Impact of Nickel Intoxication on Ovarian Histology in *Oreochromis mossambicus*

LOVELL CRISTINA SIOSON AND ANNABELLE A. HERRERA
Institute of Biology, College of Science
University of the Philippines
Diliman, Quezon City 1101

ABSTRACT

The effect of chronic sublethal concentration of nickel on ovarian histology in two-month old *Oreochromis mossambicus* was analyzed histologically. The control ovaries showed abundance of vitellogenic oocytes with smooth and intact membranes and few atretic and previtellogenic oocytes. The ovaries of the fish exposed to 0.5 mg/L nickel sulfate showed wrinkled and deformed oocytes, disrupted theca layers, abundant atretic oocytes in various stages of degeneration and several previtellogenic oocytes. Degenerative changes were worse in the ovaries exposed to 1.0 mg/L nickel sulfate. Proliferation of connective tissues and tissue scars indicated advanced necrosis.

INTRODUCTION

Natural concentrations of nickel in water, air, and soil are not considered a biological hazard. However, increased industrialization and other human activities have hastened the mobilization and transport of nickel into the environment. Consequently, these higher concentrations of nickel can be toxic to man and the biota.

Nickel is used in metal-plating, electroplating, in the production of ferroalloys, stainless steel, and alkaline storage batteries (EIFAC 1984). It is released into the environment during these manufacturing processes and during mining and smelting. The principal ores of nickel — pentlandite, garnierite and the arsenicals NiAs and $NiAs_2$ — are released into the environment by extensive ore-processing operations. Another major contributor of nickel to the environment is the combustion of fossil fuels (crude oil contains tens of milligrams of nickel per kilogram). Combustion of coal releases nickel directly into the atmosphere and indirectly into aquatic environments by drainage from fly ash storage ponds (Elwood 1977). The nickel-plating industry also releases nickel sulfate, the most important compound of nickel, in significant amounts into the aquatic environments (US EPA 1975). Schreier and Taylor (1980) reported significant concentrations of asbestos fibers in many rivers. All of these discharges increase the nickel burden on the ecosystem, leading to deterioration of water quality. In the Philippines, the concentration of nickel in freshwater was found to be 0.18 mg/L (NEPC 1982).

Aquatic organisms tend to accumulate nickel and other metals in their tissues (Darmono 1990). This accumulation is important for public health reasons since nickel can be transferred to man via edible aquatic organisms.

This study investigated possible ovarian histological changes, induced by chronic exposure to nickel, in the hardy fish, *O. mossambicus*. The ovary produces fertilizable eggs necessary for successful reproduction and the pituitary-dependent synthesis and secretion of a variety of steroid hormones that regulate the development of eggs. Thus, any environment-induced alteration in the reproductive histology and physiology of the fish will consequently affect its fecundity (Herrera 1984).

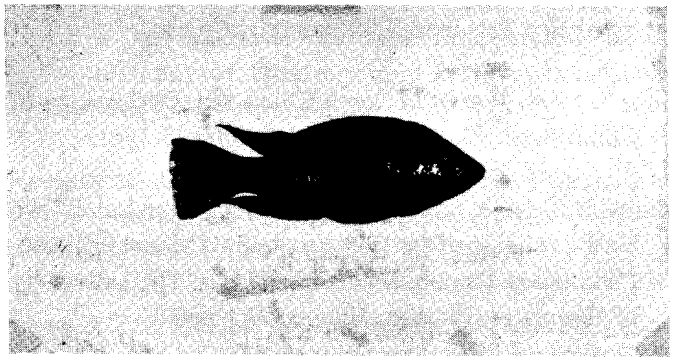

Fig. 1. Two-month old *Oreochromis mossambicus* used in the study.

Key words: *Oreochromis mossambicus*, nickel, ovary

MATERIALS AND METHODS

Two-month old freshwater fish, *O. mossambicus* (Fig. 1), were obtained from a private fishpond in Malabon, Metro Manila and used as the test specimen. The fish were acclimated to laboratory conditions five days prior to the experiment.

Sublethal concentrations of nickel sulphate, 0.5 mg/L and 1.0 mg/L, were used in this study. The treatment lasted for one month.

