Pakistan J. Zool., vol. 37(1), pp. 61-67, 2005.

Effect of Endosulfan on Histomorphology of Fresh Water Cyprinid Fish, *Cyprinion watsoni*

OMMIA KALSOOM, SAMINA JALALI AND S.A. SHAMI
Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

Abstract.- The present study is aimed at assessing effects of endosulfan, an organochlorine insecticide, on reproductive and developmental parameters of male fresh water Cyprinid fish, *Cyprinion watsoni*. The fish was exposed to 0.75 and 1 ppb endosulfan on alternate days for 30 days during early spawning season (March). The body length and body weight of 0.75ppb treated group increased significantly ($p<0.05$) compared to control but with a dose of 1 ppb, the body weight decreased and the body length increased significantly ($p<0.05$). The condition factor (K) and GSI of treated groups showed no change. The testicular weight, increased significantly ($p<0.05$) with 0.75 ppb, whereas a dose of 1 ppb did not cause any significant change in testicular weight and breadth. The testicular length, however, showed an increase. The mean diameter of spermatogonia decreased significantly in 1 ppb group. Histomorphological studies showed loosely arranged lobules, irregular nuclear and cell membrane of spermatogonia, clumping of spermatocytes and spermatids and reduction in sperm count.

Key words: Organochlorine, endosulfan, testes, GSI, spermatogenesis.

INTRODUCTION

Organochlorine (OC) insecticides have been found to impair both reproduction and development in fish (Olsson *et al*., 1999). Several investigators (Westernhagen *et al*., 1981; Hansen *et al*., 1985; Cross and Hose, 1988; Spice and Rice, 1988; Mattison and Thomford, 1989; Collier, 1992) have reported adverse reproductive effects in fish population inhabiting OC contaminated environment. Endosulfan (6, 7, 8, 9, 10, 10 hexachloro-1, 5,5a, 6,9,9a-hexahydro-6, 9-methano-2, 4,3-benzo-dioxanthiepin-3-oxide) is an OC insecticide, which is less persistent in the environment but more toxic to the fish (Pie *et al*., 1981; Matthiessen *et al*., 1982; Nowak and Ahmed, 1989; Nowak, 1990; Nowak and Julli, 1991). It has been reported to affect reproductive success in animals (Wasternhagen *et al*., 1987; Addison, 1989; Elliot *et al*., 1988; Casillas *et al*., 1991). Endosulfan treatment resulted in decreased spermatozoa counts in the cauda epididymis and reduced intratesticular spermatid counts and sperm deformity associated with an elevation in the activities of specific testicular marker enzymes which was seen in the adult male rats (Sinha *et al*., 1995). The plasma testosterone and testicular testosterone contents of the male rats have also been reported to be reduced after endosulfan treatment (Singh and Pandey, 1990).

The present report describes the effect of endosulfan on male reproductive system of *Cyprinion watsoni*, at morphological and histological levels.

MATERIALS AND METHODS

Live specimens of *Cyprinion watsoni* were collected with nets from Ramly stream in hilly areas near Quaid-i-Azam University Islamabad during early spawning period (March) (Shaikh and Jalali, 1986). The fish were acclimatized according to the laboratory conditions for at least one week. Endosulfan was administered to fish through water of aquaria. Endosulfan dose was administered on alternate days and the water was also renewed on alternate days. The size of the aquarium was 75x28x48 cm with a capacity of 75 liters of water. The fish were divided into three groups (30 in each). One group was exposed to 0.75 ppb endosulfan, the other to 1ppb endosulfan and the third group was maintained as control for 30 days.

Histological procedure

The testes of fish were dissected out, weighed and length and breadth (cm) was measured. The testes were immersed in fixative sera (Rodemer *et al*., 1986) and processed for histological sections.

0030-9923/2005/0001-0061 $ 4.00/0

Table I.- Effect of endosulfan on body weight, body length, total testicular weight, faulton's condition factor (K) and gonadosomatic index (GSI) of *Cyprinion watsoni*.

