The Bioscan 11(4): 2277-2280, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

HISTOPATHOLOGICAL EFFECTS IN GILLS OF FRESHWATER MUSSELS, *LAMELLIDENS MARGINALIS* EXPOSED TO MERCURY CHLORIDE

ABHED PANDEY*, SHANTHANAGOUDA, A. H., DEVENDRA PATHAK[1] AND AVTAR SINGH[2]
*Department of Aquaculture, College of Fisheries, GADVASU, Ludhiana - 141 004, Punjab, INDIA
[1]Department of Veterinary Anatomy, GADVASU, Ludhiana - 141 004, Punjab, INDIA
[2]Department of Food Technologies, Prince of Songkla University, Thailand - 900 112
e-mail: pandeyabhed@yahoo.com

KEYWORDS
Lamellidens marginalis
Mercury
Gill
Histopathology

Received on :
10.08.2016

Accepted on :
07.10.2016

***Corresponding author**

ABSTRACT
Freshwater mussels, *Lamellidens marginalis* exposed to 0 (control), 1, 5, 10, and 15ppm concentrations of the mercury chloride for 96 hrs. Histopathological changes in the filtering organ, gills were observed after exposure period in all the groups. The study revealed that water quality parameters such as pH (7.55±0.6), temperature (25.2±2.1ºC), dissolved oxygen (8.36±1.21 mg/l), total alkalinity (230±0.2 mg/l), total hardness (165±0.5mg/l), total ammonia (<0.24 mg/L) and nitrite levels (<0.003 mg/l) did not vary significantly in the treatments. Gill histopathology showed remarkable changes in mussels exposed to mercury in comparison to control. The lengths of gill lamellae were changed and clubbing of their shape was occurred and hyperplasia of epithelial cells was observed. Rupture of the ciliated epithelium, increase in the size of the lamellae and increase in the space between the inter-lamellar junctions were observed. Granuloma was occurred in connective tissue. In many cases, tissue rupture in connective tissue and atrophy of haemolymph channels epithelium were observed. The results indicate higher doses of mercury (Hg) resulting in massive destruction in normal architecture of gill tissue which is concentration dependent. Moreover, these alterations can be correlated with damages of organs from *L. marginalis* exposed to natural Hg contamination in aquatic biota.

INTRODUCTION

Heavy metals are the most dangerous contaminants of aquatic biota. The occurrence and accumulation of mercury in aquatic species has received much attention in the literature, not only as a potential source of mercury intoxication in humans because it is concentrated in muscle tissue, but also due to the potential toxic effects on aquatic species themselves. The episodes of the Minamata disease and itai-itai disease caused due to mercury and cadmium poisoning respectively, in Japan. Mercury (Hg) is one of the most harmful pollutants due to its high toxicity and persistence in the environment (Kehrig *et al*., 2002). Once Hg is in aquatic systems, different organisms can accumulate Hg, leading to its bio magnification through the food web (Liao *et al*., 2006). Molluscs are mainly consumers of the first order in the food chain and can accumulate a high amount of heavy metals without exhibiting marked visible physiological effects (Jurkieuwicz-Karnkowska, 1994). The freshwater mussel's filter feeding behaviour helps to remove particulates and dissolved nutrients from the water column, and is therefore useful as a bio monitor for contamination in water (Parant and Pain, 2001; Corners and Black, 2004; Wagner and Boman, 2004; Milam *et al*., 2005; Marie *et al*., 2006). They are widespread, sedentary, easy to collect and handle and good accumulators. Furthermore, they might represent a significant entrance of metals in the ecological food chain and accumulate toxicants according to bioavailable levels in the environment (Hendriks *et al*., 1998; Kraak *et al*., 1991; Bervoets *et al*., 2005). In bivalves, mantle and gills have respiratory and feeding functions (Gosling 2003) and significant potential for accumulation of heavy metals and other pollutants (Chakraborty *et al*., 2010). These organs are composed of ciliated epithelium, connective and muscular tissues and rich vessels of haemolymph (Gosling, 2003).

