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Glucuronidation and sulfonation, in vitro, of the major endocrine-active metabolites of methoxychlor in the channel catfish, *Ictalurus punctatus*, and induction following treatment with 3-methylcholanthrene

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Abstract

The organochlorine pesticide, methoxychlor (MXC), is metabolized in animals to phenolic mono- and bis-demethylated metabolites (OH-MXC and HPTE respectively) that interact with estrogen receptors and may be endocrine disruptors. The phase II detoxication of these compounds will influence the duration of action of the estrogenic metabolites, but has not been investigated extensively. In this study, the glucuronidation and sulfonation of OH-MXC and HPTE were investigated in subcellular fractions of liver and intestine from untreated, MXC-treated and 3-methylcholanthrene (3-MC)-treated channel catfish, *Ictalurus punctatus*. MXC-treated fish were given i.p. injections of 2 mg MXC/kg daily for 6 days and sacrificed 24 hr after the last dose. The 3-MC treatment was a single 10 mg/kg i.p. dose 5 days prior to sacrifice. In hepatic microsomes from control fish, the $V_{\max}$ value (mean $\pm$ S.D., $n=4$) for glucuronidation of OH-MXC was 270 ± 50 pmol/min/mg protein, higher than found for HPTE (110 ± 20 pmol/min/mg protein). For each substrate, the $V_{\max}$ values observed in intestinal microsomes were approximately twice those found in the liver. The K_m values for OH-MXC and HPTE glucuronidation in control liver were not significantly different and were 0.32 ± 0.04 mM for OH-MXC and 0.26 ± 0.06 mM for HPTE. The K_m for the co-substrate, UDPGA, was higher in liver (0.28 ± 0.09 mM) than intestine (0.04 ± 0.02 mM). Treatment with 3-MC but not MXC increased the $V_{\max}$ for glucuronidation in liver and intestine. Glucuronidation was a more efficient pathway than sulfonation for both substrates, in both tissues. The $V_{\max}$ values for sulfonation of OH-MXC and HPTE respectively in liver cytosol were 7 ± 3 and 17 ± 4 pmol/min/mg protein and in intestinal cytosol were 13 ± 3 and 30 ± 5 pmol/min/mg protein. Treatment with 3-MC but not MXC increased rates of sulfonation of OH-MXC and HPTE and the model substrate, 3-hydroxy-benzo(a)pyrene in both intestine and liver. Comparison of the kinetics of the conjugation pathways with those published for the demethylation of MXC showed that formation of the endocrine-active metabolites was more efficient than either conjugation pathway. Residues of OH-MXC and HPTE were detected in extracts of liver microsomes from MXC-treated fish. This work showed that although OH-MXC and HPTE could be eliminated by glucuronidation and sulfonation, the phase II pathways were less efficient than the phase I pathway leading to formation of these endocrine-active metabolites.

1. Introduction

The organochlorine pesticide 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane, methoxychlor (MXC) has been widely used to control a variety of insect pests. Although MXC is less environmentally persistent than the related organochlorine pesticide, DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), residues of MXC have been reported in soils, tissues of wildlife, and in household dust (Campoy et al., 2001; Rudel et al., 2003; Vorkamp et al., 2004; Wan et al., 2005). MXC metabolism by P450 isoforms both *in vitro* and *in vivo* have led to the identification of two major metabolites, formed by its sequential demethylation: the chiral mono-demethylated 2-(p-hydroxyphenyl)-2-(p-methoxyphenyl)-1,1,1-trichloroethane (OH-MXC) and the bis-demethylated 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) (Figure 1). MXC itself has negligible estrogenic activity in mammals, however both metabolites have been shown to interact with human estrogen and androgen receptors, HPTE being more potent than OHMXC (Kupfer and Bulger, 1987; Gaido et al., 1999; Gaido et al., 2000; Miyashita et al., 2004). Exposure of fish to MXC has been shown to induce vitellogenin synthesis (Schlenk et al., 1998; Hemmer et al., 2001) and affect development (Krisfalusi et al., 1998). Both OH-MXC and HPTE bound catfish estrogen receptors more tightly than unchanged MXC (Nimrod and Benson, 1997; Schlenk et al., 1998). Studies with largemouth bass estrogen receptors showed that parent MXC, OH-MXC and HPTE all acted as agonists with expressed largemouth bass ER-alpha, ER-beta-a and ER-beta-b receptors (Blum et al., 2007). The order of potency was HPTE>OH-MXC>MXC for ER-alpha and ER-beta-b, while for ER-beta-a MXC, OH-MXC and HPTE were of similar potency.

The demethylated metabolites of MXC, OH-MXC and HPTE, have phenolic hydroxyl groups that are potential substrates for the major phase II pathways, glucuronidation and sulfonation, catalyzed respectively by UDP-glucuronosyltransferase (UGT) and PAPS-sulfotransferase (SULT). Formation of glucuronide or sulfate conjugates of OH-MXC and HPTE would be expected to detoxify these endocrine-active metabolites, and facilitate their elimination. To date there have been very few studies of the phase II metabolism of OH-MXC or HPTE.

In vivo studies of MXC metabolism in goats and chickens showed that in both species, MXC was completely demethylated prior to elimination in bile, feces and urine (Davison et al., 1982; Davison et al., 1984). Glucuronide conjugates of the demethylated metabolites were identified by mass spectrometry, NMR and observation of unconjugated metabolites following glucuronidase treatment (Davison et al., 1982; Davison et al., 1984). No sulfate conjugates were reported. In a study conducted with precision cut liver slices from rat, mouse, Japanese quail, and rainbow trout, different metabolic profiles were observed from an identical 5 µM dose of MXC (Ohyama et al., 2004). Mouse and quail produced mostly OH-MXC-glucuronide and some HPTE-glucuronide, the rat formed HPTE-glucuronide and small amounts of a double conjugate of HPTE with sulfate and glucuronide. Slices from trout liver produced almost equal amounts of OH-MXC-glucuronide and HPTE-glucuronide. A study of the glucuronidation of single concentrations each of OH-MXC, 62.5 µM or HPTE, 31.3 µM, in human liver microsomes showed that both formed mono-glucuronides (Hazai et al., 2004). Up to three-fold differences in rates for each substrate were observed with three different human liver microsomal samples. Several UGT isoforms, UGT1A1, UGT1A3, UGT1A9 and UGT2B15 were shown to catalyze conjugation of OH-MXC and HPTE (Hazai et al., 2004). The different metabolic profiles for phase II metabolism of MXC in different species and even among individuals will result in varying lengths of exposure to the estrogenic metabolites and may result in individual or species-selective differences in response to identical exposure scenarios.