Parameters	Control	0.75 ppb	1 ppb
Body weight (g)	4.33±0.44	7.27±1.11	5.56±0.40
Body length (cm)	6.01±0.19	7.8±0.39^{a**c*}	6.68±0.22b*
Condition factor (K)	0.02±0.0007	0.02±0.0007^{c*}	0.02±0.00029
Gonadosomatic index	0.66±0.12	1.30±0.34	0.80±0.08
Testicular length (cm)			
Right	1.23±0.10	2.34±0.19^{a***c***}	1.58±0.14
Left	1.15±0.13	2.57±0.15^{a***c***}	1.65±0.104^{b**}
Testicular breadth (cm)			
Right	0.16±0.02	0.29±0.04^{a**}	0.22±0.02
Left	0.16±0.02	0.24±0.02^{a**c*}	0.18±0.02
Testicular weight (mg)	33.17±7.87	117.78±23.7a^{**c*}	53.09±9.72

Values (Mean±S.E), student "t" test, a, b=treated groups Vs high dose treatment, c = low dose Vs high dose treatment, P<0.05*, P<0.01**

The sections (6µm thick) were stained in hematoxylin and eosin. The slides were prepared for histomorphological studies. Ten slides from each were used for microscopic study.

Length-weight relationship

Length-weight relationship was calculated by the following formula:

$$K = \text{body wt (g)}/(\text{standard body length})^3 \text{ cm}$$

where K is Faulton's condition factor. Gonadosomatic index was calculated by Mein's formula (1927) as given below

$$GSI = \text{Total testicular wt (g)}/\text{body wt (g)} \times 100$$

RESULTS

Body weight and length

Mean body weight and length of control and endosulfan treated (0.75 ppb and 1 ppb) groups are given in Table I. The body weight and length of 0.75 ppb endosulfan treated group showed a significant (P<0.05) increase compared to control. The higher dose of endosulfan (1 ppb) showed negative effect on mean body weight and body length. The body length decreased significantly (P<0.05) with high dose compared to the low dose of endosulfan.

The condition factor and gonadosomatic index of both treated groups showed non-significant (P>0.05) difference compared to control (Table I).

Testicular weight and size

Testicular weight, length and breadth increased significantly (P<0.05) compared to control in low dose (0.75 ppb) treated group (Table I). In high dose (1 ppb) treated group there was decrease in testicular weight, length and breadth except for significant increase in the left testicular length.

Histomorphology

Control

Testis was covered by tunica albuginca on its outer surface (Fig. 1d). Testicular section showed many spermatogenic lobules (Fig. 1a, 2a, 2d). The compactly arranged cysts were present in the lobules. The cysts containing spermatogonia were visible in the peripheral area. Each spermatogonium was large and spherical compared to other cells and possessed large lightly stained spherical nucleus with distinct nucleolus (Fig. 1d, 2a, 2d). The chromatin was attached to the inner side of the nuclear membrane (Fig. 1d, 2d). Other cysts contained primary and secondary spermatocytes. Primary spermatocytes were smaller than secondary spermatcytes; their nuclei were darkly stained (Fig. 2a). Round spermatids were also observed in some of the cysts (Fig. 1a, 2a, 2d). The interstitium contained Leydig cells, connective tissue, lymphatic vessels, blood vessels and capillaries (Fig. 2d).

