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The Effect of Endosulfan on the Ovary of Bluegill Sunfish: A Histopathological Study (*Lepomis macrochirus*)

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ABSTRACT: The effects of pesticide endosulfan (an organochlorine compound), on the ovaries of bluegill fish (*Lepomis macrochirus*) were studied. Exposures for 24 hs with histological preparations at 25% (0.25 μg/L), 75 % (0.75 μg/L), and 100 % (1 μg/L) sub lethal concentrations were examined. The control contained an abundance of the different stages of oocytes (Oocytes I, II, III, and IV) and had an intact ovigerous lamellae and follicular lining. The control also contained a thick and complete ovarian wall with evident provitelline and euvitelline nucleoli. After 24 hr exposure to a 25% concentration, many Oocyte II and III cells had damaged stroma and cytoplasmic and nuclear retraction. Adhesion is pronounced at the 25% concentration, but is even more profound at the 75% concentration. Empty follicles and a unique cytoplasmic clumping can be observed in Oocyte III and IV cells in the 75% concentration. The ovaries of fish exposed to a 100% concentration display an immense amount of empty follicles along with necrosis of nuclei and expelled nuclei. As the concentration increased, the amount of atretic cells increased, and the ovarian wall became more thinned and lifted. Macrophages were more evident as the concentration increased and the sizes of the different stages of oocytes ,exposed to the different concentrations became smaller as well. This study showed that there is a clear correlation between the amount of damage seen and the amount of endosulfan.

Key words: Bluegills; Ovary; Endosulfan, Pesticide, Damage

INTRODUCTION

Endosulfan is an organochlorine pesticide that has documented effects on hormones and chemical homeostasis in gonadal tissue. It is used as an insecticide, but is not readily soluble in water and therefore bioaccumulates increasing toxicity through attaching to soil particles in ground water. Peterson and Batley (1993) noted that the two isomers of endosulfan, alpha and beta, were found to have half-lives of 3.6 days and 1.7 days respectively. In the alkaline waters of the cotton region, hydrolysis is the dominant degradation process, however, and the major proportion of endosulfan particles associate with the sediment. According to Johnson and Finley (1980), endosulfan was particularly toxic to four teleost foodfish species when exposed to 50% lethal concentrations: *Onchorhynchus mykiss, Pimephales promelas, Ictalurus punctatus,* and *Lepomis macrochirus*.

Daesik, *et al.,* 2004 studied the toxic effects of endosulfan to breeding and non-breeding mosquito fish and found that non-breeding female exposed to this pesticide had a significant greater ratio of anal fin/body length and larger thyroid follicles than did control females. Endosulfan inhibits endocrine function and also has an effect on chemical levels in the blood. When *Tilapia mossambica* (foodfish) was exposed to endosulfan an increase in calcium and magnesium resulted in hypocalcemia and hypomagnesemia in serum levels in the blood. Jerky, violent movements were noted as a direct result of this ionic imbalance (Rangaswamy and Naidu, 1989). When *Oreochromis mossambicus* (Foodfish) was exposed to endosulfan a decrease in blood pH and

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increase in CO_2 content, along with altered oxygen equilibrium curves were noted (Rangaswamy and Naidu, 1999). This alteration in blood pH has a definite correlation with chemical imbalances in body tissue after exposure. Acetyl cholinesterase activity in brain tissue in *Lepomis macrochirus* (bluegill sunfish) was measured and found to have a step-wise decrease in activity for increased endosulfan exposure durations (Dutta and Arends, 2002). Glycogen, protein and lipid contents were monitored in the gonadal tissue of freshwater bivalves and an increase in glycogen and decrease in protein and lipid levels was observed. It was theorized that when endosulfan introduced stress, the animal has to work with metabolic and endocrine processes to combat the imbalance, which may cause several level of changes (Muley and Mane, 1995). When endosulfan and malathion, another endocrine inhibitor, was introduced, steroid genesis was inhibited (Inbaraj and Haider, 1988).

Along with chemical changes, structural changes have been documented. Stage II and Stage III oocytes disappeared after exposures to endosulfan and malathion (Inbaraj and Haider, 1988). Ram and Singh (2001) noticed perinuclear ooplasmic lysis, clumping and dissolution of ovarian tissues when exposed to another endocrine inhibitor called carbofuran. Cytoplasmic clumping and the degeneration of Stage I oocytes was also noticed as effects to exposure to malathion. Atretic oocytes and the increase of nucleoli were other direct effects of increasing malathion exposure duration in *Heteropneustes fossilis* (air-breathing catfish) (Dutta, *et al.*, 1992). During a study of the effects of sewage runoff and general pesticide pollution on fish, malformation of germ cells and/or reproductive ducts was observed. Altered gamete production and appearance of intersex fish was also evident. Vitellogenin, the major component of egg yolks, had increased in intersex fish as more female tissue was formed.Vitellogenin levels were observed to be a major clue as to how much hormonal change occurred in the fish studied, which also was correlated to how much structural change took place (Jobling *et al.*, 2002).

