



Effect of Organochlorine Pesticides on Ovary weight, Ova diameter and Gonado Somatic Index of Common carp *Cyprinus carpio*.

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ABSTRACT

In the present research is to see how low doses of organochlorine pesticides (dieldrin and methoxychlor) affect Ovary size in the common carp, *Cyprinus carpio* (C. carpio). Dieldrin and Methoxychlor were tested at different doses to assess their acute toxicity. Since the impact of the pesticide on fish becomes consistent with 24h of exposure for LC₅₀ of a toxicant, which is 0.23ug/L (96hLC₅₀), and sub-lethal doses of the Dieldrin and Methoxychlor organochlorine pesticides produced various effects on the oocytes of different types. The diameter of the oocytes decreased with progressive duration of the pesticide exposure as compared to controls. In 7 days exposure no significant change in ova diameter was observed in the immature oocytes but subsequent exposure produced significant changes. The percentage of immature oocytes increased while that of mature oocytes decreased from 0 days to 21 days of exposure. The maturing oocytes underwent slight reduction in size and change shape. In this research diameter of oocytes and shape reduction observed.

KEYWORDS: Ovary, *Cyprinus carpio*, Organochlorine Pesticides, Dieldrin, Methoxychlor, LC₅₀

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I. INTRODUCTION

Numerous studies are available reporting the effects of pesticides on reproductive activities in Indian fishes. The majority of these reports deal with histopathological changes in gonads and endocrine glands involved in the regulation of reproduction following treatment with different pesticides. Pesticides are reported to cause degenerative changes in gonads and arrest gametogenic processes either by acting directly on the gonads or by interfering with the secretory activity of the hypothalamo-hypophysal-gonadal/thyroid axis that regulates various reproductive events. Secretion of hormones such as gonadotropin releasing hormone, gonotropin, growth hormone, adrenocorticotrophic hormone, testosterone etc are in general lowered leading to cessation of gametogenesis, vitellogenesis, oocytes maturation, ovulation spermiation etc. Adverse effects of pesticides have also been demonstrated on fecundity, fertilization, hatching and postembryonic development. The effects are highly variable and depend on the nature, dose and mode of application of the pesticides.

Some literature is available on the fish ovarian damage due to pesticide. In India, most of the ovarian research has been concentrated on air breathing fishes catfishes, carps with relation to effect of a number of organochlorine, carbamate and organophosphate pesticides. Kurlshrestha *et al.* (1984) worked on the exposure of endosulfon and carbaryl on the ovaries of *Channa striatus* (Bloch) and observed reduction in the number of oocytes, increased number of damaged oocytes, development of inter follicular spaces, reduction in gonado somatic Index, Fecundity, fertilization etc.

Boyd (1964) reported that, at concentration above the threshold toxicity, many organophosphate compounds caused large pregnant female of *Gambusia affinis* to abort.

Carlson (1972) reported that the carbaryl (Sevin) pesticides cause prevention of reproduction in fathead minnow, *Pimephales promelas*.

Holden (1972) reported that brown trout eggs containing aldrin failed to hatch. Hatching of blue gill eggs exposed to different doses of a formulation of fenofrop (Kuron) was not affected, but all the fry hatching from eggs exposed to the highest concentration died.

Nagler (1984) investigated sublethal effect of pentachlorophenol (PCP) on ovarian development in rainbow trout, *Salmo gairdneri* and reported increase in intrafollicular spaces, thickening of ovarian wall and follicular atresia.

Zagatto (1999) reported that the toxicity tests with embryos and larvae are valuable for assessing potential impacts on growth, reproduction and survival of organisms in polluted environments and are important tools for good environmental monitoring.

Hanson *et al.* (2009) studied the uptake and toxicity of same pesticide on three fresh water fish *Oreochromis niloticus*, *Clarias gariepinus* and *Chrysichthys nigrodigitatus* and reported that the pesticides had adverse effects on the general growth and reproduction of fishes, gonadosomatic indices also showed that the pesticides affected the development of the body the gonads and their reproduction.

The responsiveness of animals including fishes to organochlorine compounds ranges from altered metabolic activities to death (Anam and Mitra, 1995). On several occasions depending upon the level of exposure impaired reproductive activities following ingestion of organophosphate compounds have been reported in both female and male fishes.

