Persistent Organic Pollutants, and Biomarkers, in Nile Tilapia from Four Lakes in Chiapas, Mexico

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Introduction

One of the largest oil producing regions in Mexico is in the State of Chiapas, in the southeastern part of the country. In the north of the state there is a group of three lakes (Limón, Río and Enmedio) in the municipality of San Miguel, where there are more than one hundred oil wells close by, which have been seriously impacted. A fourth lake, Caracol, a few kilometers away was used as a reference site. Previous studies done in this area have demonstrated high concentrations of organic and inorganic contaminants in both Nile tilapias (*Oreochromis niloticus*) and sediments [1]. During the year 2000 several thousand tilapias O. niloticus under culture died in these lakes. As part of an integrated effort to diagnose this mass mortality, the concentrations of organic and inorganic pollutants in the sediments and in tilapias were determined, and an initial study using some biomarkers was made to determine the possible effects caused by pollutants. Sampling took place several months after the mass mortality, and thus the fish collected are survivors of this event. The fish collected were free living organisms.

Materials and Methods

Forty six live tilapias *Oreochromis niloticus* were collected in 18 sampling sites from four lakes (Caracol, Río, Enmedio and Limón) in September 2000, and eighty fish were collected in May 2002. Livers were removed and sectioned. One of these sections was shock frozen for enzymatic analysis. Another portion of the liver was immediately frozen for organic compound analysis. A section of muscle was removed and frozen for BChE activity, and bile removed with a syringe and frozen for PAH metabolite analysis. Organic compounds (total hydrocarbons, organochlorine pesticides, and polychlorinated biphenyls) in fish livers were extracted and the extracts purified and separated into fractions by column chromatography [2, 3]. Compounds were quantified by gas chromatography. Quality control and assurance procedures included running blanks and spiked blanks with each set of samples, duplicate samples and intercalibration sample IAEA-432 from the IAEA Marine Environmental Laboratory. Recoveries were in the range from 80 to 110%. CYP protein content was determined by the CO difference spectra of the reduced protein [4]. EROD activity was assayed fluorometrically [5]. CYP-1A protein was determined by electrophoresis (SDS–PAGE slab gels) [6] and CYP1A content was quantified by image analysis densitometry in a Kodak Digital Science EDAS 120 System Densitometer (Eastman Kodak, Rochester, NY). PAH metabolites (free plus conjugated) were analyzed by fixed wavelength fluorescence [7]. To decrease self-absorption and quenching of the fluorescence signal, the bile was diluted in 48% ethanol-water (v v-1). BChE activity was determined in muscle using BSChI as substrate [8]. All results are reported as medians ± interquantile range. All statistical tests were non-parametric. Significant differences between medians of various fish groups were determined by a Kruskal-Wallis one-way analysis of variance (ANOVA), using $\alpha=0.05$.

Results and Discussion

Fish collected had a mean weight and total length of 459.2 ± 187.8 g and 27.2 ± 3.7 cm, respectively. A correlation analysis between fish total length and weight shows that tilapias from lake Caracol have a higher condition index, which suggests that fish from Caracol are in better condition than fish from the other lakes. Generally, contaminant concentrations in fish liver were higher in the rainy season than in the dry season, and the concentrations in both seasons were high as compared to published results for other species in different ecosystems in southern Mexico [9, 10]. Contaminant concentrations found in this study are higher than those found in liver of the Mayan Catfish, Ariopsis assimilis, in Chetumal Bay [11] and in liver of Ariopsis felis in Tampa Bay [12]. It is interesting that catfish from both sites had tumors and other severe histological lesions, but not the tilapias from these lakes in Chiapas. The highest concentrations of total hydrocarbons and PAHs in fish liver were found in fish collected from lake Limón in May 2002, and the lowest concentrations were detected in fish captured in lake Caracol. These concentrations ranged from 0.7 to 158.4 µg g-1, and are higher than the limits established for PAH concentrations in organisms by the United States Environmental Protection Agency (USEPA) [13]. Several compounds in the 21 critical PAHs established by the USEPA for their carcinogenic potential were detected, such as benzo[a]pyrene, pyrene, naphthalene, phenanthrene, etc. Organochlorine pesticides and PCBs had a different pattern, where the maximum concentrations were detected in fish captured in lake Limón during both seasons, and in lake Río during the rainy season, and as was also the case for PAHs, the observed concentrations are higher than the criteria set by the USEPA. On the other hand, the highest concentrations of PAH metabolites in fish bile were detected in fish captured in lake Limón, the major concentrations being for the low molecular weight metabolites (1-OH naphthalene, and 1-OH phenanthrene). This indicates a petrogenic source of these hydrocarbons [14, 15]. EROD activity results showed that the highest activities were found in fish collected from lake Limón, where the mean value was 330 pmol min-1 mg-1 during the dry season, and 140 pmol min-1 mg-1 of protein in the rainy season. EROD activities showed the same behavior that hydrocarbons and PAH metabolites, with the highest concentrations found in fish livers from lake Limón during the dry season. A Spearman correlation test showed a good correlation between EROD activities with total hydrocarbons and PAH metabolites, especially with 1-OH pyrene ($r= 0.8538$; $p=0.000002$). These results suggest that CYP1A, measured as EROD activity, may transform PAHs and as a result of this process there is a high concentration of PAH metabolites in bile. EROD induction in tilapia has been well documented [16 to 21]. It has been observed that CYP1A induction in Tilapia Oreochromis niloticus can be used as a biomarker for monitoring the exposure of fish to environmental pollutants. Western blot analysis of CYP1A protein also showed statiscally significant differences between protein levels in tilapia livers collected in the different lakes. Highest protein levels were found in Limón and Río lakes. To confirm that tilapias from these lakes were induced, livers from laboratory reared tilapias were also analyzed. CYP1A levels in these tilapias were much lower than protein levels in fish from the lakes, confirming that CYP1A is over-expressed in the fish from the lakes, and thus exposed to pollutants. Butirylcholinesterase from the four lakes where statistically different, the median BChE activities in the lakes were lower than in cultured tilapias (154.86± 13.95 nmol/min/mg prot), indicating a possible source of neurotoxic pollutants not measured in this study. The lowest BChE activity was found in fish collected from lake Caracol. Previous reports in the area indicated an extended use of organophosphate pesticides, especially near this lake [22]. A Spearman test showed a significant correlation between BChE activity with fish weight and total length. Positive significant correlations were found with liver PAH concentrations and the metabolites 1-OH pyrene, 1-OH naphtalene and 1-OH benzo[a]pyrene, this behavior is the opposite to that found in [23], where anticholinesterase effects were found with some PAHs, and in [9], where there was a negative correlation between PAHs and cholinesterase activity. On the other hand, the highest BChE activities were also found in fish collected from lake Limón. A Spearman test showed significant correlations between EROD and BChE activity with fish weight, but BChE correlated positively with PAHs. AChE correlated negatively with Endosulphan, HCB and Pentachloroanisol. In general, the highest pollutant levels were found in lake Limón, particularly during the rainy season. This was confirmed by the biomarkers used. There appears to be some synergistic and antagonistic effects of pollutant concentrations on biomarker levels, which must be taken into account when monitoring results are discussed, particularly when organisms are exposed to complex mixtures of pollutants such as those found here. This study provides useful information to demonstrate that high concentrations of contaminants are causing effects on fish metabolism, and the use of tilapia as a test organism in tropical ecosystems.

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