Although there are extensive sources describing the normal structure of ovarian tissue before endosulfan exposure (Groman, 1982), this study is unique because it explores the histopathological effects of an organochlorine on ovarian tissue. Most studies that have already been completed about endosulfan's effects focus on chemical and hormonal imbalances in blood serum and other bodily liquids rather than tissue structure damage. Many insecticide studies do not focus on organochlorines either, and instead detail the effects of organophosphates such as diazinon and dimethoate. In a study conducted by Dutta and Maxwell, 2003, ovarian tissue exposed to diazinon for increasing lengths of time (24, 48, 72, 96 hr) had increasing amounts of damaged cells. Maxwell and Dutta, 2005 have correlated the changes in the ovarian follicles of diazinon exposed bluegills to the estradiol levels in the blood which led to the endocrine disruption.There are some studies as mentioned above on effects of organophosphate, such as diazinon on the ovary and testis available, but no histopathological studies of an organochlorine, such as endosulfan is found in the field of toxicology. Therefore our objective was to find out how significantly the endosulfan an organochlorine can damage the ovarian tissues of the bluegill upon exposure to its different levels of concentration.

MATERIALS & METHODS

Bluegills, *Lepomis macrochirus*, (total length 10-21.5 cm, weight 25-125 g), were obtained from a fish hatchery near Baltic, OH. Fish were acclimated to the laboratory in a 500-gallon aquarium for four weeks after being exposed to formaldehyde and salt for cleansing and pH stability. The filtration system used was a Mini Fish Farm Biofiltration and Clarification device that allowed for 3 pounds (1.36 kg) of feeding without replacement and contained 500 gallons (1890 L) of water in a 5 ft diameter polyethylene tank. Fish were fed daily to satiation with commercially balanced food sticks, tetra Doro Min (Tetra Werke, Germany).Automatically controlled fluorescent lighting was used to keep a constant 14/10h day/night cycle throughout both during acclimatizing and experimental periods. For control 10 fish were removed from the holding tank and were kept in a similar sized tank, and were subjected to the same regimen as the others. These 10 control fish were handled with separate nets, gloves and apparatus to prevent any exposure to pesticides from the experiment. Water directly from the fish's

aquarium was used for each exposure. Water temperature and dissolved oxygen concentration were as follows: temperature, 21° Celsius, pH 7; dissolved oxygen, 8.27 mg/L; total hardness 125.12 mg/L as $CaCO_3$; and alkalinity 41.78 mg/L as CaCO3. A technical grade (98%) endosulfan was obtained from Micro Flo Co. (Spark,GA).Based on the 98% lethal concentration (LC 50) of endosulfan was determined to be 1.2ug/L (Johnson and Finley,1980). A 1.0 μg/L endosulfan stock solution was prepared and fish were exposed to 25%, 75%, and 100% concentrations of the stock solution for 24 hours in glass tanks. The exposure tanks were 190 L tanks and 95 liters of water was filled into the tanks for each trial. The water was continuously aerated up until exposure and then once daily for a period of 30 min. before the repeated exposure. On the top of that feeding was stopped to ensure that the food pellets could not absorb any pesticide. The control fish were handled with separate nets, gloves and apparatus to prevent any exposure to pesticides from the experiment. Water quality tests were conducted daily prior to repeated exposure to ensure that the nitrogen levels were sufficiently low and the oxygen levels were high. The 100% concentration had 9.5 μg of endosulfan stock solution, the 75% had 7.125 μg stock solution, and the 25% had 2.375 μg stock solution. Fifteen female fish were used in each exposure tank in order to guard against premature or unexpected infection or death. Fifteen female fish were used as control from the holding tank which was not exposed. These fish were subjected to the same regimen as others. Ten fish from the control and exposure groups were used. Two ovaries from each control and the exposed fish were sampled. Therefore, 20 samples from each control and exposed fish were used for the analysis.

The exposed and control fish were anesthetize in 100 mg/L of MS222 (amino benzoate) and 100mg/L of sodium carbonate. Since 5 L of water were placed into the anesthetizing tub, 500 milligrams or .5 g of sedative and .5g of sodium carbonate were used. After the ovaries had been extracted and placed in Bouin's fixative and were allowed to remain there for at least two days, the tissues were process for the histological slides following Humason's Animal Tissue Techniques (Humason1972). The wax embedded tissues were molded into wax blocks and then cut into 6 μm sections and attached with warm water to glass slides. The slides were stained with hematoxylin and eosin and cover slips were attached with mounting solution. The oocytes were evaluated and monitored. The oocytes were also measured for oocyte diameter and percent damaged cells.Twenty ovaries sections were examined from each exposure level, including controls. The diameter was measured using an ocular micrometer under x100 and x400 magnification. The SPSS computer program was used to configure means ±Standard Deviation and 2-Tailed t-tests. The percent of the damaged oocytes was calculated from each ovary exposed to different concentrations.

RESULTS & DISCUSSIONS

Tables 1, 2 and 3 show obtained results.

Table 1. Measurements of the diameters (μm) of the oocytes of different stages after 24 h. exposure at varying concentrations (25%, 75%, and 100%) as compared to the control stage (n=20)

Variables	Mean	Standard Deviation	t-value	Sig. (2-tailed)
25% Oocyte I	1.1*10^(-2)	1.729*10^(-2)	2.012	.075
75% Oocyte I	1.4*10^(-2)	1.430*10^(-2)	3.096	.013*
100% Oocyte I	1.6*10^(-2)	8.433*10^(-3)	6.000	.000**
25% Oocyte II	2.0*10^(-2)	3.300*10^(-2)	1.917	.088
75% Oocyte II	-3.6*10^(-2)	4.248*10^(-2)	-2.680	.025*
100% Oocyte II	-5.5*10^(-2)	4.403*10^(-2)	-3.950	.003**
25% Oocyte III	7.2*10^(-2)	4.237*10^(-2)	5.373	.000**
75% Oocyte III	8.6*10^(-2)	4.531*10^(-2)	5.665	.000**
100% Oocyte III	5.8*10^(-2)	5.673*10^(-2)	3.233	.010*
25% Oocyte IV	3.1*10^(-2)	9.643*10^(-2)	1.017	.336
75% Oocyte IV	1.5*10^(-2)	7.835*10^(-2)	.605	.560
100% Oocyte IV	.1480	4.756*10^(-2)	9.840	.000**