In fish, the uptake of pesticide taken place through absorption by gills (Holden, 1962; Murphy, 1971) and by ingestion of contaminated food (Grazenda *et al.*, 1970; Macek, 1970). Residue uptake through gills is related to the metabolic rate and body size (Murphy, 1971). The fishes are directly influenced due to these toxic chemicals which cause mortality and several disease as a result the growth is ceased. The breeding capacity of fishes is also hampered due to these toxicants. Behavioral activities of an organism represents the final integrated result of a diversity of biochemical and physiological processes of behavioral patterns are known to be highly sensitive to changes in the steady state of an organism (Warner *et al.*, 1996). Lots of scientist expressed the pattern of behavioral changes varies according to fish species, concentration of chemical (Pesticide), Physico-chemical conditions and on rate of excretion of fish species. Poisons with a restorative action, the symptoms are manifested primarily through their action on the nervous system; cause a rapid loss of equilibrium in fish (Metler *et al.*, 1971).

Symptoms of furadon and Malathion toxicity were reported by Konar and Ghosh (1982). Within 24 hours of exposure to 0.5 ppm furadon, fish had slow opercular movement, lethargy and occasional jumping. Reports related to effects on fish reproduction are scarce and do not encompass the diverse range of events involved in reproduction such as the onset of puberty, gametogenesis, oocyte maturation, ovulation, spermiation, spawning, fecundity, fertilization, endocrinology of reproduction, and developmental events such as embryogenesis, hatching, and post hatching metamorphosis. Information on all these reproductive aspects is available in temperate zone fishes exposed to pesticides.

Since the lipid content of fish gonads increase tremendously during their reproductively active phase some times by more than 200 times (Lal and Singh, 1987a), lipophilic pesticide residues accumulate and increase in recrudescing fishes. These pesticides are therefore likely to interfere with gonadal activities.

Pesticide-Induced reproductive failure or dysfunction is evident from the available reports on Indian fishes (Singh and Singh, 1982b; Singh *et al.*, 1997). Pesticides have been reported to cause damage to gonads such as cytolysis of germ cells, arrest of gametogenesis, inhibition of steroidogenesis, gamete maturation, release of gamete, spawning and hatching. Reproductive toxicity indicates changes on the pattern of breeding response, on fecundity, on fertilization rate, hatchability of larvae and above all survivability of larvae, fry etc. though, it is a prime subject of research because it directly relates to productivity of fishes.

Hence, the present study was undertaken to investigate influence of the organochlorine pesticides on the reproductive activities of female common carp, *Cyprinus carpio communis*. Also, it may not be ruled out that organochlorine pesticides are potent antiacetylcholinesterase agents which phosphorylate the enzyme acetylcholinesterase resulting in accumulation of endogenous acetylcholine and consequent disruption of neuro-function.

Thus measurement of acetylcholine activities in different tissues are considered as marker of organophosphates neurotoxicity associated with brain and reproductive organs of target fish. Nevertheless those who have worked on this aspect made appreciable advances in this subject. On the basis of the lethal and sub-lethal toxicity study we can compare toxicity of these selected pesticides to other pesticides and can also use common carp as a model for other fish species. The reported results would be useful contribution in ecotoxicity risk assessment studies of these organophosphate pesticides as fish species.

II. MATERIALS AND METHODS

2.1. Test Organisms

Females of common carp (*Cyprinus carpio communis*) were used as testorganisms for several reasons. Ecotoxicological studies of common carps are of potentially great importance,as they have a wide distribution throughout India including Kashmir waterbodies. They are designated as toxicity test fish by United States Environmentalprotections agency (U.S. EPA, 1979). *Cyprinus carpio communis* is a representative of an ecologically important group.It occupies a position within a food chain leading to man. It is widely available, amendable to laboratory tests, early maintained andgenetically uniform, and There is an adequate background data on the organism (*Cyprinus carpiocommunis*) i.e. physiological, genetics, Taxonomy, Embryology etc..