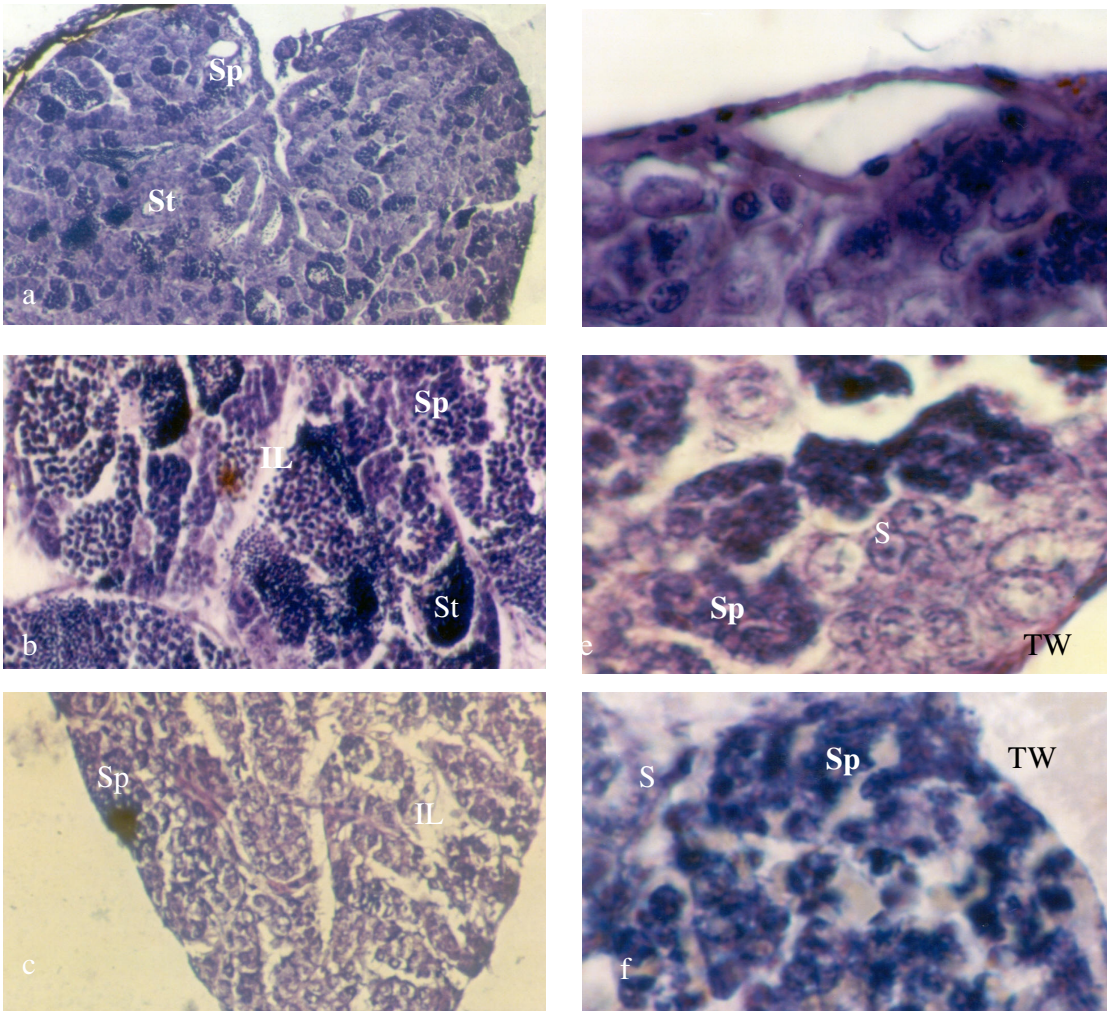

Fig. 1. *Cyprinion watsoni* testis showing (a) Control group with compact lobules of spermatogonia (S), spermatocytes (Sp) and spermatids (St) X.220.87 (b) Testis treated with 0.75ppb endosulfan has increased interlobular (IL) areas, clumping of spermatocytes and spermatids. X 441.75. (c) Testis treated with high dose endosulfan showing increased interlobular areas and clumping of spermatocytes. X 220.87. (d) Normal morphology of testicular wall (TW) and spermatgonia. (e, f) Section of testis of *Cyprinion watsoni* treated with 0.75ppb and 1ppb endosulfan showing thinning of testicular wall, disintegration of spermatogonia and clumping of spermatocytes. X 2208.77. (H.E).

Treatment group 1 (0.75 ppb)

The lobules were loosely arranged (Fig. 1b, 2b, 2e). The cyst containing spermatogonia were fewer and loosely arranged. The cell and nuclear membranes were irregular in shape and chromatin material was interspersed in the nucleus (Fig. 1e, 2b, 2e). The primary and secondary spermatocytes showed clumping (Fig. 1b, 2e, 2b) and their cell membrane and nuclear membranes were also not intact. The cysts containing spermatids increased in number and showed clumping (Fig. 1b, 2b, 2e).