***- Significant **- Highly significant**

Table 2. Percent damage of oocytes for 24 hr exposure periods with primary mode of cell change

Variables	Oocyte I	Oocyte II	Oocyte III	Oocyte IV
25% (0.25 μg/L) concentration	20%- Increased Atresia (AT)	10%-Cytoplasmic retraction (CR)	70%-Increased Adhesion (AD), (CR), and nuclear retraction (NR)	1%-karyoplasmic clumping (CK)
75% (0.75 μg/L) concentration	60%- (AD)	50%- (CR)	80%- Empty follicles (EF), (CR), (AT), (NR)	15%- (CK), follicular lining damage and looseness (DFL and LFL)
100% (1 μg/L) concentration	80%- (AT)	80%- (AT), (CR)	90%- (EF)	40%- (CK), (DFL), (LFL)

There were other types of damage present in the oocytes but only those mentioned above were observed as extensive

Table 3. Percentages of oocytes present in the different concentration exposures plus the total numbers of oocytes present in the field of the slide viewed in the microscope

Concentrations	Oocyte I	Oocyte II	Oocyte III	Oocyte IV
Control	30% (7)	20% (5)	40% (10)	10% (2)
25% (0.25 μg/L)	20% (7)	20% (7)	35% (13)	25% (9)
75% (0.75 μg/L)	20% (4)	15% (3)	30% (7)	35% (8)
100% (1.0 μg/L)	10% (4)	10% (4)	25% (12)	55% (24)
Total number of oocytes present	24	36	22	44

As seen in Fig. 1, the diameters of each stage of oocyte progressively increase in length, with Oocyte I having the smallest diameter (.052 mm) and Oocyte IV having the largest (.345 mm). There are few atretic (AT) cells, and the ovarian wall and zone radiata (Z) are intact. The ovarian wall (OW) is thick and complete. The ovigerous lamellae (OL) and follicular lining (FL) are intact as well. Provitelline (PN) and Euvitelline (EU) nucleoli are present in Oocyte III cells and these Oocyte III cells also have obvious nuclei (N) and nuclear lining. Oocyte IV cells are distinguished by a spread of yolk vacuoles (YV) and a faint nuclear lining. Oocyte III cells are the most numerous, but there are abundant quantities of Oocyte I, II and IV cells present as well. Standard teleost ovaries are paired, elongated sac-like structures that lie in the abdominal cavity, ventral to the kidneys. They are attached to the body wall by mesovarium and are free on the anterior ends. Both ovaries are usually equal in size and become enlarged during maturation. The ovigerous lamellae are the seat for the development of oocytes and oogonia are found in clusters in various stages of development in the lamellae. The ovarian wall contains nerves, lymphatic ducts, and blood vessels. During the course of this study Oocyte Stages I, II, III, and IV along with the ovarian wall, ovigerous lamellae, and follicular lining were monitored for lesions as the result of endosulfan exposure.

As the concentration of endosulfan increased the amount of damage increased.The tissue exposed to the 25% concentration (0.25 μg/L) of endosulfan (Figs. 2 and 3) had a high percentage of damaged Oocyte III cells and almost no damaged Oocyte IV cells except for the cells affected by karyoplasmic clumping (See Table 2). Most of the damage seen was in adhesion (AD) of oocytes, some karyoplasmic clumping (CK), and a higher prevalence of atretic (AT) cells. Oocyte III cells also experienced cytoplasmic retraction (CR) and nuclear retraction (NR). Many Oocyte II cells experienced cytoplasmic retraction. Oocyte IV cells experienced a slight degree of karyoplasmic clumping although there was not much damage. Lifting of the ovarian wall (LOV) occurred and therefore the ovarian wall was thinned and incomplete at several locations. Damaged stroma (DS) was evident because of the breakage of miscellaneous linings that were observed. The ovigerous lamellae (OL) and follicular lining (FL) were seen freely floating

near the oocytes, no longer attached. Breakage of the germinal epithelium (GE) and enucleated (AN) cells was also prevalent. Provitelline (PN) and Euvitelline (EU) nucleoli were still present in Oocyte III cells (Fig. 3).The 75% concentration (0.75 μg/L) experienced even further damage (Figs. 4 and 5). There were many empty follicles (EF), most of which were probably previously been Oocyte II cells. The diameter and ultimate size of the oocytes had decreased for each Oocyte stage. The follicular lining (FL) was not intact, and the ovigerous lamellae (OL) was obviously not in its rightful place. The follicular lining was damaged (DFL) (Fig. 5) and loose (LFL) (Fig. 4), and had loosely attached follicular lining remnants (RFL) that were mainly seen in empty follicles (Fig. 4). These damaged follicular linings were seen as a type of damage for many Oocyte IV cells. Damage to the ovigerous lamellae (DOL) was noted next to empty follicles. The amount of atretic cells (AT) was still greater and cytoplasmic retraction (CR) and nuclear retraction (NR) was very common in Oocyte III cells. Damaged (D) cells (Fig. 5) were noted, and were distinguished by a lack of any linings or nuclei. Also seen was a unique cytoplasmic clumping (CC) that showed patchy areas of darkness that could not be explained (Fig. 6). Karyoplasmic clumping was still evident in Oocyte IV cells, and Euvitelline (EU) and Provitelline (PN) nucleoli were present in Oocyte IV cells as well. An expelled nucleus (EN) (Fig. 5) was observed. Macrophages (M) had seemingly been introduced into this exposed tissue to fight any foreign bodies present (Fig. 6). The 100% concentration (1 μg/L) had even more cytoplasmic clumping (CC) occurrences, and a majority of empty follicles (EF). The adhesion (AD) observed was very pronounced (Fig.7). Large patches of macrophages (M) were noted, as were large amounts of atretic Oocyte I and II cells. Karyoplasmic clumping (CK) was seen in most cells that were not empty. Necrosis (NE), or loss of genetic material, of the nuclei and expelled (EN) nuclei were visible (Fig. 8). Logically, because of the large amount of empty follicles, cytoplasm was missing (MC). Ovigerous lamellae (OL) were frayed and broken. Cytoplasmic retraction (CR) was observed in many Oocyte IV cells along with karyoplasmic clumping (Figs. 7 and 8). It was difficult to discern the exact amount of damage each stage of oocyte received because of the extensive damage and difficulty in finding identifying characteristics of the different stages of oocytes. Oocyte IV cells were the most resistant to damage, (Table 3), and the number of Oocyte IV cells actually increased