Six 30-liter aquaria were used. Each aquarium contained five fish. There were two replicates for the control and for each of the two treatments. Dechlorinated tap water previously allowed to stand for three days in two 100-liter pails was used in the experiment. The fish were fed twice a day using commercially available fish flakes. Aquarium water was changed twice a week.

After one month of treatment, the ovaries were collected and processed using the standard paraffin and resin technique at the UP Natural Sciences Research Institute.

RESULTS

Behavioral changes

During the one month treatment, behavioral changes were observed in the fish exposed to nickel. After the aquarium water was changed, the control fish were immediately able to adjust, showing no behavioral changes. The fish swam actively and ate voraciously. The fish in the newly-changed nickel solution stayed at the bottom of the aquaria, were inactive, and refused to eat. It took about 1 to 2 days for the treated fish to adjust to the change. The treated fish secreted large amounts of mucus that was not observed in the control.

Histological observations

The oocytes observed were grouped into three categories (Herrera 1984). The first group, the previtellogenic oocytes, was characterized by the presence of homogeneous basophilic cytoplasm. The second group, the early vitellogenic, had peripheral yolk vesicles or cortical alveoli in the cytoplasm, indicating the start of vitellogenesis. The third group, the late vitellogenic oocytes, was characterized by the presence of yolk vesicles and yolk granules in the cytoplasm.

Control ovaries

In the control fish, the development of the oocytes was normal. The oocytes observed were mostly in the vitellogenic stage (Fig. 2), although there were several previtellogenic oocytes. Previtellogenic oocytes were observed as small basophilic cells with homogeneous cytoplasm. In the late previtellogenic oocytes, zona radiata started to form and the follicles were of two layers: the inner layer of granulosa cells and the outer layer of theca cells.

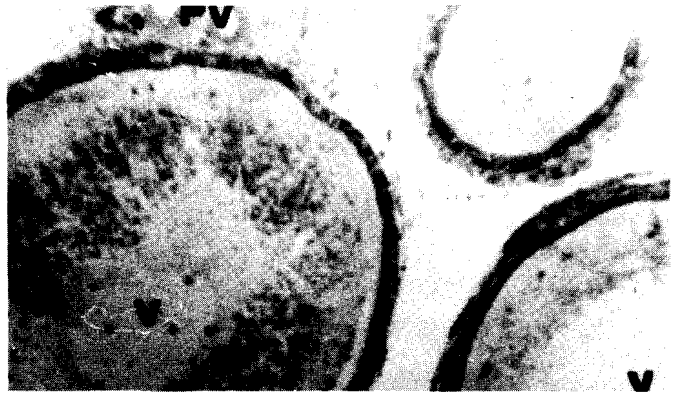

Fig. 2. Photomicrograph of control ovary of *Oreochromis mossambicus*showing vitellogenic (V) and few previtellogenic (PV) oocytes.

Early vitellogenic oocytes were bigger than previtellogenic oocytes. They showed yolk vesicles or cortical alveoli at the periphery (Fig. 3) indicating the start of vitellogenesis. Late vitellogenic oocytes were the biggest and contained yolk vesicles and yolk granules (Fig. 4). Vitellogenic oocytes were covered by well-developed intact granulosa and theca

Fig. 3. Photomicrograph of early vitellogenic oocyte of control *O. mossambicus* showing smooth outline and intact theca folliculi of granulosa (G) and theca layer (T).

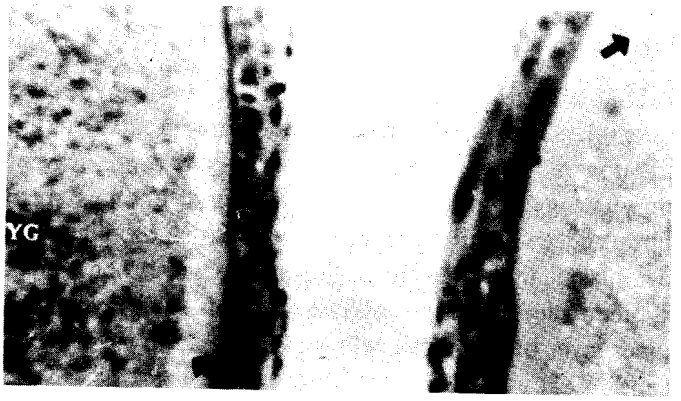

Fig. 4. Photomicrograph of two vitellogenic oocytes of control *O.mossambicus* showing yolk vesicles (↖) and yolk granules (YG).

