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Bioaccumulation and Evidence of Hormonal Disruptions in Tilapia Fish (*Oreochromis spp.*) Exposed to Sub-lethal Concentrations of Pesticides in Sinaloa, Mexico

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Authors' contributions

This work was carried out in collaboration between all authors. Author JGGR designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors GRGR and MDCCO managed the analyses of the study and performed the statistical analysis, author FMJ managed the literature searches and wrote the final draft. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Examination of endocrine disruption in tilapia fish (*Oreochromis spp.*) after exposure to sub-lethal concentrations of endosulfan, lindane, diazinon and malathion was evaluated by the quantification of 17-β-estradiol, progesterone and testosterone in fish. Female tilapia (96-110 g/fish) were exposed to 2.8, 15, 225 and 315 µg/l of endosulfan, lindane, diazinon and malathion, respectively; while, the male tilapia (same range weight) were exposed to 2.8 µg/l endosulfan and 315 µg/l of malathion separately for 21 days. The obtained results showed that the level of 17-β-estradiol and progesterone concentration in female, were significantly decreased in all treatment. Similarity, the level of testosterone

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was decreased significantly in the blood of male fish exposed to endosulfan and malathion pesticides when compared to the control fish group. The bioaccumulation of pesticides in muscular tissue of fishes were 760, 310, 41.3 and 11.9 (µg/g) for endosulfan, lindane, malathion and diazinon, respectively. Results suggest that pesticides assayed, are endocrine disruptors in tilapia, which can affect the reproduction and other biochemical and physiological functions controlled by hormones. On the other hand, pesticides accumulated in fish muscle represent a risk for public health, since tilapia is a popular food in Mexico like in others developing countries.

Keywords: Endocrine disruption; tilapia; pesticides; progesterone; testosterone; 17-β-estradiol.

1. INTRODUCTION

The tilapia (*Oreochromis spp.*) is a very important source of protein in the developing world. Its production in hatcheries, ponds, damps, etc., has grown exponentially over the past 30 years (Fig. 1) [1].

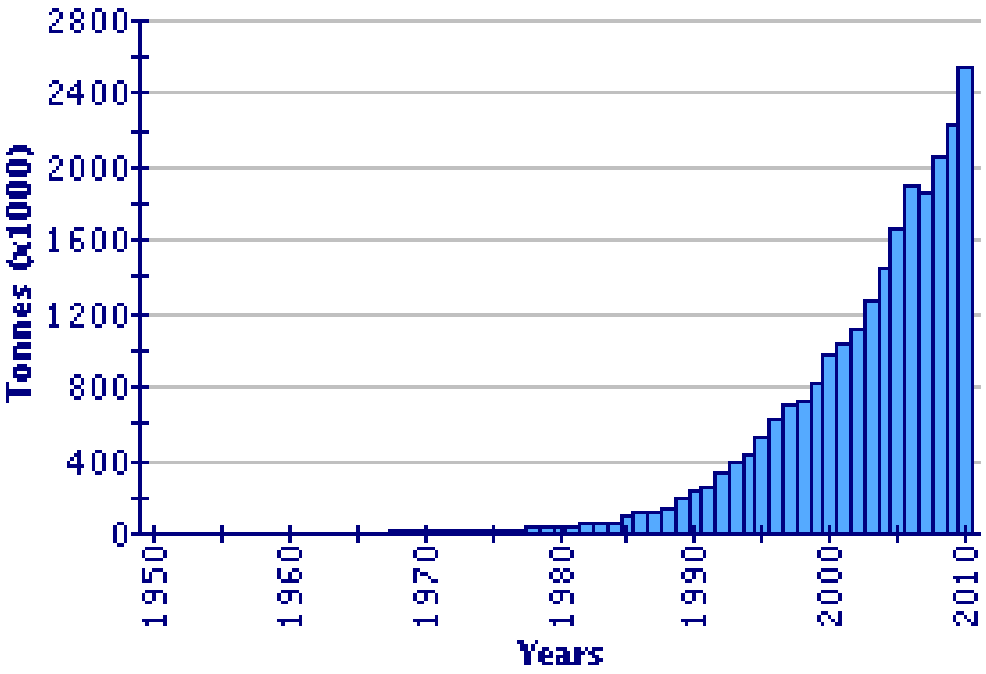

Fig. 1. Global aquaculture production of Tilapia (*Oreochromis spp.*)

