UNDER PEER REVIEW

Original Research Article

Bioaccumulation and evidence of hormonal disruptions in tilapia fish (Oreochromis spp.) exposed to sub-lethal concentrations of pesticides in Sinaloa, Mexico

ABSTRACT

Examination endocrine disruption induced in tilapia fish (*Oreochromis spp.*) caused by after exposure to sub-lethal concentrations of endosulfan, lindane, diazinon and malathion was evaluated by the quantification of 17-β-estradiol, progesterone in the blood of female and testosterone in male fish blood. 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 (96-110g/fish) were exposed to 2.8 ug/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 (*Oreochromis spp.*) were significantly decreased in all treatment. Similarity, The level of testosterone was decreased significantly in the blood of male fish exposed to endosulfan and malathion pesticides when compared th the control fish group. At the end this period, the bioaccumulation of pesticides in muscular tissue of fishes were 760, 310, 41.3 and 11.9 (ug/g) for endosulfan, lindane, malathion, and diazinon, respectively. was quantified using gas chromatography with an electron capture detector (ECD) and a flame ionization detector (FID). The hormonal concentration in blood plasma was measured using high-performance liquid chromatography (HPLC). The bioaccumulation factors were 760, 310, 41.3 and 11.9 for endosulfan, lindane, malathion, and diazinon, respectively. 17-β-estradiol and testosterone concentrations in blood were significantly lower in exposed fishes than in controls, while progesterone was lower in fishes exposed to endosulfan, lindane and malathion, and higher for fishes exposed to diazinon. Results suggest that pesticides assayed, are endocrine disruptors in tilapia, which can affect the reproduction re production 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 (Figure 1) [1].

UNDER PEER REVIEW

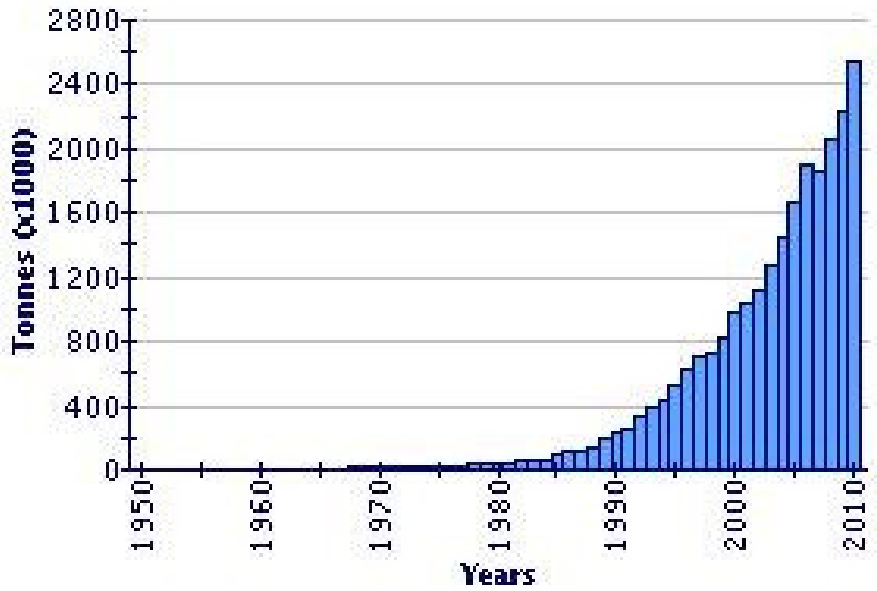

Figure 1. Global aquaculture production of Tilapia (Oreochromis spp.)