The fish is voraciously omnivorous; efficiently converting the food ingested,into flesh, grows very fast and is prone to artificial feeds. It naturally breeds inconfined waters, spawning occurs in shallow marginal weed infected areas. Breedingseason is mid January to March and again July to August. Fish for the present work the healthy female fishes were procured from the local market throughout the year 2019, for the experimentation of different parameters related to reproductiveactivities of the fish. Such as effect on GSI, ova diameter and ovary weight. The procured healthy female *Cyprinus carpio* brought to the laboratory in plastic buckets with sufficient air. The plastic buckets were opened and the fish specimens were shifted to the glass aquaria for 20 days to be acclimatized and to eliminate transport-induced stress and allow for capture induced mortalities prior to pesticide exposure.

The specimens were about 09 ± 1.05 cm in length and 50 ± 1.02 gm in weight.New supplies of fish were obtained monthly so that the fish material was seldom keptin the laboratory aquaria longer than one mont.**2**

2.2. Test Compounds/organochlorine pesticides

For the present study, Dieldrin and Methoxychlor were chosenas toxicants based partially on the probability of their having reproductive effects. These are employed routinely in the integrated farming practice to protect crops and animals from insects, weeds and diseases. The liberal use of these organophosphate pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce is posing great danger to aquatic environment. These organochlorine pesticides are more frequently used because of theirhigh insecticidal property, low mammalian toxicity, less persistence and rapidbiodegradability in the environment. Dieldrin and Methoxychlor stock solution was prepared in acetone, which was found to be nontoxic to fis

2.3. Experimental groups and dosage

After acclimatization, healthy and same sized *Cyprinus carpio* were chosen and sorted into 3 groups of 20 fishes each.

Groups	Dosage
GROUP I	Control fishes
GROUP II	Fishes exposed to 1/10th of LC50 value of Dieldrin (0.23 μ g/L) for 21 days.
GROUP III	Fishes exposed to 1/10th of LC50 value of Methoxychlor (0.23 μ g/L) for 21 days.

Fishes were exposed to their respective sub-lethal concentration of Dieldrin and Methoxychlor and maintained in these concentrations up to the stipulated period of exposure. Test medium was renewed daily, which facilitated the removal of nitrogenous waste excreted by the test fishes and for the removal of unconsumed food. 24 hours after the respective exposure, the effect on GSI, ova diameter, ovary weight of the fish were studied.

2.4. Effect on Body Weight

For the determination of changes in body weight of the fish, each fish wastaken out from all the groups separates and body weight was taken at differentintervals according to the method adopted by Singh (1987) and others. Body weightwas measured by putting individual specimens from all the groups one by one in widemouthed jar containing half-filled water. The reading was taken on digital electronicbalance as follows: Weight of fish = weight of Jar with water - weight of jar with water containing fish. Mean, S.D and ANOVA was used to determine the statistical significance of the data.

2.5. Effect of Organochlorine pesticides on GSI (Gonado Somatic Index).

To study the effect of Dieldrin and Methoxychlor organochlorine pesticides on fish, this experiment was started in the month of December when the fishes werein resting phase and ended after continuous exposure up to the month of July, when the gonads of the experimental fish were in spawning phase. The aquaria were keptin natural light and temperature conditions. At the end of the experiment, specimens were sacrificed by decapitation and the required tissues wereremoved and processed for the following investigations.

GSI (Gonado Somatic Index):

The gonado somatic index was calculated using the formula:

$$GSI = (\text{weight of gonad} / \text{weight of fish}) \times 100$$

ANOVA was used to determine the statistical significance of the data.

2.6. Ovary Weight

The ovaries were made free from the adjoining tissues and traces of extraneous fluid and then weighed on a balance sensitive to 0.1mg. The observed results were recorded separately for the control as well for Dieldrin and Methoxychlor exposed groups for all concentration and presented in the **table- 10** for both the test substances.

2.7. Ova diameter

Ova diameter is the diameter of the eggs from the three different (Anterior, middle and posterior) parts of the ovary. The diameter of 100 ova from the samples of each ovary of the fish species from different groups (Control and pesticide treated groups) was measured using stereoscopic microscope fitted within an ocular micrometer 100 and 400 magnification. The means of the measurements from the three regions was taken as the standard diameter of the ova of the particular fish.