The principal growers are China, Egypt, Philippines, Thailand, Costa Rica, Ecuador, Colombia, Honduras and Brazil. It was recently reported that tilapia is one of three main fishery products of Latin America and the Caribbean [2]. In Mexico, the estimated production of tilapia for 2012 is about 75000 metric tons [3] and new projects have been initiated for the production of tilapia in Chiapas and Sinaloa States (provinces). In Sinaloa, the use of

pesticides to control agricultural pests, exo-parasites in cattle and domestic animals and vectors of diseases like dengue and malaria, has increased considerably during the last decades, causing environmental contamination and an endless number of negative effects for non-target species, as well as for human health [4,5]. Due to their physical-chemical properties (low reactivity, little solubility, etc.), many pesticides can directly enter aquatic organisms by diffusion through the skin and by the processes of breathing and feeding [6,7,8].

In the Sinaloa State, Mexico, many agricultural fields are located close to tilapia hatcheries and places where they are cultured (damps, ponds, etc.). Therefore, pesticide residues from the agricultural fields can enter aquatic environments and lead to exposure of aquatic organisms, which eventually can affect the endocrine systems of organisms and those of subsequent generations, which are more vulnerable because their organ and neural systems are developing [9,10]. The pesticides or their residues can interfere with production, transport, metabolism, reception, and alteration of hormones, which regulate the homeostasis of the organisms, i.e. disrupt the endocrine system. The endocrine system also regulates part of the sexual and reproduction processes [11,12,13]. However, many substances such as chemical and pharmaceutical products, heavy metals, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), dioxins and furans, among others, have been reported as endocrine disruptors (EDs) [11,12,13,14,15,16,17]. Therefore, the objective of this study was to investigate the potential impacts of pesticides currently used in Sinaloa, Mexico as (EDs) on tilapia. This could help to prevent health hazards, since humans rely on tilapia as one of the main sources of protein, especially when it is commercially produced in bodies of water which could be polluted by pesticides.

2. MATERIALS AND METHODS

2.1 Tilapia Exposure to Pesticides

Healthy tilapia (*Oreochromis spp*.) length 14-19 cm and weighting 96-110 g/fish were obtained from the aquaculture laboratory of Instituto Tecnológico de Mazatlán. The tilapia fish were sorted according to weight and sex. The sex identification in tilapia was done by observing the genital papilla; the males have two openings in the papilla, whereas females have three [18]. After acclimation period (56 days), the female tilapia was separated from the male and distributed randomly in glass aquaria (50Lx30Wx40H cm.) each, at a rate 3 fish/aquarium, containing 30 l of aerated water. The female aquaria were divided into five groups with three replicates per each group. The first group was free from any pesticide and maintained as a control female group. The second, third, fourth and fifth groups were exposed to 2.8, 15.0, 225.0 and 315.0 µg/l of endosulfan, lindane, diazinon and malathion, respectively for 21 days. The pesticide solutions were prepared dissolving each one in acetone and 3 ml of each solution was added to aquariums. While the male aquariums, were divided into three groups with three replicates per each group. The first group was free from any pesticide and maintained as a control male group. The second and third groups were exposed to 2.8 and 315.0 µg/l of endosulfan and malathion respectively for a period of 21 days. Controls consisted of fishes of the same sizes, weights and sex and 3 mL of acetone (the same volume of the pesticide solutions) was added. During this period, commercial food (Purina®) was supplied twice daily, at a rate of 5% the gross weight of the tilapias. The water temperature fluctuated between 26 and 27 $^{\circ}$C and the salinity between 3 and 5 PSU. Constant aeration of the aquariums was maintained and the periods of illumination were

normal hours of day and night. At the end of the experimental period, blood was extracted, then the fishes were sacrificed and the dorsum-lateral tissue dissected.