Treatment group II (1 ppb)

The testicular wall showed decrease in thickness (Fig. 1f). Spermatogonia containing cysts were fewer and loosely arranged compared to control and 0.75 ppb group. They had irregular cell membrane and nuclear membrane (Fig. 2f). Number

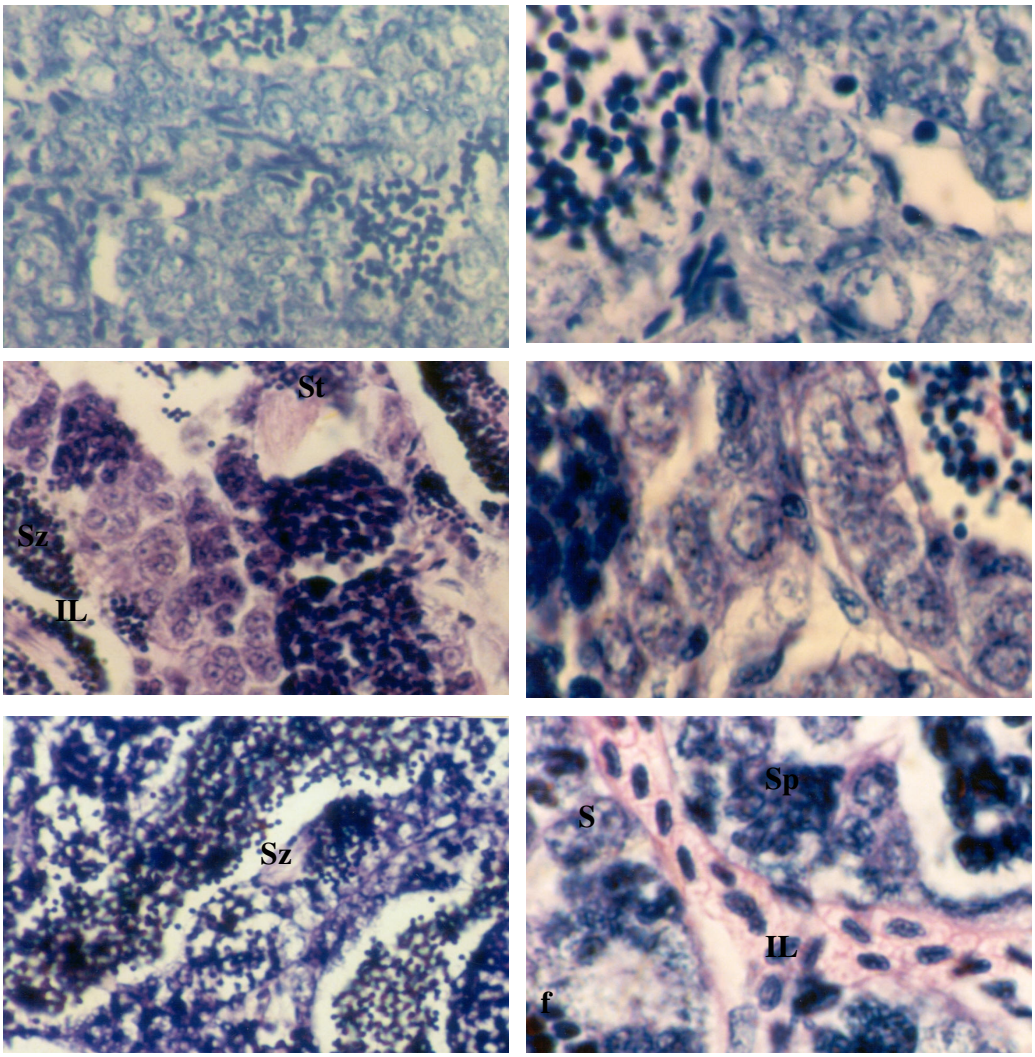

Fig. 2. *Cyprinion watsoni* (a) showing normal and compact lobules of spermatogonia (S), spermatocytes (Sp) and spermatids (St). (b) Testis treated with low dose endosulfan has increased interlobular areas (IL), some spermatozoa (Sz), disintegration of spermatogonia, clumping of primary spermatocytes (P.Sp), secondary spermatocytes (S.Sp) and spermatids. (c) Testis treated with high dose endosulfan showing increased interlobular areas, some spermatozoa, clumping of secondary spermatocytes and spermatids. X.883.5 (d) Control group with compact lobules of normal spermatogonia and spermatids. (e) Tesis treated with 0.75ppb endosulfan has increased interlobular areas, disintegration of spermatogonia, clumping of spermatocytes and spermatids. (f) Fish testis treated with high dose endosulfan showing increased interlobular areas, disintegration of spermatogonia and spermatocytes and clumping of spermatocytes. X 2208.77. (H.E).

of primary spermatocytes, secondary spermatocytes and spermatids increased due to arrest of spermatogenic cycle and showed clumping like that in 0.75ppb group (Fig. 1f, 2c, 4f).