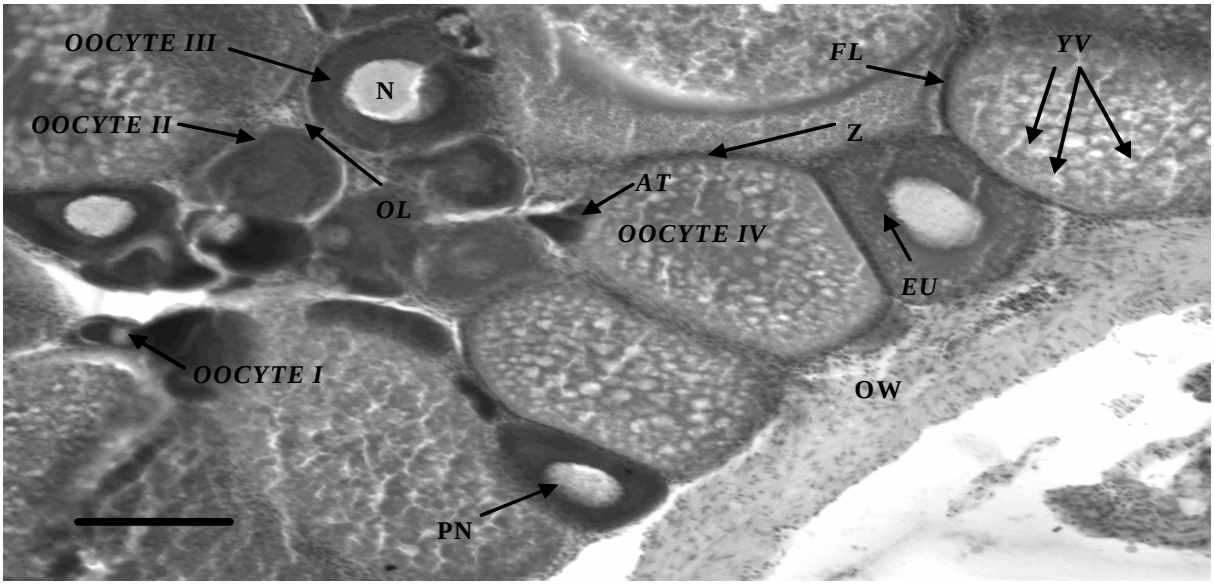

Fig. 1. Represents the control ovary at 100x magnification. Each stage of oocyte (Oocyte I, II, III, and IV) are present in abundance. The Ovarian Wall (OW) is thick and intact. A large nucleus (N) can be seen in Oocyte III cells, and provitelline (PN) and euvitelline (EU) nucleoli can also be noticed on Oocyte III cells. The follicular lining (FL) and zona radiata (Z) are distinct and intact. Numerous yolk vacuoles (YV) are present on the mature Oocyte IV cells. Scale Bar=2 cm