2.2 Hormones in Blood Plasma

Blood samples (0.5 ml) were taken from the caudal vein of non-anaesthetized fish by sterile syringe containing EDTA as an anticoagulant. Plasma was obtained by centrifugation at 3000 rpm for 15 min and non-haemolyzed plasma was saponified with NaOH-Ethanol (55ºC, 10 min.) to separate the hormones from the lipids. The hormones were extracted from the saponified solution with n-hexane by vigorous agitation for 5 minutes. The extracts were taken to dryness and re-dissolved in 1 mL of acetone plus 0.5 mL of a hormone mixture solution (containing progesterone, testosterone and 17-β-estradiol each with concentration of 0.5 ng/ml) to fortify the samples. The identification and quantification of hormones in the extracts was carried out by high performance liquid chromatography (HPLC) using a Shimadzu LC-10A, coupled to an UV-Vis detector Shimadzu SPD-10A and a column Supelcosil-LC-18-T (15cm x 4.6mm, 3µm) from Supelco® [19]. The mobile phase was acetonitrile-water (50-50 v/v) at a 1ml per min flow rate, during 18 min. The absorbance was recorded at 205 and 270 nm wavelength and as hormone standards, the same mixture solution used to fortify the samples was used.

2.3 Pesticides in Muscular Tissue

The dorsum-lateral muscle (5 g) of each all treatment and control fishes was dissected and then crushed using a tissue homogenizer (Omni TH®). The pesticides from the crushed tissues were extracted by successive additions of n-hexane and anhydrous sodium sulfate as dehydrating agent in a beaker, vigorously agitated and the liquid decanted to centrifuge tubes and centrifuged to separate the tissue debris. Each extract was separated from dehydrated tissue mass by centrifugation (822 g, 15 min.)The extracts were clean-up passing the extracts through packed columns of Alumina, Florisil, Silica-gel and anhydrous sodium sulfate (3+3+3+1.5 g). The cleaned extracts were concentrated to dryness using a rotary evaporator (Brinkmann®) and re-dissolved in 2ml of iso-octane, following the protocol proposed in [5]. The identification and quantification of pesticides in the samples was performed by gas chromatography (GC) with an electron capture detector (ECD) and a flame ionization detector (FID). The column used was an Equity5 (30m X 0.25 mm I.D., 0.25 µm thickness) from Supelco® and N_2 (99.99 % purity) as carrier gas. The pesticides detection limit by this method ranged from 0.001 to 0.005 (µg/g-tissue). Details of the analyses are presented in [5,20].

2.4 Statistical Analyses

All experiments were performed in triplicate and a SYSTAT software (Version 11, SPSS) was used to calculate the means and standard deviations of each experiment data, also ANOVA tests were performed for the comparison between treatments and controls and a Fisher Least Significant Difference, with significant level at $P < .05$ was calculated for each experiment, such as recommended by Sokal and Rohlf [21].

3. RESULTS AND DISCUSSION

3.1 Results

It can be seen from absorbance values shown in (Fig. 2), that for hormone standards 17-β-estradiol and progesterone, the response were higher at 270 nm wavelength compared to 205 nm, whereas for testosterone, response was lower at 270 than at 205 nm. Therefore, the analyses of hormones in blood samples were done using both wavelengths.