III. RESULTS AND DISCUSSION

The effects of pesticidal contamination of wildlife habitats may be expected to be proportional to the toxicity of the compounds, the rate and manner of application, persistence of the basic chemical and/ or any toxic metabolites, and the extent to which these substances are stored in animal tissues or concentrated by successive elements of wildlife food chain. Measurement of these effects under field conditions is difficult, but the need for field studies may be reduced or eliminated by controlled laboratory tests. An attempt has been made in the present investigation to demonstrate influences of organochlorine pesticides (Dieldrin and Methoxychlor) on the reproductive organs of female common carp, *Cyprinus carpio*.

3.1. Effects of Dieldrin and Methoxychlor (Organochlorine pesticides) on Ovary weight of test animal, *Cyprinus carpio*

In the present investigation there was found no significant change in the ovarian weight until 7 days of the pesticide treatment. The decrease in the weight of ovary was observed only on day 7 in pesticide exposed fishes and increase the weight which continue until the end of the exposure period. The decline in the ovarian weight was highest on the 7th day of exposure, which coincided, with the pre-spawning phase of the experimental fish. With the Dieldrin treatment the ovarian weight did not deviate from the control value until after about 14 days of exposure. However, from 21st day onwards until the termination of the experiment ovarian weight varied significantly from the respective control value and exposure time.

Similarly, with Methoxychlor treatment there was significant deviation from the control value on increasing the exposure time and to the pesticide. The following table-1 and Figure-1 shows the deviation of ovary weight from control values in pesticide treated fish.

Table-1. Analysis by mean and standard deviation showing ovary weight in grams in control and Dieldrin and Methoxychlor treated groups of *Cyprinus carpio*

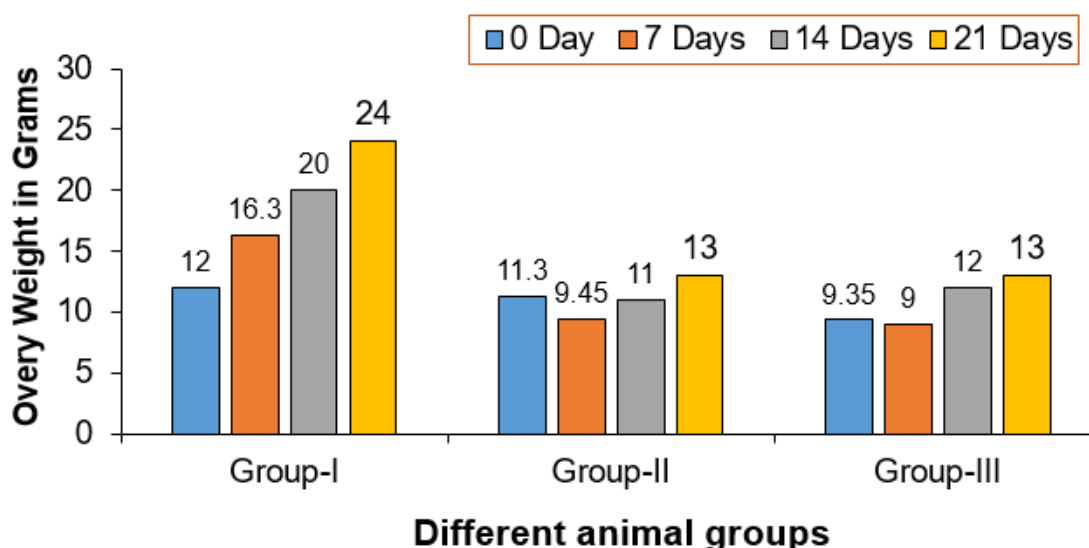
Test Animal Group	Ovary Weight in Grams			
	Day-0	Day-7	Day-14	Day-21
Group-I	12.01 ± 2.34	16.34 ± 3.42	20.48 ± 2.5	24.74 ± 4.3
Group-II	11.34 ± 3.5	9.45 ± 2.12	11.0 ± 3.32	13.32 ± 2.65
Group-III	9.35 ± 1.35	9.0 ± 0.15	12.5 ± 2.9	13.4 ± 1.80

Group-1: Control;

Group-II: Fishes exposed to 1/10th of LC50 value of Dieldrin (0.6µg/L) for 21 days;

Group-III: Fishes exposed to 1/10th of LC50 value of Methoxychlor (0.6µg/L) for 21 days

Figure-1. Analysis by mean and standard deviation showing ovary weight in grams in control and Dieldrin and Methoxychlor treated groups of *Cyprinus carpio*



3.2.