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 in 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 to 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, with the view to reinforce regulation and policies in the management of these pesticides. 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. MATERIAL AND METHODS

2.1 Tilapia exposure to pesticides

Healthy Juveniles of tilapia (*Oreochromis spp.*) length 14-19 cm and weighting 96-110g/fish were obtained from the aquaculture laboratory of Instituto Tecnológico de Mazatlán. The tilapia fish organisms 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 a conditioning period (56 days), the female tilapia was separated from the male and distributed randomly in galss aquaria each at a rate 3 fish/ aquarium that containing aerated water. The female aquaria were divided into five groups with three replicates each per group The first group was free any pesticides 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. While. The male aquarium were divide into three groups with three replicates each per group. The first group was free any pesticides and maintained as a control male group The second and third groups were exposed to 2.8 and 315.0 ug/lof indosulfan and malathion respectively for a period of 21 days . . Separate cohorts of male and female tilapias (3 per

UNDER PEER REVIEW

aquarium) of sizes between 14 to 19 cm and weights from 96 to 110 g were exposed to sub-lethal concentrations of the following pesticides: endosulfan (2.8 µg/L); lindane (15.0 µg/L); diazinon (225.0 µg/L) and malathion (315.0 µg/L) for a period of 21 days, with daily application of 3 mL of pesticide solutions in acetone to each aquarium. However, due to insufficient of tilapias male, the males were exposed only to malathion and endosulfan. Controls consisted of fishes of the same sizes, weights and sex, and 3 mL of acetone (the

UNDER PEER REVIEW

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 °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 exposure period, blood was extracted, then the fishes were sacrificed and the dorsum-lateral tissue dissected.

Hormones in blood plasma

At the end of the experiment, blood samples were taken from the caudal vein of non anaesthetized fish by sterile syringe. 0.5 ml of the blood containing EDTA as an anticoagulant Plasma was obtained by centrifugation at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for further steroid analyses.

The blood from each fish was extracted by puncturing the caudal fin vein using a syringe impregnated with an anticoagulant, ethylenediaminetetraacetic acid (EDTA). The blood samples were centrifuged (822 g, 15 min.) to separate the plasma,. and 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.

Pesticides in muscular tissue

The dorsum-lateral muscle (5 g) of each all treatment exposed fish 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. Details of the analyses are presented in [5].

2.4. Statistical Analyses.

All experiments were performed by triplicate and a SYSTAT software (Version 11, SPSS) was used to calculate averages 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. (Should be added the reference) such as indicated by Sokal and Rohlf [20].

3. RESULTS AND DISCUSSION

Results

It can be see from data given in As can be observed in (Fig. 2), the hormone standards of 17-β-estradiol and progesterone concentration in the blood of female tilapia were showed higher response at 270 nm wavelength compared to 205 nm, whereas testosterone was registered lower response at 270 than at 205 nm. Therefore, the analyses of hormones in blood samples were done using both wavelengths.

