).

Discussion

Evaluation of Rainbow trout as Surrogate for Coho Salmon Biotransformation and Toxicity

Coho salmon, steelhead trout and other species of Pacific salmonids have declined markedly in the western United States, largely as a result of deterioration or loss of critical freshwater and coastal habitats (Huntington et al., 1996; Dauble and Watson, 1997). The widespread contamination of surface waters and sediments in western river systems is a limiting factor for the recovery of some of these stocks (Domagalski et al., 1997; Wentz et al., 1998). The rainbow trout is typically used as surrogate species to evaluate the impacts of contaminants on other species of cold-water fish including salmonids which are threatened and/or endangered. It is often assumed that being of the same genus, chemicals would have similar mechanisms of action and toxic effects. However, LC_{50} values vary significantly between salmonids with rainbow trout values usually more sensitive (Mayer and Ellersieck, 1986). LC_{50} values for FTH in rainbow trout were slightly lower (0.75–1.2 mg/L) than salmon (1.0–1.7 mg/L) (Bawardi et al., 2007; Lavado et al., 2009). PTH was approximately 2-fold more toxic in rainbow trout relative to salmon (Macek and McAllister, 1970; Mayer and Ellersieck, 1986), and CPF was more than 5-fold more toxic in rainbow trout (Barron and Woodburn, 1995).

The acute toxicity of OPs are caused by acetylcholinesterase inhibition, which is significantly enhanced in phosphorothiolate pesticides after bioactivation to oxon metabolites. Inhibition occurs through irreversible phosphorylation of serine residues within the active site of the enzyme. Formation of the oxon metabolite enhances the phosphorylation. Thus, species differences in oxon formation may be an important mechanism explaining the enhanced sensitivity of rainbow trout. Of the 3 tissues examined, the liver generally had the highest rates of catalysis. Comparing the enzymatic activities with other fish species, the hepatic biotransformation of CPF in both salmonids was similar to what has been described in goldfish (Smith et al., 1966), mosquitofish and previous studies in rainbow trout (Metcalf, 1974) with TCP as the principal metabolite. Hepatic biotransformation in humans and mice had much higher catalytic efficiency (130 and 30 for CPO and TCP production, respectively) compared to the two salmonids species from this study (Sultatos and Murphy, 1983; Foxenberg et al., 2007). These results indicate mammals may be more efficient in the activation of chlorpyrifos compared to salmonids. In each salmonid, the catalytic efficiency

towards dearylation of CPF and PTH was not different, but the desulfuration efficiency towards POX formation was up to 6-fold higher than CPO production implying a far greater detoxification potential for CPF than for parathion in both salmonid species. Although rainbow trout and coho salmon showed higher catalytic efficiency of PTH activation compared to CPF, each salmonid species was up to 10–13 lower (oxon production) compared to humans and rodents (Foxenberg et al., 2007).

Generally, the apparent binding affinities (K_m) for FTH, CPH and PTH for microsomal biotransformation did not differ between rainbow trout and coho salmon, indicating that the enzymes responsible for this metabolism may be structurally similar in both fish species. The primary differences between species were observed in V_{max} , which is typically influenced by expression content of enzyme or through inhibition or stimulation by another substrate or regulating material. Rainbow trout generally had up to 2-fold higher velocity (V_{max}) than coho salmon for the biotransformation of the three OPs, which led to higher catalytic efficiencies particularly for oxon formation, which provides a mechanistic basis for the greater toxicity in rainbow trout relative to coho salmon.

Structural Differences of OPs and Chemical-Chemical Toxicity Comparisons

While species-specific differences in toxicity corresponded well with hepatic biotransformation, chemical-specific patterns of toxicity were not related to hepatic biotransformation. For example, PTH underwent oxidation to the oxon more readily in both species, but the LC_{50} values of PTH (3–5 mg/L) are much higher relative to the other compounds (6–51 μ g/L CPF; 0.75–1.7 mg/L FTH) (EPA, 1986; Howard, 1989). The differences may be explained by physicochemical parameters of the three compounds (CPH $\log K_{ow} = 5.0$ vs. 3.5–3.8 for the other compounds). Another possibility may be the formation of novel metabolites or biotransformation pathways not detected using the methodology of the current study.

For example, one potential mechanism explaining the higher toxicity of FTH relative to PTH may be *S*-oxygenation. Although *S*-oxygenation reduces the toxicity of thio-ether organophosphate pesticides (Henderson et al., 2004), the *S*-oxygenation of FTH creates a chiral center with *S*- and *R*-oxides. However, desulfuration of the sulfoxide, or *S*-oxygenation of the oxon, to the *R*-fenoxon sulfoxide is a critical bioactivation pathway for FTH (Gadepalli et al., 2007). Fenoxon sulfoxide was not observed (<0.3 pmol/min/mg protein) in either species in the current study, but the metabolite was previously observed in hepatic incubations from rainbow trout maintained under hypersaline conditions (Lavado et al., 2009). Whether formation of this most potent AChE inhibitor occurs in other critical tissues (i.e. brain) may potentially explain the relatively higher toxicity compared to PTH.

