J. Ecophysiol. Occup. Hlth. 11 (2011) 9-19

Organochlorines, organophosphate bioaccumulation and reproductive dysfunction in fish captured from polluted river Gomti during pre-monsoon

S. K. Nigam, Jyoti Singh[1], Ashwarya Luxmi Singh[2], V. K. Das[3] and P. B. Singh

Ganpat Sahai Postgraduate College, Sultanpur-228001 India, [1]T. D. College, Jaunpur-222 002 India, [3]KNIPSS, Sultanpur-228 002 India

Abstract: *The present investigation was performed to monitor the HCHs (HCH isomers), DDTs (DDT and its metabolites), chlorpyrifos, plasma levels of estradiol-17β (E2) and gonadosomatic index (GSI) between the sampling sites of the non-polluted ponds of Gujartal, Jaunpur (reference site) and the polluted river Gomti (Jaunpur), affecting the reproductive physiology of some edible female catfish during pre-monsoon season. The HCHs, DDTs and chlorpyrifos were measured by gas liquid chromatography (GLC) and hormone by Enzyme-Linked Immunosorbent Assay (ELISA). The results indicated that the presence of HCHs, DDTs and chlorpyrifos was very high in the catfish captured from polluted river as compared from the conspecific fish captured from reference site. The GSI and E2 declined in female catfish of polluted site when compared to same species from reference site. Results have also indicated that catfish contained high bioaccumulation of HCHs, DDTs and chlorpyrifos which was beyond permissible limit as compared to the female catfish of the reference site. In conclusion, the fishes of river Gomti were highly polluted when compared with the fish of reference site as was evident from high levels of tissue bioaccumulation of HCHs, DDTs, chlorpyrifos and decreased levels of plasma E2 inhibiting the reproductive physiology of these species at receptor level. However, the levels exceeded maximum residue limits (MRL) as recommended by Codex, hence it is suggested that the fish containing pesticide residues beyond permissible limit should be avoided for the food purposes.*

Key Words: Catfish, Estradiol-17β, HCHs, DDTs, Chlorpyrifos Bioaccumulation, Reproduction, Steroidogenesis, River pollution.

Introduction

Despite the ban on some persistent lipophilic organochlorines such as HCHs (HCH isomers), DDTs (DDT-1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane and its metabolites), and organophosphate (chlorpyrifos) in most of the industrialized nations like India, the occurrence of high concentrations of these compounds in ecosystems has prompted the need for the measurement of bioaccumulation in fishes of riverine systems. The use of organochlorines and organophosphates have been banned or restricted in most developed countries but are still being used in India to eradicate vector-borne diseases and for the enhancement of agricultural production (Dale *et al.,* 1965; Edward 1970; El Beit *et al.,* 1981; Paasivirta *et al.,* 1988; Ramesh *et al.,* 1989; Ahmad *et al.,* 1996). Some researchers have reviewed the tissue concentration of pesticides in fish (Kulshrestha *et al.,* 1995; Kime 1998). The data indicated that fish, birds, reptiles, mammals and other species inhabiting polluted environments with a number of known and unknown synthetic compounds (Senthilkumar *et al.,* 1999a b, 2001) suffer from reproductive problems (Colborn and Clemment, 1992; Colborn *et al.,* 1993; Singh and Singh, 2008a,b; Singh *et al*., 2008a,b,c; Han *et al.,* 2010; Barrett and Munkittrick., 2010;

Email: pratap_b_singh@rediffmail.com

Barrett *et al.,* 2010; Hanson *et al.,* 2011) and acts as endocrine disruptors (Ankley *et al.,* 2009; Olujimi *et al.,* 2010).

Tissue concentration of pesticides in wild captured fish have been reported by several workers (Letherland and Sonstegard, 1982; Von Westernhagen *et al.,* 1987; Hellou *et al.,* 1993; Kannan *et al.,* 1995; Sharma *et al.,* 1995; Singh *et al.,* 1997; Kumari *et al.,* 2001; Singh and Singh, 2008a,b; Singh *et al.,* 2008a,b,c). Several reports are available for the study of various pesticide residue analysis in various fish. Some of these reports give explanation about the anti-steroidogenic nature of these toxicants which also showed that these toxicants act as endocrine disruptors (Hilmy *et al.,* 1983a, b; Verma, 1991; Singh *et al.,* 1994; Kime and Singh 1996; Sahagun *et al.,* 1998; Singh and Canario, 2004; Harding *et al.,* 2006; Barse *et al.,* 2007; Lokman *et al.,* 2010; Pankhurst, 2010). Bioaccumulation of insecticide residues in Ganges river, dolphin (Kannan *et al.,* 1994; Senthilkumar *et al.,* 1999a; Kumari *et al.,* 2002) and fish, as well as in food stuffs (Tanabe *et al.,* 1991; Kannan *et al.,* 1995) have also been reported. Most of the studies are restricted to total pesticide residues. Since pesticide causes reproductive and metabolic dysfunctions hence it is necessary to monitor the tissue bioaccumulation of HCHs, DDTs, chlorpyrifos and plasma levels of estradiol-17β (E2) in some edible catfish of the non-polluted ponds of Gujartal, Jaunpur (considered as reference site) and the polluted river of Gomti of eastern Uttar Pradesh of north India. This study aims to compare the insecticide bioaccumulation in tissues and plasma levels of E2 in relation to reproduction during pre-monsoon season. Considering above facts, persistent chemicals like organochlorine and organophosphate insecticides may reside in the rivers eventually being incorporated in the food chain. This necessitates monitoring of residues of toxic chemicals in order to keep wild fish species in the riverine resources edible for human beings.

