ISSN 2515-8260 Volume 07, Issue 09, 2020

Monocrotophos toxicity induced hormonal and biochemical disruption in liver and ovary of *Cyprinus carpio communis*

Sarabjeet Kaur

Assistant professor, Department of Zoology, Mehr Chand Mahajan DAV College for Women, Chandigarh

Abstract

The organophosphorus pesticide, monocrotophos induced toxicity in fish's adobe has been studied to access the biochemical and hormonal disruption caused in the liver and ovary of the fish. Monocrotophos pose serious risk to biochemical parameters, enzyme activities and level of hormones in the fishes. A direct relationship between concentrations of monocrotophos, elevated cholesterol levels and depressed estradiol concentrations have been traced in the study. The elevated levels of cholesterol in the ovary apparently reflect the reduced rate of its utilization for steroid synthesis by the ovary as significant effect of pesticide. The decreased estradiol level causes decreased production of vitellogenin as well as hampers the development of oocytes in ovary of Cyprinus carpio communis which was also evident in the experiments.

Key words: Cholesterol, Estradiol, Pesticide, Toxicity, Ovary

Introduction

The organophosphorus pesticide like monocrotophos with multiple beneficiary qualities has attracted farmers to use these compounds in pest control on crops like paddy, cotton, citrus fruits and vegetables. But these compounds are found to be highly toxic to fishes (Bhatia *et al.*, 2002).

The pesticides have hazardous effects on the non-target aquatic species such as fishes, which are of great significance to man. At primary level these chemicals get accumulated in planktons which forms the food of fishes and thus are ultimately deposited in the soft and hard part of the fishes. Their ultimate effect is change in growth rate, drastic fall in reproductive ability and fecundity, besides several morphometric alterations. Consequently, some fish species have disappeared from these polluted aquatic habitats (Tandon *et al.*, 1980). Fishes have no evolutionary adaptation to these recently synthesized, xenobiotic material (Ryder, 1988).

Measurement of contaminants in fish has concentrated on muscle tissue since the aim has generally been to protect the health of consumer rather than that of fish. Little information is available on the effects of the pollutants on the developmental stages of fish egg, which develop inside the body of the fish. These changes may be responsible for the low fecundity, abnormal development of the eggs responsible for either no fertilization or poor fertilization

of the eggs. In both the cases, the ultimate result is the formation of either deformed embryos, which will die after some time and low auto stocking of fish stocks in the nature.

Fish are ideal sentinels and test organisms to assay various stress factors and toxic exposures as fish has the tendency to bioaccumulate various contaminants and play a significant role in food web biomagnification. These endpoints exposures are important because they integrate endogenous and exogenous factors that can link biochemical and physiological processes and can provide insights into individual and community level effects of environmental contamination.

The monocrotophos induced toxicity has been studied to access the biochemical and hormonal disruption caused in the fish tissues. Monocrotophos pose serious risk to biochemical parameters and enzyme activities in fishes. The histological and ultra-structural alterations in oocytes detected in the present study are the results of the altered biochemical processes in the fishes. The altered biochemical parameters have further led to endocrine disruption in the fish. Endocrine system is a control system of the body which responds to the external threats and evoke a stress response .These stimuli cause brain to release gonadotrophin releasing hormone which in turn stimulate pituitary to produce gonadotrophins (GtH-I and GtH-II). GtH-I stimulate the thecal layer of the ovary to convert cholesterol to testosterone which is aromatized to 17β estradiol by the granulosa layer of the ovary. This hormone stimulates the liver to synthesize yolk precursor protein, vitellogenin. Vitellogenin is transported to ovaries and incorporated in developing oocytes as yolk.