Pond-reared aquaculture species such as the channel catfish are at particular risk of exposure to agricultural chemicals, because they are often farmed in proximity to crop-producing fields.

Thus it is important to understand the factors affecting the formation and detoxication of endocrine-active xenobiotics in this species. In the channel catfish, biotransformation of MXC to its endocrine-active demethylated metabolites occurs readily in liver and intestinal microsomes (Schlenk et al., 1997; Stuchal et al., 2006). Treatment of catfish with a polycyclic aromatic hydrocarbon, 3-methylcholanthrene (3-MC) increased the rate of demethylation of MXC in liver and intestinal microsomes (Stuchal et al., 2006), suggesting that co-exposure to polycyclic aromatic hydrocarbons and MXC may increase the exposure of the fish to the estrogenic metabolites. In some fish, however, exposure to polycyclic aromatic hydrocarbons results in induction of UGT measured with p-nitrophenol as aglycone (Andersson et al., 1985), and there was a recent report that polycyclic aromatic hydrocarbons induce SULT activity towards 9-hydroxybenzo(a)pyrene in catfish (Gaworecki et al., 2004). If 3-MC also induces the glucuronidation and sulfonation of MXC metabolites, the net effect of co-exposure of fish to polycyclic aromatic hydrocarbons and MXC may be increased elimination and reduced overall exposure to OH-MXC and HPTE. The objectives of this study were to assess the relative contributions of glucuronidation and sulfonation to the phase II elimination of the endocrine-active metabolites of MXC, and to examine the effect of exposure to 3-MC or MXC on these pathways.

2. Materials and Methods

2.1 Chemicals

Methoxychlor was obtained from ICN Biomedical (Aurora, OH). OH-MXC and HPTE were prepared by boron tribromide-catalyzed demethylation of MXC as described previously (Stuchal et al., 2006). The OH-MXC and HPTE products were separated by silicagel column chromatography and further purified by re-crystallization from ether:heptane. Reverse phase HPLC with UV detection at 245 nm was used to verify the purity was >99% using a 25 cm × 4.6 mm C₁₈ Discovery column with a pore size of 5 μm (Supelco, Bellefonte, PA). The mobile phase was isocratic acetonitrile:water (70:30) at 1 mL/min. Uridine 5'-diphospho-α-D-glucopyranosuric acid (UDPGA), sodium salt, and 3-MC were obtained from Sigma Chemical Co. (St. Louis, MO). The 3'-phosphoadenosine-5'-phosphosulfate (PAPS) was obtained from Dr. S. S. Singer, University of Dayton, Ohio. The ¹⁴C-UDPGA (313 mCi/mmol) and ³⁵S-PAPS (1.82 or 2.67 Ci/mmol) were purchased from PerkinElmer (Boston, MA). The radiolabeled UDPGA was diluted with unlabeled material to a specific activity of 5 mCi/mmol for use in assays. The tetrabutylammonium sulfate (PIC® A) was obtained from Waters Corporation (Milford, MA). The 3-hydroxy-benzo(a)pyrene (3-OH-BaP) and benzo(a)pyrene-3-β-D-glucuronide were obtained from the NCI chemical carcinogen reference standard repository (Midwest Research Institute, Kansas City, MO). All other chemicals used were of the purest grade available and were purchased from commercial suppliers.

2.2 Animals and tissue preparation

Adult male and female channel catfish, body weight 1100 to 2700 g, were held in dechlorinated city water and maintained on a purified diet for at least two weeks before use in this study (James et al., 1997). Groups of four to eight fish were untreated, treated with MXC, 2 mg/kg by i.p. injection daily for six days prior to sacrifice on the seventh day, or treated with 3-MC, 10 mg/kg by i.p. injection on day 1 prior to sacrifice on day 4 or 5. Catfish were sacrificed as approved by the University of Florida Institutional Animal Care and Use Committee, and liver and intestinal mucosa removed as described previously (Stuchal et al., 2006). Cytosol and washed microsomal sub-cellular fractions were isolated by differential centrifugation and stored in aliquots at -80°C under nitrogen until use (James et al., 1997).

2.3 Glucuronidation assays, OH-MXC and HPTE

For the two demethylated MXC metabolites, a radiochemical method was used based on ion-pair extraction of the glucuronide conjugates (James et al., 1994). For each substrate, assay conditions were optimized for time, protein, ratio of Brij 58 to microsomal protein, and saturating concentrations of UDPGA. Assay tubes contained, in a volume of 0.08 mL, OH-MXC or HPTE (variable concentrations), 0.1 M Tris pH 7.6, 50 mM MgCl₂, liver microsomes (0.1 mg protein for OH-MXC, 0.2 mg protein for HPTE) or intestinal microsomes (0.05 mg protein for OH-MXC and HPTE). The microsomes were mixed with an optimal ratio of Brij 58 and placed on ice for 20 min before use. In all experiments, the OH-MXC and HPTE were added to tubes in ethanol solution (0.005 mL) and the ethanol was removed under nitrogen before addition of other components. Incubated blanks with no substrate and zero time blanks with substrate were included. The tubes were set in a 35°C shaking water bath for one minute. This temperature was selected so the results could be compared with previous studies of monooxygenase activity (Stuchal et al., 2006). Catfish monooxygenase activity continues to increase up to at least 40°C (Nyagode, 2007): activity at 35°C is approximately double that at 22°C. To start the reaction, ¹⁴C-UDPGA (normally 1 mM for liver and 300 μM for intestine) was added to each tube for a total volume of 0.1 mL. After zero minutes (blank) or 30 minutes (samples and blank with no added substrate) the reaction was stopped by the addition of 0.1 mL of a 1:1 mixture of 2.5% acetic acid and 0.25 mM PIC® A, 0.3 mL ice cold water, and 2 mL water-saturated ethyl acetate. The tubes were vortex-mixed and phases separated by centrifugation. The ethyl acetate layer, containing the tetrabutylammonium ion pair of the glucuronide conjugate, was transferred to clean tubes. Two more extractions were performed with 1 mL ethyl acetate, and the extracts pooled. Aliquots (1 mL) of each pooled ethyl acetate extract were mixed with Ecolume scintillation cocktail and counted on a Beckman Coulter (Fullerton, CA) LS 5000TD liquid scintillation counter. From the total extracted radioactivity, and the known specific radioactivity of ¹⁴C-UDPGA, the rate of glucuronidation was calculated as pmole product/min/mg protein.