DISCUSSION

The current study was conducted to evaluate the effect of endosulfan on testicular histology of fish *Cyprinion watsoni* because no information is available on the adverse effect of endosulfan on the testicular histology of fish *Cyprinion watsoni*. The fish size is an important determinant of reproductive success in fish (Collier, 1992). The present study showed that the mean body size (length) of the fish treated with 0.75 ppb and 1 ppb endosulfan

increased significantly ($P<0.05$) compared to control. Mean body weight showed significant ($P<0.05$) increase in fish treated with 0.75 ppb endosulfan compared to control. However, strangely enough fish treated with a high dose (1 ppb) indicated non-significant ($P>0.05$) increase in body weight.

Condition factor is a generalized indicator of the overall health or "plumpness" of a fish and can reflect the integrated effect of nutritional status and metabolic stress (Adams and Mclean, 1985). In this study there was no significant ($P>0.05$) difference in the condition factor (K) with low as well as high dose. The simplest measure of gonadal dysfunction is its gonadosomatic index (GSI) in control and treated fish (Kime, 1995). In the present study no significant ($P>0.05$) difference was noticed in the control and endosulfan treated groups. Johnson *et al*. (1998) observed a decrease in the GSI of English sole (*Parophrys vetulus*) from Dawamish waterway contaminated with aromatic hydrocarbons and PCBs than in the fish from Sinclair Inlet.

Mean testicular weight of the fish treated with 0.75 ppb endosulfan showed a significant increase ($p<0.05$) compared to control. However, there was no change in testicular weight compared to control when it was treated with 1ppb endosulfan. Mean testicular length and breadth of fish testis treated with 0.75ppb endosulfan showed a significant ($p<0.05$) increase compared to the control. However, treatment with high dose (1 ppb) showed significant ($p<0.05$) increase in the length and breadth of left testis but this increase was not seen in the right testis. In contrast to this study, Sinha *et al*. (2001), while studying effect of endosulfan on spermatogenesis in rats, investigated that testis showed significant decrease in weight in treated groups. Singh and Pandey (1990) observed that endosulfan did not alter the body weight and testicular weights of the treated rats. Aromatic hydrocarbons and PCBs have been associated with reproductive failure in fish in control laboratory exposures (Roweet *et al*., 1983; Chen and Soustegart, 1984). Deleterious effects of PCBs on various aspects of male reproduction in rats have been found (Sager, 1983; Sager *et al*., 1987, 1991). The effects include hypothyroidism in treated animals (Gray *et al*., 1993; Ness *et al*., 1993; Li *et al*., 1994), and also increase in adult testis size (Cooke and Meisami, 1991; Cooke *et al.*, 1991).

Treatment of 0.75 ppb endosulfan caused thinning of testicular walls compared to control. The cysts and lobules were loosely arranged compared to control. Irregular cell membrane and nuclear membrane was observed in spermatogonia. Histological examination also revealed disintegration of some of the spermatogenic cells within the cysts of some lobules. Similar results (disintegration of spermatogenic cells) were observed by Sangalang *et al*. (1981) while studying the effect of 2.5 µg/g PCB diet on *Gadus morhua*. In the present study primary and secondary, spermatocytes were more affected. Their number increased and showed clumping and irregular cell membrane. PCB (Aroclor, 1242) caused disintegration of spermatogonia and spermatocytes and clumping of spermatocytes in the testis of fish *C. watsoni* (Ishaq, 2001). Clumping of early spermatocytes was also observed by Sangalang *et al*. (1981). High dose treatment had increased number of spermatids and clumping of spermatocytes. The sperm count reduced which may be due to arrest of spermatogenic cycle. Sinha *et al*. (1995) suggested that endosulfan caused impairment in testicular functions, thereby influencing intratesticular spermatid count and causing low sperm production and sperm deformity.