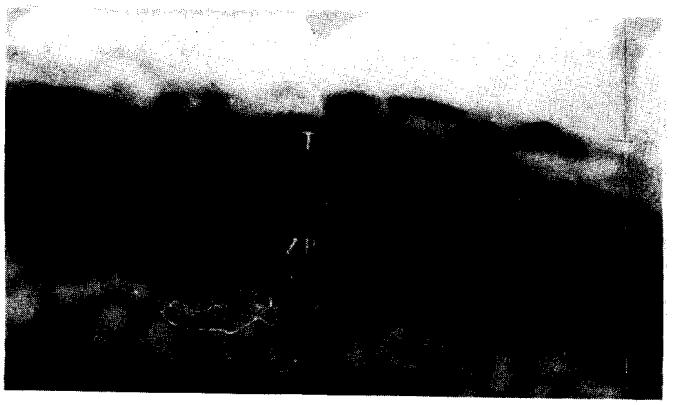

Fig. 5. Portion of a vitellogenic oocyte of control *O. mossambicus* showing enlarged granulosa cells (G).

layers (Fig. 5). The special theca cell type was much larger than ordinary theca cells. Atretic oocytes were rarely observed. The ovarian membrane was intact and the ovary maintained its histological integrity.

Ovaries exposed to 0.5 mg/L nickel sulphate

In the treated fish, normal development of the oocytes was interrupted by chronic sublethal exposure to nickel. The treated fish ovaries looked like spent ovaries with abundant atretic cells and previtellogenic oocytes (Figs. 6–10). Some oocytes are in early or advanced atresia and yolk granules are found scattered in the ovarian cavity.

Ovaries exposed to 1.0 mg/L nickel sulphate

The ovaries of fish exposed to 1.0 mg/L contained few vitellogenic oocytes. Furthermore almost all vitellogenic oocytes were degenerating. The oocytes were wrinkled

Fig. 6. Photomicrograph of a portion of the ovary of *O. mossambicus* after exposure to 0.5 mg/L nickel showing deformed vitellogenic oocyte (D) and abundance of previtellogenic oocytes (PV).

Fig. 7. Photomicrograph of the ovary of *O. mossambicus* after exposure to 0.5 mg/L nickel showing an oocyte with bizarre shape (B) and an oocyte with unusual growth (UG).

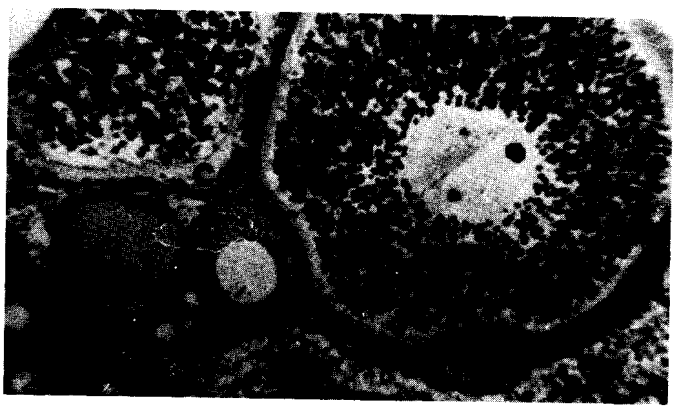

Fig. 8. Photomicrograph of the ovary of *O. mossambicus after* exposure to 0.5 mg/L nickel showing a cell with clear layer (C) between the zona pellucida and the granulosa layer.

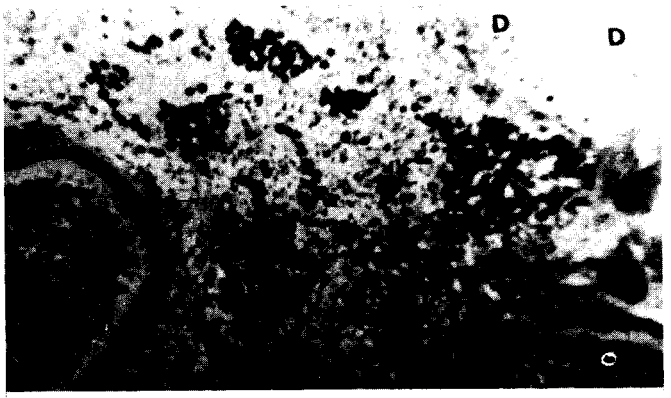

Fig. 9. Photomicrograph of the ovary of *O. mossambicus* after exposure to 0.5 mg/L nickel, showing scattered yolk granules (YG) and debris (D).