Materials and Methods

The original research reported herein was conducted under ethical guidelines for the treatment of animals in behavioral research and teaching (Anonymous, 1998) established for animal usage by Tilak Dhari College, Jaunpur (UP). For the comparative study of HCHs, DDTs, chlorpyrifos and plasma estradiol-17β levels, tissues were collected from the reference site and polluted river Gomti during breeding season to assess the status of pollutants causing reproductive dysfunctions. Same fish species were captured from reference site as well as polluted site during pre-monsoon (breeding season-prespawning phase). Fish were captured by drag net with the help of local fishermen from each sampling site. Total 10 fish were used in this study from all sites. Blood was taken by caudal vein in separate heparinized culture tubes for pesticide residue and hormone assay and fish were kept in the ice-box and brought to laboratory for the collection of brain, liver and ovary. Tissues were kept at -20^{0}C till subsequent analysis. Within two days all the samples were extracted and cleanup for the analysis and sent to Indian Institute of Toxicology Research Centre (IITR), Lucknow (UP) for analysis. The heparinized blood was centrifuged at 10,000 rpm for 15 min at 4^{0}C and plasma was separated for hormone assay. The gonadosomatic index (GSI) was calculated by the formula- gonad weight x 100 / body weight. The extraction method was applied as described by Singh and Singh (2008). The quantitative analyses of OC (HCHs and DDTs) were performed by Gas Liquid Chromatography (Nucon 5765) equipped with ^{63}Ni electran capture detector (ECD). The GLC column (6 inch x 1/8 inch i.d.) filled with 80-100 mesh, Gas Chrome coated with a mixture of 1.5 / SP-2250 and 1.95% SP-2401. Oven temperature was 190^{0}C. The injector and detector temperature were kept at 250^{0}C. Nitrogen IOL-AR grade I was kept as carrier gas (flow rate 60 ml/min). The volume of injection for each unknown samples and standard were 2-5 ml depending upon concentration of pesticides in samples. Pesticides were estimated from individually resolved peak of samples with corresponding peaks of standards. The extraction and cleanup procedure was followed as described by Lino and Silveira (1994).

The quantitative analysis of Organochlorines (isomers of HCH), metabolites of DDT and Chlorpyrifos were performed by Gas liquid chromatography (Nucon 5765) equipped with 63 electron capture detector (ECD-GC) for the identification of Organochlorines. The GLC column (6 inch × 1/8 inch I'd) filled with 80-100 mesh, Gas chrome coated with a mixture of 1.5/ SP- 2250 and 1.95%SP – 2401. The oven temperature was programmed to 60°C (1 minute hold) to 160°C (10 minute hold) at the rate 20°C / minute and then to 270°C (15 minute hold) at the rate of 20°C / minute, at 190°C. The injector and detector temperature was kept at 250°C. Nitrogen, Indian Oil Limited Analytical Reagent (IOC- AR) was kept at carrier gas (flow rate 60 ml./ minute). The volume for injection for each unknown sample and standard was 2-5µl. depending upon the concentration of insecticide in the samples. Insecticides were estimated from individual resolved peak of samples with corresponding peaks of the standard. The conformation of Chlorpyrifos was done on NPD detector in same column and conditions. The methodology used for the extraction of free steroid hormone was same as described by Singh and Kime (1995), and the method was followed as given in ELISA kit (DiaMetra, Italy). Values were expressed as ng/ ml plasma and ng/g (ppb) of tissues, Mean ± SEM, n=5. Results were analysed by Student's t test (Bruning and Kintz, 1977) and analysis of variance (ANOVA TW) by Microsoft Excel tool pack data analysis (ANOVA two factor with replication).

Results and Discussion

Analysis of variance have revealed that the tissue concentrations of isomers of HCH, metabolites of DDT and chlorpyrifos varied in the female catfish collected from that of reference site and polluted river Gomti (Figures 1- 6).

The catfish captured from the river Gomti has high bioaccumulation of α, β, γ and δ isomers of HCH in liver, brain and ovary as compared to the catfish of the control site. The percentage of γ-HCH of the ΣHCH was high in the female catfish in liver, brain and ovary with low percentage of β and δ isomers during pre-monsoon. The catfish captured from the river Gomti has showed high bioaccumulation of α, β, γ and δ isomers of HCH in studied tissues as compared to same fish species tissues captured from reference site. The preferential order of tissue bioaccumulation of ΣHCH was same for the catfish (liver > ovary > brain) captured from the river Gomti (Fig. 1). The catfish captured from the river Gomti and reference site showed high bioaccumulations of *p,p*'-DDE, *o,p*'-DDT, *p,p*'-DDD and *p,p*'-DDT metabolites of DDT in liver, brain and ovary as compared to the catfish from reference site. Similarly, the tissue bioaccumulation of organophosphate (chlorpyrifos) was also very high in female cat fish captured from polluted river Gomti as compared to that of reference site of same species. The percentage of bioaccumulation was different in different tissues. It was very high in polluted fish as compared to reference site (Fig. 1-5).