Treatment with 1 ppb endosulfan revealed that lobules changed their organization and the cysts and lobules are loosely arranged *i.e.* the interlobular space increased compared to control. The cysts containing spermatogonia decreased and they had irregular cell and nuclear membrane. The number of primary and secondary spermatocytes containing cysts increased in number. Similar results were given by Sangalang *et al*. (1981) while studying the effects of 5 µg/g PCB diet on *Gadus morhua*. Similar results were obtained by Ishaq (2001) while studying the effect of 10 mg Arochlor 1242 (PCB) on the testis of *Cyprinion watsoni*. High dose (1 ppb) increased the number of cysts containing spermatids and they also showed clumping like spermatocytes. Unlike the present study Sinha *et al*. (1995, 2001) observed reduction in the spermatid and sperm count in testis while studying the effect of endosulfan on testis of rats but this shows

increase in seprmatid count. Dalsenter *et al*. (1999) also reported decrease in sperm production with high dose of endosulfan (3.0 mg/kg, body weight) in rats.

It is concluded from this study that low dose (.75 ppb) treatment of endosulfan had positive effect on increase in testicular length, breadth and weight, while treatment with high dose (1 ppb) endosulfan had no significant effects on these parameters. This suggests that high dose of endosulfan (>.75 ppb) does not bring in major changes in morphology of the testes, but it does have more harmful effects on histology of the testes.

REFERENCES

ADAMS, S. M. AND MCLEAN, R.B., 1985. Estimation of large mouth bass. Micropterus salmonids lacepede, growth using the liver somatic index and physiological variables. *J. Fish Biol*., **26**: 111-126.

ADDISON, R.F., 1989. Organochlorines and marine mammals reproduction. *Can. J. Fish. Sci*., **46**: 360-368.

ASPLUND, L., ATHANSIANDOU, M., SJODIN, A., BERGMAN, A. AND BORJESON, H., 1999. Organohalogen substances in muscles, egg and blood from healthy Baltic Salmon (*Salmo salar*) and Baltic Salmon that produced offspring with the M74 syndrome. *Ambio*, **28**: 67-76.

CASILLAS, T.K., MISITANO, D., JOHNSON, L.L., RHODES, L.D., COLLIER, T.K., STEIN, J.E., McCAIN, B.B. AND VARANASI, U., 1991. Reproductive success of female English sole (*Parophrys ventulus*) from urban and non-urban area of Puget Sound, Washington. *Mar. environ. Res*., **31**: 99-122.

CHEN, T. AND SOUSTEGART, R. 1984. Development of a rapid, sensitive and quantitative test for the assessment of the effects of xenobiotics on reproduction in fish. *Mar. environ. Res*., **14**: 429-430.

CLEMENS, H. P. AND REED, C. A., 1967. Long term gonadal growth and maturation of gold fish (*Carassius auratus*) with pituitary injections. *Copeia*, **1967**: 465-466.

COLLIER, T. K., 1992. Field studies of reproductive success in English sole (*Perophrys vetulus*). Correlation with bioindicators of maternal contaminants exposure. *Sci. Total Environm*., **116**: 169-185.

COOKE, P.S. AND MEISAMI, E., 1991. Early hypothyroidism in rats increases adult testis and reproductive organ size but does not change testosterone levels. *Endocrinology*, **129**: 237-243.

COOKE, P. S., HESS, R. A., PORCELLI, J. AND MEISAMI, E., 1991. Increased sperm production in adult rats following transient neonatal hypothyroidism. *Endocrinology*, **129**: 244-248.

CROSS, J.N. AND HOSE, T.E., 1988. Evidence of impaired reproduction invite croaker (*Genyonemus lineatus*) from contaminated areas of Southern California. *Mar. environ. Res*., **24**: 185-188.

DALSENTER, P. R., DALLEGRAVE, E., MELLO, J. R. B., LANGELOH, A., OLIVIERA, R. T. AND FAQI, A. S., 1999. Reproductive effects of endosulfan on male offspring of rats exposed during pregnancy and lactation. *Human exp. Toxicol*., **18**: 583-589.

EKELUND, R., 1989. Bioaccumulation and biomagnification of hydrophobic persistent compounds as exemplified by hexachlorobenzene. In: *Chemicals in the aquatic environment. Advanced hazard assessment* (eds. L. Lander and S. Verlag,), Heidelberg, pp. 128-149.

ELLER, L.L., 1971. Histomorphological lesions in the cut-throat trout, *Salmo salmo clarki*, exposed the insecticide endrin. *Am. J. Path*., **64**: 321-336.

