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Toxicokinetics of Pyrene in Tilapias *Oreochromis niloticus* Following an Intraperitoneal Administration

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Pyrene is one of the most abundant compounds of a complex mixture of chemicals collectively called polycyclic aromatic hydrocarbons (PAHs) that pose a great risk to the health of any organism. PAHs are ubiquitously distributed in the environment at high concentrations, specifically in the majority of coastal lagoons of the Gulf of Mexico; they are present in sediments and water representing a source of exposure to wildlife and aquacultured organisms. PAHs contamination can cause immunotoxic effects (Payne 1989), lesions in kidney and liver, and also metabolic, behavioral, and reproductive changes in fish (Ostrander 1990). Furthermore, long-term exposures in organisms to low levels of some PAHs have caused cancer in laboratory animals (Baumann 1989; ATSDR 1995). However, little information is available about the distribution of PAHs in fishes, and the kinetic parameters are necessary to establish the main target organs and thus those most susceptible to suffer damage in these species.

Although pyrene poses no carcinogenic potential, it provides a useful model to study the kinetics and distribution of PAHs. Pyrene is found more concentrated in polluted lakes and wastewaters than in continental areas (Xiaoxing et al. 2001). Therefore, due to the worldwide distribution of PAHs, particularly of pyrene, in aquatic environments, its multiple exposure ways, metabolic rates, different uptake forms and accumulation and its relevance as model compound for PAH in fish, we investigated the toxicokinetics of pyrene in tilapia (*Oreochromis niloticus*), a species of great economic importance in the Gulf of Mexico region.

MATERIALS AND METHODS

Tilapias, weighting 150 - 250 g were obtained from our fish farm (Cinvestav). Fish were maintained at 32° - 35 °C in tanks containing 500 L of aerated water, under flow-through conditions with natural photoperiod. To evaluate the pyrene blood elimination rate, 50 fish were injected intraperitoneally (*i.p.*) with pyrene (20 mg kg^{-1} body weight) dissolved in corn oil (1 mL kg^{-1}) and 10 control fish received corn oil only (vehicle control). Blood samples were taken from three fish by caudal punction from 2 hr and onwards, up to 120 hr after injection and blood samples were placed in heparinized eppendorff tubes and shaken until their analysis.

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Pyrene blood concentration was determined according to a procedure described by Namandary et al. (1996). An aliquot of blood was mixed with 1N sulfuric acid and distilled water free of hydrocarbons and vigorously shaken. The mixture was vortexed and extracted twice with hexane containing 9,10-dihydroantracene as an internal standard. The tubes were shaken all night on a mechanical shaker (Eberbach Co. Ann Arbor, MI), and later were centrifuged to separate the organic and aqueous layers. The hexane extract was removed and put in a clean centrifuge tube. Quantification was performed by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph equipped with a 30 m x 0.25 mm (0.33 μm film thickness) HP-5 (5 % phenyl-methyl silicone) capillary column working in the splitless mode, with a flame ionization detector (FID) at a temperature of 300° C. The initial temperature of the oven was 60° C, programmed at 6° C min^{-1} to a final temperature of 290° C that was held during 20 min, using nitrogen as a carrier gas. Pyrene was identified and quantified using standards from Ultra Scientific (USA). Quality assurance of the analytical procedure including the addition of internal standards and the analysis of the blank used in the procedure was performed for each set of samples. Toxicokinetic analysis was performed assuming a two-compartment model. All parameters described in this study and fitting were carried out using the WinNonlin version 3.0 software (Pharsight. Corp. Mountain View California).