The GSI was significantly decreased in the catfish captured from the river Gomti when compared from the conspecific captured from the reference site. Similarly, plasma levels of E2 also noticed decline in the wild populations of captured catfish from polluted river Gomti when compared to the same species from the reference site. The decrease in plasma E2 and

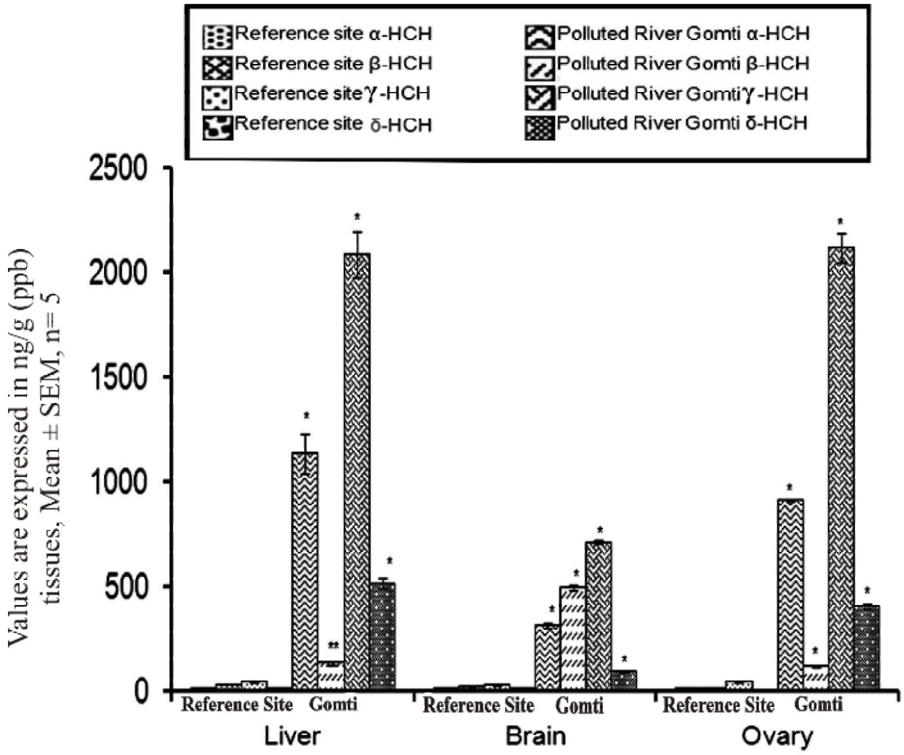

Fig. 1. Comparison of isomers of ΣHCH in fish tissues namely liver, brain and ovary of captured female catfish (*Bagarius bagarius*) between reference site and same species from polluted river Gomti during pre-monsoon season. Results were compared from reference site fish to polluted fish by Student's *t*-test. The level of significance (P) - * $P< 0.001$; ** $P<0.05$. ANOVA (TW) - tissue : $F = 825.54$, $P< 0.001$; pesticides : $F = 159.43$, $P< 0.01$; tissue ´ pesticide : $F = 87.55$, $P< 0.005$.

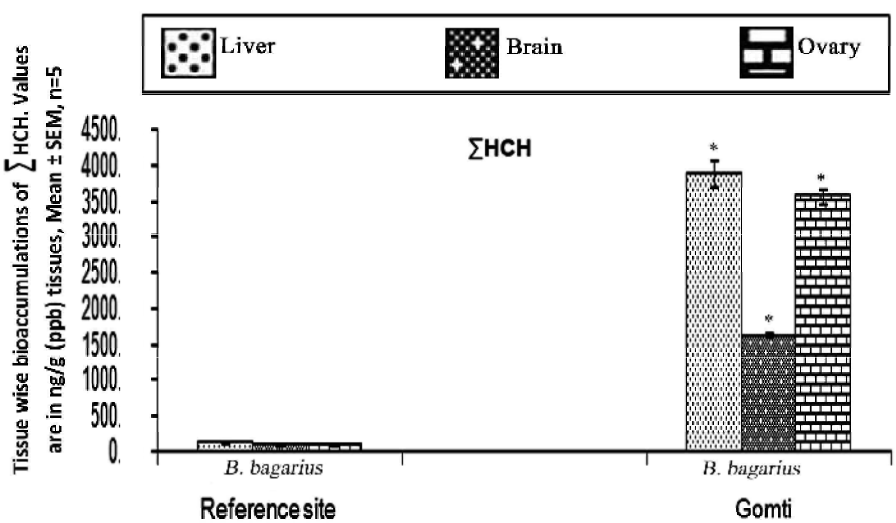

Fig. 2. Comparison of ΣHCH in liver, brain and ovary in female catfish (*B. bagarius*) captured from reference site and conspecific from polluted river Gomti. Results were compared by student's *t* – test. The level of significance (P) - * $P< 0.001$. ANOVA (TW) – tissue: $F = 1587.83$, $P < 0.05$; pesticide: $F = 93.30$, $P< 0.001$; tissue × pesticide: $F = 89.23$, $P< 0.001$.

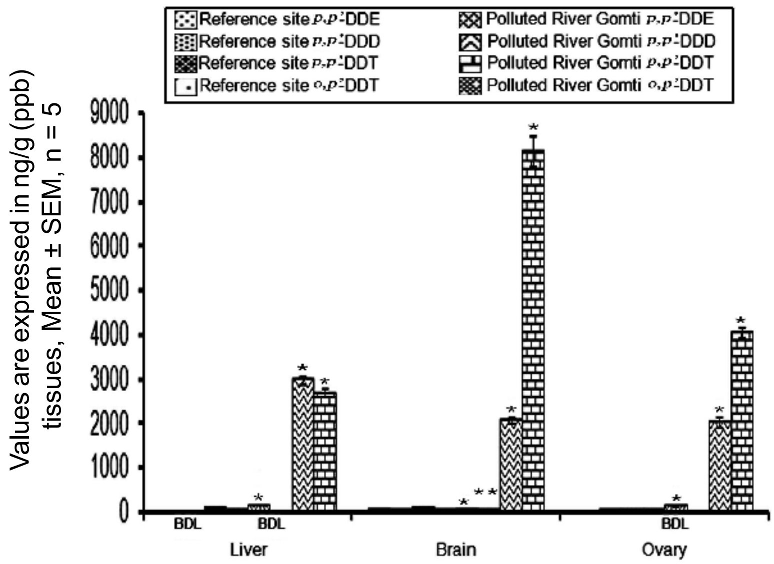

Fig. 3. Comparison of metabolites of DDT liver, brain and ovary of captured female catfish (*Bagarius bagarius*) between reference site and polluted river the Gomti during pre-monsoon. BDL- below detection limit. Results of metabolites of DDT of fish tissues from reference site versus fish captured (conspecific) from rivers the Gomti were compared by Students t- test. The level of significance (P) - * $P< 0.001$; ** $P< 0.025$. ANOVA (TW): tissues $F = 1441.39$ $P< 0.01$; metabolites of DDT $F = 114.02$ $P< 0.001$; tissue × metabolites of DDT $F = 157.98$ $P< 0.05$.