K_m and V_{max} values for formation of OH-MXC-glucuronide and HPTE-glucuronide by liver and intestinal microsomes were determined by separately varying ¹⁴C-UDPGA and OH-MXC or HPTE. When varying ¹⁴C-UDPGA, a final concentration of 1 mM OH-MXC was incubated with 0.1 M Tris pH 7.6, 50 mM MgCl₂, liver microsomes (0.1 mg protein) or intestinal microsomes (0.05 mg protein). With liver microsomes, final ¹⁴C-UDPGA concentrations of 100, 200, 300, 500, and 750 μM were employed to determine K_m values. For intestinal microsomes, final ¹⁴C-UDPGA concentrations of 10, 25, 50, 100, and 300 μM were used. To examine the kinetics of glucuronidation of OH-MXC and HPTE for control and MXC treated fish, final OH-MXC or HPTE concentrations of 50, 100, 200, 300, 500 and 750 μM were employed to determine K_m values. For 3-MC treated fish, final OH-MXC and HPTE concentrations of 100, 200, 400, 600, and 750 μM were used. In all studies of aglycone kinetics, the concentration of ¹⁴C-UDPGA was 1mM for liver microsomes and 300 μM for intestinal microsomes.

To verify that the assay method was effectively extracting the tetrabutylammonium ion-pairs of the ¹⁴C-glucuronide conjugates of OH-MXC and HPTE into ethyl acetate and leaving the unreacted co-substrate, ¹⁴C-UDPGA, in the aqueous phase, and to demonstrate glucuronide product formation, separate portions of the pooled ethyl acetate extracts from incubations with each substrate were evaporated to dryness under nitrogen. The residues were dissolved in 0.1 mL of Tris buffer, pH 6.0, or Tris buffer, pH 6.0 containing 7,500 units per mL of β-glucuronidase and incubated at 37°C for 14 hr, at which time 0.1 mL ethanol was added. Following centrifugation, portions of the treated ethyl acetate extract residues (0.04 mL) as well as portions of the aqueous phase from the incubations (0.005 mL) were applied to the pre-adsorbent phase of LK5DF plates (Whatman, Florham Park, NY). Plates were developed in a

solvent system containing 1-butanol:acetone:acetic acid: ammonium hydroxide:water, 50:25:9:0.75:15 by volume. Dried plates were placed in a Packard Instant Imager to visualize and quantitate the radioactivity by electronic autoradiography.

2.4 Glucuronidation assay, 3-OH-BaP

The glucuronidation of 3-OH-BaP was assayed by monitoring the fluorescence of the product, BaP-3-glucuronide, as described previously (James et al., 1997). The effect of OH-MXC on the glucuronidation of 3-OH-BaP was examined. Varying concentrations of OH-MXC and a single concentration of 3-OH-BaP, 0.5 μ M, were added to assay tubes from methanol solution and the solvent removed under nitrogen. The remaining assay components, 0.1M Tris-Cl buffer, pH 7.6, 5 mM $MgCl_2$, 0.025 mg hepatic microsomes and water to 0.4 mL were added and the tubes placed in a water bath at 35 °C. The reaction was started by adding 0.1 mL UDPGA, final concentration 1 mM. After 5 min the reaction was stopped by adding 2 mL methanol. Following precipitation of the protein, 2 mL clear supernatant was added to 0.5 mL 1M NaOH and the fluorescence measured at excitation/emission wavelengths of 300/421 nm. A standard curve of authentic BaP-3-glucuronide was used to calculate product formation.

2.5 Sulfotransferase assays, OH-MXC and HPTE

An ion-pair extraction method similar to that described above for glucuronosyltransferase was used to assess the sulfonation of OH-MXC and HPTE, and was optimized for channel catfish hepatic and intestinal cytosol with each substrate. Briefly, various concentrations of OH-MXC or HPTE dissolved in methanol were added to assay tubes and the solvent evaporated under nitrogen. A reaction mixture, 80 μ L, consisting of 0.4% BSA, 50 mM Tris-HCl (pH 7.0), 0.05 mg cytosolic protein and water were added to the tubes which were then placed in a 35°C water bath. The reaction was started by adding 20 μ L ^{35}S -PAPS, 1.5 μ Ci, with unlabeled PAPS to a final saturating concentration of 20 μ M. Blanks contained no OH-MXC or HPTE. Tubes were incubated for 20 min with gentle shaking then the reaction was terminated by adding 400 μ L of an ice-cold 1:1:6 (v/v) mixture of 2.5% acetic acid: 0.2 mM PIC® A: water. The sulfated products were extracted three times into 1.5 mL water-saturated ethyl acetate, by vortexing then centrifuging for 10 min to separate the phases. The organic phases from each extraction were pooled for each tube. The total amount of sulfate conjugates per sample was determined by measuring, in duplicate, the radioactive counts in a 500 μ L aliquot of the ethyl acetate phase, extrapolating for the total volume of extract and converting the counts into pmole from the known, decay-corrected, specific radioactivity of the ^{35}S -PAPS. The remaining ethyl acetate extract was dried with anhydrous sodium sulfate, 2 mL transferred to a clean tube, the solvent evaporated to dryness under nitrogen and the residue dissolved in 40 μ L of methanol. This solution was then spotted either on RP-18F254S reverse-phase TLC plates (Merck, Darmstadt, Germany) and developed using a 4:1 methanol:water (v/v) solvent system as previously described (Sacco and James, 2005) or on the pre-adsorbent lane of LK5D normal-phase TLC plates (Whatman, Florham Park, NJ) and developed using a solvent system of 1:1:4 (v/v) methanol:ethylacetate:dichloromethane containing 0.6mL per 60 mL of a 5 mM aqueous solution of tetrabutylammonium hydrogen sulfate. Separated radioactive spots were visualized and quantified by autoradiography using a Packard Instant Imager (Meriden, CT). The identity of the sulfated product from OH-MXC and HPTE was determined by comparing R_f values with those determined previously (Sacco and James, 2005), or by comparing incubations containing substrate with incubations containing no substrate. The percentage fraction of radioactivity that represented the sulfate conjugate of the OH-MXC or HPTE was multiplied by the known total amount of sulfate conjugate formed (obtained by direct counts of the ethyl acetate extracts as described above) to quantify product formation in each sample.

2.6 Sulfotransferase assay, 3-OH-BaP

A fluorescence method was used to assay the sulfonation of 3-OH-BaP as described previously (James et al., 1997; James et al., 2001). The kinetics of 3-OH-BaP sulfonation were examined in hepatic and intestinal cytosol from control and 3-MC-induced fish by varying the concentration of 3-OH-BaP in assays from 20 to 400 nM in the presence of a constant concentration of PAPS, 20 μ M. The effect of varying concentrations of OH-MXC, 20 to 200 μ M on 3-OH-BaP sulfonation was examined with 0.1 μ M 3-OH-BaP as substrate.