UNDER PEER REVIEW

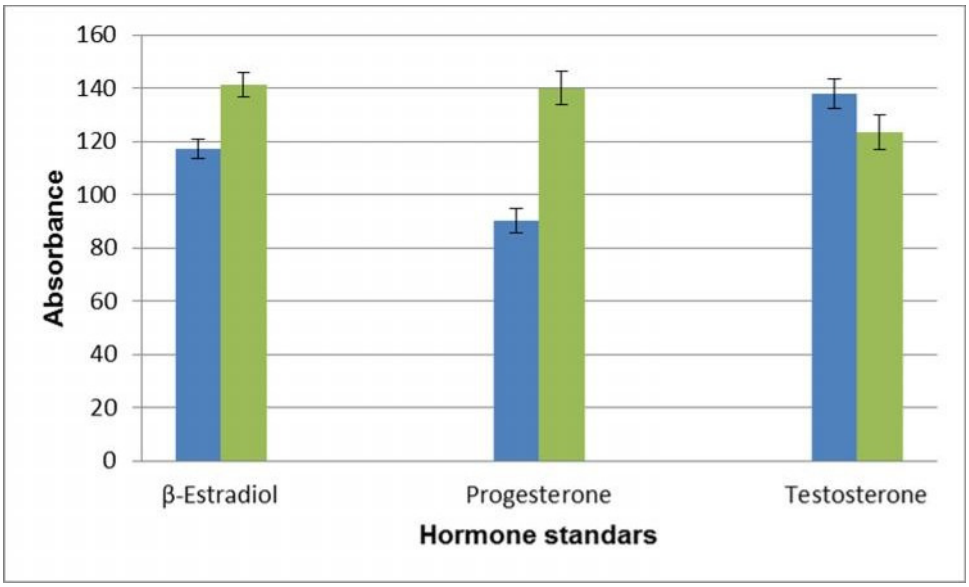

Figure 2. Absorbance values of hormone standards recorded at 205 nm (blue bars) and 270 nm (green bars) of wavelengths

The 17-β-estradiol concentrations in blood of female tilapia exposed to pesticides are shown in (Fig 3.). The present study showed that level of 17-β-estradiol were lowered significantly in the blood of female *Oreochromis* after exposed to all mention pesticides when compared the control group *a*t both wavelengths the concentration of 17-β-estradio in the fe was significantly lower (*P* values ranged from .00 to .03) in exposed fishes than those of controls.

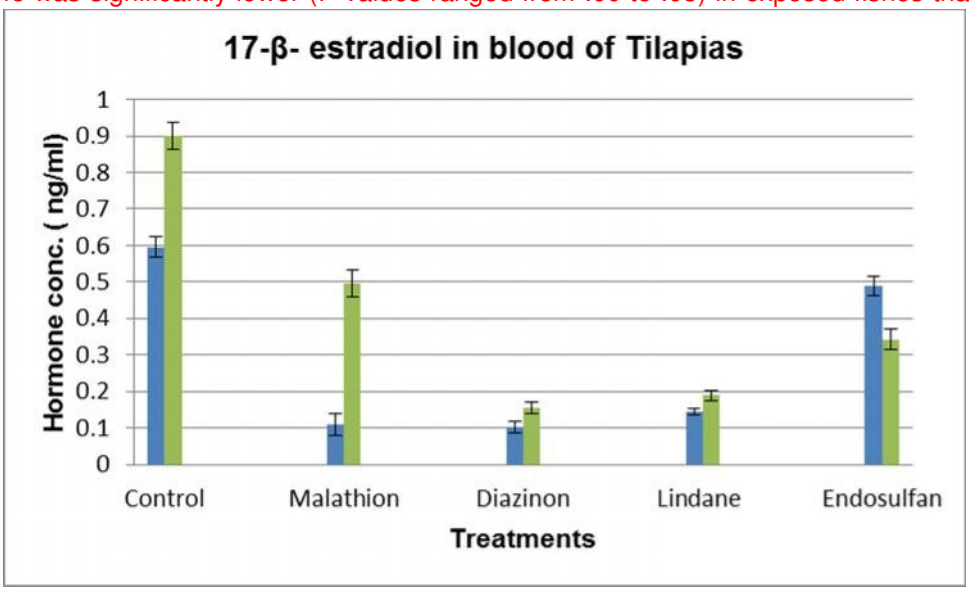

Figure 3. 17-β-estradiol in blood of Tilapias exposed to sub-lethal concentration of pesticides. Blue bars correspond to values calculated from absorbance lectures at 205 nm, and green bars to those at 270 nm.

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 for all pesticides assayed (*P* values ranged from .000 to .009). Whereas at 270 nm, the concentrations were significantly lower in fishes exposed to all pesticides except the female fish exposed to sublethal dose of diazinon was lower non significantly when compared to the control group. malathion, lindane and endosulfan (*P* values ranged from .000 to .007) than control, whereas for diazinon was slight higher (*P* =.054).