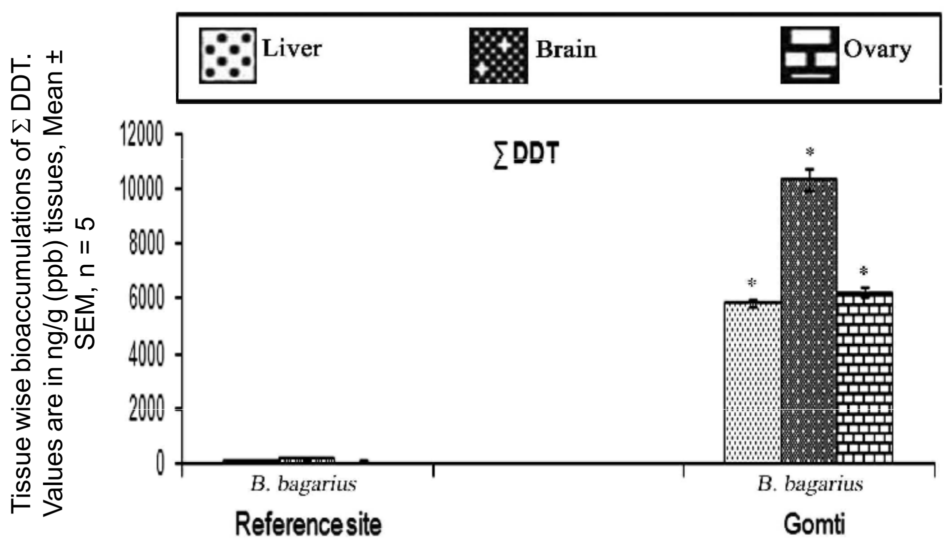

Fig. 4. Comparison of Σ DDT in liver, brain and ovary in female catfish captured from reference site (*B. bagarius*) and conspecific from polluted river Gomti. Results were compared by student's *t* – test. The level of significance (P) - * $P< 0.001$. ANOVA (TW) – tissue: $F = 2143.06$, $P < 0.001$; pesticide: $F = 82.50$, $P< 0.05$; tissue × pesticide: $F = 79.59$, $P< 0.025$.

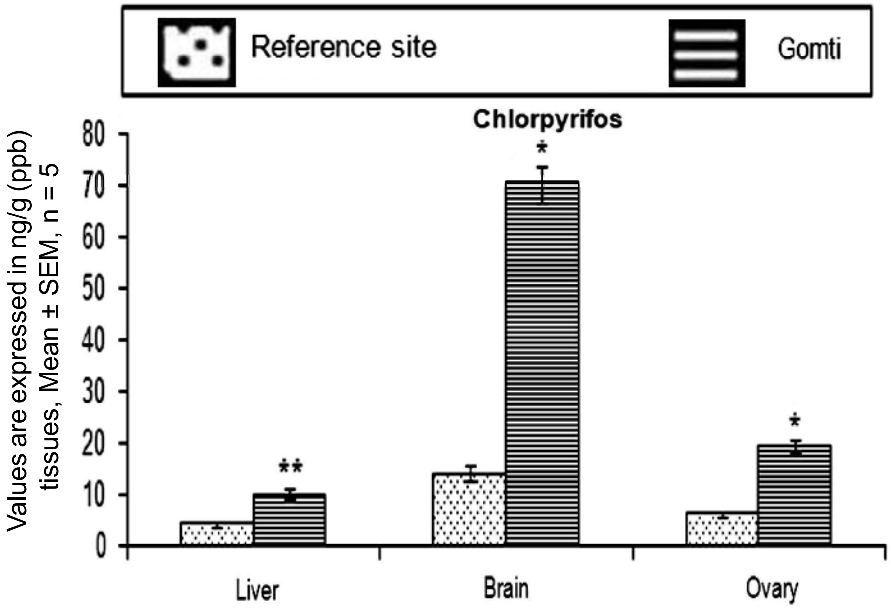

Fig. 5. Comparison of Chlorpyrifos in liver, brain and ovary in female catfish captured from reference site (*Bagarius bagarius*) and conspecific from polluted river Gomti. Results were compared between reference site to polluted fish by student's *t*- test. The level of significance (P) - * P<0.001; * * P< 0.005. ANOVA (TW): tissues F = 342.83 P< 0.05; pesticides of Chlorpyrifos F = 258.24 P< 0.001; tissue × pesticides of Chlorpyrifos F = 134.17 P< 0.001.