2.7 HPLC detection of MXC metabolites in channel catfish liver microsomes

Microsomes from 4 control and 7 MXC-treated fish were examined for residues of MXC by extraction and HPLC. Control liver microsomes (10 mg), MXC treated liver microsomes (10 or 20 mg) and control liver microsomes (10 mg) spiked with 0.25 mg each of MXC, OH-MXC and HPTE were placed in separate test tubes. Acetic acid (30%) was added to a pH of 5. The microsomes were sonicated with a hand sonicator for a twenty second burst and then extracted three times with 2 mL ethyl acetate per extraction. The pooled ethyl acetate fractions were dried with anhydrous sodium sulfate, evaporated under nitrogen and reconstituted in 1 mL 70:30 acetonitrile/water. Samples were analyzed by HPLC with UV detection at 245 nm on a C₁₈ reverse phase column with a 5 μ m pore size, 25 cm $\times$ 4.6 mm Discovery C₁₈ preceded by a 2 cm $\times$ 4 mm C₁₈ guard column (Supelco, Bellefonte, PA). The mobile phase was isocratic acetonitrile:water (70:30) at 1 mL/min.

2.8 Data analysis

Using Prism 4 software (GraphPad Software, San Diego, CA, USA), kinetic parameters were determined by fitting data to the appropriate equation; either the Michaelis-Menten equation (1) or the Hill equation (2). Eadie-Hofstee plots were used to diagnose any atypical kinetics.

$$v = V_{\max} [S] / (K_m + [S]) \quad (1)$$

$$v = V_{\max} [S]^h / (S_{50}^h + [S]^h) \quad (2)$$

The efficiency of an enzyme was calculated by dividing $V_{\max}$ by K_m . Parameters were compared within treatments and between organs by ANOVA analysis, followed by the Tukey's multiple comparison test, or by Student's t-tests as were considered appropriate.

3. Results

3.1 Glucuronidation of OH-MXC and HPTE

3.1.1 Assay validation and conditions—The tetrabutylammonium ion-pairs of either the OH-MXC- or the HPTE-¹⁴C-glucuronide conjugates were separated from unreacted ¹⁴C-UDPGA by extraction into ethyl acetate, and the product quantitated from the amount of radiolabel extracted. Analysis by TLC of the ethyl acetate and aqueous phases from assays verified that the ion-pairs of the OH-MXC-glucuronide and the HPTE-glucuronide were quantitatively extracted into ethyl acetate, with no glucuronide conjugate of OH-MXC or HPTE detected in the aqueous phases (Figure 2, lanes 1 and 6). The unreacted cosubstrate, UDPGA, remained in the aqueous phase, and no unreacted UDPGA was found in the ethyl acetate phase (Figure 2, aqueous phases in lanes 3 and 8, ethyl acetate phases in lanes 1 and 6). The OH-MXC-glucuronide and HPTE-glucuronide were shown to be susceptible to hydrolysis by β -glucuronidase (Figure 2, lane 2 for OH-MXC-glucuronide and lane 7 for HPTE-glucuronide). If OH-MXC or HPTE were incubated with ¹⁴C-UDPGA in the absence of microsomes, no product was formed and no ¹⁴C was extracted into the ethyl acetate phase from ¹⁴C-UDPGA (data not shown). When hepatic or intestinal microsomes were incubated with ¹⁴C-UDPGA in the absence of substrate, very small amounts of glucuronide were formed

(Figure 2, lane 4), as discussed further in section 3.2 below. Rates were corrected for the small amount of glucuronide formed in the absence of added substrate.

The formation of OH-MXC- glucuronide in channel catfish was linear up to 45 min and 0.1 mg protein per 0.1 mL assay volume in the liver, and 45 min and 0.2 mg protein in the intestine. In subsequent assays, incubation times of 30 min were used for liver and intestine, with 0.1 mg protein for liver and 0.05 mg for intestine. The glucuronidation of HPTE was linear up to 60 min and 0.2 mg protein per 0.1 mL assay volume in the channel catfish liver microsomes and 45 min and 0.3 mg protein in the intestinal microsomes. In further studies of HPTE glucuronidation, incubation times of 60 min were used for liver and intestine, with 0.2 mg protein for liver and 0.05 mg for intestine. The optimal ratio of Brij:microsomal protein for maximum glucuronidation activity with both substrates was 0.2:1 for liver and 0.5:1 for intestine, and these ratios were used for subsequent studies.

3.1.2 Kinetic studies—To ensure that a saturating amount of ^{14}C -UDPGA was used for examining the kinetics of the aglycone, the apparent K_m , and V_{max} values for UDPGA were determined for both intestinal and hepatic microsomes, with OH-MXC as substrate (Table 1). The data fit the Michaelis-Menten equation for both tissues. The intestinal K_m for UDPGA was significantly lower ($p < 0.01$) than the K_m with liver microsomes. For all subsequent assays, including those with HPTE, the amounts of UDPGA used were saturating for the respective tissue and were 1 mM in liver and 0.3 mM in intestine.

The kinetics of glucuronidation of OH-MXC were examined in hepatic and intestinal microsomes from control and treated catfish (Table 2). The variation of rate with substrate concentration fit the Michaelis-Menten equation for all samples. Liver microsomes from 3-MC-treated catfish had significantly higher apparent V_{max} values ($p < 0.05$) than the control or MXC-treated fish, but similar K_m values, resulting in significantly higher glucuronidation efficiency ($P < 0.01$). For the intestine, control and MXC-treated fish had similar kinetic parameters, whereas 3-MC-treated fish had significantly higher apparent K_m ($p < 0.05$) and V_{max} ($p < 0.01$) values than control fish intestinal microsomes. A comparison of the intestinal and hepatic data revealed that for each treatment group, the intestine displayed a significantly higher apparent V_{max} ($p < 0.05$) than the corresponding fish liver.

The kinetics of glucuronidation of HPTE were determined in channel catfish liver microsomes only (Table 3). As was found with OH-MXC, the kinetics of HPTE fit the Michaelis-Menten equation. The 3-MC-treated catfish had a significantly higher apparent K_m ($p < 0.01$) and V_{max} ($p < 0.05$) than the control fish values. Kinetic constants were not determined for HPTE glucuronidation in the intestine due to the limited yield of intestinal microsomes from each fish. There was no significant difference ($p > 0.05$) between control and treated catfish in the rates found at 1 mM HPTE, although there was considerable individual variability in the control and 3-MC-treated groups (Table 3). A comparison of the intestinal and hepatic data revealed that the fish intestinal microsomes displayed significantly higher rates at 1 mM HPTE ($p < 0.05$) than the apparent V_{max} values for the corresponding fish liver microsomes.