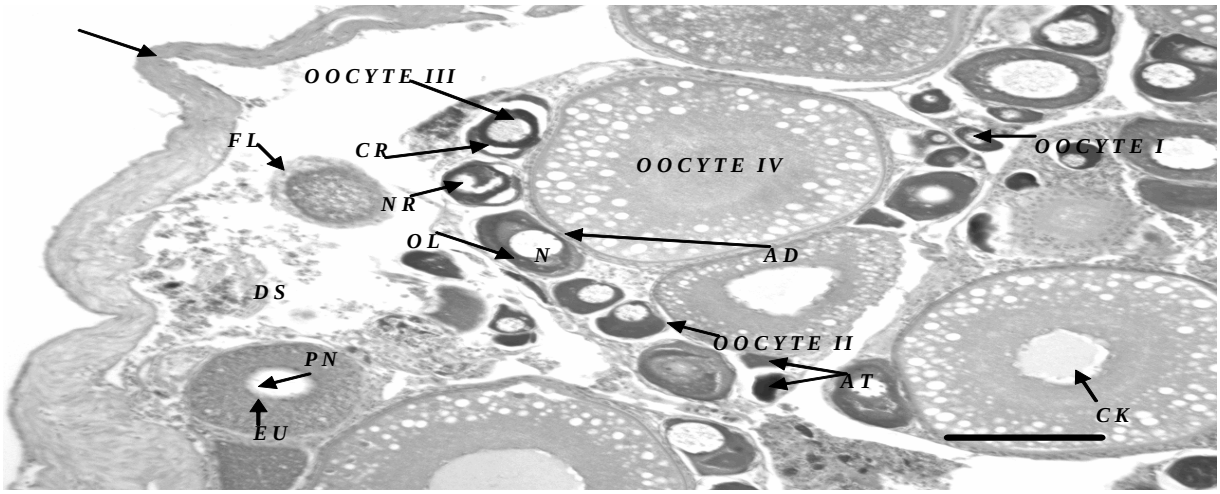

Fig. 2. After a 24 hr exposure to a 25% concentration (0.25 µg/L) of endosulfan some slight damage can be observed. A lifting of the ovarian wall (LOV) has occurred. The follicular lining (FL) and ovigerous lamellae (OL) are no longer firmly attached to the follicles and can be seen floating around the cells. Although all types of oocyte stages are present (I, II, III, and IV), these cells are no longer wholly intact. Nuclear retraction (NR) and cytoplasmic retraction (CR) are taking place in Oocyte III cells. Damaged Stroma (DS) is the result of broken linings and epithelium. Adhesion (AD) between ovarian follicles is present and there is also an increasing number of atretic (AT) cells. Karyoplasmic clumping (CK) is noticed in some Oocyte IV cells. Provitelline (PN) and Euvitelline (EU) nucleoli are still obvious and the nuclei (N) of the oocyte III cells are still generally unharmed. (x100), scale bar=2 cm

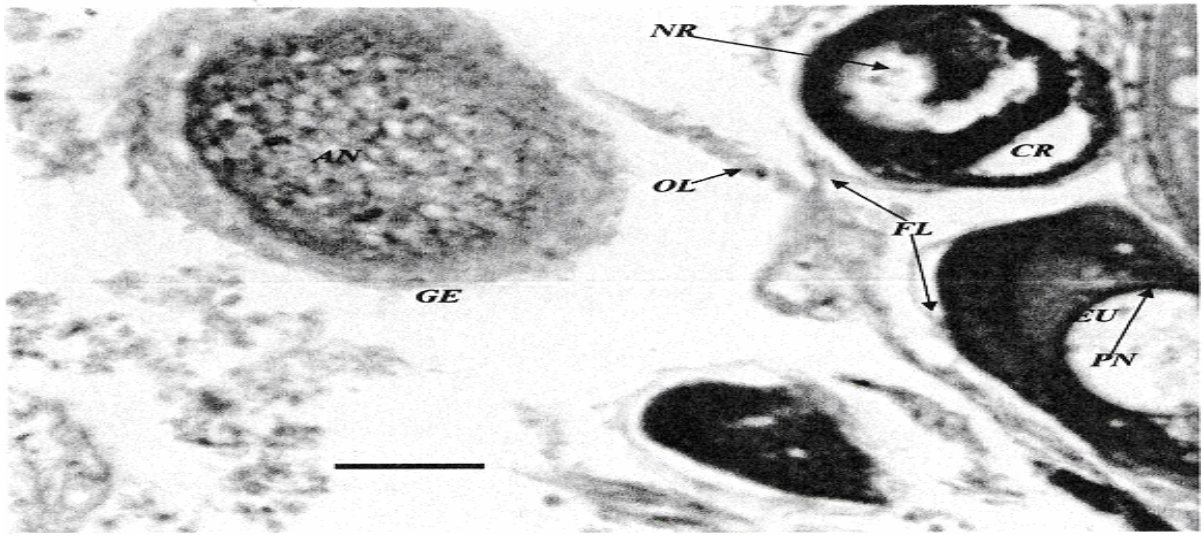

Fig. 3. The 25% concentration of endosulfan (0.25 µg/L) at a higher magnification (x400) shows further damage than was seen at the (x100) magnification. An anucleated (AN) cell is surrounded by a blurry and indistinct germinal epithelium (GE). The free-floating ovigerous lamellae (OL) and follicular lining (FL) can be seen clearly unattached to their respective cells. Nuclear retraction (NR) and cytoplasmic retraction (CR) are more pronounced even though the provitelline (PN) and euvitelline (EU) nucleoli are present. Scale bar=2 cm

with increased exposure. Oocyte III cells had the most obvious damage and were the most vulnerable to damage in each concentration.

CONCLUSION

The quality of the natural surroundings of fish has an important role in their development and reproduction. Even slight changes in concentration of certain chemical compounds can negatively affect the histological properties of fish. As seen in this study, marked deterioration and damage occurs even after a 24-hr exposure to 0.25g/L (25%) concentration. In a recent study, Pandey (1988) observed that when ovaries of the