Simultaneously, to determine the distribution and transformation of pyrene, the same 3 fish that were collected from the group mentioned above at 24, 48, 72, 96 and 120 hr, were killed by a sharp blow on the head. They were then dissected, and livers, intestines, and bile were collected and frozen. Pyrene and 1-OH pyrene concentrations were assessed in: a) liver and intestine, and b) bile.

a) Pyrene and 1-OH pyrene concentrations in livers and intestines were extracted according to a procedure described by Sericano et al. (1990) and Wade et al. (1993). Tissues were homogenized in methylene chloride and sodium sulfate. The extraction was repeated 3 times. Methylene chloride was filtrated to eliminate the sodium sulfate, concentrated in Snyder system condenser, and transferred to a Kuderna-Danish tube to concentrate the extract to a final volume of 1 mL using a continuous stream of nitrogen. The extract was fractionated by partially deactivated aluminum oxide/silica gel column chromatography, and sodium sulfate was added evenly over the silica gel to remove humidity. The extract was eluted from the column using hexane. The final solvent was evaporated again with a Snyder column condenser, and concentrated in a continuous stream of nitrogen to 200 μL. Pyrene was injected and quantified by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph as described above. Later, the extract was evaporated to dryness under a stream of nitrogen and the residues were redissolved in 100 μL ethanol/water. 1-OH pyrene was analyzed and quantified by fluorescence, using a Shimatzu 5301 Spectrofluorometer. The optimal wavelength pairs for 1-OH pyrene was 346/384 nm, (excitation /emission). 1-OH pyrene (99% purity) was obtained from Sigma Chemical Co. (St. Louis, MO, USA), and used to calculate the concentrations.

b) Free pyrene concentration in bile was extracted 3 times with methylene chloride and the extract was concentrate to a final volume of 100 µL. The extract was changed by hexane and pyrene was quantified by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph as described above. 1-OH pyrene concentrations in bile were determined as described by Ass et al. (1998). Bile was diluted with 48 % ETOH. The sample was shaken 2 to 3 times and 1-OH pyrene was quantified by fluorescence using a Shimatzu 5301 Spectrofluorometer. The optimal wavelength pairs for 1-OH pyrene was 341/383 nm, (emission/excitation). 1-OH pyrene (99% purity) was used to calculate the concentrations.

Pyrene and 1-OH pyrene concentrations were expressed as mean ± one standard deviation (s.d.). Since data were normal and homocedastic, all statistical tests were parametric. Significant differences between means of different treatment groups were determined by analysis of variance (ANOVA, $\alpha = 0.05$) and means were contrasted using Dunnett's t-test. Statistical analyses were carried out using Statistica, version 5.5, 1999 edition (Statsoft, USA).

RESULTS AND DISCUSSION

The toxicokinetic analysis showed that the distribution and elimination of pyrene could be adjusted to a two compartments model where the observed and predicted values are shown in Figure 1. The results from the curve-fitting of pyrene showed that the elimination of this compound has at least two steps. The average half-life in the first phase (α) was 4.5 hr, and the average terminal half-life in the second phase (β) was 636.2 hr. The results of K_{10}, K_{12}, and, K_{21} were 0.41 h^{-1}, 0.42 h^{-1}, and 0.004 h^{-1}, respectively, while the value of Alpha and Beta were 0.15 h^{-1} 0.0011 h^{-1}.

The area under the blood level-time curve (AUC), required to calculate the bioavailability, was evaluated using WinNonlin software where the area obtained for the time interval from 0 to 120 hr ($AUC_{0 \rightarrow 120}$) was 222.16 µg h mL^{-1} while the $AUC_{0 \rightarrow \infty}$ was 679.54 µg h mL^{-1}. The highest pyrene concentration (C_{max}) extrapolated for time 0 in blood, was 27.69 µg mL^{-1}, and the pharmacokinetic parameters were the mean of pre-exponential coefficient, A and B, with values of 27.14 and 0.55 µg mL^{-1}, respectively. The clearance value (Cl) was 0.029 L h^{-1} kg^{-1} and the steady state volume of distribution (V_{dss}) was 20.02 L kg^{-1}.