Comparison of the kinetics of glucuronidation of OH-MXC and HPTE in liver microsomes from control and MXC-treated channel catfish showed no differences in apparent K_m values ($p > 0.05$) but significantly different apparent V_{max} values ($p < 0.001$) for the two MXC metabolites (Tables 2 and 3). The same comparison with 3-MC-treated catfish showed differences ($p < 0.05$) in both K_m and V_{max} values for the two aglycones. In all treatment groups, the V_{max} values for HPTE glucuronidation were significantly lower than for OH-MXC glucuronidation in liver ($p < 0.05$). In intestinal microsomes, the rate of conjugation of HPTE, measured at 1 mM HPTE, was about half the intestinal apparent V_{max} value for OH-MXC ($p < 0.05$).

3.2 Residues in hepatic microsomes from MXC-treated fish

There was a possibility that residual unconjugated metabolites of MXC may be present in microsomes from MXC-treated fish. Rates of glucuronide formation without addition of substrate were compared in liver microsomes from MXC-treated and control or 3-MC-treated catfish. MXC-treated fish produced an average of 11.1 ± 7.9 pmoles glucuronide/min/mg protein (mean $\pm$ S.D., $n = 7$), significantly more ($p < 0.05$) than control fish, 4.2 ± 0.8 ($n = 5$) but not significantly different from 3-MC-treated fish, 5.2 ± 2.3 ($n = 4$). Individual MXC-treated fish showed greater variability than controls in the amount of glucuronide produced from hepatic microsomal substrate blanks (Figure 3). By subtracting the average rate in the absence of substrate in hepatic microsomes from control fish from each rate measured in the absence of substrate in microsomes from the MXC-treated fish, and substituting the resulting rate into the Michaelis-Menten equation using the K_m and V_{max} values of control fish obtained for OH-MXC or HPTE (Tables 2 and 3), we estimated that the amount of glucuronide formed by liver microsomes from MXC-treated fish in the absence of substrate was consistent with residues of 1-17 μ M OH-MXC or 2-38 μ M HPTE in assays. These values were used as a rough guide to spike control liver microsomes with MXC, OH-MXC and HPTE as standards. HPLC of microsomal extracts revealed several UV₂₄₅ peaks in MXC treated microsomes that were not observed in the control microsomes (Figure 4). The chromatogram in Figure 4B is from the microsomes that showed the highest rate of glucuronidation in the absence of substrate. When compared with the control microsomes spiked with standards of MXC and its metabolites (Figure 4C), the MXC treated microsomes showed a clear peak at the retention time of HPTE, a smaller peak at the retention time of OH-MXC and little or no evidence of a peak at the retention time of unmetabolized MXC. No attempt was made to quantitate the amounts of unconjugated metabolites in this study.

3.3 Sulfonation of OH-MXC and HPTE

3.3.1 Assay conditions and validation—As shown previously for other substrates (Varin et al., 1987; Wang et al., 2004), the ion-pair extraction method was effective in separating the sulfate conjugates of OH-MXC or HPTE, which were extracted into the ethyl acetate phase, from the unreacted PAPS, which remained in the aqueous phase (data not shown). In validating the assay, it was found that in the presence or absence of added substrate both hepatic and intestinal cytosol formed several sulfate conjugates which were extracted as ion-pairs into ethyl acetate (Figure 5, lane 1). Thus, TLC of the ethyl acetate extracts was routinely performed to separate the sulfate conjugates of OH-MXC and HPTE from those formed from components of cytosol. Figure 5 lane 2 shows that the formation of the sulfate conjugates from hepatic cytosol components was suppressed by the addition of a high concentration (800 μ M) of OH-MXC.

Sulfonation of OH-MXC and HPTE by hepatic or intestinal cytosol fractions was linear for up to 6 hr incubation time. Formation of product was linear with up to 0.2 mg protein per 0.1 mL incubation volume for both tissues. An incubation time of 1 hour min with 0.05 mg protein was selected for most assays.

3.3.2 Kinetic studies—The kinetics of sulfonation of OH-MXC and HPTE were examined in hepatic and intestinal cytosol fractions from control and treated fish, with TLC separation of products. The rates of formation of the sulfate conjugates at different concentrations of substrate fit the Michaelis-Menten equation and the results are shown in Tables 4 and 5. Rates and efficiencies of sulfonation of both OH-MXC and HPTE were lower than glucuronidation of these substrates. Within a treatment group, intestine showed higher apparent V_{max} values than liver ($p < 0.05$). Treatment with 3-MC increased the apparent V_{max} approximately two-fold and the efficiency of sulfonation of OH-MXC almost three-fold ($p < 0.05$) in liver and intestine. For HPTE, apparent V_{max} was increased by 3-MC treatment in both tissues ($p < 0.05$),

but in liver, because apparent K_m was also increased, there was no change in efficiency of HPTE sulfonation ($p>0.05$). Treatment with MXC affected the K_m of HPTE sulfonation in liver ($p<0.05$), but did not affect V_{max} .

For comparison, the kinetic parameters of sulfonation of 3-OH-BaP were examined in the same fish. For most samples, the kinetic curves with 3-OH-BaP fit both the Michaelis-Menten and Hill plots (no significant deviation from model, as assessed by Graph-Pad software). Some samples fit only the Hill plot, and for all fish cytosol fractions, the data fit the Hill plot better than the Michaelis-Menten equation. Hill plot parameters are shown in Table 6. Treatment with 3-MC increased the apparent V_{max} and efficiency of sulfonation of 3-OH-BaP in both liver and intestine ($p<0.05$). As was observed with OH-MXC and HPTE as substrates, sulfonation of 3-OH-BaP was higher in intestine than liver.

3.4 Inhibition of glucuronidation and sulfonation by OH-MXC

To assess the inhibitory potency of OH-MXC, varying concentrations of OH-MXC were added to assays to measure the glucuronidation or sulfonation of 3-OH-BaP. OH-MXC inhibited the glucuronidation of 3-OH-BaP in a concentration-dependent manner (Figure 6). The IC_{50} was $44 \pm 9 \mu M$ (mean $\pm$ S.D., $n=4$). The ability of OH-MXC to inhibit 3-OH-BaP sulfonation was examined, however OH-MXC was a very weak inhibitor with IC_{50} values of $170 \pm 10 \mu M$ (data not shown).

