Bull Environ Contam Toxicol (2008) 81:543–547
DOI 10.1007/s00128-008-9556-9

Vitellogenin Induction and Increased Plasma 17*β*-Estradiol Concentrations in Male Nile Tilapia, *Oreochromis niloticus*, Exposed to Organochlorine Pollutants and Polycyclic Aromatics Hydrocarbons

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Received: 5 September 2007 / Accepted: 1 September 2008 / Published online: 12 September 2008

Abstract Vitellogenin (Vtg), 17*β*-estradiol (E2) and testosterone (T) were used as biomarkers of endocrine disruption in mature male nile tilapia (*Oreochromis niloticus*) from three lakes (Rio, Enmedio and Limon) in Chiapas, Mexico. Vitellogenesis induction was found in tilapias from Rio and Limon, moderately high E_2 levels in Rio and Limon tilapias, compared with controls (cultured tilapias). Significant correlations between benzo(*a*)pyrene (BaP) metabolites and hexachlorobenzene (HCB) with Vtg and E_2 were found. The results of this study indicate that endocrine disruption exists in tilapias from Rio and Limon lakes, and that exposure to HCB and B*a*P could be causing these alterations.

Keywords Vitellogenin · Estradiol · Hexachlorobenzene · Benzo(a)pyrene · Endocrine disruption · Tilapia

Recently a group of compounds that can cause adverse effects in humans and wildlife have received much attention, the endocrine disruptors (EDs), which are a structurally diverse group of compounds that Kavlock et al. (1996) define as "exogenous agents that interfere with production, release, transport, metabolism, binding, action or elimination of the natural hormones". They include synthetic and natural compounds. Compounds commercially manufactured as organochlorine pesticides (Dichlorodiphenyltrichloroethane (DDT), Chlordecone, Endosulfan, Chlordane and Lindane), industrial products (Polychlorinated biphenyls (PCBs) and phthalates), metals (cadmium, tin, lead and mercury) and pharmaceuticals (ethinylestradiol contraceptive pills), or are produced as a byproduct or degradation product, for example alkyl phenols, can be classified as synthetic EDs (Colborn et al. 1993). Natural EDs are synthesized by animals or plants, i.e. natural estrogens (17*β*-estradiol (E2), estrone and estriol synthesized in mature females) and the phytoestrogens (isoflavones, lignans and coumestans synthesized by plants and fungi) that can be found in some aquatic environments.

The xenoestrogens are the most studied, they are synthetic compounds that can mimic natural estrogens causing adverse effects in the development, sexual maturation and reproduction processes (Kavlock et al. 1996). Multiple adverse effects due to exposure to xenoestrogens have been reported, Ankley et al. (1998) in their overview mention effects such as bird eggshell changes, embryo mortality, and reduced fertility, in fish feminization (vitellogenin induction and oocytes presence in testis), delayed sexual maturation, reduced gonadal growth, and altered steroidogenic capacity. Moreover, many adverse effects, for example hormone-dependent cancer (breast, prostate, ovaries and testicles), cryptorchidism (condition in which the testes do not descend into the scrotum) and hypospadias (displacement of urethral opening from the tip to the ventral side of the penis) in humans exposed to environmental estrogens have been reported (Rivas et al. 2004).

Vitellogenin (Vtg) is a sensitive and powerful biomarker of effect to estrogen receptor (ER) agonists in oviparous vertebrates (Ankley et al. 1998). This lipoprotein is the precursor of the egg yolk proteins phosvitin and lipovitellin, and it is normally present in mature female fish. In male fish the Vtg production is an abnormal process, since male fish