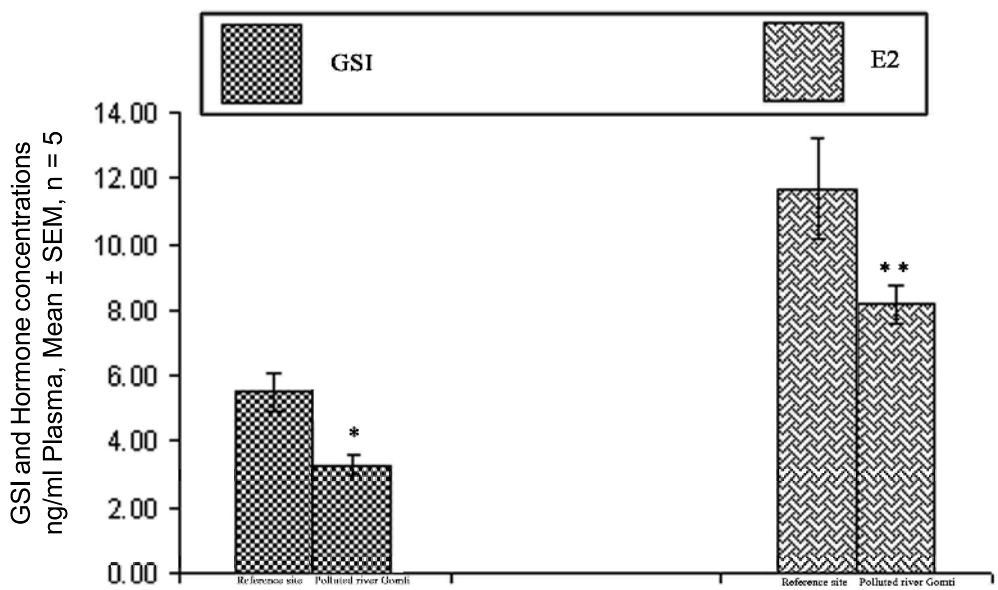

Fig. 6. Comparison of GSI and Plasma level of estradiol - 17β (E2) during pre-monsoon season in female catfish from reference site (*Bagarius bagarius*) and conspecific fish from polluted river Gomti, Jaunpur. Results were compared from reference site fish to polluted fish by student's *t*- test. The level of significance (*P*)- * P< 0.005; ** P< 0.025.

GSI indicated positive correlation during pre-monsoon season in captured catfish from the river (Fig. 6).

Present studies have demonstrated that ΣHCH has preferential order of bioaccumulations in the tissues of catfish (liver > ovary > brain) during pre-monsoon season of the river Gomti. The percentage of tissue bioaccumulations of γ-HCH isomer (lindane) was detected more than the other isomer which may be due to isomerization of α- and β-HCH. This transformation could have been caused by bacterial activity and ultraviolet radiation in the water column which ultimately sink to the sediments and finally get bioaccumulated in fish tissues. These findings get support from the report of Lenardon *et al.* (1984). Further it has been also demonstrated by El Beit *et al.,* (1981) that γ-HCH is more resistance to biological and chemical degradation under aerobic conditions.

Some researchers have shown that isomer α- and β-HCH have very high mammalian toxicity (Tomizawa, 1977), β-HCH toxicity in *Poecilia reticulata* (Wester *et al.,* 1985) and γ-HCH toxicity in *Heteropneustes fossilis* (Singh *et al.,* 1993). In river Gomti, the preferential order of organochlorine bioaccumulation was high in the catfish than the carp and ΣDDT was higher (catfish) than the ΣHCH (carp) indicating the higher levels of pollution in the river Gomti as reported by Singh and Singh, 2008a,b. Above findings are in relevance with the results of Kaphalia *et al.,* (1986) where these authors have shown that carnivorous fish have higher levels of insecticides than herbivores. The presence of HCHs and DDTs have been reported in river Gomti (Singh *et al.,* 2005) which may be one of the major factors for bioaccumulation of different HCHs and DDTs in liver, brain and ovary. Aditya and Chattopadhyaya (2000) have reported the accumulation of pesticide residue occurred in the order (muscle>testis>ovary) when adult *Labeo rohita* were sub-lethally (1/5th 96h LC 50) exposed to methyl parathion during prespawning, spawning and post-spawning phase. They suggested that the prespawning ovary appears to be an important organ of methyl parathion bioconcentration. Our findings in the present investigations showed high bioaccumulation of HCH and DDT in catfish ovaries in the polluted river Gomti thus support the above observations.

Results indicated that organochlorines (HCH and DDT) and organophosphate (chlorpyrifos) bioaccumulation was higher in catfish captured from wild habitat in comparison to same species from reference site. Some researchers (Singh *et al.,* 1988a,b,c; Singh *et al.,* 1997) have reported that nearly 85% of the total DDT produced in India is used for mosquito control, therefore the levels of total DDT observed could be attributed to municipal waste water inflow from the residential area into the river which might have caused bioaccumulation in various tissues of fish ultimately reaching human beings through the food chain. The compound of DDT is also used for the control of sand flies (*Phleobotamus argintepes* and *P. papatasi*), the vector of kala-azar disease, in areas near river Gomti and Ganga. Disposal of wastes from several insecticide manufacturing factories located along the rivers Gomti and Ganga have also contributed to pesticidal contamination (Haldar *et al.,* 1989; Singh *et al.,* 2005). Presence of HCHs and DDTs in bioaccumulation suggests its increased use in India recently.