Material and Methods

The live sexually mature female *Cyprinus carpio communis* having the total fish length of 15 cm (±2 SD) and weight of 171 gm (±7.9 SD) were brought to the laboratory from Government Fish Seed Farm at Bagrhiani Phagon Majra village on Sirhind road, District Fatehgarh Sahib (Punjab). The fish were acclimatized to laboratory conditions in dechlorinated tap water for 15 days in a glass aquarium measuring 4’9” × 1’ 5” × 1’0.5” which was fitted with filters and aerators and were fed floating type food. The LC_{50} of technical grade of monocrotophos having cyclohexane as solvent to *Cyprinus carpio* showed LC_{50} as 0.37 ppm. The sublethal concentration 0.052 ppm, 0.074 ppm and 0.123 ppm were prepared in dechlorinated water (APHA, 1998) for toxicity testing.

The quantitative estimation of Cholesterol in the treated and control ovaries and liver of fish was carried out by procedure given by Zlatkis *et al*., 1953. The competitive immunoenzymatic colorimetric method for quantitative determination of 17β Estradiol concentration in plasma was used at normal and sublethal concentrations.

Results and Discussion

The LC_{50} of monocrotophos to *Cyprinus carpio* showed LC_{50} as 0.37 ppm. The *Cyprinus carpio communis* treated with monocrotophos at three sublethal concentrations (0.052 ppm, 0.074 ppm and 0.123 ppm) was sacrificed on 30^{th}, 45^{th} and 60^{th} day and analyzed for cholesterol level in ovary and liver of the fish. This was further corelated to the estradiol level in the blood plasma of the fish.

ISSN 2515-8260 Volume 07, Issue 09, 2020

Effect of administration of monocrotophos on Cholesterol estimation (mg/g weight of tissue) in ovary and liver of *Cyprinus carpio communis*.

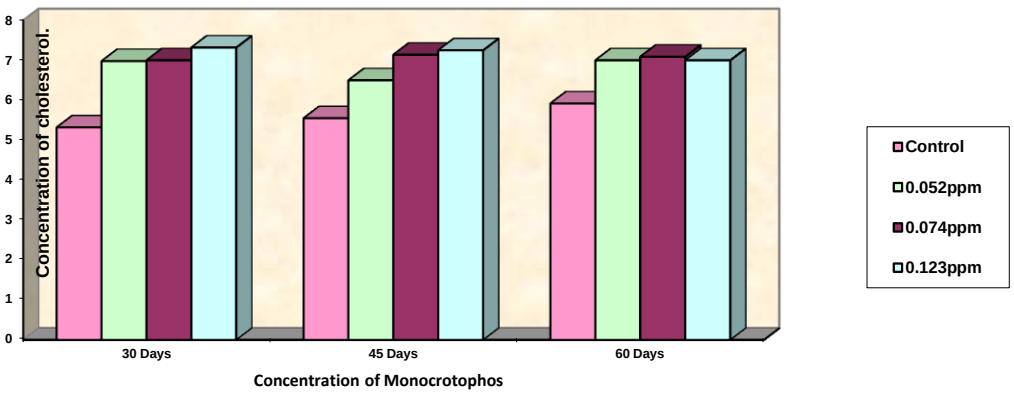
The cholesterol level of the ovary in response to monocrotophos treatment has been found to the higher than those of their respective control as shown in the table below. The increase in ovary has been observed to be by 31%, 32% and 38% w.r.t control on 30th day 17%, 29%, and 31% w.r.t control on 45th day and 18%, 20% and 18% w.r.t control on 60th day of treatment at 0.052 ppm, 0.074 ppm and 0.123 ppm of monocrotrophos. The cholesterol in the liver increased 38%, 20%and 18% w.r.t control on 30th day, 35%, 20%and 15% w.r.t control on 45th day and 37%, 20%and 16% w.r.t on 60th day of monocrotophos exposure.