Histological approach is the most valuable tool for assessing the action of toxicants at tissue level providing data concerning tissue damage and also it manifests structural and functional changes (Sprague, 1971) in tissues and organs. The histopathological studies show that heavy metal caused tissue damage in aquatic organisms. It has been previously noted in a variety of animals by many workers, in crabs exposed to mercury (Vernberg and Vernberg, 1972), in the fish *Clarias batrachus* exposed to mercury and cadmium (Selvanathan *et al*., 2013), in the fish *Gymnotus carapo* exposed to mercury chloride (Vergilio *et al*., 2012), in the fish *Punctius sophore* exposed to mercury (Khangarot and Somani, 1980) and in the fish *Poecilia reticulate* exposed to methyl mercury chloride (Wester and Canton, 1992). In the fish *Puntius ticto* exposed to dimethoate pesticides (Marutirao, 2012). Whereas, in the freshwater mussel *Lamellidens marginalis* exposed to nickel (Andhale *et al*., 2011), cadmium (Yasmeen *et al*., 2012; Yasmeen and Mane, 2013), arsenic (Shamsundar and Sureshchandra, 2013). Keeping in view of the fact that histopathological investigations have been proved to be a sensitive tool to detect effects of chemical compounds within

the target organ of fish and mussels in laboratory experiments (Santhakumar *et al*., 2001; Ortiz *et al*., 2003; Olojo *et al*., 2005; Cengiz, 2006; Cengiz and Unlu, 2006; Rao *et al*., 2006; Figueiredo-Fernandes *et al*., 2007; Velmurugan *et al*., 2007), the present study focussed on mercury induced histopathology of the vital organ, gills of *Lamellidens marginalis* as because a large number of water bodies in India are contaminated with sub-lethal to toxic mercury concentrations. These alterations can also be correlated with damages of target organs from *L. marginalis*.

MATERIALS AND METHODS

Lamellidens marginalis were collected from Allahabad, India and transported to the College of Fisheries, GADVASU, Ludhiana. Before the start of the experiment the mussels were acclimatized and depurated in the freshwater for two weeks with the constant supply of oxygen. Water renewed daily till the inception of the exposure experiment. Water quality parameters (pH, temperature, dissolved oxygen, total alkalinity, total hardness, total ammonia and nitrite levels) were monitored fortnightly during the study period according to standard methods of APHA (1998). Mercuric chloride ($HgCl_2$) was purchased from Sigma Aldrich. To determine the range of tolerance of mussels against the mercury they were exposed to different concentrations of mercury including 0 (control), 1, 5, 10, and 15ppm of the mercury. Each treatment had 3 replicates and each replicate contained 10 animals in 50L of water. During the experiment, water was renewed after every 24h to restore mercury level. The mussels were exposed for 96 h and the mussels were not fed during the experiment. After 96h, mussels were removed and killed by cutting abductor muscles and gills were dissected out and cut into pieces and fixed in Bouin's fixative for 24h and processed according to standard procedure of routine micro technique (Nikalje *et al*., 2012, Marutirao, 2012). The tissues were processed for wax sectioning. The sections were cut at 5.0-7.0 µm and stained with haematoxylin and eosin. The observations were made under Nikon microscope and wide and narrow field eyepieces.

RESULTS AND DISCUSSION

The study revealed that during the experimental period, water quality parameters such as water pH (7.55 ± 0.6), temperature (25.2 ± 2.1°C), dissolved oxygen (8.36 ± 1.21 mg/L), total