Consistent with previous studies, a stereoselective preference for *S*-sulfoxide (65%) was observed in liver microsomes from trout (Schlenk et al., 2004), but also from salmon. However, a racemic mixture of sulfoxides was observed in gill from both species with no oxidation in olfactory tissues. Potential reasons for the tissue-specific differences likely involve differences in tissue-specific expression of CYP or FMO, which catalyze the *S*-oxidation of FTH (Lavado et al., 2009). Preliminary studies of 3 FMO genes in these tissues indicated significant differences in expression under hypersaline conditions (Lavado et al., unpublished).

Tissue-specific Differences in Extrahepatic OP Biotransformation

Measurable biotransformation was observed for all 3 compounds in the gills of both species. With the exception of PTH, where very low rates of transformation were observed, rates of catalysis tended to be higher in gills of rainbow trout. FTH oxon was observed in gill

incubations of trout, but not in coho salmon indicating a species-specific activation presumably through CYP. A recent evaluation of CYP mRNA and catalytic activities in gills of salmon indicated limited expression (Matsuo et al., 2008) relative to trout (Buhler and Wang-Buhler, 1998). Since salmon tend to undergo osmotic change more regularly in their life history relative to rainbow trout (with the notable exception of anadromous steelhead trout), CYP-mediated biotransformation of xenobiotics in gills may be impaired. When rainbow trout were acclimated to hypersaline conditions CYP-mediated oxygenation of FTH was diminished, but non-CYP catalyzed hydrolysis was enhanced in gills indicating hypersaline conditions may impair CYP in gill of salmonids (Lavado et al., 2009). Additional studies are needed to better understand the impact of environmental influences on CYP and other oxygenation pathways in anadromous and catadromous fish species.

The only metabolites observed for FTH and PTH in olfactory tissues were hydrolysis products. In contrast, biotransformation of CPF was not observed in olfactory tissues of either species. Olfactory tissues are critical targets for xenobiotics. Recent studies have indicated a direct link between olfactory impairment, behavior and likely population impacts of coho salmon (Sandahl et al., 2004; Sandahl et al., 2005; Tierney et al., 2010). The lack of CPF biotransformation in olfactory tissue suggests that alterations in this tissue due to CPF may not be caused by oxon formation or may occur by another unidentified metabolite formed in tissues outside of the olfactory bulbs or non-AChE-mediated pathway. Preliminary studies using microarray methods indicate that several signal transduction pathways may be involved in the response (Gallagher et al., submitted). Olfactory tissue does carry enzymes such as CYP known to oxygenate CPF (Matsuo et al., 2008), but it is unclear why these isoforms do not seem to be active. Hydrolysis of FTH and PTH in olfactory tissues was catalyzed primarily through NADPH-dependent and NADPH-independent processes. The latter reaction observed with FTH was similar between the species and would likely be catalyzed by microsomal carboxylesterases. The NADPH-dependent reaction was previously observed in trout and appears to be CYP-independent (Lavado et al., 2009). The NADPH-dependent reaction in coho salmon was much less than that observed in trout. Due to biomass limitations, CYP inhibitors were not used in the current study. Thus, it is unclear whether the same enzymatic phenomenon occurs in salmon. Additional studies are needed to characterize this hydrolytic reaction in both species and in multiple tissues.

In summary, hepatic biotransformation to oxon relative to hydrolytic metabolites largely explained species-specific differences in toxicity to OPs, but failed to correlate with chemical-chemical toxicities. Biotransformation in salmonids possessed similar apparent K_m , but enzymes in rainbow trout had higher velocities leading to greater catalytic efficiencies for oxon formation and subsequent toxicity. Thus, rainbow trout tend to be a conservative model for acute toxicity of OP in coho salmon. However, additional studies are needed with more structurally diverse compounds to better characterize the chemical-chemical differences in potency in each species.

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Figure 1.

Metabolites produced by biotransformation of fenthion (100 μ M) in microsomal fractions isolated from liver, gills and olfactory tissues from rainbow trout (*O. mykiss*) (A) and coho salmon (*O. kisutch*) (B). Data are presented as pmol/min/mg protein and as mean $\pm$ SD ($n=6-8$ individuals for liver and gills; $n=3$ replicates for each pooled sample for olfactory tissues). MMTP: 3-methyl-4-(methylthio)-phenol. bdl: below detection limit (<0.3 pmol/min/mg protein). Different letter indicates significant differences between tissues ($p<0.05$, One-way ANOVA, Tukey's test). * indicates significant difference between species ($p\leq 0.05$)

Figure 2. Metabolites produced by biotransformation of chlorpyrifos (100 μ M) in microsomal fractions isolated from liver, gills and olfactory tissues from rainbow trout (*O. mykiss*) (A) and coho salmon (*O. kisutch*) (B). Data are presented as pmol/min/mg protein and as mean $\pm$ SD (n=6–8 individuals for liver and gills; n=3 replicates for each pooled sample for olfactory tissues). *bdl*: below detection limit (<0.1 pmol/min/mg protein). Different letter indicates significant differences between tissues ($p < 0.05$, One-way ANOVA, Tukey's test). * indicates significant difference between species ($p \leq 0.05$)