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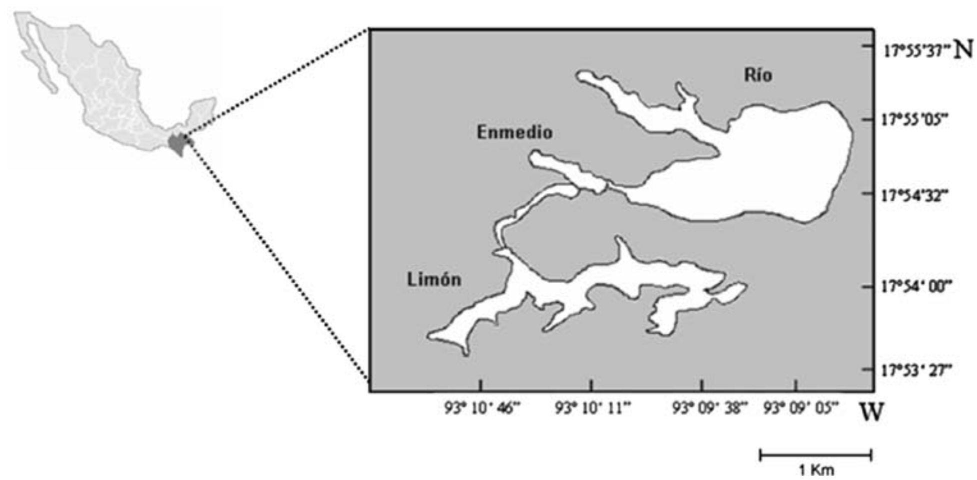

Fig. 1 Location of San Miguel lakes system in Reforma, Chiapas, Mexico

have the Vtg gene, and exposure to environmental estrogens can trigger the gene's expression, leading to Vtg accumulation in the blood (Tyler et al. 1999). Plasma 17β-estradiol and testosterone (T) levels have been used as biomarkers of effect for exposure to xenoestrogens. Testosterone levels in fish decreased in plasma due to exposure to xenoestrogens, and high E_2 levels, presumably due to a feedback inhibition in steroids synthesis (Ankley et al. 1998).

This study was done in the San Miguel lakes system in Chiapas, Mexico. It is a system formed by three lakes: Rio, Enmedio and Limon (Fig. 1). They are located in the north of the state. In this zone, the Mexican oil company (PEMEX) has 111 oil wells in operation, and a gas processing complex (CCPG Cactus); moreover around the lakes there are agricultural zones. In previous studies in these lakes, Gold-Bouchot et al. (2006) reported high concentrations of pesticides, polycyclic aromatic hydrocarbons (PAHs) and total hydrocarbons in liver of tilapias, compared with fish of other lakes from Mexico.

The objective of this study was to determine if male tilapias from the lakes in San Miguel show endocrine disruption by using Vtg induction and sexual steroids (E_2 and T) alterations, and to determine if they are correlated with organochlorine pesticide concentrations in liver and PAH metabolites in bile. This study is part of a larger study on the environmental pollution in the lakes in San Miguel. Concentrations of organochlorine pesticides and PAH metabolites in bile used in this study to find correlations with Vtg, E2 and T, were measured in the same male fish. Results of pollutants in all fish (male and female) and other biomarkers where reported by Gold-Bouchot et al. (2006).

Materials and Methods

In May 2002, reproductively mature male nile tilapias (*Oreochromis niloticus*) were collected from each lake at San Miguel (9 in Río, 10 in Enmedio y 9 in Limon). Thirteen mature male nile tilapias from a farm at Cinvestav Merida were used as a control group. Fish length and weight ranges were 19.5–37 cm and 142–1056 g, respectively. Blood was collected from the caudal vein using a heparinized syringe, centrifuged to obtain plasma and frozen for later analysis of Vtg, E_2 and T. After blood extraction, fish were killed and livers were removed and frozen for organochlorine pesticide analysis. Bile was collected using a syringe and frozen for PAH metabolites analysis.