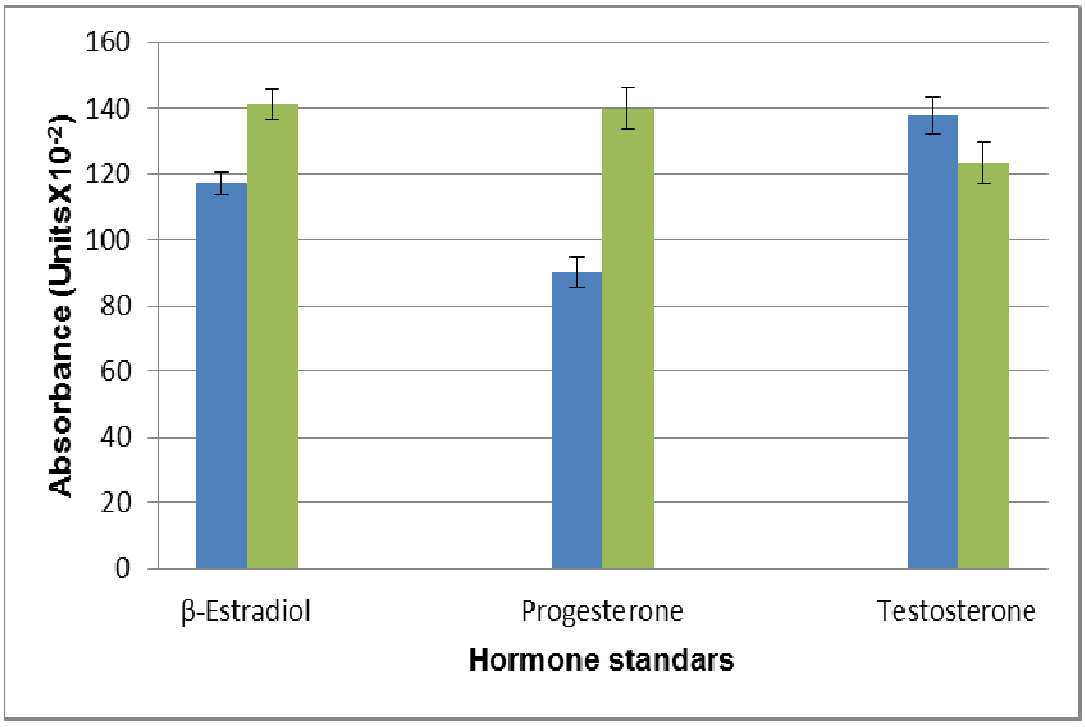

Fig. 2. Absorbance values of hormone standards (0.5 ng/ml) recorded at 205 nm (blue bars) and 270 nm (green bars) of wavelengths

As shown in (Fig. 3.), the present study showed that concentrations of 17-β-estradiol, were lowered significantly in the blood of female *Oreochromis spp*., after exposed to all pesticides assayed, when compared to the control group at both wavelengths (*P* values ranged from .00 to .03).

The progesterone concentrations are show in (Fig. 4). The values measured at 205 nm, were significantly lower in exposed fishes to all pesticides assayed when compared to control group (*P* values ranged from .000 to .009). Whereas at 270 nm, the concentrations were significantly lower in fishes exposed to all pesticides (*P* values ranged from .000 to .007), except the female fish exposed to sublethal dose of diazinon was not significantly lower, when compared to the control group.