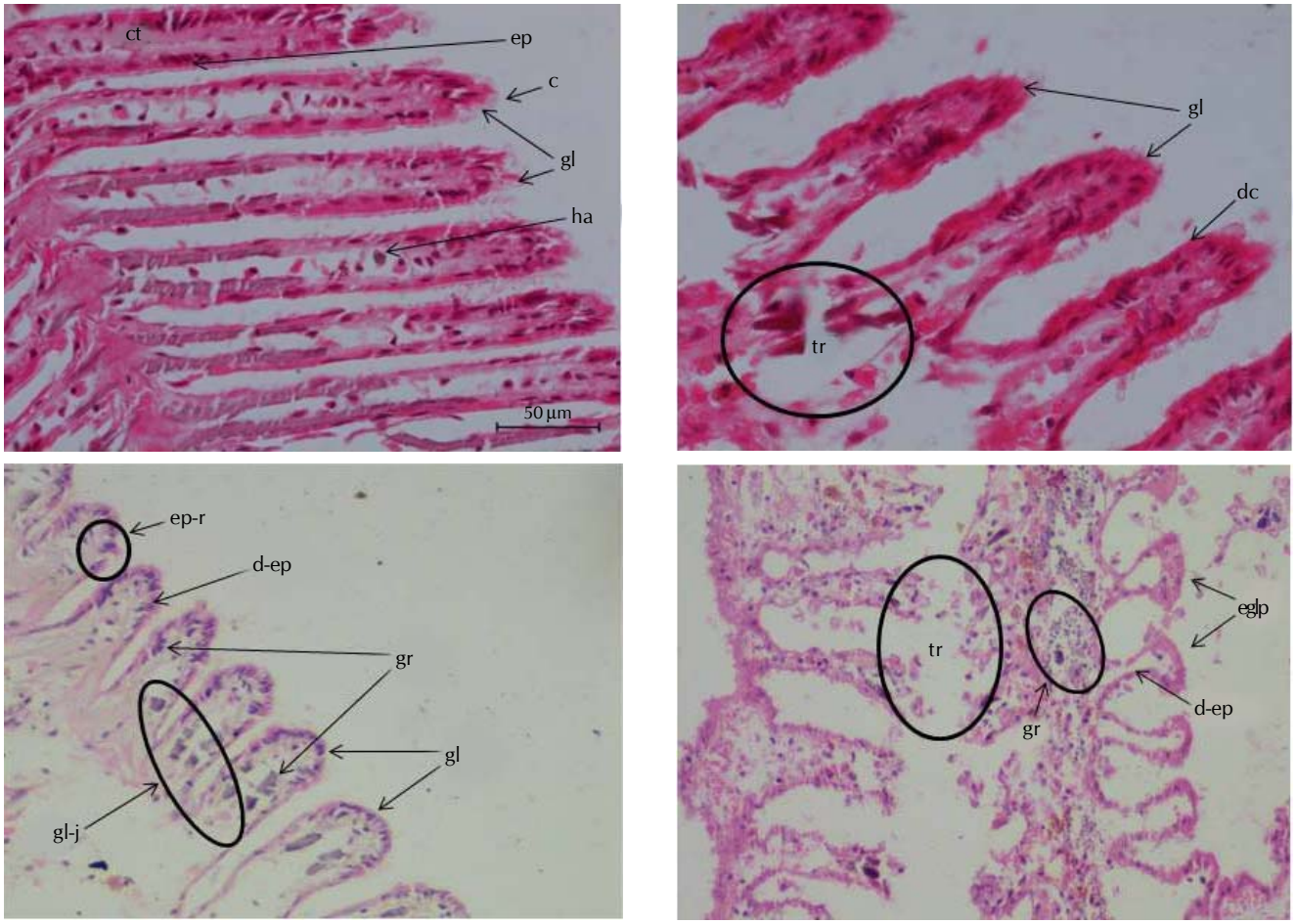

Figure 1: Micrograph of transverse sections (5-7 µm thikness, stained with H and E) through gill. Control (A) and mercury exposed (B, C and D) spcimens. gl: lamellae, ct: connective tissue; c: cilia, ep: epithelium; ha: haemocyte; tr: tissue rupture; dc: damaged cilia; ep: epithelium rupture; d-ep: damaged epithelium; gl-j: junction of adjacent gill lamellae