Plasma Vtg was extracted as plasma alkaline-labile phosphorous (P-ALP) according to Wallace and Jared (1968), and analyzed by using a commercially available kit (Sigma 670-C). Plasma levels of E_2 and T were measured by using enzyme immunoassays (EIA), with commercial kits (Bio Check BC-1111 and BC-1115). Detection limits calculated for Vtg, E_2 and T in serum where 0.2 μg/mL, 6 pg/mL and 50 pg/mL, respectively. Organochlorine pesticides were measured according to Sericano et al. (1990). Polycyclic aromatics hydrocarbons (PAHs) metabolites were analyzed by fixed wavelength fluorescence using a Shimadzu model 5301 spectrofluorometer (Aas et al. 1998). The detections limits calculated for PAH metabolites in bile were 0.045 μg/mL.

Organochlorine pesticides in liver and B*a*P and phenanthrene in bile were identified and quantified using standards from Ultra Scientific; hidroxy-pyrene and hidroxy-naphthalene were identified and quantified using standards from Sigma Aldrich. Differences between groups were evaluated by one-way ANOVA after data transformation (Log); if the ANOVA is significant, an HSD Post-hoc test for unequal N ($p < 0.5$) was used. Results are reported as medians ± one interquartile range. Non-parametric Spearman correlation coefficients were used to determine the correlations between concentrations of organochlorine pesticides and PAH metabolites with Vtg, E_2 and T.

Results and Discussion

Vtg induction in male fish was found in tilapias, reported here as Vtg concentrations in plasma, from Rio (45 ± 30 µg/mL) and Limon (52 ± 42 µg/mL) (Fig 2a); these results indicate exposure to environmental estrogens (Tyler et al. 1999). These environmental estrogens are agonists, binding to the estrogen receptor in liver from male fish and causing Vtg gene expression.