Effects of test substances, Dieldrin and Methoxychlor (Organochlorine pesticides) on ova diameter of test animal, *Cyprinus carpio*

In the present investigation sub-lethal doses of the Dieldrin and Methoxychlororganochlorine pesticides produced various effects on the oocytes of different types. The diameter of the oocytes decreased with progressive duration of the pesticide exposure as compared to controls. In 7 days exposure no significant change in ova diameter was observed in the immature oocytes but subsequent exposure produced significant changes. The percentage of immature oocytes increased while that of mature oocytes decreased from 0 to 21 days of exposure (Table-2). The maturing oocytes underwent slight reduction in size and deformity in shape in all treated groups. The yolk vesicles may be damaged and interfollicular spaces increased.

Table-2. Analysis by mean and standard deviation showing the egg diameter in control, Dieldrin and Methoxychlor treated groups of *Cyprinus carpio*

Duration of Pesticide Exposure	Oocyte Maturity	Egg diameter in microns in test animal groups		
		Group-I	Group-II	Group-III
Day-0	Immature	65.75 ± 4.30	47.9 ± 7.44	45.0 ± 4.76
Day-7	Immature	71.87 ± 5.80	37.7 ± 6.67	41.88 ± 5.33
	Maturing	155.57 ± 24.5	104.22 ± 9.33	101.55 ± 11.22
Day-14	Immature	77.68 ± 7.13	43.18 ± 8.70	41.44 ± 6.77
	Maturing	159.06 ± 44.37	112.10 ± 12.67	108.67 ± 16.6
	Mature	264.56 ± 35.45	144.44 ± 14.33	140.33 ± 12.2
Day-21	Maturing	124.33 ± 8.75	121.34 ± 10.0	118.3 ± 9.33
	Mature	304.45 ± 22.5	280.67 ± 22.3	264.77 ± 21.6

Group-1: Control;

Group-II: Fishes exposed to 1/10th of LC50 value of Dieldrin (0.6µg/L) for 21 days;

Group-III: Fishes exposed to 1/10th of LC50 value of Methoxychlor (0.6µg/L) for 21 days

3.2. Effects of different concentrations test substances, Dieldrin and Methoxychlor (Organochlorine pesticides) on Gonado Somatic Index of test animal, *Cyprinus carpio*

Cyprinus carpio breeds almost throughout the year with peak periods from January to April and again from July to August. Gonado somatic index (GSI) of species has been widely used to indicate the maturity and periodicity of spawning of the fish. The GSI increases with the maturation of the fish and is maximum during the peak period of maturity. It decreases abruptly after spawning. Gonado somatic indices were calculated for control group and pesticide treated groups separately and is tabulated in tables 3 and Figure-3. In control group