The results revealed that pyrene injected *i.p.* in tilapias was distributed rapidly in the blood 2 hr post-treatment. Our results agree with Kennedy and Law (1990), who also found that pyrene, in the blood of trout exposed to dissolved pyrene was immediately absorbed. They reported that uptake data could be fitted to a two-compartment toxicokinetic model, with rapid distribution and mixing of the chemical in the tissues after being absorbed into the blood.

Pyrene concentration in blood decreased 12 hr after injection; however; pyrene was widely distributed in liver and intestine. This phenomenon was observed

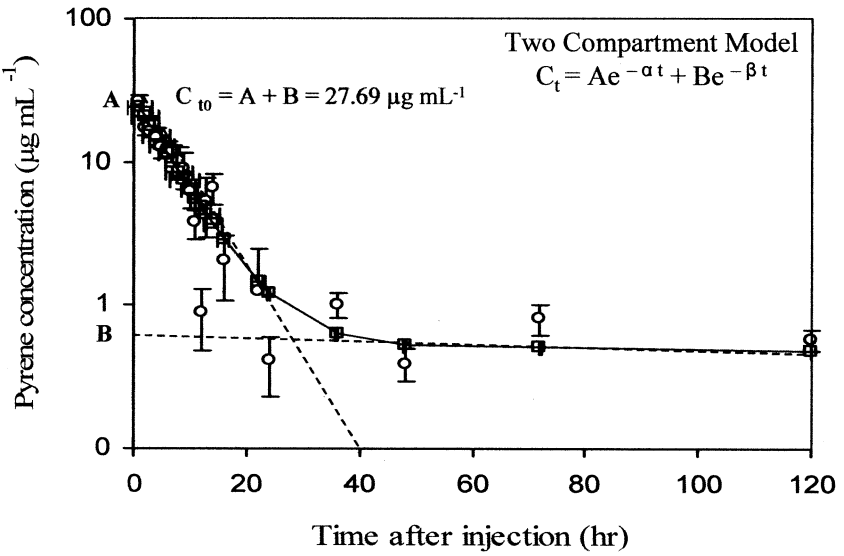

Figure 1. Pyrene concentration in blood of tilapias injected intraperitoneally with 20 mg kg -1 body weight of pyrene.

when the highest concentrations of pyrene were found in intestine and liver, 24 hr later that pyrene disappeared from the blood (Fig. 2). It could be due to pyrene's log K_{ow}, which facilitates absorption in various tissues. Our data agree with Withey et al. (1991), who reported that rats injected *i.v.* with pyrene accumulated mostly in liver then, kidney and lung. Barron et al. (1987) also reported that trout tissues had different blood perfusion rates and tissue-blood partition coefficients for pyrene. The clearance obtained in this study was low, about 0.029 L h^{-1} kg^{-1} and this parameter may help to explain pyrene's long time of elimination. Our data are consistent with those described by Namdari and Law (1996) who reported a higher rate of pyrene uptake and a slower rate of elimination in trout exposed to dissolved pyrene.

On the other hand, mean concentration of pyrene in liver, intestine and bile increased during the first 24 hr after injection (Fig. 2), and decreased gradually 48 hr post-treatment. In contrast, the highest concentration of the metabolite 1-OH pyrene in liver and bile were found 72 hr post-treatment (Fig. 2). It is important to mention that concentrations of 1-OH pyrene reported in this study must be taken cautiously; because the concentration 1-OH pyrene could be the sum of the free pyrene and the conjugated metabolites, and in this study the conjugate was not determined. Low et al. (1994) showed that trouts exposed to dissolved pyrene transformed it mainly to free 1-OH pyrene, and the main pyrene metabolites in the bile were conjugated with glucoronic acid or sulphate.