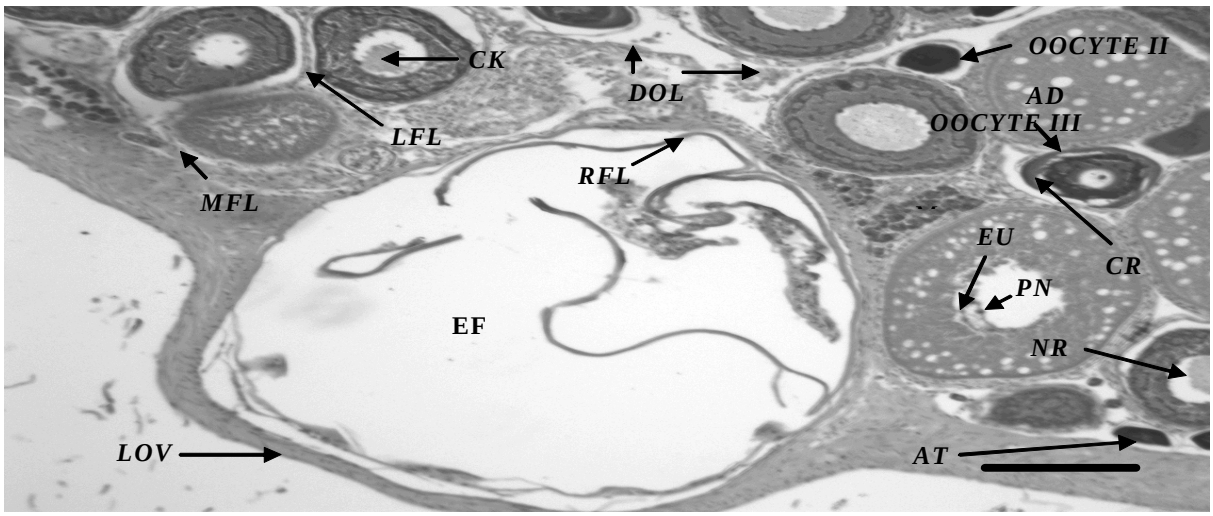

Fig. 4. Represents the 75% (0.75 µg/L) concentration. There is extensive follicular lining damage. Remnants of follicular lining (RFL) are seen floating in the empty follicle (EF). Follicular lining has also been loose (LFL) and is missing (MFL) in some areas. Macrophages (M) probably are ingesting atretic materials or contents of ruptured follicles. The ovarian wall is lifted (LOV) around the empty follicle. Damaged ovigerous lamellae (DOL) and increases in atresia (AT) and adhesion (AD) can be easily seen. Cytoplasmic retraction in Oocyte III cells as well as nuclear retraction (NR) and karyoplasmic clumping (CK) in Oocyte IV cells have made damage more apparent. Euvitelline nucleoli (EU); Provitelline nucleoli (PN). (100x), scale bar=2cm

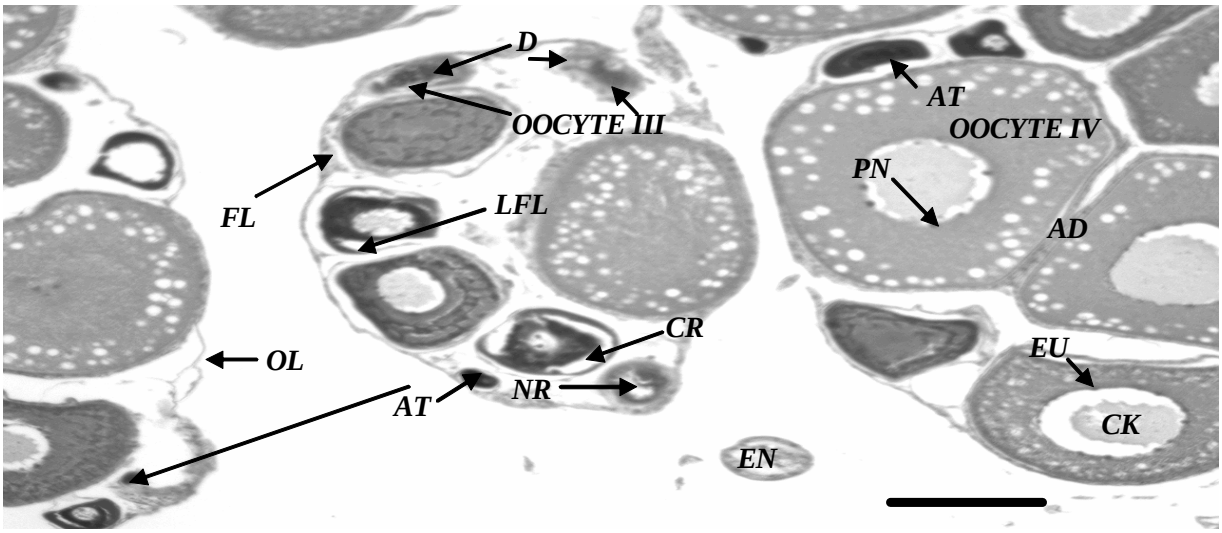

Fig. 5. Shows another view of the 75 % concentration (0.75 µg/L) of endosulfan exposure. Adhesion (AD); Atretic cells (AT); Cytoplasmic retraction (CR); Karyoplasmic Clumping (CK); Damaged (D) cells with indiscernible stages of maturity are seen; An expelled nucleus (EN) is also noticed; Euvitelline (EU) nucleoli; Follicular lining (FL); Damaged follicular lining (LFL); Nuclear retraction (NR); Ovigerous lamellae (OL); and Provitelline (PN). (x100), scale bar=2 cm

freshwater fish *Colisa fasciatus* was treated with a 1 ppm concentration of endosulfan ovarian activity was retarded and the diameter of stage II and stage III oocytes was severely reduced. In the present study, results concurred with Pandey's study, and also Showed that as the amount of endosulfan present in the water increased, so did the amount of damage. The damage that occurred was different for the different stages of oocyte cells, and shows that endosulfan affects not only the entire ovary but also each individual follicular cell. One of the major modes of evaluating damage was the increase in atresia that was seen in Oocyte I and II cells. Changes in gonadotropic hormones or the imbalance of hormones and steroids is suggested to be a driving force in the