4. Discussion

4.1 Assay method

As has now been shown with several xenobiotic substrates that have a single phenolic group and no other polar substituents (Varin et al., 1987; James et al., 1994; Wang et al., 2004; Sacco and James, 2005), addition of an ion-pair reagent in the assay stop solution, followed by extraction of the conjugate ion pair into ethyl acetate, is a rapid method that can be used successfully to assay rates of glucuronidation and sulfonation of suitable substrates. Extraction alone was suitable for measuring rates of glucuronidation of OH-MXC and HPTE, since background rates of glucuronide formation in microsomes from control or 3-MC-treated fish in the absence of added substrate were low, typically between 1 and 5% of the apparent V_{max} values in the presence of OH-MXC or HPTE. For studying sulfonation, however, it was necessary to analyze the extracts by TLC to separate the xenobiotic-sulfate conjugates. This was because OH-MXC and HPTE were very poor substrates for sulfonation, so that sulfonation of endogenous components was not negligible compared to sulfonation of the xenobiotics, and because both compounds inhibited the sulfonation of components in cytosol in dose-dependent fashion (Figure 5 and data not shown). The nature of the endogenous substrates for glucuronidation and sulfonation is not known, but may include steroid hormones and related compounds.

4.2 Kinetics of glucuronidation of OH-MXC and HPTE

The UDPGA dependency was studied for the glucuronidation of OH-MXC. It was found that a higher concentration of UDPGA was needed for maximal activity with liver microsomes compared with intestinal microsomes (Table 1). The concentration of UDPGA found in liver tissue from untreated catfish was 0.14 mM (James et al., 2007), half the measured K_m for OH-MXC glucuronidation in liver. This suggests that the physiologically available concentration of UDPGA is insufficient to support maximal glucuronidation in the liver. In contrast, the concentration of UDPGA in intestinal mucosa, 0.17 mM (James et al., 2007) was four times higher than the K_m for UDPGA in intestinal microsomes, indicating that the physiological concentration of UDPGA is such that glucuronidation should proceed at close to saturating rates in the intestine.

The kinetic data for glucuronidation of OH-MXC and HPTE in the liver showed similar aglycone K_m values for the two MXC metabolites, in the high μM range. K_m values in this range are not unusual for xenobiotic aglycones (King et al., 2000), suggesting a low affinity of these substrates for glucuronidation, however these K_m values are considerably higher than the 1 μM K_m value for 3-hydroxy-benzo(a)pyrene found with channel catfish intestinal microsomes (James et al., 2001). Comparison of the V_{max} values for OH-MXC and HPTE shows that V_{max} is higher for OH-MXC, and suggests that OH-MXC will be more readily glucuronidated than HPTE in the catfish liver. This may be important in the context of toxicity, since HPTE is a more potent xenoestrogen than OH-MXC (Schlenk et al., 1998; Blum et al., 2007). Although we were not able to determine kinetic data for both substrates in the intestine, the glucuronidation of OH-MXC and HPTE showed higher maximal rates in the intestine than the liver. This suggests that if catfish ingest food containing residues of OH-MXC or HPTE, there is likely to be pre-systemic glucuronidation of these residues in the intestine, which may reduce the systemic uptake of these contaminants by the catfish. The only other fish species for which phase II metabolism of MXC has been reported, the rainbow trout, produced glucuronide conjugates of both OH-MXC and HPTE in precision-cut liver slices incubated at 15°C, and these were the major metabolites found (Ohyama et al., 2004).

4.3 Kinetics of sulfonation of OH-MXC and HPTE

The maximal rates of sulfonation of OH-MXC and HPTE were very low in both the intestine and the liver, suggesting that the catfish sulfotransferase enzymes cannot readily sulfonate these compounds. The K_m values for sulfonation were in the high μM range, evidence that the affinity of the catfish sulfotransferase enzymes for these substrates was low. The V_{max} values for microsomal glucuronidation were more than 10-fold higher than V_{max} values for cytosolic sulfonation, while the K_m values for sulfonation and glucuronidation of each substrate were of the same order of magnitude (Tables 2-5). Thus, even at low concentrations of OH-MXC or HPTE, glucuronidation will probably be the predominant phase II conjugation pathway in catfish. This has been shown for at least one other substrate in the catfish: benzo(a)pyrene-7,8-dihydrodiol was a very poor substrate for sulfonation compared with glucuronidation in intestinal cytosol or microsomes from the channel catfish (van den Hurk and James, 2000). Studies of benzo(a)pyrene-7,8-dihydrodiol metabolism in an isolated perfused channel catfish liver confirmed that although glucuronides of both hydroxyl groups were identified, no sulfate conjugates were formed (James et al., 2004).

4.4. Induction of sulfonation and glucuronidation

An important finding in the context of environmental exposure to mixtures of chemicals was the finding that treatment with a single 10 mg/kg dose of 3-MC, 5 days before sacrifice of catfish, caused an increase in V_{max} values for sulfonation and glucuronidation of both OH-MXC and HPTE in liver and intestine. Previous studies showed that the V_{max} for cytochrome P450-dependent monooxygenation of MXC was increased in 3-MC-induced catfish, compared with untreated controls (Stuchal et al., 2006), so the finding that V_{max} values for both phase II pathways were increased suggests that catfish co-exposed to MXC and polycyclic aromatic hydrocarbons may exhibit an overall increased biotransformation of MXC, and perhaps more rapid elimination. The finding that glucuronidation was increased by 3-MC treatment was not unexpected, as there are other reports of induction of glucuronidation by polycyclic aromatic hydrocarbons, both in fish, including catfish (Andersson et al., 1985; Clarke et al., 1992; Gaworecki et al., 2004) and in mammals (Bock et al., 1990). The 3-MC-induced increases in sulfotransferase activities of OH-MXC and HPTE, both very poor substrates for sulfonation, prompted a study of the effect of 3-MC on the sulfonation of a better substrate, 3-OH-BaP. The V_{max} for sulfonation of 3-OH-BaP was increased by 3-MC treatment more than 4-fold in liver and 2-fold in intestine, suggesting induction of one or more forms of sulfotransferase in catfish liver and intestine. At least one form of phenol sulfotransferase has been purified from

catfish liver and intestine (Tong and James, 2000). The purified enzymes were very efficient catalysts of 3-OH-BaP sulfonation, exhibiting K_m values of 0.05 and 0.07 μM in liver and intestine, respectively, and V_{max} values of 192 and 218 nmol/min/mg protein. Given the similarity of the S_{50} values found in the present study with cytosol (Table 6) to the K_m values observed for the purified sulfotransferase, it is possible that 3-MC induced this enzyme in the catfish liver and intestine. There is preliminary evidence for additional forms of sulfotransferase in the catfish liver (Merritt and James, 2005). Induction of sulfotransferase by polycyclic aromatic hydrocarbons has not been shown in mammalian species, indeed there is evidence that exposure to polycyclic aromatic hydrocarbons or 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin downregulates SULT1A1 and SULT2A in rats (Runge-Morris and Kocarek, 2005). In fish, however, treatment of catfish or mummichog with 3-MC or environmental exposure of mummichog to polycyclic aromatic hydrocarbons was shown to induce sulfotransferase measured with 9-hydroxybenzo(a)pyrene (Gaworecki et al., 2004). Further investigation of the regulatory regions of catfish sulfotransferase genes will be needed to determine the reason for the increase in activity of sulfotransferase with OH-MXC, HPTE and 3-OH-BaP in 3-MC-treated catfish.