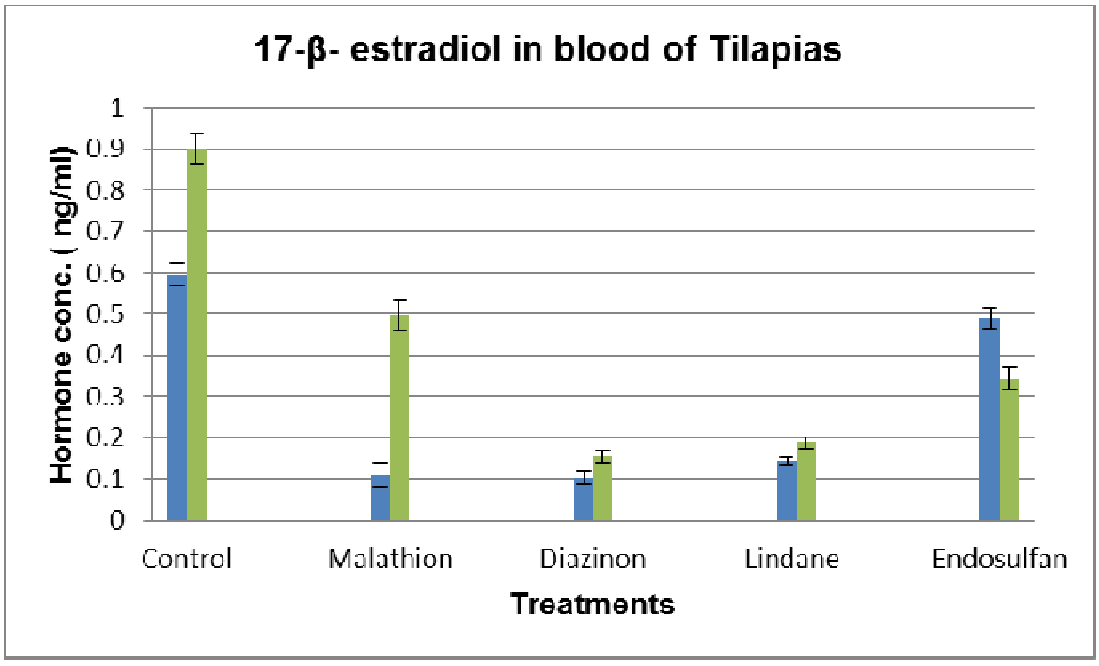

Fig. 3. 17-β-estradiol in blood of Tilapia exposed to sub-lethal concentration of pesticides. Blue bars correspond to values calculated from absorbance readings at 205 nm and green bars to those at 270 nm

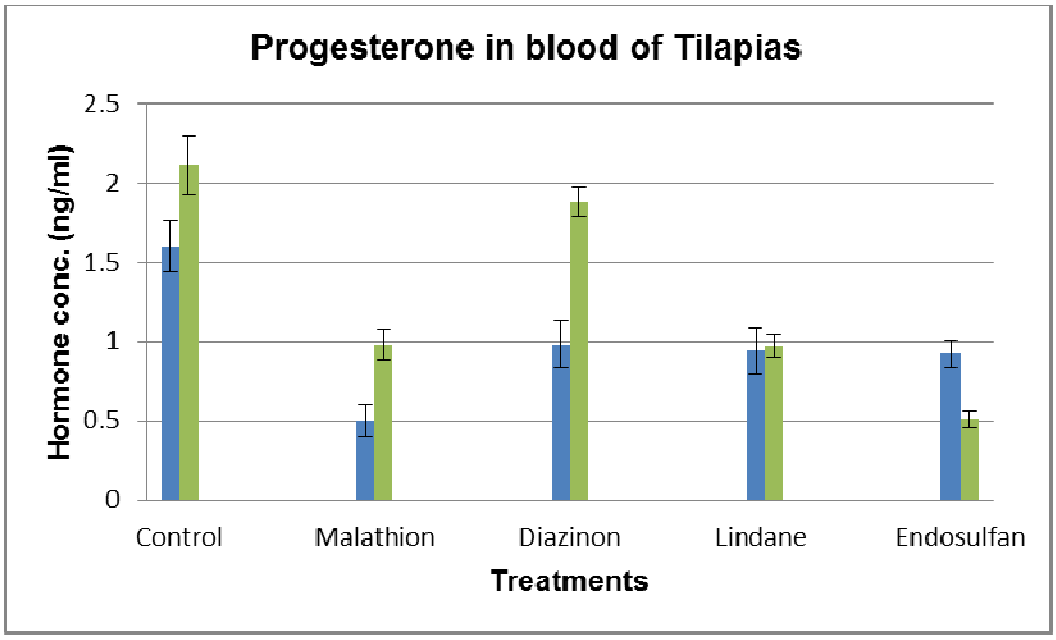

Fig. 4. Progesterone in blood of Tilapia exposed to sub-lethal concentration of pesticides. Blue bars correspond to values calculated from absorbance readings at 205 nm and green bars to those at 270 nm

In (Fig. 5), is illustrates the effect of chronic malathion or endosulfan toxicity on the testosterone concentration in the blood of male tilapia, throughout experimental period. The level of testosterone, showed a significant decrease ($P \leq 0.01$) in all fishes intoxicated with malathion or endosulfan at both wavelength.