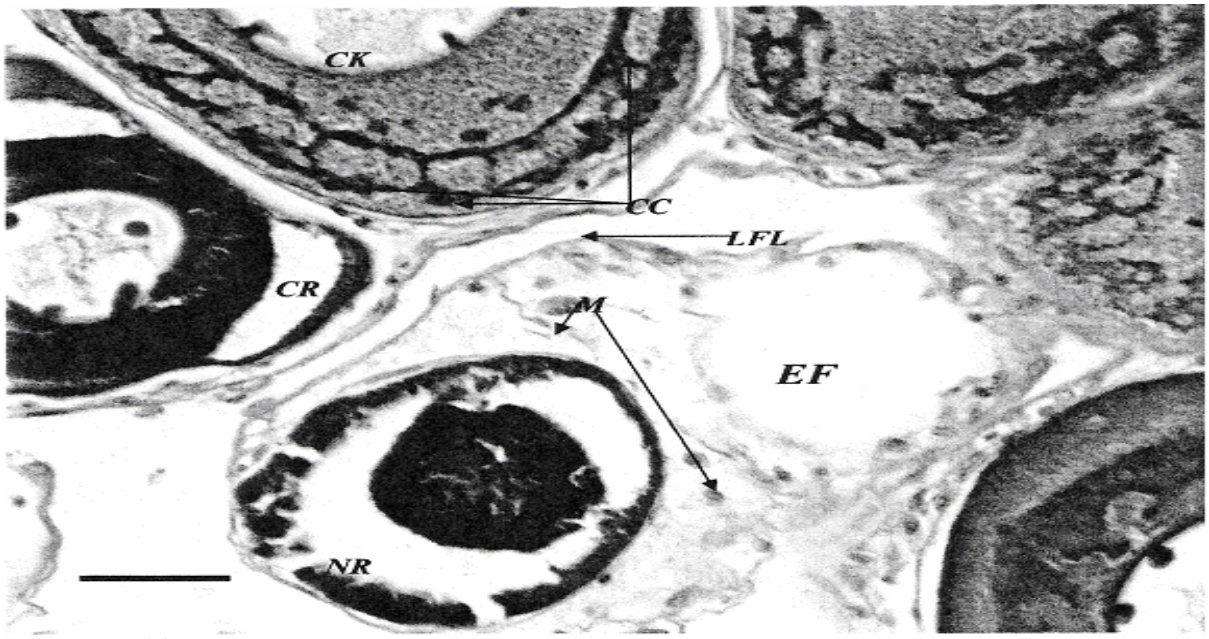

Fig. 6. In this representation of the 75% (0.75 µg/L) concentration exposure a unique cytoplasmic clumping (CC) is observed. An empty follicle (EF) is noticed. Karyoplasmic retraction (CK); Cytoplasmic retraction (CR); Loose follicular lining (LFL); Nuclear retraction (NR). (400x), scale bar=2 cm

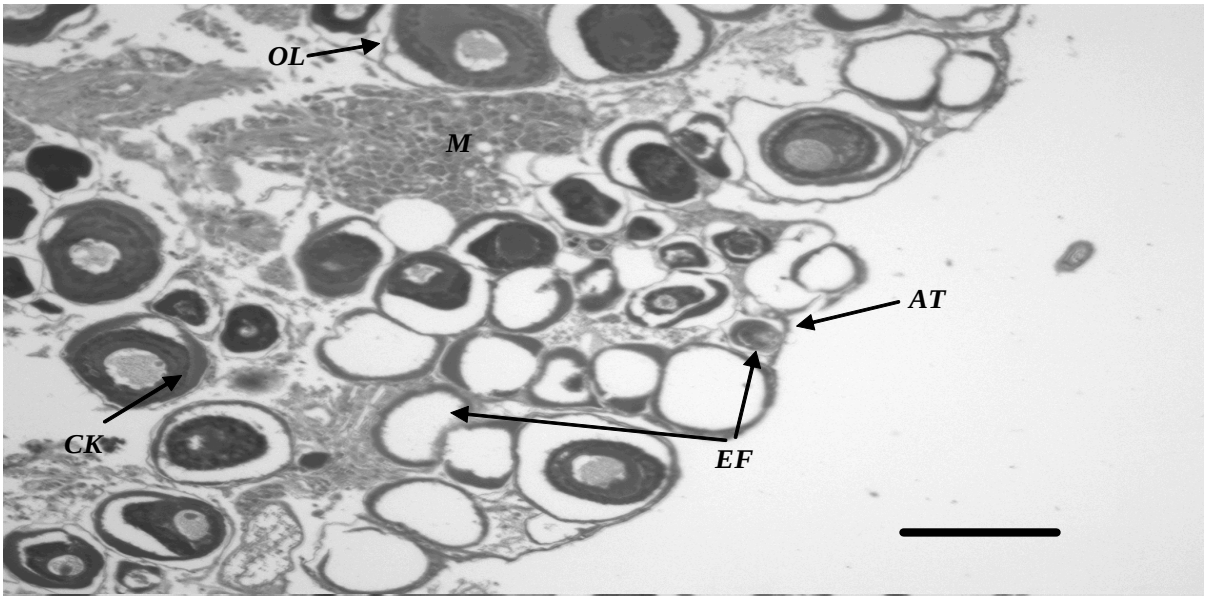

Fig. 7. Is a depiction of the 100% concentration (1 µg/L) exposure. Numerous empty follicles (EF) are apparent. There is a patch of macrophages (M) present as well. Atretic cells (AT); Karyoplasmic clumping (CK); Ovigerous lamellae (OL). (100x), scale bar=2 cm

formation of follicular atresia. Any disturbance in environmental, endocrinological, and metabolic factors may initiate the atresia of oocytes. This increase in atresia can then be correlated with a reduced fecundity (Agarwal and Singh, 1990). When ovaries of albino mice were exposed to endosulfan, atresia disrupted the estrous cycle and atretic cells were observed to have formed as a direct result of endosulfan's effects on the hypothalamo-hypophysial-ovarian axis (Hiremath and Kaliwal, 2002). Therefore, any endocrine inhibitor, such as endosulfan, can supposedly cause atresia and a consequent reduction in fish fecundity.