		30 days				45 days				60 days		
Organ	**Control**	**0.052**	**0.074**	**0.123**	**Control**	**0.052**	**0.074**	**0.123**	**Control**	**0.052**	**0.074**	**0.123**
Ovary	5.32± 4.02	6.98 ± 7.09 **x**	7.00 ± 8.40 **x**	7.32 ± 8.04 **x**	5.55± 5.13	6.50 ± 4.36 **x**	7.14 ± 5.42 **x**	7.25 ± 3.69 **x**	5.92± 4.04	7.00 ± 5.04 **x**	7.09 ± 3.92 **x**	7.00 ± 4.08 **x**
Liver	2.52± 3.01	3.48 ± 3.04 **x**	3.02 ± 3.15 **x**	2.98 ± 3.04 **x**	2.60± 3.11	3.52 ± 2.96 **x**	3.12 ± 2.78 **x**	3.00 ± 2.64 **x**	2.59± 2.96	3.54 ± 3.12 **x**	3.10 ± 3.04 **x**	3.00 ± 2.97 **x**

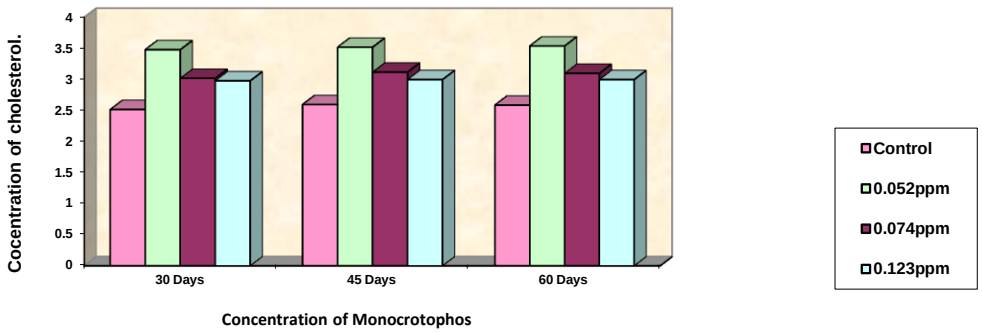
The results are expressed as mean ± S.D for n=3

x Significant w.r.t control ($p < 0.05$)

≠ Non Significant



ISSN 2515-8260 Volume 07, Issue 09, 2020



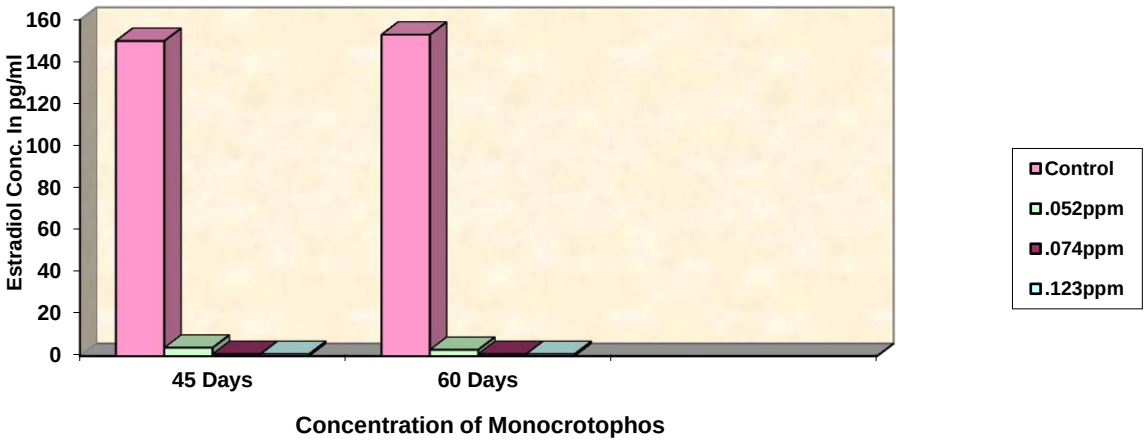
Variation in the cholesterol (mg/g weight of tissue) of ovary and Liver of *Cyprinus carpio communis* upon exposure to sub lethal doses of monocrotophos is clearly depicted in the graph. The elevated levels of cholesterol in the ovary apparently reflect the reduced rate of its utilization for steroid synthesis by the ovary as significant effect of pesticide. The increase in cholesterol might be due to its accumulation in less active ovary. The elevation of the tissue cholesterol and triglycerides may be attributed to enhanced cholesterol and triglycerides synthesis or reduced cholesterol and triglyceride catabolism (Fatma and Nahed, 2008). The similar type of results on cholesterol level has been earlier reported by Singh and Singh (1980) in *Heteropneustes fosillis* on treatment with cythion and hexadrin; Jaroli and Sharma (2005) in *Channa punctatus* on treatment with chlorpyrifos; Desai *et al*. (2002) in *Channa punctatus* by nickel. Singh and Singh (1978) reported a significant decrease in total gonadotrophin output with a resultant suppression of ovarian activity in *Heteropneustes fossilis* after the fish has been exposed to pesticides at the different concentrations. Kapur *et al.* (1978) have observed reduced 3β HSD acitivity in the gonads of *Cyprinus carpio* in response to fenitrothion treatment at sublethal doses.