4.5 Comparisons of conjugation and demethylation pathways

In the environment, catfish are likely to be exposed to parent MXC through contact with run-off from contaminated soil, contact with contaminated sediment, and ingestion of MXC-exposed prey. They may be additionally exposed to the demethylated metabolites through ingestion of MXC-exposed prey organisms that have biotransformed MXC. Recent studies have reported parent MXC concentrations of 0.7 ng/g in seafood from Southern China (Guo et al., 2007), between <0.35 ng/g (limit of detection) and 2 ng/g in freshwater fish captured in the Colorado river (Hinck et al., 2007), between 8 and 17 ng/g in alligator eggs from central Florida lakes (Rauschenberger et al., 2007) and 534 ng/g in fat from crocodile scute in Costa Rica (Rainwater et al., 2007). MXC was found in 5 of 36 soil samples taken from agricultural areas in British Columbia, and the average MXC concentration in these samples was 0.23 mg/kg (Wan et al., 2005). Concentrations of MXC metabolites have not been reported from field studies, but it is likely that demethylated metabolites will be present in MXC-exposed wildlife. The present study showed that glucuronidation of OH-MXC and HPTE proceeded more rapidly than sulfonation, so that even at low concentrations of these MXC metabolites, glucuronidation will probably be the predominant phase II conjugation pathway in catfish. In contrast to the high K_m values for conjugation, the K_m values for demethylation of MXC in control, MXC-treated and 3-MC-treated fish ranged from 3 to 6 μM , and demethylation proceeded readily with efficiencies of 40 $\mu\text{L}/\text{min}/\text{mg}$ in liver microsomes from control and 3-MC-treated catfish (Stuchal et al., 2006). This study showed that the efficiencies of the conjugation pathways were less than 5 $\mu\text{L}/\text{min}/\text{mg}$ for glucuronidation and less than 1 $\mu\text{L}/\text{min}/\text{mg}$ for sulfonation. The K_m values for both conjugation pathways were considerably higher than expected concentrations of OH-MXC and HPTE produced from MXC metabolism in fish exposed environmentally to the pesticide, suggesting that detoxication of these endocrine-active metabolites through conjugation may be relatively inefficient in the channel catfish. Our finding that microsomes isolated from the livers of channel catfish exposed to MXC contained residues of unconjugated OH-MXC and HPTE (Figure 4) further suggested relatively inefficient conjugation of these MXC metabolites.

The possibility that OH-MXC could inhibit glucuronidation and sulfonation was examined, since studies of sulfonation showed that high concentrations of OH-MXC inhibited the sulfonation of endogenous components in cytosol. With 3-OH-BaP as substrate, OH-MXC was only a weak inhibitor of glucuronidation or sulfonation. It is unlikely that concentrations of OH-MXC in MXC-exposed fish will be high enough to significantly inhibit conjugation of other substrates.

In summary, this work has demonstrated that the phase I demethylated metabolites of MXC are substrates for glucuronidation and sulfonation in the channel catfish liver and intestine and that glucuronidation proceeds more rapidly than sulfonation. Sulfonation and glucuronidation of OH-MXC and HPTE exhibit higher maximal rates in the intestine than the liver. While both pathways are induced following exposure to the polycyclic aromatic hydrocarbon, 3-MC, these phase II pathways are less efficient than the demethylation pathway leading to their formation.

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Figure 1.
Pathways of MXC metabolism

Figure 2.

TLC of ethyl acetate extracts and aqueous phases following incubation of hepatic microsomes with OH-MXC, no substrate or HPTE. The ethyl acetate extracts were evaporated to dryness under nitrogen and the residues reconstituted in ethanol as described in the methods section. Lane 1, ethyl acetate extract of an incubation with OH-MXC, showing the glucuronide conjugate; lane 2, β -glucuronidase-treated ethyl acetate extract from an incubation with OH-MXC, showing hydrolysis of the glucuronide conjugate; lane 3, aqueous phase from an incubation with OH-MXC, showing UDPGA but no glucuronide conjugate of OH-MXC; lane 4, ethyl acetate extract from an incubation with no substrate, showing a very faint band with similar retention to glucuronide conjugates; lane 5, aqueous phase from incubations with no

substrate, showing only unreacted UDPGA; lane 6, ethyl acetate extract from an incubation with HPTE, showing the glucuronide conjugate; lane 7, β -glucuronidase-treated ethyl acetate extract from an incubation with HPTE, showing hydrolysis of the glucuronide; lane 8, aqueous phase from an incubation with HPTE, showing unreacted UDPGA but no glucuronide conjugate.

Figure 3.

Glucuronidation of endogenous components in microsomes. Microsomes were incubated with ^{14}C -UDPGA in the absence of exogenous aglycone. Compared with microsomes from control catfish, microsomes from MXC-treated catfish showed higher formation of glucuronides in the absence of exogenous substrate, $p < 0.05$. Microsomes from 3-MC-treated fish were not different from controls, $p > 0.05$.

Figure 4.

HPLC of extracts of microsomes, with UV₂₄₅ detection. Panel A shows an extract from control microsomes, panel B an extract of microsomes from MXC-exposed fish and panel C an extract of control microsomes spiked with standards for MXC, OH-MXC and HPTE.

Figure 5.

Radiochromatogram showing TLC separation of the products of incubation of ^{35}S -PAPS with hepatic cytosol in the presence and absence of exogenous substrate. Arrows indicate the sulfate conjugates. Lane 1 shows sulfate conjugates formed from endogenous compounds present in hepatic cytosol; lane 2 shows the sulfate conjugate formed by incubation of hepatic cytosol with OH-MXC, 800 μM ; lane 3 shows sulfate conjugates formed in the presence of OH-MXC, 50 μM , and hepatic cytosol; and lane 4 shows sulfate conjugates formed by incubation HPTE, 100 μM , with hepatic cytosol and ^{35}S -PAPS.