alkalinity (230 ± 0.2 mg/l), total hardness (165 ± 0.5mg/L), total ammonia (< 0.24 mg/l) and nitrite levels (< 0.003 mg/l) did not vary significantly in the treatments and were recorded within the recommended range as mentioned by Janakiram (2003) for freshwater mussel culture throughout the experimental period. The gill or a ctenidium of *L. marginalis* consists of two gill plates or demibranchs. Each gill plate is formed of two similar flaps or lamellae. Lamellae are formed of numerous gill filaments which contain holes called ostia. The gill filaments are covered by different kinds of cilia and supported by two chitinous rods. The space between two lamellae of a gill plate contains blood vessels. Histological condition of gill in control group of bivalves is showed in figure 1. The epithelium of gill lamellae was healthy and the length of them was normal and equal. The connective tissue at the base of gill lamellae was integrated. In inner parts, Haemolymph channels were observed in a regular fashion and the spaces between these channels were composed of connective tissue. Acute exposure to mercury at 1ppm resulted in change in lengths of gill lamellae, hypoplasia of epithelial cells and interlocking clumps of cilia which covers gill filaments were also damaged. The epithelial cells and connective tissue cells have lost their cellular structure. Epithelium became oedematic, necrotic and vacuolated. Whereas organism exposed to5ppm mercury displayed elongated gill filaments with a swollen lumen (black colour ring). The shape disruption was mainly due to necrosis. Severe loss of epithelium was observed. In spite of severe denudation, small tufts of cilia were seen on the lateral sides. In many cases, tissue ruptures in connective tissue and atrophy of haemolymph channels structure were observed. Granuloma (appearance of cells with yellow to brown colour) was occurred in connective tissue. Whereas organism exposed to 10ppm mercury displayed change in the length of gill lamellae and clubbing of their shape was occurred and hypoplasia of epithelial cells was observed. In inner parts, swelling and hyperplasia of the haemolymph channels epithelium were observed. The exposed organisms showed total loss of gill architectures and damaged inter-lamellar junctions. In connective tissue, disintegration of regular structure of cells was occurred and tissue rupture was observed. Cytoplasm showed disintegration due to swelling in respiratory epithelium. The shape disruption was mainly due to necrosis. Severe loss of epithelium was observed. Gill is important organ of bivalves that involved in respiration of organism. Assessment of histological alterations is an important method adopted to assess the impacts of pollution. Organs such as gills were in direct contact with the surrounding environment and are the major target organs for toxicants (Oliva *et al*., 2009).Histopathology of gill of metal-exposed bivalves showed clear signs of damages that were not observed in control group. The results of the present study is in agreement with Gulbhile (2006) who studied mercuric chloride exposure on *Lamellidens corrianus*, the lamellae of gill showed various changes such as rupture of the ciliated epithelium, increase in the size of lamellae, increase in space between the inter lamellar junction and increase in space between the water tube and inner lamellar junctions. Normal structure of gills totally damaged or disturbed due to mercuric chloride showing fusion and atrophy of secondary gill lamellae, displacement and necrosis of outer layer of gill lamellar epithelium. Similar to our results, changes in length and shape of gill lamellae were reported in different studies (Chakraborty *et al*., 2010; Montaser *et al*., 2010). Nikalje *et al*. (2012) studied histopathological changes in liver of freshwater major carp, *Labeo rohita* after acute and chronic exposure to textile mill effluent. Whereas, Marutirao (2012) studied histopatholgical changes in the gills of *Puntius ticto* (Ham) under dimethoate toxicity. In the present study histopathologic al analysis showed morphological alterations in gills of Hg-exposed *L. marginalis* revealing differences in lesions according to increases in levels of Hg. Higher doses of mercury (Hg) concentrations resulting in massive destruction in normal architecture of gill tissue which is concentration dependent. These results help to characterize the mechanism of Hg-induced pathogenesis. Moreover, these alterations can also be correlated with damages of target organs from *L. marginalis* exposed to natural Hg contamination in aquatic biota.

ACKNOWLEDGEMENT

The authors are thankful to the UGC, Government of India, New Delhi for financially supporting this work. The authors are also thankful to Dean, College of Fisheries, GADVASU for providing the facilities to carry out this work.

REFERENCES

Andhale, A. V., Bhosale, P. A. and Zambare, S. P. 2011. Histopathological study of nickel induced alterations in the fresh water bivalve, *Lammellidens marginalis*. *J. Experimental Sciences*. **2(4):** 01-03.

APHA (American Public Health Association) 1998. Standard Methods for the Examination of Water and Wastewater, WEF and AWWA, 20th Edition, USA.

Bervoets, L., Voets, J., Covaci, A., Chu, S. G., Qadah, D., Smolders, R., Schepens, P. and Blust, R. 2005. Use of transplanted zebra mussels *(Dreissena polymorpha)* to assess the bioavailability of micro contaminants in Flemish surface waters. *Environ. Sci. Technol.* **39:** 1492-1505.