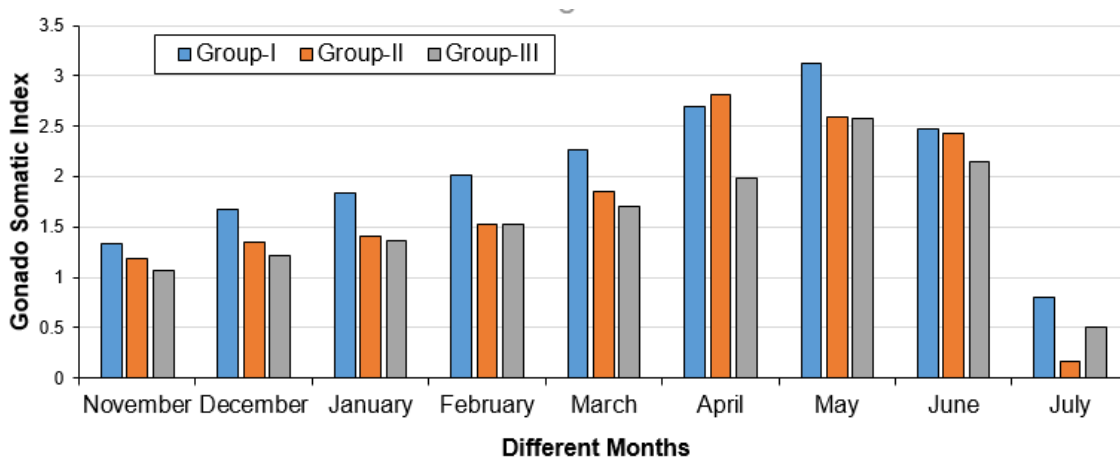
fishes GSI was found to increase gradually from 1.34± 0.35 (November), 1.67±0.36 (December), 1.84±0.24 (January), 2.02±0.41 (February), 2.26±0.29 (March), 2.70±0.42 (April), 3.13±0.35 (May), 2.47±0.37 (June) and 0.80±0.25 (July). A sudden increase of GSI during April and May period was indicative of onset of spawning activity. In the month of June to July GSI values decreased from 2.47±0.37 to 0.80±0.25 was observed and thus showing cessation of IST spawning act.

In Dieldrin treated groups GSI was found to increase gradually, but the increase was comparatively less than control groups. Also there was found variation in increase with different concentrations of the same pesticide. In these exposed groups the GSI was found to range from 1.19±0.35 (November), 1.35±0.35 (December), 1.41±0.35 (January), 1.53±0.33 (February), 1.85±0.12 (March), 2.81±0.22 (April), 2.59±0.37 (May), 2.43±0.37 (June), 0.17±0.10 (July) for the Dieldrin concentration of 0.6µg/L. Thus in case of all the Dieldrin treated groups there was increase in the GSI value but increase was less than the control groups. GSI values in exposed.

Table-3. Monthly changes in the mean gonado somatic indices in control as well as pesticide (Dieldrin and Methoxychlor) treated groups of the female, *Cyprinus carpio*.

Months	Gonado Somatic Index (GSI) in Different Groups		
	Group-I (Control)	Group-II (Dieldrin treated, 0.6µg/L)	Group-III (Methoxychlor treated, 0.6µg/L)
November	1.34±0.35	1.19±0.35	1.07±0.38
December	1.67±0.36	1.35±0.35	1.21±0.31
January	1.84±0.24	1.41±0.35	1.37±0.34
February	2.02±0.41	1.53±0.33	1.52±0.08
March	2.26±0.29	1.85±0.12	1.70±0.06
April	2.70±0.42	2.81±0.22	1.98±0.23
May	3.13±0.35	2.59±0.37	2.58±0.27
June	2.47±0.37	2.43±0.37	2.15±0.25
July	0.80±0.25	0.17±0.10	0.51±0.33

Figure-3. Diagrammatic representation of the quantitative values (mean in vertical bar) of Gonado somatic index of female common carp after exposure of Dieldrin and Methoxychlor in November to July months.



Similarly, in the Methoxychlor treated groups the decrease in GSI values was found as compared to control groups. There was slight increase observed from 1.07±0.38 (November), 1.26±0.31 (December), 1.81±0.34 (January), 1.96±0.08 (February), 2.19±0.06 (March), 2.45±0.23 (April), 2.81±0.27 (May), 2.50±0.25 (June), 0.43±0.33 (July) for the Methoxychlor concentration of 0.6µg/L. It may be also noted that GSI did not deviate from the control value significantly until after 14 days of exposure for both the test substances every month. However, 21 days onwards until the termination of the experiment GSI values varied significantly from the respective control values. The tables-3 and Figure-8 show the deviation of GSI from control values in pesticide treated fish for Dieldrin and Methoxychlor respectively.