Tanabe *et al.* (1991) and Kannan *et al.* (1995) have reported the bioaccumulation of organochlorines in several species of fish collected from fish market in Bankok, eastern and southern Asia and Oceania without considering the sex hormones. These authors have indicated the range of ΣHCH and ΣDDT including other insecticides which did not exceed MRL. The present studies have indicated that the catfish captured from the river has significant decreased GSI and plasma levels of E2 as compared to the same species of reference site. This might be an indication that the bioaccumulated HCHs and DDTs organochlorine and chlorpyrifos which was beyond permissible limit, was a major factor to decline the plasma E2 levels and vitellogenesis in liver during gonadal recrudescence (Fig. 6). Decrease in plasma sex hormones have also been reported by γ-HCH exposure in catfish and carp (Singh *et al.,* 1993; Singh *et al.,* 1994; Singh and Canario 2004). Another study of Kime and Singh (1996) has also indicated *in vitro* that γ-HCH decreased the hormone biosynthesis and its glucuronide and sulfates in the goldfish responsible for pheromonal behavior and spawning. Some researchers have reported that DDT caused the inhibition of reproduction (Burdic *et al.,* 1964; Burdic *et al.,* 1972). The isomer *p,p*'-DDT has been reported as anti-androgenic (Mills *et al.,* 2001; Kristensen *et al.,* 2006). It is likely that these isomers bind with estrogen receptor site of oocytes and cause dysfunction by affecting steroidogenesis ultimately decreasing the GSI of both catfish and carp inhabiting rivers of Gomti and Ganga. In addition to above, Kelce *et al.* (1994, 1995) have also demonstrated that Vinclozodin and *p,p*'-DDE alter androgenic expression at androgen receptor radiated mechanism. Here it might be that the metabolite *p,p*'-DDE of DDT affected estrogen receptor of gonads and inhibited the steroidogenesis during the gonadal growth. It is suggested that catfish have higher bioaccumulation of ΣDDT than ΣHCH and chlorpyrifos captured from the river Gomti. The river Gomti is highly polluted as compared to reference site. The bioaccumulation of HCHs, DDTs and chlorpyrifos in ovary, brain and liver indicated that these insecticides also affected pituitary level hence endocrine disruption occurred via hypothalamo-hypophyseal ovarian axis and affected the reproductive physiology. It is suggested that fish containing pesticide residues beyond the permissible limit must be avoided as food by the human beings as have been reported (Codex, 1996; PFA, 1954). It is also imperative to monitor insecticide pollution level in both Gomti and Gangetic ecosystem. Such measures would minimize their use in the catchment area and protect the riverine fishes from the adverse impact of insecticidal pollution on fish reproduction.

Acknowledgements

A grant-in-aid from UGC to PBS is greatly appreciated. The authors are grateful to Indian Institute of Toxicology Research (IITR), Lucknow for GLC and excellent IITR library facilities.

References

Aditya, A.K. and Chattopadhyaya, S. (2000) Accumulation of methyl parathion in the muscle and gonad of *Labeo rohita. J. Environ. Biol.*, **21,** 55-57.

Anonymous (1998) Guidelines for the treatment of animals in behavioral research and teaching. *Anim. Behav.,* **55**, 251-257.

Ahmad, S., Ajmal, M. and Nomani, A.A. (1996) Organochlorines and polycyclic aromatic hydrocarbons in the sediments of Ganga river (India). *Bull. Environ. Contam. Toxicol.,* **57**, 794-802.

Ankley, G.T., Bencic, D.C., Breen, M.S., Collette, T.W., Conolly, R.B., Denslow, N.D., Edwards, S.W., Ekman, D.R., Reyero, N.G., Jenson, K.M., Lazorchak, J.M., Martinovic, D., Miller, D.H., Perkins, E.J., Orlando, E.F., Villeneuve, D.L. Wang, R.L. and Watanabe, K.H. (2009) Endocrine disrupting chemicals in fish: Developing exposure indicators and predictive model of effects based on mechanism of action. *Aquat. Toxicol.*, **92,** 168-178.

Barrett, T.J. and Munkittrick, K.R. (2010) Seasonal reproductive patterns and recommended sampling times for sentinel fish species used in environmental effects monitoring programs in Canada. *Environ. Rev.,* **18**, 115-135.

Barrett, T.J., Lowell, R.B., Tingley, M.A. and Munkittrick, K.R. (2010) Effects of pulp and paper mill effluents on fish : a temporal assessment of fish health across sampling cycles. *Eviron. Toxicol. Chem.,* **29,** 440-452.

Barse, A.V., Chakrabarti, T., Ghosh, T.K., Pal, A.K. and Jadhao, S.B. (2007) Endocrine disruption and metabolic changes following exposure of *Cyprinus carpio* to diethyl phthalate. *Pest. Biochem. Physiol.,* **88,** 36-42.

Bruning, J.L. and Kintz, B.L. (1977) Computational Handbook of Statistics, Second Edition, Freeman, Chicago, IL, USA.

Burdic, G.E., Harris, E.J., Dean, H.J., Walker, T.M., Skea, J. and Colby, D. (1964) The accumulation of DDT in the lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.,* **93**, 127-136.

Burdic, G.E., Dean, H.J., Skea, J., Karcher, R. and Frisa, C. (1972) Effect of rate and duration of feeding DDT on reproduction of salmonid fishes reared and held under controlled conditions. *N.Y. Fish Game J.*, **19**, 97 -115.

Codex (1996) Food chemicals Codex. Committee on Food Chemicals Codex 4th edition. Food and Nutrition Board, Institute of Medicine, National Academy of Sciences, National Academy Press, Washington D. C.

Colborn, T. and Clemment, C. (1992) Chemically induced alterations in sexual functional development. The Wildlife Human Connection Princeton Scientific Publishing, Princeton, N J.

Colborn, T., Vom Saal, F.S. and Soto, A.M. (1993) Developmental effects of endocrine disrupting chemicals in wildlife and humans. *Environ Health Perspect.*, **101**, 378-384.

Dale, W.E., Copeland, M.F. and Hayes, W.J.Jr. (1965) Chlorinated insecticides in the bodies of peoples in India. *Bull World Health Org.*, **33**, 471-477.