Cengiz, E. I. and Unlu, E. 2006. Sub lethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquito fish, *Gambusia affinis*: a microscopic study. *Environ.Toxicol. Pharmacol.* **21:** 246-253.

Chakraborty, S., Ray, M. and Ray, S. 2010. Toxicity of sodium arsenite in the gill of an economically important mollusc of India. *Fish and Shellfish Immunology*. **29:** 136-146.

Corners, D. E. and Black, M. C. 2004. Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Archives of Environment Contamination and Toxicology*. **46:** 362-371.

Figueiredo-Fernandes, A., Ferreira-Cardoso, J. V., Garcia-Santos, S., Monteiro, S. M., Carrola, J., Matos, P. and Fontainhas-Fernandes, A. 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus* exposed to waterborne copper. *Pesqui. Vet. Bras*. **27(3):** 103-109.

Gosling, E. 2003. *Bivalve molluscs: biology, ecology and culture*. Blackwell, New York. p. 443.

Gulbhile, S. D. and Zambare, S. P. 2013. Caffeine Supplementation on Arsenic Induced Histopathological Alterations In The Gills of Freshwater Bivalve, *Lamellidens corrianus* (Lea). *International J. Advanced Research in Pharmaceutical and Biosciences*. **3(3):** 15-25.

Gulbhile, S. D. 2006. Caffeine (1,3,7 Trimethylxanthine) supplementation: possible recovery in mercury and arsenic induced alteration in the freshwater bivalve, *Lammelideins corrianus*. Ph.D. Thesis, Dr. *Babasaheb Amedkar Marathwad University, Aurangabad, Maharashtra, India.*

Hendriks, A. J., Pieters, H. and de Boer, J. 1998. Accumulation of metals, polycyclic (halogenated) aromatic hydrocarbons, and biocides in zebra mussel and eel from the Rhine and Meuse rivers. *Environ. Toxicol. Chem.* **17:** 1885-1898.

Janakiram, K. 2003. Freshwater Pearl Culture Technology Development in India. *J. Applied Aquaculture*. **13(3-4):** 341-349.

Jurkieuwicz-Karnkowska, E. 1994. Molluscs and heavy metals in freshwater and terrestrial environments. *Wiadom. Ekologiczne*. **40(3):** 127-141.

Kehrig, H. A., Costa, M., Moreira, I. and Malm, O. 2002. Total and methyl mercury in a Brazilian estuary, Rio de Janeiro. *Mar. Pollut. Bull.* **44:** 1018-1023.

Khangarot, B. S. and Somani, R. C. 1980. Toxic effect of mercury on the gills of a freshwater teleost, *Puntius sophore* (Hamilton), *Curr. Sci.* **49:** 832-834.

Kraak, M. H. S., Scholten, M. C. T., Peeters, W. H. M. and Dekock, W. C. 1991. Biomonitoring of heavy-metals in the Western-European rivers Rhine and Meuse using the fresh-water mussel *Dreissena polymorpha*. *Environ. Pollut*. **74:** 101-114.

Liao, C. Y., Fu, J. J., Shi, J. B., Zhou, Q. F., Yuan, C. G. and Jiang, G. B. 2006. Methyl mercury accumulation, histopathology effects, and cholinesterase activity alterations in medaka (*Oryzias latipes*) following sub lethal exposure to methyl mercury chloride. *Environ. Toxicol. Pharmacol.* **22:** 225-233.

Marie, V., Baudrimont, M. and Boudou, A. 2006. Cadmium and zinc bioaccumulation and methallothionein response in two freshwater bivalves (*Corbicula fluminea* and *Dreissena polymorpha*) transplanted along a polymetallic gradient. *Chemosphere*. **65:** 609-617.

Marutirao, G. R. 2012. Histopatholgical changes in the gills of *Puntius ticto* (Ham) under dimethoate toxicity. *The Bioscan*. **7(3):** 423-426.

Milam, C. D., Farris, J. L., Dwyer, F. J. and Hardesty, D. K. 2005. Acute toxicity of six freshwater species (glochidia) to six chemicals: Implications for Daphnids and *Utterbackia imbecillis* as surrogates for protection of freshwater mussels (Unionidae). *Archives of Environment Contamination and Toxicology*. **48:** 167-173.