UNDER PEER REVIEW

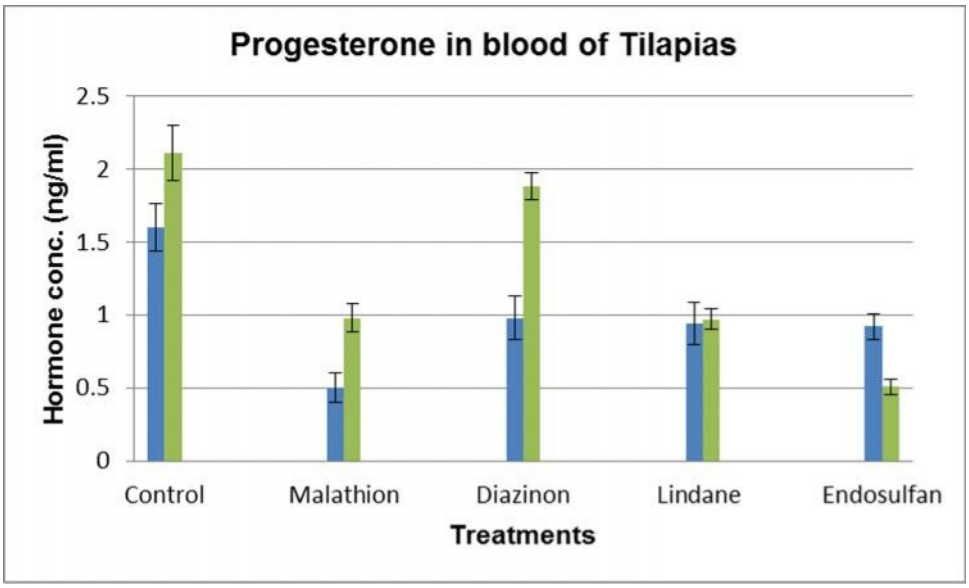

Figure 4. Progesterone in blood of Tilapias exposed to sub-lethal concentration of pesticides. Blue bars correspond to values calculated from absorbance lectures at 205 nm, and green bars to those at 270 nm.

Fig (5) 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 ≤ 0.01) in fish intoxication with malathion or endosulfan at both wavelength.

Although testosterone was measured only in tilapia exposed to malathion and endosulfan, the hormone values at both wavelength were significantly lower (*P* values ranged from .000 to .001) in fishes exposed than those in controls (Fig. 5).

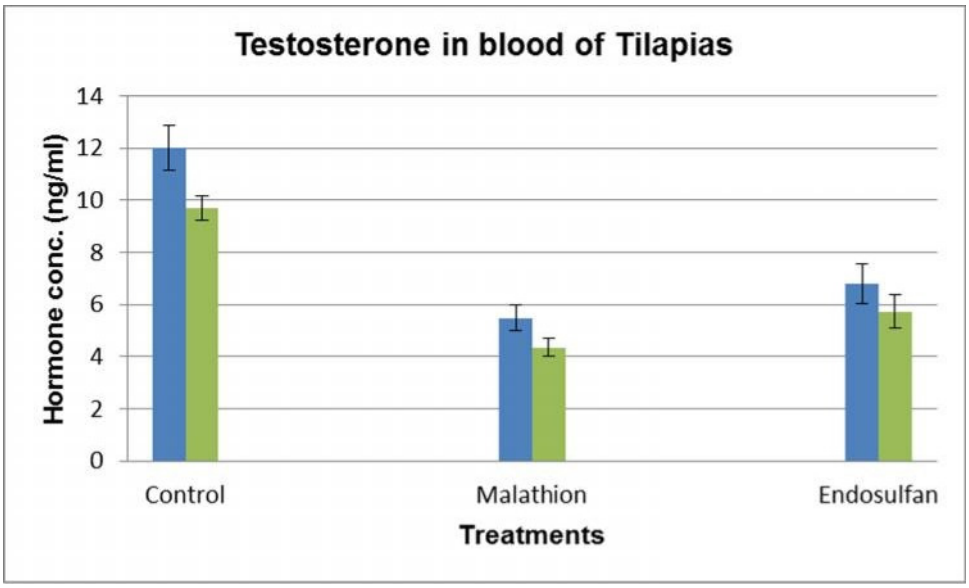

Figure 5. Testosterone in blood of Tilapias exposed to sub-lethal concentration of pesticides. Blue bars correspond to values calculated from absorbance lectures at 205 nm, and green bars to those at 270 nm.