Most of nutrients required for the development of oocytes in ovary of fish are synthesized in liver and then transported to ovary. So, any alteration in the enzyme activity and other nutrients in ovary because of pesticide toxicity are expected to initially originate from liver of the fish. It has been shown by many workers (Bushan *et al*., 2002) that insecticides mainly affect liver of fishes. This is because of its relatively slow blood flow as compared to cardiac output as well as much closer association of hepatocytes to biliary system that is found in mammals (Hinton and Lauren, 1990). Due to these reasons the liver is taken as target organ for subjecting to biochemical analysis as it is directly or indirectly linked to biochemical processes occurring in ovary of the fish.

EFFECT OF MONOCROTOPHOS ON PLASMA ESTRADIOL CONTENT OF COMMON CARP

The *Cyprinus carpio communis* treated with monocrotophos was sacrificed on 45th and 60th day at three concentrations (0.052 ppm, 0.074 ppm and 0.123 ppm) to detect the estradiol level in blood plasma by RIA. There was drastic fall in its level in treated fish as compared to the control fish as shown in the graph.

ISSN 2515-8260 Volume 07, Issue 09, 2020



The control fish has 153 pg /ml of estradiol whereas treated fish showed 3 pg/ml or even less than 1 pg/ml of estradiol. This can be due to the impairment in nerve conduction by monocrotophos as the information from brain is required to synthesize the aromatize enzyme in ovary that converts the testosterone to estradiol (Khan and Law, 2005).

The main factor causing the increase in ovarian weight during the reproductive development of fish is the deposition of yolk into the developing eggs. Abnormal development of ovary can be caused by lack of stimulation by pituitary hormones, failure of steroid synthesis or direct cellular damage. The most observed effect in the ovary is a decrease in numbers of large yolky eggs together with increased number of immature oocytes, suggesting a primary effect on pituitary function. Similar types of observations have been reported by other workers resulting in lowering of estradiol levels in blood serum. Some of them are lowered estradiol level by diazinon in *Lepomis macrochirus* (Lal, 2007); TCDD in *Danio rerio* (Heiden *et al*., 2005); cypermethrin in *Heteropneustes fossilis* (Singh and Singh, 2008); aquatic herbicides in rainbow trout (Xie *et al*., 2005); chemical contaminants in fishes in Colorado river (Susan *et al*., 2001); various pesticides in Indian fishes (Lal, 2007).

Inhibition of ovarian steroid synthesis has been demonstrated for the heavy metals cadmium and lead and hormone levels were also depressed in plasma of fish exposed to the effluent of pulp mills or taken from great Lakes and other areas rich in PCBS and PAHS (Susan *et al*., 2001). Male and female gonads differ in that only the ovary has an active aromatize enzyme that converts testosterone to estradiol, Inhibition of this ovarian aromatize activity in female fish will decrease estrogen synthesis and have consequential effects on the ability of the liver to synthesize yolk proteins, leading to retarded growth of the oocytes (Khan and Law, 2005). Such aromatase inhibiting activity has been demonstrated for imidazole fungicides (Monod *et al*., 1993) and tributylin (TBT) which was widely used in antifouling paints in boats (Fenske, 2005). These compounds may also affect steroid feedback to the pituitary in both sexes since this is dependent upon aromatize activity (Scholz and Kluver, 2009).