The present work also showed that the prolonged exposure of *Cyprinus carpio* to different concentrations of Dieldrin and Methoxychlor organochlorine pesticides in water induces a variety of anomalies in feeding behavior, food utilization and body weight of the fish. The significant reduction in the body weight in experimental animal, *Cyprinus carpio* exposed for Dieldrin and Methoxychlor indicates that the fish were severely stressed in both the cases. The suppressive effect on food consumption due to toxicant cannot be ruled out. Studies with other pesticide compounds like methylparathion on the fish *Tilapia mossambica* (Siva Prasada, 1980) and malathion, Sevin and lindane on the same species (Basha, 1980) showed a decrease in the body weight. Ghatak and Konar, 1991, also reported the feeding rate of *Tilapia* was reduced significantly when exposed to pesticide at various concentrations. Similarly, Ponmani et al., 1997, observed significant reduction in feeding rates and body weight of *Cyprinus carpio* exposed to sub-lethal concentrations of monocrotophos.

Also O' Brien, R.D. (1976) reported that the acetylcholinesterase inhibition decreased the feeding rate due to impairment of impulse transmission. Pal and Konar, 1987, reported that methylparathion of various digestive enzymes. A reduced body weight may also be attributed to an increased activity associated with attempt to avoid the contaminated waters, or an increased expenditure of energy on chemical detoxification and tissue repair.

The variations in body weight of fish exposed to organochlorine pesticides (Dieldrin and Methoxychlor) should be due to loss of some constituents other than water. Similar observations in relation with weight changes were made in *Tilapia mossambica* treated with different pesticides like, Malathion, methyl parathion and parathion (Kabeer et al., 1981). They observed that 96 decrease in the body weight can partly be attributed to the loss of ions from the body. In the present context also, the same situation can be expected. However, changes in body weight are more in fish exposed to different concentration of Methoxychlor than in fish exposed to different concentration organophosphate pesticide Dieldrin.

The teleost ovary undergoes a seasonal reproductive cycle which may, for convenience, be divided into four main phases: Vitellogenesis, involving the major growth phase of the ovary during which ovarian secretion of oestradiol stimulates hepatic synthesis of vitellogenin which in turn, is incorporated into the developing oocytes. Oocyte maturation, during which the germinal vesicle migrate to the periphery of the oocytes and breaks down under control of pituitary gonadotrophins and ovarian progestrogens. Ovulation and Spawning and Postspawning in which the gonads regress in preparation for the next reproductive cycle.

Clearly the stage at which the fish are exposed to the pesticides and the duration of such exposure will determine to a large extent the effect on the ovary. Results from the previous investigations have suggested that the long term exposure of fish to pesticides invariably lead to the antigonadal influences such as decrease in ovarian weight, smaller, less developed oocytes and fewer large mature oocytes and an increase in the numbers of the atretic follicles and oocytes frequently contained less yolk granules (Sukumar and Krpaganapathy, 1992). Kulshrestha et al., 1984 worked on the exposure of endosulfan and carbaryl pesticides on the ovaries of *Channa straitus* (bloch) and observed reduction in the number of oocytes, increased number of damaged oocytes, development of interfollicular spaces and decreased gonado somatic index. Shukla et al., 1984, noted decreased ovarian activity and atretic oocytes in *Sarotherodon mossambica* exposed to melathion, and Ghosh et al., 1985 has described on damage in ovary viz., degeneration of follicular wall, ooplasm and connective tissue due to melathion toxicity on *Heteropneustes fossils*. In the present investigations a significant reduction in the ovarian weight was observed following exposure of Dieldrin and Methoxychlor organochlorine compounds to experimental animal for 21 days. According to Kiling (1986) total follicular atresia, inhibition of vitellogenesis and disruption of reproductive endocrine functions due to the pesticide exposure could have caused significant decrease in ovarian weight.

Thus it appears logical to summarize that the decrease in ovarian weight in *Cyprinus carpio* may be due to the decrease in number of mature follicle, increase in number of atretic follicles and decreased levels vitellogenesis. Oocyte maturation in teleost fish is a necessary condition for successful ovulation. This phase of oocytes development is initiated by gonadotrophin, which induces both migration of the germinal vesicles to the periphery of the oocyte and the follicular synthesis of a maturation inducing steroid (which is often considered to be 17, 20 β -dihydroxy-4-pregnen-3-one). Results from the previous investigations have suggested that organochlorine and organophosphate pesticide exposure to fish caused necrosis and fibrosis of ovarian connective tissue, dilation of blood vessels, decrease in oocytes diameter and increase in intrafollicular space in *Rasbora deniconius* and *Channa straitus* (Kulshrestha and Arora, 1984; Rastogi and Kulshrestha, 1990).