ELLIOT, J.E., NORDSTROM, R.J. AND KEITH, J.A., 1988. Organochlorine and egg shell thinning in Northern Gannets (*Sula bassanus*) from Estern Canada 1968-1984. *Environ. Pollut*., **81**: 102.

GRAY, L.E., JR., OSTBY, J., MARSALL, R. AND ANDREW, S.J., 1993. Reproductive and thyroid effects of low level polychlorinated biphenyl's (Aroclor 1254) exposure. *Fundam. appl. Toxicol*., **20**: 288-294.

HANSEN, P.D., VONWESTERNHAGEN, H. AND ROSENTHAL, H., 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. *Mar. environ. Res*., **15**: 59-76.

HERZBERG, A. M. 1986. Accumulation and toxicity of endosulfan in the common carp (*Cyprinus carpio*) and Saint Peter's fish (*Oreochromis aureus*). Israeli *J. Aquacult. Bamigdeh*, **38**: 99-107.

HOLM, G., NORRGREN, L., ANDERSSON, T. AND THUREN, A., 1993. Effect of exposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in three spinned stick leback, *Gasterosteus aculeatus. Aquat. Toxicol*., **27**: 35-50.

ISHAQ, M. 2001. *Reproductive toxicity of polychlorinated biphenyl (Aroclor 1242) in male* Cyprinion watsoni. M.Phil thesis, Department of Biological Sciences, Quaid-i-Azam University, Islamabad.

JOHNSON, L.L., MISITANO, D., SOL, S.Y., NELSON, G.M., FRENCH, B., YLITALO, G.M. AND HAM, T., 1998. Contaminant effects on ovarian development and spawning in Rock sole from pudget sound, Washington. *Trans. Am. Fish. Soc*., **127**: 375-392.

KIME, D. E., 1995. The effect of pollution on reproduction of fish. *R. F. Biol. Fish*., **5**: 52-96.

LI, M. H., ZHAO, Y. D. AND HANSEN, L.G. 1994. Multiple dose toxicokinetic influence on the estrogenicity of 2, 2′, 4,4′, 5,5′-hexachlorobiphenyles. *Bull. environ. Contam. Toxicol*., **53**: 583-590.

MATTISON, S.R. AND THOMFORM, P.J., 1989. The mechanism of action of reproductive toxicants. *Toxicol. Path*., **17**: 364-376.

MATTHIESSEN, P., FOX, P.J., DOUTHWAITE, R.J. AND WOOD, A.B., 1982. Accumulation of endosulfan residues in fish and their predators after aerial spraying for the control of tsetse fly in Botswana. *Pestic. Sci*., **3**:

39-48.

MEIN, V.A., 1927. Observations on the yearly variation of the ovaries of the perch (*Perca fluviatilis*). *Russk. Zool. Zh*., **7**: 4.

NESS, D. K., SCHANTZ. S. L., MOSHTAGHIAN, J. AND HANSEN, L. G. 1993. Effect of potential exposure to specific PCB congeners on thyroid hormone concentration and thyroid histology in the rat. *Toxicol. Lett*., **68**: 311-323.

NOWAK, B., 1990. Residues of Endosulfan in liver of wild cat fish from cotton growing area. *Environ. Monitor. Assess.*, **14**: 347-351.

NOWAK, B., 1991. Accumulation of endosulfan by cat fish after acute exposure to sublethal concentrations. *Verh. Internat. Verein. Limnol*., **24**: 2560-2562.

NOWAK, B. AND AHMAD, N., 1989. Residues in fish exposed to sublethal doses of endosulfan and fish collected from cotton growing area. *J. environ. Sci. Hlth*., **24**: 97-109.

NOWAK, B. AND JULLI, M., 1991. Residues of endosulfan in wild cat fish from cotton growing area in New South Wales, Australia. *Toxicol. environ. Chem*., **33**: 151-67.

NOWAK, B. AND SINDERAM, R. M. S., 1991. Toxicity and bioaccumulation of endosulfan to mosquito fish (*Gambusia affinis*). *Verh. Internat. Verein. Limnol*., **24**: 2327-2329.