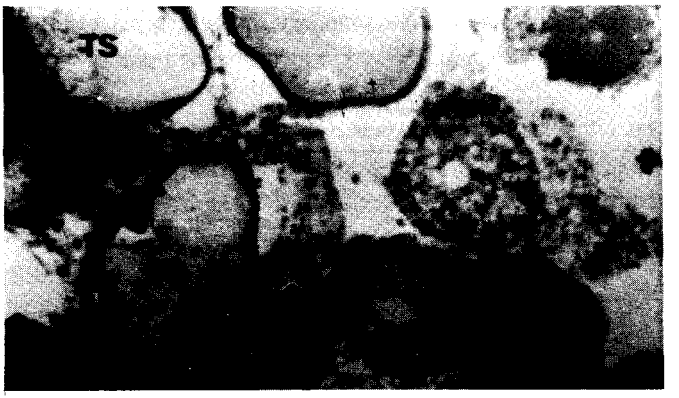

Fig.12. Photomicrograph of the ovary of *O. mossambicus* after exposure to 1.0 mg/L nickel showing abundance of tissue scars (TS).

Fig. 10. A portion of a vitellogenic oocyte of 0.5 mg/L nickel-exposed *O. mossambicus* showing disorganized theca folliculi (STF).

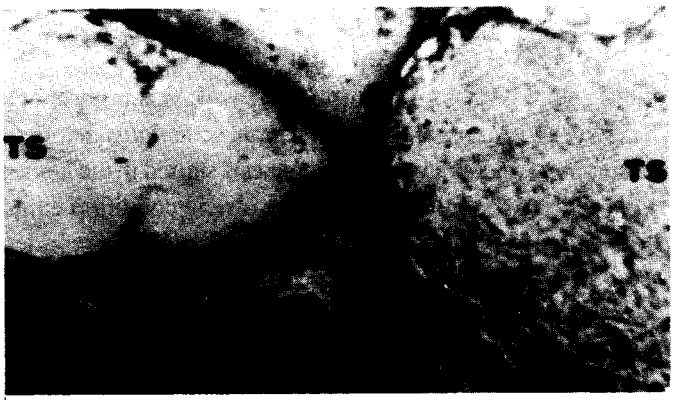

Fig. 13. Photomicrograph of the ovary of *O. mossambicus* after exposure to 1.0 mg/L nickel showing tissue scars (TS).

Fig. 11. Photomicrograph of the ovary of *O. mossambicus* after exposure to 1.0 mg/L nickel showing wrinkled and deformed oocytes. Connective tissues abound and tissue scars (TS) appear.

and deformed (Fig. 11). Connective tissues were abundant and tissue scars appeared (Fig. 12–13). In several oocytes, a portion of the theca was lifted off, the granulosa were stratified, and the zona pellucida was wrinkled.

DISCUSSION

The stages of egg development in *O. mossambicus* are the same as those described by Herrera (1984, 1989). The smaller gametogenic cells are oocytes in the first growth phase or previtellogenic stage and the bigger gametogenic cells are oocytes in the second growth phase or vitellogenic stage. The vitellogenic phase is made up of the yolk vesicle and yolk granule stages.

O. mossambicus exposed to chronic sublethal concentration of nickel exhibited extensive damage in the ovary (Table 1). Degeneration and yolk resorption were widespread in

Table 1. A comparative summary of the ovarian histology of the control and nickel-ex thusposed *Oreochromis mossambicus*