The presence of pyrene (without transformation) in the bile during the first 24 hr showed that pyrene was rapidly distributed after injection and immediately there

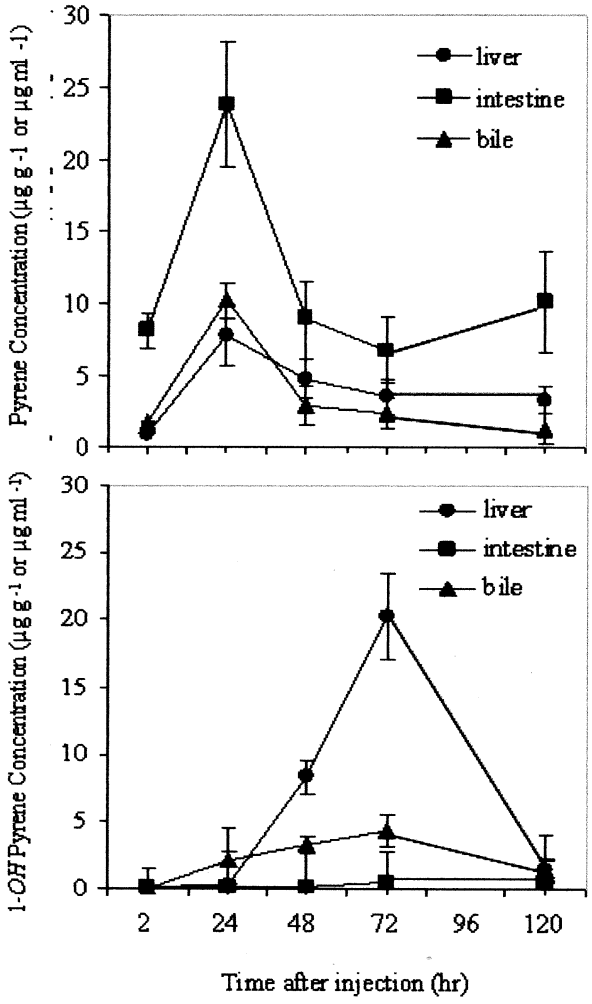

Figure 2. Pyrene and 1-OH pyrene concentration in liver, intestine and bile of tilapias injected intraperitoneally with 20 mg kg^{-1} body weight of pyrene. (All concentrations are given as means ± one S.D; n=3 each point)

was a high transformation increasing the 1-OH pyrene concentration. In this study, the metabolite 1-OH pyrene was used as indicator of transformation metabolism. The behavior of the metabolite in bile was similar to Ethoxyresorufin-*O*-deethylase (EROD) activity behavior reported by Zapata et al. (2002), where they found maximum EROD activities 3 days after injection. EROD activities dates reported in Zapata et al. (2002) were used in this work to correlate enzymatic activities with 1-OH pyrene concentration. The correlation between EROD activities and the metabolite 1-OH pyrene is showed in Figure 3,

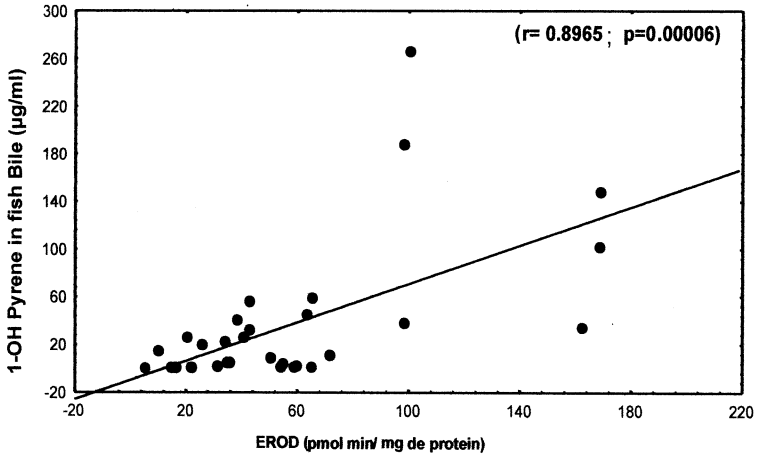

Figure 3. Spearman correlation between EROD activity in liver and the metabolite 1-OH pyrene in bile of tilapias injected intraperitoneally with pyrene (20 mg kg $^{-1}$ body weight).

suggesting that high concentration of the metabolite was due to the high power of transformation of the enzymes as measured by EROD activities.