Figure 6. Inhibition of glucuronidation of 3-OH-BaP by OH-MXC in hepatic microsomes from control catfish. The sigmoidal dose-response curve shown is typical of results with catfish hepatic microsomes.

Table 1

Apparent kinetic values for UDPGA in the glucuronidation of OH-MXC, 1 mM in intestinal and hepatic microsomes

Tissue	K_m μ M	V_{max} pmol/min/mg protein
Liver	280 ± 92	248 ± 36
Intestine	$39 \pm 16^*$	363 ± 189

Values shown are mean $\pm$ SD, n=4* Intestine significantly different from liver, $p < 0.01$

Table 2

Apparent kinetic values for glucuronidation of OH-MXC in hepatic and intestinal microsomes from control and treated catfish. The concentration of UDPGA was 1 mM for liver and 0.3 mM for intestine

Tissue	Treatment	K _m μ M	V _{max} pmol/min/mg	Efficiency μ L/min/mg
Liver	Control	324 $\pm$ 43	269 $\pm$ 49	0.83 $\pm$ 0.11
	MXC	224 $\pm$ 48	269 $\pm$ 22	1.25 $\pm$ 0.21
	3-MC	234 $\pm$ 62	399 $\pm$ 90 ^a	1.73 $\pm$ 0.27 ^a
Intestine	Control	175 $\pm$ 30	536 $\pm$ 144 ^b	3.05 $\pm$ 0.51 ^b
	MXC	191 $\pm$ 15	484 $\pm$ 158 ^b	2.49 $\pm$ 0.66 ^b
	3-MC	309 $\pm$ 87 ^c	1026 $\pm$ 207 ^{b,c}	3.42 $\pm$ 0.45 ^b

Values are mean $\pm$ S.D., n=4 per treatment.

MXC: catfish were treated ip with 2 mg/kg MXC daily for 6 days and sacrificed on day 7

3MC: catfish were treated ip with a single injection of 10 mg 3-MC and sacrificed after 4-5 days.

^aSignificantly different from liver control, p<0.05

^bIntestine significantly higher than corresponding liver value, p<0.05

^cSignificantly different from intestine control, p<0.05

Table 3

Apparent kinetic values obtained for HPTE glucuronidation in channel catfish liver, and rate at 1 mM HPTE in intestine, for control, methoxychlor treated, and 3-methylcholanthrene treated catfish. The concentration of UDPGA was 1 mM for liver and 0.3 mM for intestine

	K_m μ M	Liver V_{max} pmole/min/mg	Efficiency μ L/min/mg	Intestine Rate pmole/min/mg
Control	262 $\pm$ 64	108 $\pm$ 19	0.43 $\pm$ 0.12	280 $\pm$ 134 ^c
MXC Treated	286 $\pm$ 52	119 $\pm$ 20	0.42 $\pm$ 0.09	252 $\pm$ 74 ^c
3-MC Treated	431 $\pm$ 52 ^a	210 $\pm$ 16 ^b	0.49 $\pm$ 0.03	435 $\pm$ 164 ^c

Fish were treated as described in Table 2.

Values shown are mean $\pm$ S.D., n=4 fish per treatment group.

^aSignificantly different from control, p<0.01.

^bSignificantly different from control, p<0.05.

^cRate significantly different from liver V_{max} , p<0.05.

Table 4

Apparent kinetic values for the sulfonation of OH-MXC in hepatic and intestinal cytosol from control and 3-MC-induced fish

Tissue	Treatment	Km μ M	Vmax pmol/min/mg	Efficiency μ L/min/mg
Liver	Control	180 $\pm$ 17 (4)	6.7 $\pm$ 2.5 (4)	0.04 $\pm$ 0.02 (4)
	3-MC	77 $\pm$ 14 ^a (6)	11.6 $\pm$ 1.7 ^a (6)	0.16 $\pm$ 0.04 ^a (6)
Intestine	Control	267 $\pm$ 72 (4)	12.6 $\pm$ 3.4 (4)	0.05 $\pm$ 0.01 (4)
	3-MC	170 $\pm$ 38 ^b (8)	24 $\pm$ 6.9 ^{a,b} (8)	0.14 $\pm$ 0.02 ^a (8)

values shown are mean $\pm$ S.D (N)

^a significantly different from corresponding organ control, p<0.05

^b intestine significantly different from liver, p<0.05

Table 5

Apparent kinetic values for the sulfonation of HPTE in hepatic and intestinal cytosol from control and treated channel catfish

Tissue	Treatment	Km μ M	Vmax pmol/min/mg	Efficiency μ L/min/mg
Liver	Control	33 $\pm$ 15	17.5 $\pm$ 4.4	0.60 $\pm$ 0.24
	MXC	117 $\pm$ 12 ^a	22.4 $\pm$ 2.6	0.19 $\pm$ 0.04
	3-MC	75 $\pm$ 15	35.4 $\pm$ 4.6 ^a	0.48 $\pm$ 0.06
Intestine	Control	120 $\pm$ 30 ^b	29.6 $\pm$ 4.7 ^b	0.26 $\pm$ 0.08
	MXC	148 $\pm$ 40	28.5 $\pm$ 5.1	0.20 $\pm$ 0.03
	3-MC	99 $\pm$ 10	63.5 $\pm$ 4.6 ^{a,b}	0.64 $\pm$ 0.08 ^a

values shown are mean $\pm$ S.D; N=4

^a significantly different from corresponding organ control, p<0.05

^b intestine significantly different from liver, p<0.05

Table 6

Apparent kinetic values from the Hill plot for the sulfonation of 3-OH-BaP in hepatic and intestinal cytosol from control and treated channel catfish

Tissue Treatment	S_{50} μ M	Vmax pmol/min/mg	Hill coefficient	Efficiency μ L/min/mg
Liver				
Control	0.11 ± 0.01	103 ± 41	2.7 ± 0.4	930 ± 370
3-MC	0.16 ± 0.02	478 ± 70^a	2.4 ± 0.2	$2,980 \pm 380^a$
Intestine				
Control	0.14 ± 0.02	612 ± 203	1.8 ± 0.1	$4,310 \pm 1,150$
3-MC	0.14 ± 0.01	$1200 \pm 85^{a,b}$	2.2 ± 0.2	$8,600 \pm 230^{a,b}$

Values are mean $\pm$ S.D., n = 4

^a 3-MC-treated fish significantly higher V_{max} and efficiency values than control fish.

^b Intestine significantly higher V_{max} and efficiency values than liver.