OLSSON, P.E., WESTER LUND, L., THE, S.J., BILLSON, K., BERG, A.A., TYSKLIND, M., NILSON, J., ERIKSSON, L.O. AND HINTON, D.E., 1999. Effect of maternal exposure to estrogen and PCB on different life stages of Zebra fish (*Danio rerio*). *Ambio*, **28**: 100-106.

PANDEY, A.C., 1988. Impact of endosulfan on behaviour and dynamics of oocyte development in the teleostean fish, *Trichogaster fasciatus. Ecotoxicol. Environ. Saf*., **2**: 221-225.

PIC, F.E., DEBEER, P.R. AND VAN DYK, L.P., 1981. Organochlorine insecticide residues in birds and fish from the Transvaal, South Africa. *Chemosphere*, **10**: 1243-1251.

ROWEET, D. W., SPRAGUE, J. B. AND HEMMING, T. A., 1983. Sublethal effects of effluents from a petroleum refinery. *Aquat. Toxicol*., **2**: 149-159.

RODEMER, E.S., IHMER, A. AND WARTENBERG, H., 1986. Gonadal development of chick embryo following microsurgically caused agencies of mesonephros and using interspecific quail-chick chimaeras. *J. Embryol. exp. Morph*., **98**: 269-285.

SAGER, D. B., 1983. Effect of postnatal exposure to polychlorinated biphenyls on adult male reproductive function. *Environ. Res.*, **31**: 76-94.

SAGER, D. B., GIRARD, D. AND NELSON, D., 1991. Early postnatal exposure to PCB sperm function in rats. *Environ. Toxicol. Chem.*, **10**: 737-746.

SAGER, D. B., SHEH. SCHROEDER. W. AND GIRARD. D., 1987. Effect of early postnatal exposure to polychlorinated biphenyls (PCBs) on fertility in male rats. *Bull. environ. Contam. Toxicol.*, **28**: 946-953.

SANGALANG, G. B., FREEMAN, H. C. AND CROWELl, R., 1981. Testicular abnormalities in Cod (*Gadus morrhu*) fed Aroclor 1254. *Arch. environ. Contam. Toxicol*., **10**: 617-626.

SCHOETTGER, R. A. 1970. *Toxicology of thiodan in several fish and aquatic invertebrates. Investigations in fish control, 35*. United States Department of the interior, fish and wildlife service, Bureau Sport, Fisheries and Wildlife, Washington, D. C., pp. 31.

SHAIKH, S. A. AND JALALI, S., 1986. Seasonal changes in the ovary of the Cyprinid fish, *Cyprinion watsoni. Pakistan J. Zool*., **23**: 19-25.

SINGH, S. K. AND PANDEY, R. S., 1990. Effect of subchronic endosulfan exposures on plasma gonadotrophins, testosterone, testicular testosterone and enzymes of androgen biosynthesis in rat. *Indian J. exp. Biol*., **25**: 953-956.

SINHA, N., ADHIKARI, N. AND SAXENA, D. K., 2001. Effect of endosulfan on the enzymes of polypathway in rat Sertoli germ cell coculture. *Bull. environ. Contam. Toxicol*., **67**: 821-827.

SINHA, N., NARAYAN, R., SHANKER, R. AND SAXENA, D. K., 1995. Endosulfan induced biochemical changes in testis of rats. *Vet. Human Toxicol*., **37**: 547-549.

SPICES, R.B. AND RICE, D.W., 1988. Effect of organic contaminants on the reproduction of starry flounder (*Platichthys stellatus*) in San Fronsisco Bay. II. Reproductive success of fish captured in San Fransisco Bay and spawned in the laboratory. *Mar. Biol*., **98**: 191-200.

WESTERNHAGEN, H.V., ROSENTHAL, H., DETHLEFSEN, V., ERNST, W., HARMS, U. AND HANSEN, P.D., 1981. Bioaccumulation substances and reproductive success in Baltic flounder (*Platichthys flesus*). *Aquat. Toxicol*., **1**: 85-99.

WESTERNHAGEN, H.V., SPERLING, K.R., JANSSEN, D., DETHLEFSEN, V., CAMAREN, P., KOCAN, R., LANDELT, M., FURSTENBERG, G. AND KRENLING, K., 1987. Anthropogenic contaminants and reproduction in marine fish. *Der. Biol. Anstalt. Helgoland*, **3**: 70.

(Received 3 May 2003, revised 4 April 2004)