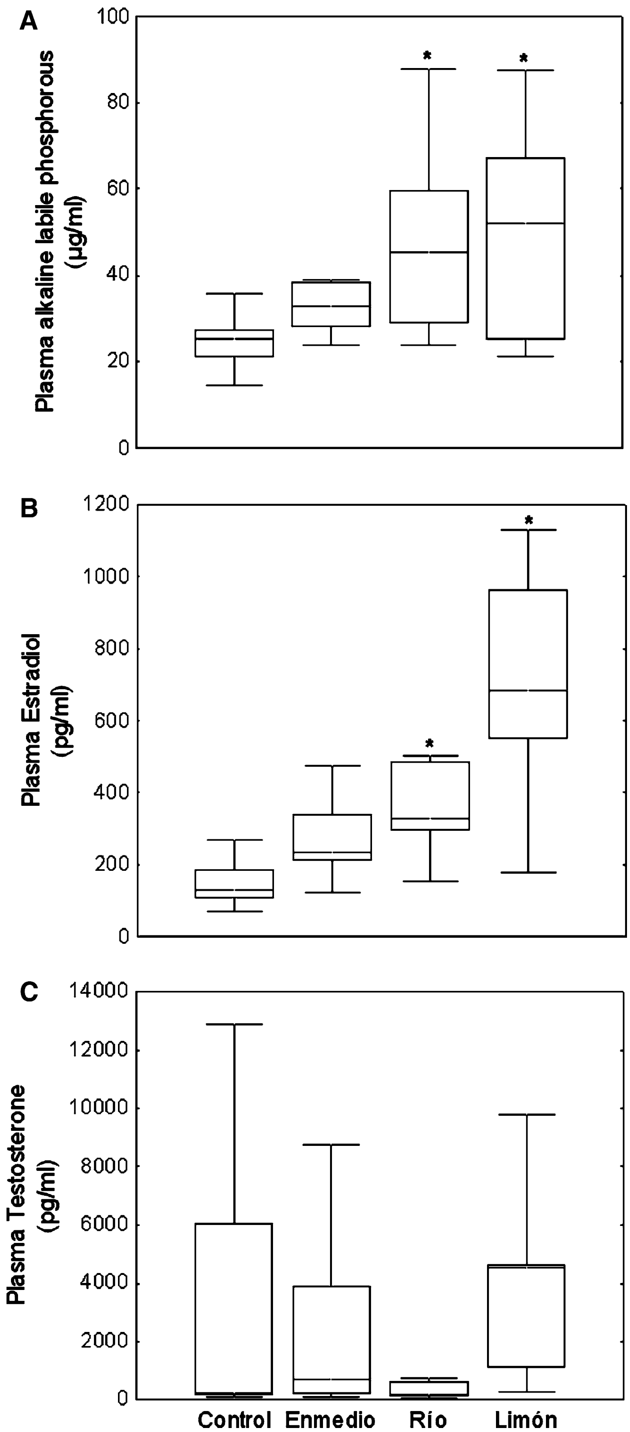

Fig. 2 Plasma Vtg (**a**), E_2 (**b**) and T (**c**) levels in male nile tilapia (*Oreochromis niloticus*) from three lakes in San Miguel Chiapas Mexico, and control group. *Indicates a significant difference ($p < 0.05$) from the control group

High E_2 levels were also found in fish from Rio (326 ± 187 pg/mL) and Limon (684 ± 412 pg/mL) (Fig. 2b). Vtg induction and high E_2 levels in male tilapias agree with results reported by Folmar et al. (1996) in male carp (*Cyprinus carpio*) captured near a treatment plant and exposed to environmental estrogens and by Leaños-Castañeda et al. (2002) in male nile tilapia (*O. niloticus*) treated with *o,p′*-DDT. Correlation between Vtg induction and high E_2 levels (Fig. 3) was tested, and showed good correlation ($r = 0.6316$; $p = 0.000009$), suggesting that both alterations could be caused by similar mechanisms, very likely exposure to environmental estrogens. Ankley et al. (1998) reported that estrogen agonists can interfere with the feedback inhibition synthesis of E_2, leading to increased steroidogenesis and increased E_2 levels. There were no significant differences between the T levels of lake fish and those of the control group (Fig. 2c). In several studies decreased T levels in fish exposed to environmental estrogens were reported (Folmar et al. 1996; Leaños-Castañeda et al. 2002), although in this study there was no significant difference.

Significant correlations between plasma Vtg and E_2 levels with the pesticide hexachlorobenzene (HCB) in liver (Fig. 4) and B*a*P metabolites in bile (Fig. 5) were found. Ezendam et al. (2004) have reported effects on the immune and reproductive system (interferences with estrogen metabolism), hepatic porphyria and neurotoxicity in rats exposed to HCB. Jarrell et al. (1998) reported significant correlations between HCB levels in serum from women accidentally exposed in Turkey with spontaneous abortion.

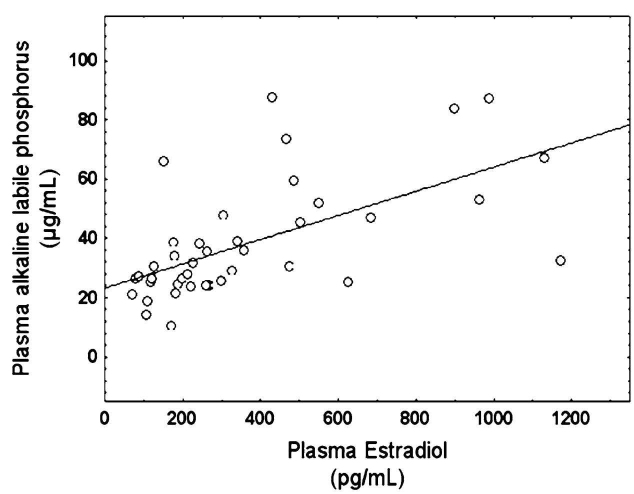

Fig. 3 Correlation between E_2 and Vtg ($r = 0.6316$; $p = 0.000009$) in plasma, from male nile tilapias (*Oreochromis niloticus*) from three lakes in Chiapas, Mexico