Figure 3. Metabolites produced by biotransformation of parathion (50 μ M) in microsomal fractions isolated from liver, gills and olfactory tissues from rainbow trout (*O. mykiss*) (A) and coho salmon (*O. kisutch*) (B). Data are presented as pmol/min/mg protein and as mean $\pm$ SD (n=6–8 individuals for liver and gills; n=3 replicates for each pooled sample for olfactory tissues). *bdl*: below detection limit (<0.3 pmol/min/mg protein). Different letter indicates significant differences between tissues ($p < 0.05$, One-way ANOVA, Tukey's test). * indicates significant difference between species ($p \leq 0.05$)

Table 1

Kinetic characterization of fenthion, chlorpyrifos and parathion metabolites obtained from microsomal fractions isolated from liver from rainbow trout (*O. mykiss*) and coho salmon (*O. kisutch*) incubated with each pesticide¹.

Substrate Metabolite	Rainbow Trout			Coho salmon		
	K _m (μM)	V _{max} (pmol/min/mg protein)	V _{max} /K _m	K _m (μM)	V _{max} (pmol/min/mg protein)	V _{max} /K _m
Fenthion						
Fenoxon	115.7 ± 21.1	82.8 ± 4.6	0.72	120.3 ± 15.9	74.3 ± 9.3	0.61
S-fenthion sulfoxide	264.4 ± 38.2	173.4 ± 9.6	0.66	259.3 ± 43.2	82.2 ± 21.1**	0.32**
R-fenthion sulfoxide	293.3 ± 48.6	78.6 ± 5.2	0.27	273.8 ± 33.3	32.1 ± 2.2**	0.12**
MMTP (NADPH-independent)	292.0 ± 66.6	53.7 ± 4.8	0.18	321.0 ± 35.6	53.3 ± 8.2	0.16
MMTP (NADPH-dependent)	86.6 ± 37.8	199.2 ± 24.4	2.06	75.3 ± 28.3	113.1 ± 9.3*	1.50*
Chlorpyrifos						
Chlorpyrifos-oxon	145.3 ± 12.8	101.4 ± 3.8	0.70	145.3 ± 13.8	60.8 ± 2.3**	0.42**
3,5,6-Trichloropyridinol	143.5 ± 28.8	262.2 ± 20.9	1.82	141.8 ± 19.6	111.7 ± 6.1**	0.79**
Parathion						
Paraoxon	50.3 ± 11.8	218.9 ± 13.0	4.35	45.3 ± 8.2	132.5 ± 16.3**	2.92**
p-Nitrophenol	59.7 ± 8.7	106.0 ± 4.1	1.77	56.1 ± 6.9	75.4 ± 7.7*	1.34*

MMTP: 3-methyl-4-(methyltio)-phenol.

¹Data are presented as mean ± SD (n=6–8 individuals with 3 replicates for each one).

Significant differences between species indicated by * ($p < 0.05$; Student's *t* test) and ** ($p < 0.01$; Student's *t* test).

Table 2

Percentages of conversion and metabolite formation of each organophosphate insecticide in rainbow trout and coho salmon.

Substrate Metabolite	% Conversion with respect to the Total substrate amount in the incubation	Rainbow Trout	Coho salmon	
		%Metabolite formation	% Conversion with respect to the Total substrate amount in the incubation	% Metabolite formation
Fenthion				
Fenoxon	9.9 ± 0.5	14.1 ± 0.7	8.9 ± 1.1	20.9 ± 2.6
S-fenthion sulfoxide	20.8 ± 1.1	29.5 ± 1.6	9.8 ± 2.5	23.1 ± 5.9
R-fenthion sulfoxide	9.4 ± 0.6	13.3 ± 0.9	3.8 ± 0.3	8.9 ± 0.7
MMTP (NADPH-independent)	6.4 ± 0.6	9.0 ± 0.8	6.4 ± 0.9	15.1 ± 2.1
MMTP (NADPH-dependent)	23.9 ± 2.9	33.9 ± 4.1	13.5 ± 1.1	31.8 ± 2.6
Chlorpyrifos				
Chlorpyrifos-oxon	18.2 ± 0.7	27.8 ± 1.1	10.9 ± 0.4	35.1 ± 1.3
3,5,6-Trichloropyridinol	47.2 ± 3.7	72.2 ± 5.6	20.1 ± 1.1	64.8 ± 3.5
Parathion				
Paraoxon	52.5 ± 3.1	67.4 ± 3.9	31.8 ± 3.9	63.7 ± 7.8
p-Nitrophenol	25.4 ± 1.0	32.6 ± 1.3	18.1 ± 1.8	36.3 ± 3.6

MMTP: 3-methyl-4-(methyltio)-phenol.