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.

UNDER PEER REVIEW

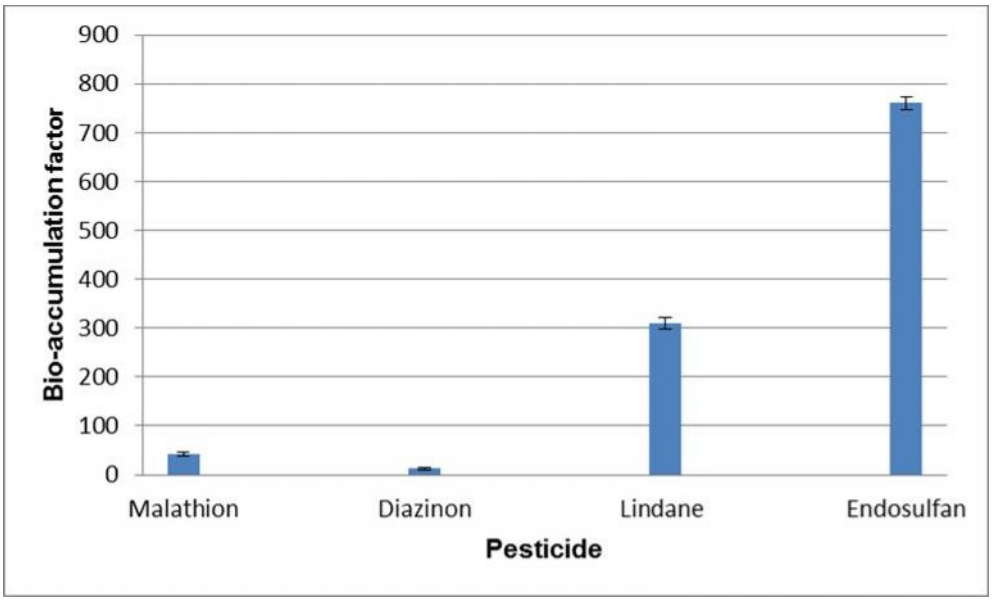

Figure 6. Bioaccumulation factors of pesticides in the dorsolateral tissue of tilapias exposed to sub-lethal concentration of pesticides.

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 [20, 21]. Similarly, the significantly decreased testosterone in Nile tilapia *O. niloticus*, specially the male may be as a results of direct damage of butataf on the leydig cells Shalaby (22). 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 17 &-hydrolase and 17-20 layse Flodstrom (23)

In previous studies, the pesticides utilized in this work have been reported in water, sediments, shrimps and fishes of coastal ecosystems of Sinaloa [24, 25, 26]. 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 [27] and has been reported as an endocrine disruptor [28]. 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 [27]. 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. 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, 27].

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 (*Oreochromis spp.*) like other vertebrate fishes, response 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 hormonal levels in the blood, the branchial blood flow, the muscular activity and induction of vitellogenesis [29].

UNDER PEER REVIEW

4. CONCLUSION

From the results can be concluded that pesticides assayed are endocrine disruptors in tilapia fish, and consequently, important endocrine functions are impaired, such 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.

COMPETING INTERESTS

The authors declare to have no conflict of interests

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.