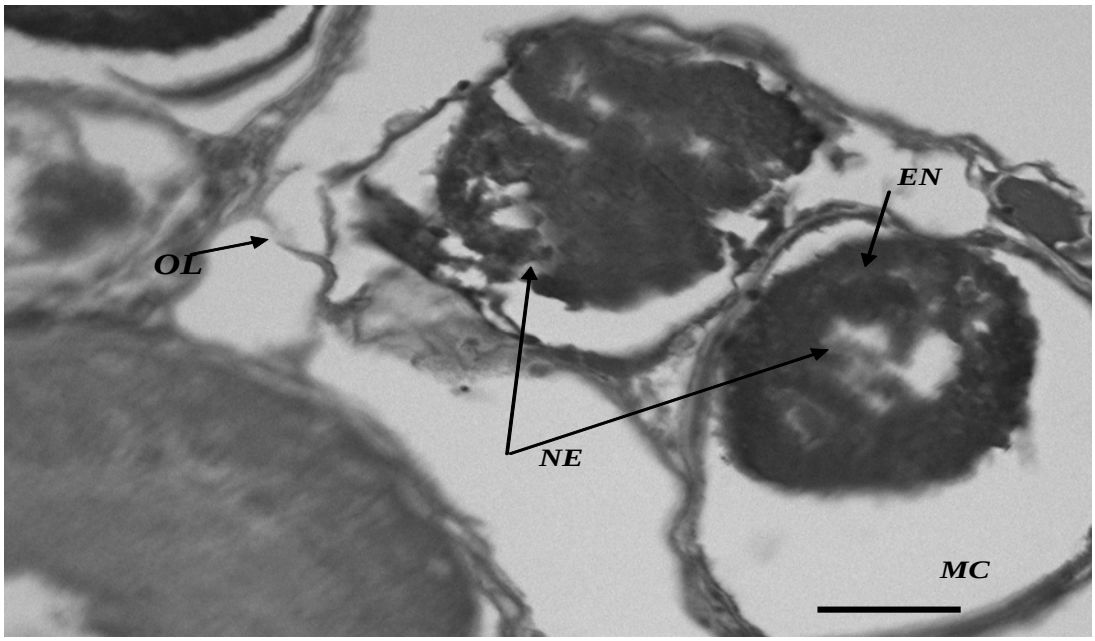

Fig. 8. Represents the most severe damage seen (100% concentration). This is a (400x) magnification and shows necrosis (NE) or lack of genetic material and missing cytoplasm (MC). Expelled nucleus (EN); Ovigerous lamellae (OL). Scale bar=2 cm

In the present study, the most severe follicular atresia was observed in stage I and II oocytes, but there were a few stage III and IV oocytes that were also atretic, especially in greater concentration exposures.Other forms of damage that were seen in the present study include reduction in size of oocytes, increased deformities (cytoplasmic retraction, nuclear retraction, lifting of ovarian wall, etc.), and development of interfollicular spaces (due to adhesion and empty follicles). When a study using two endocrine inhibitors, carbaryl and endosulfan, was conducted on the freshwater fish *Channa striatus* the changes in oocyte formation mentioned above were observed and concluded to be correlated with concentration amount and exposure time. Sub lethal doses of each of these endocrine inhibitors showed that endosulfan's doses were more toxic than those of carbaryl (a carbamate) (Kulshrestha and Arora, 1984). This is seemingly true, because even though the present study exposed fish to endosulfan for only 24 hours, the damage observed was substantial. The cytoplasmic clumping and extreme amounts of empty follicles seen in the 100% (1.0 μg/L) concentration can be used as an example of this claim. As a result of all the damage that is incurred upon the ovary during exposure to endosulfan, it is logically assumed that any changes incurred will also change the viability and normality of offspring. Necrosis, or lack of genetic material, makes the formation of viable eggs difficult (Dutta and Maxwell, 2003). Abnormal fry can result due to lack of genetic material in the many empty follicles seen in the 75% (0.75μg/L) and 100% (1μg/L) concentrations and due to the karyoplasmic clumping in stage IV oocytes. Since the stage IV oocytes, which are mature oocytes, are critical for fertilization purposes, assumptions could be made that since the Oocyte IV cells are largely intact with little percentage damage that fertilization could continue without peril. However, this is not true and even if the slightly damaged Oocyte IV cells were to allow for complete fertilization, reproduction would still be affected and abnormal fry would still be produced (Dutta and Maxwell, 2003). In a study conducted by Gormley and Teather (2003) Japanese medaka (*Oryzias latipes*) was exposed to different concentrations of endosulfan for 24 hr exposure times and eggs were found to take longer to hatch. The resulting fry were smaller at 1 week of age and had decreased mobility at 2 weeks of age.

When these abnormal fry reached sexual maturity these individuals produced fewer eggs.Therefore, the damage and changes that

occurred in the different stages of oocytes with increased endosulfan exposure concentrations confirm that water and soil quality is very important to the development and reproduction of fish. The observations of deformities and follicular spaces that occurred due to sublethal concentrations show that reduced fecundity and therefore abnormal offspring can result as a consequence of any endocrinological effects that the many pesticides of the world, including endosulfan, cause.

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