Observations	Control	Nickel Sulfate Concentration	
		0.5 mg/l	1.0 mg/l
Vitellogenic oocytes number shape zona pellucida granulosa theca layer	few deformed; some with bizarre shape wrinkled	very few deformed wrinkled stratified not recognizable	 stratified lifted from granulosa layer
Atretic oocytes	very few	abundant	abundant
Tissue scars	absent	absent	present
Yolk granules scattered in the ovarian cavity	absent	present	present
Proliferation of connective tissue	not evident	evident	evident

vitellogenic oocytes. Resorption of yolk was reported by Saksena and Agarwal (1986) in *Chana punctatus* exposed to mercury. In this study, vitellogenesis started in the largest cells prior to the treatment, and the atretic condition could be attriobuted to the action of nickel. Kling (1981) reported total atresia of late vitellogenic oocytes in the ovaries of lebaycid- treated *Tilapia leucostica.* That was also observed by Nath and Kumar (1990) in the ovaries of *Colids fasciatus* exposed to nickel. Atresia of early vitellogenic oocytes was reported in sumithion-treated ovaries of *Garra mullya* (Pawar and Katdare 1983) and in pesticide-treated ovary of *Colisa lalia* (Sukumar and Karpagaganapathy 1992). Both early and late vitellogenic oocytes that underwent wrinkling and deformity were observed by Rastogi and Kulshrestha (1990) in pesticide-treated *Rasbora daniconius.* The same deleterious effects of nickel were found in this study.

Abundance of previtellogenic oocytes and the very few numbers of early vitellogenic oocytes compared to the control were also observed in fish exposed to 0.5 mg/L and 1.0 mg/L nickel sulphate. Similar observations have been reported in mercury-treated (Ram and Sathyanesan 1983; Kirubagaran and Joy 1988) and pesticide-treated (Rastogi and Kulshrestha 1990) fishes.

Several authors tried to explain the observed growth retardation of the oocytes in fish treated with heavy metals and pesticides. Ram and Sathyanesan (1983) suggested suppression of the activity of pituitary gonatrophs and somatotrophs in *Channa punctatus* exposed to mercury. Pituitary gonatrophs secrete the pituitary gonadotropin, an important element for the teleost oocytes to undergo its prolonged growth phase (Nagahama 1983). Pituitary gonadotrophs induce estrogen synthesis by theca and granulosa cells (Herrera 1984). Low level of gonadotropin secretion caused by exposure to mercury resulted in the inhibition of proliferation and growth of oocytes and resorption of yolk in C. punctatus (Saksena and Agarwal 1986). This low level of gonadotropin is due to reduced estrogen synthesis which is necessary for vitellogenesis. Lead accumulated in the brain of *Anabas testudines* caused altered hypothalamo-hypophysial-ovarian function resulting in decreased reproductive potential of the fish (Tulasi et al. 1989).

Kapur et al. (1978) proposed the decline in the level of ovarian 3- b-hydroxysteroid dehydrogenase (b-HSDH), a principal enzyme involved in steroidogenesis in the ovary. Such decline of the level of 3-b-HSDH decreased ovarian steoidogenesis and consequently produced insufficient endogenous gonadotropin, resulting in atretic ocytes (Pawar and Katdare 1983) and decrease in number of early and late vitellogenic oocytes (Saxena and Garg 1978). The observed proliferation of the steroidogenic cells, the granulosa and theca cells, might be due to the effort of the fish to produce more estrogen for egg growth and development.

Kumar and Mukherjee (1988) reported accumulation of ovarian and hepatic cholesterol (precursor of steroids) in both phenol and sulfide treated fish. They suggested that the accumulation of ovarian cholesterol was the result of interfered production of ovarian steroids and that the accumulation of hepatic cholesterol indicated the decline of steroid production in the liver, thereby interfering with

vitellogenesis. The mechanism involved for cholesterol accumulation may include alteration of gonadotropin control via hypothalamo-hypophysial-ovarian axis (Kumar and Mukherjee 1988).