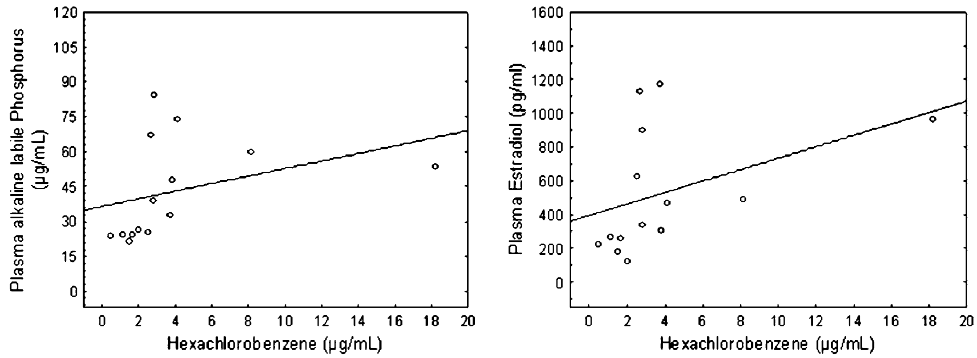

Fig. 4 Correlations between hexachlorobenzene with Vtg ($r = 0.7942$; $p = 0.00006$ (**a**)) and E_2 ($r = 0.6395$; $p = 0.01378$ (**b**)) in male nile tilapias (*Oreochromis niloticus*) from three lakes in Chiapas, Mexico

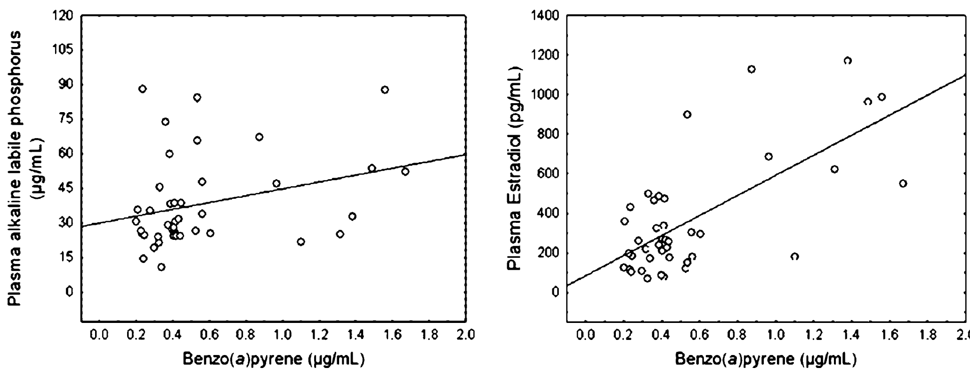

Fig. 5 Correlations between 1-OH Benzo(a)pyrene metabolites in bile with Vtg ($r = 0.3086$; $p = 0.4956$ (**a**)) and E_2 ($r = 0.4763$; $p = 0.00164$ (**b**)) in plasma male nile tilapias (*Oreochromis niloticus*) from three lakes in Chiapas, Mexico

These studies in rats and humans suggest the possible estrogenic effects produced by exposure to HCB, but in fish have not been reported to the best of our knowledge.

Reproductive toxicity in vitro by B*a*P has been studied, but information about the estrogenic effects in vivo by B*a*P in fish was not found. However, in the metabolism and biotransformation of B*a*P in fish by phase I enzymes, hydroxylated metabolites are generated (Krahn et al. 1987), and positive estrogenic activity has been demonstrated for hydroxy-B*a*P metabolites (Nishihara et al. 2000); this could explain the observed correlations of BaP with Vtg and E_2.

Our findings indicate endocrine disruption in fish from Río and Limón lakes, as expressed by vitellogenin induction and increased plasma E_2 levels, and suggest estrogenic effects in fish by exposure to HCB and B*a*P. Further studies are necessary to better understand the possible estrogenic activities of these compounds in fish.

Acknowledgments The authors wish to thank PEMEX (Producción y Exploración, Refinación, y Petroquímica) for financial support, to Dr. Rene Capurro Filograsso for revising an earlier version, and to Jorge Dominguez Maldonado for his technical assistance.

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