Hence, it can be concluded that the disturbance in biochemical parameters and hormones might be responsible for observed atresia of follicles as growth of follicle is principally

dependent on level of gonadotrophin. There is strong evidence that pesticide effects the functioning of steroid enzyme system in gonads of *Cyprinus carpio* (Kapur *et al*., 1978).
The present study revealed direct relationship between concentrations of monocrotophos, elevated cholesterol levels and depressed estradiol concentrations. The decreased estradiol level causes decreased production of vitellogenin as well as hampers the development of oocytes in ovary of *Cyprinus carpio communis.* Vitellogenesis and oocyte maturation is largely dependent on ovarian steroid hormones which need cholesterol for their synthesis and are controlled by gonadotrophins secreted from pituitary gland. Testosterone in female fish represents a substrate for estradiol -17β production (Kagawa *et al*., 1982) and then maintain oocytes. Once the oocytes are fully grown, final oocyte maturation occurs and is mediated by maturation inducing hormones (C_{21} steroids) produced by ovarian follicle prior to ovulation. During oocyte growth, estradiol – 17β, secreted by ovarian follicle act on liver to produce vitellogenin. This is produced by hepatocytes, released into bloodstream, sequestered by developing oocytes and cleaved to lipovitellin and phosphovitin (Pereira et al., 2005).
Hence, the check must be applied on the optimum usage of the pesticide in the agricultural fields especially in the vicinity of water bodies.

References

1. Bhatia, N.P., Sandhu, G.S. and Johal, M.S. 2002. Endosulfan induced changes in blood chemistry of *Heteropneustes fossilis. Poll. Res*., **21(3):** 323-327.
2. Tandon, K.K., Mohindru, A. and Johal, M.S. 1980. A note on the fisheries of Budha Nalla. *Pb. Fish. Bull*., **4(2):** 45-47.
3. Ryder, R.A. 1988. Effects of contaminants loadings on fisheries yield from large lakes. pp. 169-173. *In*: Toxic Contamination in Large Lake. Lewis Publishers, Michigan, USA.
4. APHA. 1998. Calculating analyzing and reporting results in toxicity tests. pp-820-824. *In*: Standard Methods for the Examination of Water and Wastewater. (eds. S.C. Lenore., E.G. Arnold and D.E. Andrew). American Public Health Association, Washington.
5. Fatma, A.S.M. and Nahed, N.S. 2008. Environmental pollution induced biochemical changes in tissues to *Tilapia zillii, Solea vulgaris and Mugil capito* from lake Quran, Egypt. *Glob. Veter*., **2(6):** 327-336.
6. Zlatkis, A., Zak, B., and Boyle, A.J. 1953. J. Lab, clin.Med.,**41:**486.
7. Singh, H. and Singh, T.P. 1980. Effect of two pesticides on lipid and cholesterol contents of ovary, liver and blood in *Hetropneustes fossilis*. *Environ. Poll*., **23:** 9-17.
8. Jaroli, D.P. and Sharma, B.L. 2005. Effects of organophosphate insecticide on the organic constituents in liver of *Channa punctatus. Asian. J. Exp. Sci*., **19(1):** 121-129.
9. Desai, H., Nanda, B. and Panigrahi, J. 2002. Toxicological effects on some biochemical parameters of freshwater fish, *Channa punctatuzs* under stress of nickel. *J. Enviorn. Biol*., **23:** 275-277.