As a result of impaired steroid metabolism, particularly in the liver, heavy metals and pesticides alter lipid metabolism since steroids play an important part in the regulation of lipid metabolism in teleosts in relation to reproduction (Singh and Singh 1992). 1,2,3,4,5,6-hexachlorocyclohexane (b-BHC), a pesticide, arrests hepatic lipidogenesis, as well as translocation of liver lipid to the ovary by impairing gonadotropin-releasing hormone and gonadotropin acting through the hypothalamo-hypophysial-ovarian axis in *Heteropneustes fossilis* (Singh and Singh 1992). As a consequence, there is a reduction in the hepatic diglyceride level (precursor of phospholipid) that brings about reduction in phospholipids (precursor of vitellogenin) and, consequently, interference in vitellogenesis. The liver is heavily damaged by nickel in *Clarias batrachus* (Herrera, personal communication). The interference in vitellogenin synthesis may have caused the arrest of growth of oocytes at previtellogenic stage. These oocytes did not have vitellogenin to undergo vitellogenesis. Proliferation of connective tissues was observed in the treated fish in this study. Rastogi and Kulshrestha (1990) have reported the same findings in pesticide-treated *Rasbora daniconius*. Connective tissues proliferate in degenerating organs much like cirrhosis in atretic liver. Macrophages are carried by connective tissues to necrotic organs. The widespread tissue scar in the 1.0 mg/L dose indicated more advanced atresia in the ovary.

Kirubagaran and Joy (1988) attribute the atretic changes caused by mercury to *Clarias batrachus* as a direct action of mercury on the ovary. Kumar and Pant (1984) suggest direct action of heavy metals on the ovary of *Puntius conchonius*. Zinc damages mainly younger oocytes while copper and lead damage older oocytes. All three metals induce atresia in the ovary. Furthermore, the action of pesticides on the oocytes of *P. conchonius* is direct and selective to specific oocyte stages (Kumar and Pant 1988).

The possible action of heavy metals on the cellular level is their binding to sulfhydryl groups on the surface membrane protein of the cell such as ATPase, and thereby affecting cell permeability (Trump and Jones 1975; Gerber et al. 1980). The cell membrane increases permeability to cations and water. There are volume shifts in the cell and the cell dilates which may lead to rupture.This explains the scattering of yolk granules where vitellogenic oocytes are necrotic. Ca^{++} may enter the cell leading to calcification on the electron transfer site of the mitochondria. Production of ATP is then lowered.

In a cyanobacterium, *Nostoc muscorum,* nickel acts on the Ca^{++}- dependent ATPase that causes hydrolysis of ATP, and the Mg^{++}-dependent ATPase that leads to altered transmembrane movement of vital ions (Asthana et al. 1982).

Sublethal concentrations of mercury and lead were also found to inhibit enzymes such as acid and alkaline phosphatase, glucose-6-phosphatase, lipase, and urease in the ovaries of fishes (Sastry and Agrawal 1979 a, b).

A possible mechanism for the inhibition of the mentioned enzymes, including 3-b-HSDH and ATPase, is the already mentioned high affinities of heavy metals for binding to sulfhydryl groups of proteins since enzymes are proteins. Since almost all enzymes have sulfhydryl groups, then at some concentration, heavy metals inhibit the function of these enzymes (Trump and Jones 1975).

Thus, it is possible that the mode of action of nickel in inducing histological alterations is similar to the other metals and pesticides. Nickel may induce alteration in the hypothalamo-hypophysial-ovarian axis, thereby suppressing the activity of gonadotrophs and somatotrophs and altering steroid and lipid metabolism, or it may directly act on the oocytes. However, more studies are needed to confirm these possibilities.

CONCLUSION

Chronic sublethal exposure of *O. mossambicus* to nickel has deleterious impact on ovarian histology. This has consequences on the reproductive potential of this fish species.

ACKNOWLEDGMENTS

The authors thank the UP NSRI and the UP ORC for their invaluable support to this study.

REFERENCES

Asthana, R. K., Singh, S.P. and R. K. Singh. 1992. Nickel effects on phophatase uptake, alkaline phophatase and ATPase of a cyanobacterium. Bull Environ Contam Toxicol 48:45–54.

Darmono, D. 1990. Uptake of cadmium and nickel in banana prawn (*Peneus merquiensis* de Man). Bull Environ Contam Toxicol 45:320–328.

Elwood, J. W. 1977. Environmental Sciences Division, Oak Ridge. European Inland Fisheries Advisory Commission. 1984. Water quality criteria for European freshwater fish. Report on nickel and freshwater fish. EIFAC Tech. Pap. No. 45.

Herrera, A.A. 1984. Histogenesis of the pituitary in relation to gonadal differentiation in *Tilapia nilotica*. Unpublished Ph.D. Dissertation. University of the Philippines, Diliman, Q.C.