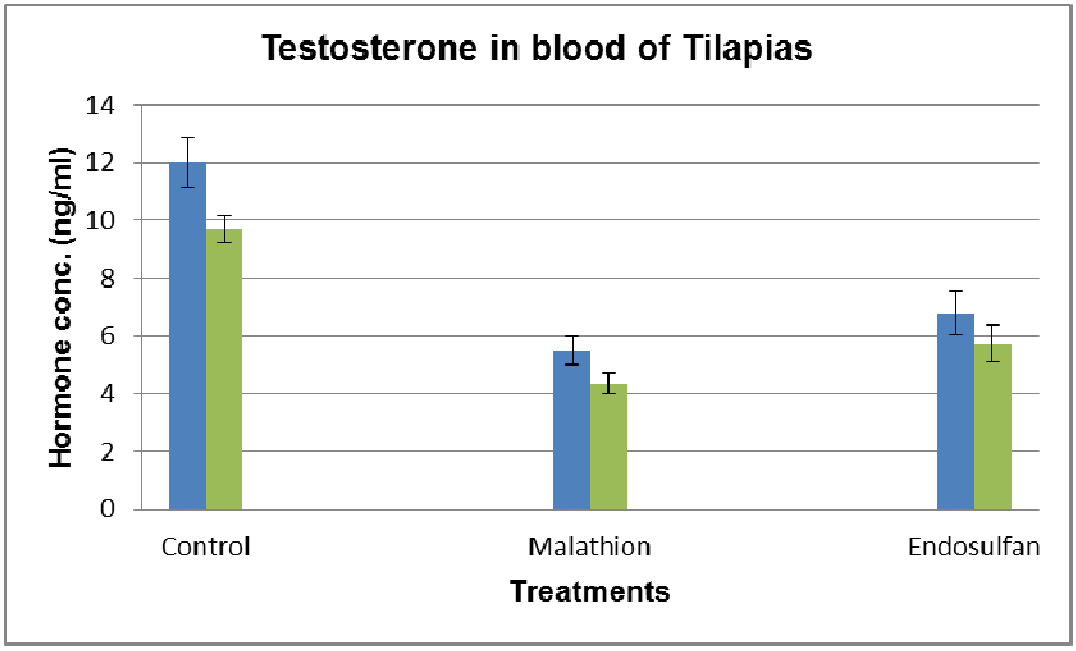

Fig. 5. Testosterone in blood of Tilapia exposed to sub-lethal concentration of pesticides. Blue bars correspond to values calculated from absorbance readings at 205 nm and green bars to those at 270 nm

The bioaccumulation factors (ppm in tissue/ppm in the water) of pesticides in the dorsum-lateral tissue of fishes exposed are shown in (Fig. 6). The organochlorine pesticides presented bioaccumulation factors much higher than those of organophosphorus pesticides: 760 for endosulfan and 310 for lindane compared with 41.3 for malathion and 11.9 for diazinon. The pesticides in tissue of control fishes were under detection limit, i.e., lower that 0.001 µg/g-tissue; therefore, there was no bioaccumulation factor in control fishes.

3.2 Discussion

The four pesticides assayed caused reduction in the hormonal levels in blood, particularly 17-β-estradiol. They may therefore be considered endocrine disruptors. This is in agreement with their listing in the European Census of Endocrine Disruptors [22,23]. Similarly, the significantly decreased testosterone in Nile tilapia *O. niloticus*, specially the male may be as results of direct damage of herbicide butataf on the leydig cells, [24]. Also, butataf may alter androgen biosynthesis mediated by cytochrome P-450 system of interstitial cells of the a testis which is required for function of 17α-Hydroxylase and 17,20 Lyase, [25].

In previous studies, the pesticides utilized in this work have been reported in water, sediments, shrimps and fishes of coastal ecosystems of Sinaloa [26,27,28]. It is also well

documented that they are widely used in the agricultural fields of this region [4]. Endosulfan is an organochlorine pesticide widely used in Sinaloa due its great efficiency and low cost. Its residence in the soils is reported between 60 to 800 days [29] and has been reported as an endocrine disruptor [30]. Lindane (γ- hexachlorocyclohexane) is also an organochlorine pesticide used in agricultural fields of Sinaloa. Although it is legal, its use has been restricted in Mexico. Its toxicity is moderate, but it is very persistent in the environment and can cause injuries to the nervous, endocrine and reproductive systems [28]. Diazinon is an organophosphorous pesticide that is highly toxic but not persistent. It reportedly does not accumulate in animal tissues and is eliminated by organisms in few days [29]. However, in this work its bioaccumulation factor in fish tissue during the exposure time, was 11.9. Malathion is also an organophosphorus pesticide widely used in Sinaloa, particularly in sanitary campaigns for controlling dengue´s vector (*Aedes spp*. mosquitoes). The majority of data on their toxic effects on fauna have been generated in studies with fishes and amphibians [15,30].