10. Kapur, K., Kamaldeep, K. and Toor, H.S. 1978. The effect of fenitrothion on reproduction of *Cyprinus carpio*: A biochemical study. *Bull. Environ. Contam. Toxicol*., **20:** 438-42.
11. Singh, H. and Singh, T.P. 1978a. Gonadotrophic concentration and ovarian activity in response to pesticides in *Heteropneutes fossilis*. *All india Symp.Comp. Endocrinol. Bhubhaneshavar*., **3:**40-41.
12. Bushan, P.B., Singh, M.K. and Rani, M. 2002. Dimethoate and monocil toxicity on concentration of protein and amino acid in the serum and liver of *Channa punctatus. Nat. Environ. Pollut. Tech.,* **1:** 147-150.
13. Hinton, D.E., and Lauren, D.J.1999. Integrative histopathological approaches to the detection of environmental stressors on fishes. *Amer. Fish. Soc.Symp*., **8:**51-65.
14. Khan, M.Z. and Law, F.C.P. 2005. Adverse effects of pesticides and related chemicals on enzyme and hormonal systems of fish, amphibians, and reptiles. *Proc. Pak. Acad. Sci.*, **42(4):** 315-323.
15. Lal, B. 2007. Pesticide induced reproductive dysfunction in Indian fishes. f*ish.Physio.Biochem*., **33(4):** 455-462.
16. Heiden, T.K., Carvan, M.J. and Hutz, R.J. 2006. Inhibition of follicular development, vitellogenesis and serum 17β-estradiol concentrations in zebrafish following chronic, sublethal dietary exposure to 2,3,7,8-tetrachlordibenzo-P-dioxin. *Toxicol. Sci.*, **90(2):** 490-499.
17. Singh, P.B. and Singh, V. 2008a. Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17β and 11-ketotestoster one, and sperm motility in *Heteropneustes fossilis* (Bloch). *Chemos*., **72(3):** 422-431.
18. Lal, B. 2007. Pesticide induced reproductive dysfunction in Indian fishes. *Fish.Physio.Biochem*., **33(4):** 455-462.
19. Susan, L., Schantz, S.L., Gasior, D.M., Polverejan, E., McCaffrey, R.J., Sweeney, A.M., Humphrey, H.E.B. and Gardiner, J.C. 2001. Impairments of memory and learning in older adults exposed to polychlorinated biphenyls via consumption of Great Lakes. Fish. *Environ. Hlth. Persp*., **109:** 605-611.
20. Xie, L., Thrippleton, K., Irwin, M.A., Siemering, G.S., Mekebri, A., Crane, D., Berry, K. and Schlenk, D. 2005. Evaluation of estrogenic activities of aquatic herbicides and surfactants using rainbow trout vitellogenin assay. *Toxicol. Sci*., **87(2):** 391-398.
21. Scholz, S., and Kluver, N. 2009. Effects of endocrine disrupters on sexual and gonadal development in fish. *Sex.Dev*., **3:** 136-151.
22. Fenske, M., Aerle, R.V., Brock, S., Tyler, C.R. and Segnes, H. 2002. Development and validation of homologous zebrafish vitellogenin by Enzyme Iinked Immunosorbent Assay and its application for studies on estrogenic chemicals. *Comp. Biochem. Physiol*., **129:** 217-323.
23. Monod, G., Mones, A. and Fostier, A. 1993. Inhibition of ovarian microsomal aromatase and follicular estradiol secretion by imidazole fungicides in rainbow trout. *Mar. Environ. Res*., **35:** 147-152.

ISSN 2515-8260 Volume 07, Issue 09, 2020

24. Kapur, K., Kamaldeep, K. and Toor, H.S. 1978. The effect of fenitrothion on reproduction of *Cyprinus carpio*: A biochemical study. *Bull. Environ. Contam. Toxicol*., **20:** 438-42.
25. Pereira, J.J., Allen, R.M., Kuropat, C., Luedke, D. and Seenefelder, G. 2005. Effects of cadmium accumulation on serum vitellogenin levels and hepatosomatic and gonadosomatic indices of winter flounder (*Pleuronectes americanus*). *Arch. Environ. Contam. Toxicol*., **24(4):**427-431.
26. Kagawa, H. Young, G., Adachi, S. and Nagahama, Y. 1982. Estradiol 17β production in amago salmon *Oncorhynchus rhodurus* ovarian follicles: Role of the thecal and granulosa cells. *Gen. Comp. Endocrinol*., **44:** 440-448.