The percentage of immature oocytes increased while that of mature ones decreased from 0 to 21 days of exposure. Collectively our results demonstrate an inhibitory influence of organochlorine pesticides on the female gonads, thus it appears logical to summarize that the decrease in ova diameter may be due to effect of organochlorine pesticides on vitellogenesis, gonadotrophin formation like physiological processes. Hence, our observations are supported by the similar results demonstrated in previous investigations.

Seasonal cycles in the gonadal development and the breeding behavior have been conclusively shown to be regulated through several external factors including photoperiod, temperature and other physical and chemical factors. These are known to serve as proximate factors and act through brain, pituitary and gonadal axis to control the reproductive behavior of the fish (Nikolsky, 1963; Hoar, 1965a; Love, 1970 and de Vlamming, 1972). Earlier reports have indicated that *Cyprinus carpio*, undergoes changes with respect to its breeding behavior (Raina, 1978). In *Cyprinus carpio*, the ovaries are typically cyst ovarian and ova develop within the ovarian sac being liberated directly to the exterior through a small oviduct.

The ovaries are paired and situated in the posterior-dorsal part of the body cavity covered by a thin walled peritoneum and attached by a mesovarium. Posteriorly the ovaries are fused but the paired nature can still be recognized. The shape, size and color of the ovaries varies with the stages of maturation. During their immature stages they occupy about 3/4th of the length of the abdominal cavity but as they grow, they become distended and on maturation occupy the entire abdominal cavity. Histologically each ovary is made up of large number ova within the ovarian sac. The walls of the sac present a number of folds the ovigerous lamellae. These were seen to be lined by germinal epithelium where the oocytes developed and were budded off into the cavity of the ovarian sac.

The breeding season of the carp extends from March and early April to the middle of June and may therefore be regarded as a spring breeder. The ovaries show a series of cyclic changes in the morphology and histology which represents the various maturation stages and are related to the gonad somatic index of the experimental fish. The various recognizable seasonal changes are:

During September, October and November the ovaries are in the maturing stage. The ovigerous lamellae are full of small rounded microscopic oocytes. The cytoplasm at this is deeply staining. The nuclear membrane is smooth and peripheral vacuoles are present in some oocytes. The blood supply becomes conspicuous and increases considerably. Along with these visible changes in the histomorphology of the ovaries, the gonad somatic index also exhibits a linear increase. It was found to gradually increase from 1.34 ± 0.35 in November to 3.13 ± 0.35 in April showing a three fold increase. The eggs appeared to be fairly advancing.

During the period of December, January and February the ovaries does not show an active histomorphological transformation, the ovaries remain rather quiescent and rate of maturity is slow. The oocytes attain a large size and the ovaries look highly packed. The yolk attains the granular form and both granular and non-granular yolk is present in oocytes. Two zones of the yolk are distinguishable; outer with large yolk plates the inner with smaller ones. The vitellogenesis is also very slow during this period. During the post-spawning or spent period (July- August) the ovaries are shrunken flaccid and blood shot, occupying very little space in the body cavity of the fish. The ovaries are marked with dark red spots, which are due to degenerating ova on the surface of the ovary and also show a high degree of vascularity.

IV. CONCLUSION

The exposure doses of the two organochlorine pesticides (Dieldrin and Methoxychlor) caused less significant mortality of the experimental fish, female of *Cyprinus carpio* but did manifest signs of physiological distress. However, they were both potent enough to cause significant reproductive impairment in terms of specific damage to ovarian tissue.

The Ganado somatic index increased in all control and pesticide treated groups during the investigation but reduction in Gonadosomatic index was observed on exposure to the test substances, Dieldrin and Methoxychlor as compared to control groups. It may be also noted that the reduction in GSI values was maximum at highest concentrations of both the organochlorine pesticides in series and reduction was treatment time dependent. Competing Interests

Authors have declared that no competing interests exist.

Author's Contributions

'Author A' performed the experiments and wrote the first draft of the manuscript. 'Author B' designed the study and finalized the draft of the manuscript.

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