Montaser, M., Mahfouz, M. E. and El-Shazly, S. A. M. 2010. Toxicity of heavy metals on fish at Jeddeh Coast KSA: metallothionein expression as a biomarker and histopathological study on liver and gill. *World J. Fish and Marine Sciences.* **2(3):** 174-185.

Nikalje, S. B., Muley, D. V. and Angadi, S. M. 2012. Histopathological changes in liver of freshwater major carp, *Labeo rohita* after acute and chronic exposure to textile mill effluent. *The Bioscan*. **7(2):** 215-220.

Oliva, M., Garrido, M. C., Sales, M. D. and Gonzalez de Canales, M. L. 2009. Sub lethal and lethal toxicity in juvenile Senegal sole (*Solea senegalensis*) exposed to copper: A preliminary toxicity range-finding test. *Exp. Toxicol. Pathol.* **61:** 113-121.

Olojo, E. A. A., Olurin, K. B., Mbaka, G. and Oluwemimo, A. D. 2005. Histopathology of the gill and liver tissues of the African catfish, *Clarias gariepinus* exposed to lead. *Afr. J. Biotechnol*. **4(1):** 117-122.

Ortiz, J. B., De Canales, M. L. G. and Sarasquete, C. 2003. Histopathological changes induced by lindane (ā- HCH) in various organs of fishes. *Sci. Mar.* **67(1):** 53-61.

Parant, M. and Pain, S. 2001. Potential use of multi xenobiotic defence mechanism (MXDM) in *Dreissena polymorpha* as a biomarker for the monitoring of freshwater pollution. *Water Research*. **35:** 3743-3748.

Rao, J. V., Begum, G., Jakka, N. M., Srikanth, K. and Rao, R. N. 2006. Sub lethal effects of profenofos on locomotor behavior and gill architecture of the mosquito fish, *Gambusia affinis*. *Drug Chem. Toxicol.* **29:** 255-267.

Santhakumar, M., Balaji, M. and Ramudu, K. 2001. Gill lesions in the perch, *Anabas testudineus* exposed to monocrotophos. *J. Environ. Biol.* **22(2):** 87-90.

Selvanathan, J., Vincent, S. and Nirmala, A. 2013. Histopathological changes in fresh water fish *Clarias bactrachus* mercury and cadmium. *International J. Life Science and Phrama Research*. **3(2):** 11-21.

Shaikh, Y. and Mane, U. H. 2013. Changes in Hepatopancreas of the Bivalve Molluscs *Lamellidens marginalis*e posed to Acute Toxicity of Cadmium in Summer. *World J. Fish and Marine Sciences*. **5(4):** 437-440.

Shaikh, Y., Suryawanshi, G. D. and Mane, U. H. 2012. Histopathological changes in hepatopancreas of *Lamellidens marginalis* exposed to cadmium in winter. *International Multidisciplinary Research J.* **2(6):** 41-43.

Sprague, J. B. 1971. The measurement of pollutant toxicity to fish III, sub lethal effect and safe concentration. *Water Res.* **5:** 245-266.

Velmurugan, B., Selvanayagam, M., Cengiz, E. I. and Unlu, E. 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *J. Environ. Sci. HealthB*. **42(2):** 157-163.

Vergilio, C. S., Carvalho, C. E. and Melo, E. J. 2012. Accumulation and histopathological effects of mercury chloride after acute exposure in tropical fish *Gymnotus carapo*. *J. Chemical Health Risks*. **2(4):** 01-08.

Vernberg, W. B. and Vernberg, F. J. 1972. The synergistic effect of temperature salinity and mercury on survival and metabolism of the adult fiddler crab *Uca pugilator*. *Fish Bull*. **70:** 415-420.

Wagner, A. and Boman, J. 2004. Bio monitoring of trace elements in Vietnamese freshwater mussels. *Spectrochimica Acta Part B*. **59:** 1125-1132.

Wester, P. W. and Canton, J. H. 1991. Histopathological Effects in *Poecilia reticulata* (Guppy) Exposed to Methyl Mercury Chloride. *Toxicologic Pathology*. **20(1):** 81-92.