REFERENCES

1 FAO Yearbook. “Fishery and Aquaculture Statistics”. Food and Agriculture Organization of the United Nations. Rome. 2012, Accessed 19 October 2013.
Available: http://www.fao.org/docrep/015/ba0058t/ba0058t.pdf

2 El estado mundial de la pesca y la acuicultura. Departamento de Pesca y Acuicultura de la FAO Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma. 2010. Spanish. Accessed 11 October 2013.
Available: http://www.fao.org/docrep/013/i1820s/i1820s.pdf

3 CONAPESCA, Comisión Nacional de Pesca y Acuacultura. Mexico D.F. 2012, Accessed 10 October 2013
Available: http://www.sagarpa.gob.mx/saladeprensa/boletines2/Paginas/2012B657.aspx

4 Beraud Lozano JL, Galindo Reyes JG, Covantes Rodríguez C. Jornaleros y medio-ambiente: Los agroquímicos en la agricultura sinaloense. 1st ed. Ediciones de la Universidad Autónoma de Sinaloa; 2008. Spanish

5 Reyes-Montiel NJ, Santamaría-Miranda A, Rodríguez-Meza GD, Galindo-Reyes JG, González-Ocampo HA. Concentrations of Organochlorine Pesticides in Fish (Mugil Cephalus) from a Coastal Ecosystem in the Southwestern Gulf of California. Biol Environ: Proc. of the Royal Irish Academy. 2013;113-B(3):1-11.

6 Pitarch AE. Desarrollo de metodología analítica para la determinación de plaguicidas organofosforados y organoclorados en muestras biológicas humanas. Departamento de Ciencias Experimentales. Química Analítica. Tesis Doctoral. Universitat Jaume I de Castellón. España. 2001, Accessed 10 September 2013.
Available: http://www.tdx.cat/bitstream/handle/10803/10403/pitarch.pdf?sequence=1

7 Hernández Rivera SA, Martínez Gándara YJ. Biotransformación de malation por cepas de Rhizobium aisladas de *Desmodium tortuosum* (SW) DC. Foresta Veracruzana. 2005;7(2):53-58. Accessed 11 September 2013. Spanish
Available: http://www.redalyc.org/pdf/497/49770209.pdf

8 Badii HM, Landeros J. Plaguicidas que afectan a la salud humana y la sustentabilidad. CULCYT/Toxicología de plaguicidas. 2007;4(19):21-34. Accessed 14 September 2013. Spanish
Available: http://www2.uacj.mx/IIT/CULCYT/marzo-abril2007/6Art_MBadii.pdf

9 Fossi M, Casini S, Marsili L. Endocrine Disruptors in Mediterranean top marine predators. Environ. Sci. Pollut. R. 2006;13(3): 204-7.

UNDER PEER REVIEW

10 Schmutzler C, Bacinski A. The UV filter benzophenone 2 interferes with the thyroid hormone axis in rats and is a potent in vitro inhibitor of human recombinant thyroid peroxidase. Endocrinology %R 10.1210/en.2006-1280: en.2006-1280, 2007.

11 Pait AS, Nelson JO. Endocrine disruption in fish. An assessment of recent research and result. NOAA technical memorandum NOS NCCOS CCMA 149. Silver Spring, MD: NOAA, NOS, Center for Coastal Monitoring and Assessment 55 pp, 2002. Accessed 1 September 2013.
Available: http://aquaticcommons.org/2177/1/NOS_TM149.pdf

12 Vrijheid M, Armstrong B. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup. Environ. Med. 2003; 60(8):543-550.

13 Chacón MO, Cuevas CF, de la Fuente OC, Díaz AF, Huaquin ML. Disrupción endocrina e imposex. Avances en Ciencias Veterinarias. 2007;22:42-48. Spanish

14 Khan IA, Thomas P. Disruption of Neuroendocrine Control of Luteinizing Hormone Secretion by Aroclor 1254 Involves Inhibition of Hypothalamic Tryptophan Hydroxylase Activity. Biol. Reprod. 2001;64(3):955-964.

15 Sandoval MT, Pérez-Coll CSY, Herkovits J. Efectos tóxicos del malatión y glacoxan® sobre los embriones de Bufo arenarum. Acta Toxicol. Argent. 2004;12(Supl.):1-46. Spanish

16 Kitamura S, Suzuki T. Comparative Study of the Endocrine-Disrupting Activity of Bisphenol A and 19 Related Compounds. Toxicol. Sci. 2005;84(2):249-259.