Edward, C.A. (1970) Persistent pesticides in the environment. Critical reviews in Environment Control, CRC, Cleaveland, Ohio, USA.

El Beit , I.O.D., Wheelocka, J.V. and Cotton, D.E. (1981) Factors affecting soil residues of dieldrin, endosulfan [equation gamma]-HCH dimethoate and pyrolan. *Ecotoxicol. Environ. Saf.,* **5**, 135-160.

Han, S., Choi, K., Kim, J., Ji, K., Kim, S., Ahn, B., Yun, J., Choi, K., Kim, J.S., Zhang, X., and Giesy, J.P. (2010) Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna and Moina macrocopa. Aquat.Toxicol*., **98**, 256-264.

Hanson, R., Dodoo, D.K., Essumang, D.K., Blay, J. and Yankson, K. (2011) The Effect of some selected pesticide on the growth and reproduction of rresh water *Oreochromis niloticus*, *Chrysicthys nigrodigitatus* and *Clarias gariepinus*. *Bull. Environ. Contamin.Toxicol.,* **79**, 544-547.

Harding. A.K., Daston, G.P., Boyd, G.R., Lucier, G.W., Safe, S.H., Stewart, J., Tillitt, G.E. and Van Der Kraak, G. (2006) Endocrine disrupting chemicals research programme of the US Environmental Protection Agency: summary of the peer-review report. *Environ. Hlth. Perspect*., **114**, 1276-1282.

Haldar, P., Raha, P., Bhattacharya, P., Choudhury, A. and Adityachaudhury, N. (1989) Studies on the residues of DDT and endosulphan occurring in Ganga water. *Ind. J. Environ. Hlth.,* **31**, 156-161.

Hellou, J., Warren, W.G. and Payne, J.F. (1993) Organochlorines including polychlorinated biphenyls in muscles, liver and ovaries of Cod *Gadus morhua. Arch. Environ. Contam. Toxicol.,* **25**, 497-505.

Hilmy, A.M., Badawi, H.K. and Shabana, M.B. (1983a) Organochlorine pesticide residues in 12 freshwater Egyptian fish species with special emphasis on *Anguilla vulgaris* and *Mugil cephalus*. *Comp. Biochem. Physiol.,* **76C**, 63-171.

Hilmy, A.M., Badawi, H.K. and Shabana, M.B. (1983b) Physiological mechanism of toxic action of DDT and endrin in two euryhaline freshwater fishes, *Anguilla vulgaris* and *Mugil cephalus*. *Comp. Biochem. Physiol.,* **76C**, 173-179.

Kannan, K., Tanabe, S. and Tatsukawa, R. (1994) Biodegradation capacity and residue pattern of organochlorines in Ganges river dolphin from India. *Toxicol. Environ. Chem.,* **42**, 249-261.

Kannan, K., Tanabe, S. and Tatsukawa, P. (1995) Geographical distribution and accumulation features of organochlorine residues in fish in tropical Asia and Oceania. *Environ. Sci. Technol.,* **29**, 2673-2683.

Kaphalia, B.S., Nigam, U., Mehrotra, S. and Seth, T.D. (1986) Freshwater fish: An indicator of pesticide contamination in the aquatic environment. *J. Environ. Biol.,* **7**, 1-9.

Kelce, W.R., Lambright, C.R., Gray, L.E. and Robert, K.P. (1994) Vinclozodin and pp-DDE alter androgenic gene expression: *In vivo* confirmation of an androgen receptor mediated mechanism. *Toxicol. Appl. Pharmacol.,* **142**, 192-200.

Kelce, W.R., Stone, C.R., Laws, S.C., Gray, L.E., Kemppainen, J.A. and Wilson, E.M. (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature,* **375**, 581-585.

Kristensen, T., Baatrup, E. and Bayley, M. (2006) p,p'-DDE fails to reduce the competitive reproductive fitness in Nigerian male guppies. *Ecotoxicol. Environ. Saf.,* **63,** 148-157.

Kime, D.E. (1998) Endocrine disruption in fish. Kluwer Academic Publishers, Massachusetts, USA.

Kime, D.E. and Singh, P.B. (1996) *In vitro* effects of g hexachlorocyclohexane on *in vitro* biosynthesis and metabolism steroids in goldfish, *Carassius auratus*. *Ecotoxicol. Environ. Saf.,* **34**, 165-173.

Kulshrestha, S.K., Krishan, G. and Jain, A. (1995) Effect of pesticides on fishes: A review of studies in India. *J. Natcon.,* **7**, 145-188.

Kumari, A., Sinha, R.K. and Gopal, K. (2001) Quantitative estimation of DDT residues in some freshwater fishes of river Ganga at Patna, Bihar. *Environ. Ecol.,* **19**, 396-399.

Kumari, A., Sinha, A.K., Gopal, K. and Lata, S. (2002) Concentration of organochlorines in Ganges river dolphins from Patna, Bihar. *J. Environ. Biol.,* **23**, 279-281.

Lokman, P.M., Kazeto, Y., Ozaki, Y., Lijri, S., Tosaka, R., Kohara, M., Divers, S.L., Matsubara, H., Moore, L.G. and Adachi, S. (2010) Effects of reproductive stage, GH, and 11- ketotestosterone on expression of growth differentiation factor-9 in the ovary of the eel, *Anguilla australis*. *Reprod.* **139**, 71-83.

Lenardon, A.M., De Hevia, M.I.M., Fuse, J.A., DE Nochetto, C.B. and Depeteris, P.J. (1984) Organochlorine, organophosphorus pesticides in the Parana river (Argentina). *The Sci. Total Environ.,* **34**, 289-297.