In conclusion, pyrene was rapidly absorbed in the blood and distributed into the tissues. Total elimination of pyrene from the fish body was very slow, probably because pyrene can still be adsorbed in fatty tissues. The metabolite 1-OH pyrene is a good marker for pyrene metabolism, and to understand pyrene biotransformation. Future work could be focused to evaluate the uptake, the accumulation and the excretion of pyrene and the relationship with EROD activities.

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REFERENCES

Aas E, Beyer J, Goksoyr A (1998) PAH in fish bile detected by fixed wavelength fluorescence. Mar Environ Res 46:225-228

Agency for Toxic Substances and Disease Registry (ATSDR) (1995) Toxicological profile for polycyclic aromatic hydrocarbons (PAHs). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, pp1-7

Baumann PC (1989) PAH, metabolites, and neoplasia in feral fish populations. In: Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. (Edited by U. Varanasi) CRC Press. Boca Raton, FL, USA, pp 269-289

Barron MG, Tarr BD, Hayton WL (1987) Temperature-dependent of cardiac output and regional blood flow in rainbowt trout, *Salmo gairdneri* Richardson. J Fish Biol 31:735-744

Kennedy CJ, Law FCP (1990) Toxicokinetics of selected polycyclic aromatic hydrocarbons in rainbow trout following different routes of exposure. Environ Toxicol Chem 9:133-139

Law FCP, Meng JX, He YT, Chui YC (1994) Urinary and biliary metabolites of pyrene in rainbow trout (*Oncorhynchus mykiss*). Xenobiotica 24:221-229

Namdari R, Law FC P (1996) Toxicokinetics of waterborne pyrene in rainbow trout (*Oncorhynchus mykiss*) following branchial or dermal exposure. Aquat Toxicol 35:221-235

Ostrander GK, Anderson JJ, Fisher JP, Landolt ML, Kocan RM (1990) Decreased performance of rainbow trout *Oncorhynchus mykiss* emergence behaviors following embryonic exposure to benzo(a)pyrene. Fish Bull 88:551-555

Payne JF, Fancey LF (1989) Effect of polyaromatic hydrocarbons on immune responses in fish. Change in melanomacrophage centers in flounder *(Pseudopleuronectes americanus)* exposed to hydrocarbon-contaminated sediments. Mar Environ Res 28:431-435

Sericano JL, Atlas EL, Wade TL, Brooks JM (1990) NOAA´s Status and Trends Mussel Watch Program: Chlorinated pesticides and PCBs in oysters (*Crassostrea virginica*) and sediments from Gulf of Mexico, 1986-1987. Mar Environ Res 29:161-203

Wade TL, Brooks JM, Kennicutt MC II, McDonald TJ, Sericano JL, Jackson TJ (1993) GERG trace organic contaminant analytical techniques. In: Sampling and Analytical Methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects 1984-1992, Vol IV, Comprehensive descriptions of trace organic analytical methods, NOAA Technical Memorandum NOS ORCA 71, pp 121-139

Withey JR, Law FCP, Endrenyi I (1991) Pharmacokinetics and bioavailability of pyrene in the rat. J Toxicol Environ Health 32:429-447

Xiaoxing L, Takashi K (2001) Dynamics analysis for the distribution of polycyclic aromatic hydrocarbons in rice. J Health Scie 47:446–451

Zapata-Perez O, Gold-Bouchot G, Ortega A, Lopez T, Albores A (2002) Effect of pyrene on hepatic cytochrome P450 1A (CYP1A) expression in Nile Tilapia (*Oreochromis niloticus*). Arch Environ Contam Toxicol 42:477-485