Kapur, K., Kumaldeep, K. and H. S. Toor. 1978. Effect of fenitrothion on reproduction of teleost fish, *Cyprinus carpio* Communis Linn.: A biochemical study. Bull Environ Contam Toxicol 20:438–442.

Kirubagaran, R. and K.P. Joy. 1988. Toxic effects of mercuric chloride, methylmercuric chloride and emisan 6 (an organic fungicide) on ovarian recrudescence in the catfish *Claria batrachus* (L). Bull Environ Contam Toxicol 41:902–909.

Kling, D. 1981. Total atresia of the ovaries of *Tilapia leucosticta* (Cichlidae) after intoxication with the insecticed lebaycid. Esperentia. 37:73–74.

Kumar, S. 1988. Comparative sublethal ovarian pathology of some pesticides in the teleost, *Puntius conchonius* Hamilton. Bull Environ Contam Toxicol 41:227–232.

Kumar, S. and D. Mukherjee. 1988. Phenol and sulfide changes in the ovary and liver of sexually maturing common carp, *Cyprinus carpio*. Aquat Toxicol

Kumar, S. and S. C. Pant. 1984. Comparative effects of the sublethal poisoning of zinc copper and lead on the gonads of the teleosts *Puntius conchonius*. Hum Toxicol Lett 23:189–194.

Nagahama, Y. 1983. The functional morphology of teleost gonads. Edited by Hoar, W. S., Randall, D. J. and E. M. Donaldson. Fish Physiology. Academic Press, New York. pp. 223–276.

Nath, K. and N. Kumar. 1990. Gonadal histopathology following nickel intoxication in the giant gourami, *Colisa fasciatus* (Bloch and Schneider), a freshwater tropical perch. Bull Environ Contam Toxicol 45:299–30

National Environmental Protection Council Report. 1982.

Pawar, K. R. and M. Katdare. 1983. Effect of sumithion on the ovaries of freshwater fish *Garra mullya* (Sykes). Curr Sci 52:784–785.

Ram, R. N. and A. G. Sathyanesan. 1983. Effect of mercuric chloride on the reproductive cycle of the teleostean fish *Channa punctatus*. Bull Environ Contam Toxicol 30:24–27.

Rastogi, A. and S. K. Kulshrestha. 1990. Effect of sublethal doses of three pesticides on the ovary of a carp minnow *Rasbora daniconius*. Bull Environ Contam Toxicol 45:742–747.

Saksena, D. N. and A. Agarwal. 1986. Effect of mercuric chloride intoxication on ovarian activity of a teleostean fish, *Channa punctatus*. Int J Zool 7:1–6 (Abstract only)

Sastry, K. V. and M. K. Agrawal. 1979a. Mercuric chloride-induced enzymological changes in kidney and ovary of a teleost fish *Channa punctatus*. Bull Environ Contam Toxicol 22:38–43.

_____. 1979b. Effects of lead nitrate on the activities of a *Heteropneustes fossilis*. Bull Environ Contam Toxicol 22:55–59.

Saxena, P. K and M. Garg. 1978. Effect of insecticidal pollution on ovarian recrudescence in the fresh water teleost *Channa punctatus* (B1). Indian J Exp Biol 16:689–691.

Singh, P. B. and T.P. Singh. 1992. Impact of g-BHC on lipid class levels in the freshwater catfish, *Heteropneustes fossilis*. Bull Environ Contam Toxicol 48:23–30.

Sukumar, A. and P. R. Karpagaganapathy. 1992. Pestcide-induced atresia in ovary of a freshwater fish, *Colisa lalia* (Hamilton-Buchanan). Bull Environ Contam Toxicol 48:457–462.

Trump, B. J., Jones, R. T. and S. Sahaphong. 1975. Cellular effects of mercury on fish kidney tubules. Ribelin, W. E. and G. Migaki (eds.). *The Pathology of Fish.* The University of Wisconsin Press. London. pp. 585–609.

Tulasi, S. J., Reddy, U. M. and J. V. Ramana Rao. 1989. Effects of lead on the spawning potential of the freshwater fish, *Anabas testudines*. Bull Environ Contam Toxicol 43:858–863.

United States Environmental Protection Agency: Preliminary investigation on effects on the environment of boron, indium, nickel, selenium, tin, vanadium and their compounds. Vol III, Nickel. U.S. Department of Commerce National Technical Information Service. PB242. 197.