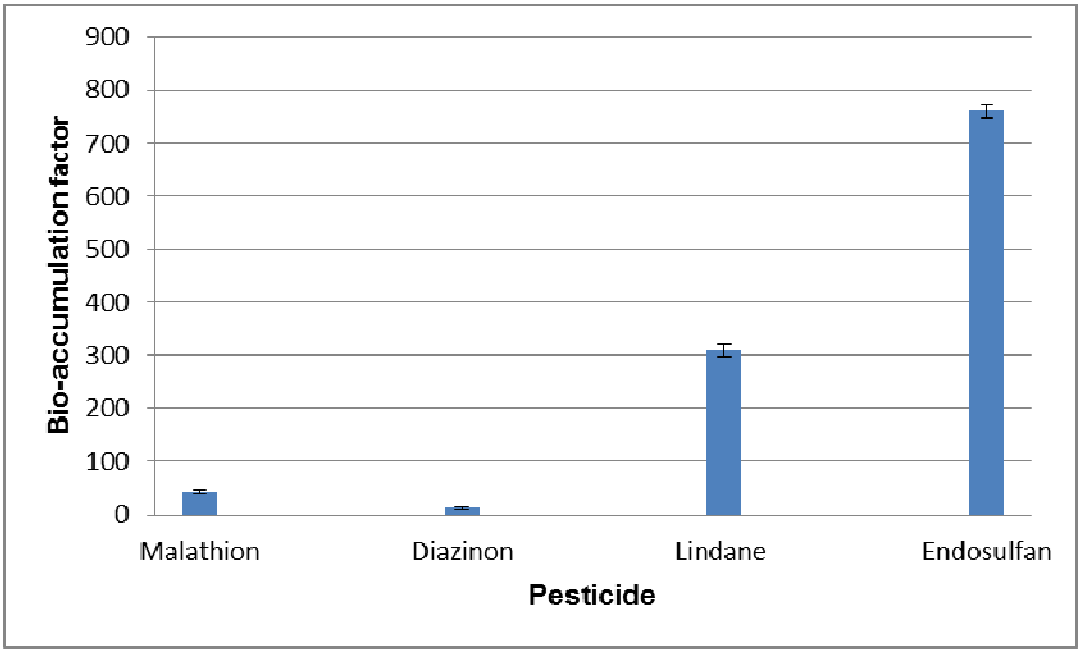

Fig. 6. Bioaccumulation factors of pesticides in the dorsolateral tissue of tilapias exposed to sub-lethal concentration of pesticides

Several pesticides have been identified as endocrine disruptors in other vertebrate fishes, including atrazine, 2,4-D, DDE, DDT, diazinon, diuron, endosulfan, fenthrothion, glyphosate, lindane, parathion and permethrine; for instance, it has been shown that DDT and methoxychlor have xenoestrogenic activity, meaning that they are chemically similar to estrogens and trigger hormonal responses in exposed organisms, including the induction of vitellogenesis in young male fishes [11]. In normal conditions, only the females are able to produce vitellogenin, due to the action of hormone 17-β-estradiol. It has been previously reported, that tilapia and other vertebrate fishes such as cutthroat trout, responding to stressing compounds (pesticides, PCBs and other pollutants) can produce several biochemical and physiological responses in nervous and endocrine systems, such as

increasing or reducing, the branchial blood flow, the muscular activity and induction of steroid hormones in blood, [31].

4. CONCLUSION

From the results it can be concluded that pesticides assayed are endocrine disruptors in tilapia fish and consequently, important endocrine functions are impaired, such as growing, sexual maturity, reproduction, etc. On the other hand, the pesticides accumulated in tilapia´s tissue represent a risk in public health, since this organism is a popular food in Mexico and other developing countries.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

The authors declare to have no conflict of interest exits.

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