17 Crane HM, Pickford DB, Hutchinson TH, Brown JA. The effects of methamizole on developmental of the fathead minnow, *Pimephales promelas*, from embryo to adult. Toxicol. Sci. 2006;93(2):278-285.

18 Eknath AE, Bentsen HB, Ponzoni RW, Rye M, Nguyen NH, Thodesen JY, Gjerde B. Genetic improvement of farmed tilapias: Composition and genetic parameters of a synthetic base population of Oreochromis niloticus for selective breeding. Aquaculture. 2007;273:1-14.
19 Wilson P. Development and validation of a liquid chromatographic metho d for the simultaneous determination of estradiol, estriol, estrone, and progesterone in pharmaceutical preparations. J AOAC Int. 2009;92(3):846-54.

20 Argemi F, Cianni N, Porta A. Disrupción endocrina: perspectivas ambientales y salud pública. Acta Bioquim. Clin. Latinoam. 2005;39(3):291-300. Accessed 19 October 2013. Spanish
Available: http://www.redalyc.org/pdf/535/53539304.pdf
21 Annex 6. List of 146 substances evaluated in the Expert meeting (European Commission). Last updated: 03/10/2013 Accessed 30 October 2013.
Available: http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm#priority_list

22 shalabyA, Mousa M and Hanan Tag El Dian. Toxicological effect of butataf herbicide on some physiological aspects and the reproductive performance of Nile TILAPIA Oreochromis niloticus. Egypt. J. Aquatic. .Biol &Fish.Vol 11, No 2:145-163 2007
23 Flodstrom, S.; Hemming, H.; Warngard, L. and Ahlborgs, U. G. Promotion of altered hepatic foci development in rat liver, cytochrome P- 450 enzyme induction and inhibition of cell – cell communication by DDT and some structurally related organohalogen pesticides. Carcinogenesis., 11:1413- 1417.

24Galindo Reyes JG, Fossato VU, Villagrana Lizárraga LC, Dolci F. Pollution Pesticides in Water, Sediment and Shrimp From a Coastal Lagoon Into the Gulf of California. Mar. Pollut. Bull. 1999;38(9):837-841

25 Galindo Reyes JG, Villagrana Lizárraga LC, Lazcano AM. Environmental Condition and Pesticide Pollution of two Coastal Ecosystems in the Gulf of California. Ecotox. Environ. Safe. 1999;44:280-286.

26 Galindo Reyes JG, López Guillén ME, Jorge Y, Verdugo AM. Plaguicidas, y sus efectos crónicos en larvas de camarones. Estudio Realizado en el Sur de Sinaloa. Industria Acuícola. 2006;2(2):12-15. Spanish.
Available: http://www.industriaacuicola.com/revista2_2.htm

27K. Verschueren, Handbook of environmental data on organic chemicals, Van nostrand reinhold. New York, USA; 1996.
28 Liu W, Zhu LS. Assessment of the genotoxicity of endusolfan in earthworm and White clover plants using the comet assay. Arch. Environ. Contam. Toxicol. 2009;56:742-746.

29Iwama KG, Afonso BLO, Todgham A, Ackerman P, Nakano K. Are hsps suitable for indicating stressed states

UNDER PEER REVIEW

in fish? J. Exp. Biol. 2004; 2007: 15-19.

Comments:-

-Most words and phrases in red color in most paragraphs should be deleted

- The sentences or phrases that have been blue color should be added
- The paragraph in dissection that have been green color should be added
- The references that have been green color should be added to references of manuscript.
- Should be write of reference for statistical analyses

Note:

Should be measured accumulated the pesticides in the in control group and compared of pesticides accumulated between the control group and toxic groups

The manuscript accept after major essential review.

- level of interest of manuscript: An article of important in its field.
- Quality of written English: Acceptable.
- Statistical analysis: should be add the reference which used for analysis .