Letherland, J.F. and Sonstegard, R.A. (1982) Bioaccumulation of organochlorines by yearling coho salmon (*Oncorhynchus kisutch* Walbaum) fed diets containing Great Lakes coho salmon, and the pathophysiological responses of the recipients. *Comp. Biochem.Physiol.,* **72C**, 91-99.

Lino, C.M. and Noronha da Silveira, M.I. (1994) Chlorpyrifos, ethion, fenitrothion and methidathion residues in chicken. *Bull. Environ. Contam. Toxicol.,* **52**, 425-431.

Mills, L.J., Gutjahr-Gobell, R.E., Haebler, A., Horoitz, D.J.B., Jayaraman, S., Pruell, R.J., McKinney, R.A., Gardner, G.R. and Zaroogian, G.E. (2001) Effect of estrogenic (o,p'-DDT; octylphenol) and anti androgenic (p,p'-DDE) chemicals on indicators of endocrine status in juvenile male summer flounder (*Paralichthys dentatus*). *Aquat. Toxicol.,* **52**, 157-176.

Olujimi, O.O., Fatoki, O.S., Odendaal, J.P. and Okonkwo, J.O. (2010) Endocrine disrupting chemicals (phenol and phthalates) in the South African environment : A need for more monitoring. *Water SA* **36**, 671-682.

Pankhurst, N.W. (2010) The endocrinology of stress in fish : An environmental perspective. General and comparative Endocrinology, In Press available online 03 Aug.

Paasivirta, J., Palm, H., Paukku, R., Akhabuhaya, J. and Lodenius, M. (1988) Chlorinated insecticide residues in Tanjanian environment. *Chemosphere,* **17**, 2055-2062.

PFA (1954) The Prevention of Food Adulteration Act Universal Law Publishing Co. Pvt. Ltd. India. **2006**, 109-119.

Ramesh, A., Tanabe, S., Taksukawa, R., Subramaniun, A.N., Palanichamy, S. Mohan, D. and V.K. Venugopalan (1989) Seasonal variations in organochlorine insecticide residues in air from Porto Novo, South India. *Environ. Pollut.,* **62**, 213-222.

Sahagun, A.M., Tem, M.T., Garcia, J.J., Sierra, M., Fernandes, N. and Fiz, M.S. (1998) Organochlorine pesticide residues in muscles tissues of rainbow trout, *Oncorynchus mykiss* taken from four fish farms in Leon, Spain. *Food Addit. Contam.,* **15**, 501-505.

Senthil Kumar, K., Kannan, K., Sinha, R.K., Tanabe, S. and Giesy, J.P. (1999a) Bioaccumulation profiles of polychlorinated biphenyl cogeners and organochlorine pesticides in Ganges river dolphins. *Environ. Toxicol. Chem.,* **18**, 1511-1520.

Senthilkumar, K., Watanabe, M., Kannan, K., Tanabe, S. and Subramanian, A.N. (1999b) Isomer-specific patterns and toxic assessment of polychlorinated biphenyls in resident and wintering migrant birds and bat collected from south India. *Toxicol. Environ. Chem.,* **71**, 221-239.

Senthilkumar, K., Kannan, K., Tanabe, S. and Subramanian, A.N. (2001) Accumulation of persistent organochlorine pesticides and PCBs in sediments, aquatic biota, birds, eggs and bat collected from south India. *Environ. Sci. Poll. Res.,* **8**, 35-47.

Sharma, A., Verma, D.K., Sharma, M., Singh, R.P. and Khaprde, S. (1995) Bioconcentration potential of body muscle of seven fresh water fishes to DDT and its isomers: a gas chromatographic investigation. *Proc. Acad. Environ. Biol.,* **4**, 55-58.

Singh, P.B., and Kime, D.E. (1995) Impact of ã-Hexachlorocyclohexane on the *in Vitro* production of steroids from endogenous and exogenous precursors in the spermiating roach, *Rutilus rutilus*. *Aquat. Toxicol.,* **31**, 231-240.

Singh, P.B. and Singh, V. (2008a) Bioaccumulation of hexachlorocyclohexane, dichlorodiphenyltri-chloroethane, estradiol 17β in catfish and carp during the pre-monsoon season in India. *Fish Physiol. Biochem.*, **34**, 25-36.

Singh, P.B., Singh, V. and Nayak, P.K. (2008a) Pesticide residues and reproductive dysfunction in different vertebrates from north India. *Food. Chem. Toxicol.,* **46**, 2533-2539.

Singh, P.B. and Canario, A.V.M. (2004) Reproductive endocrine disruption in the freshwater catfish, *Heteropneustes fossilis*, in response to the pesticide g-hexachlorocyclohexane. *Ecotoxicol. Environ. Saf.,* **58**, 77-83.

Singh, P.B., Sahu, V., Singh, V., Nigam, S.K and Singh, H.K. (2008b) Sperm motility in the fishes of pesticide exposed and from polluted rivers of Gomti and Ganga of North India. *Food. Chem. Toxicol.*, **46**, 3764-3769.

Singh, P.B. and Singh, V. (2008b) Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17beta and 11-ketotestosterone, and sperm motility in *H. fossilis.* (Bloch). *Chemosphere*, **72,** 422 – 453.

Singh, P.B., Kime, D.E. and Singh, T.P. (1993) Modulatory actions of *Mystus* gonadotropin on g-BHC-induced histological changes, cholesterol and sex steroid levels in *Heteropneustes fossilis. Ecotoxicol. Environ. Saf.,* **25**, 141-153.

Singh, P.B., Kime, D.E., Epler, P. and Chyb, J. (1994) Impact of g hexachlorocyclohexane exposure on plasma gonadotropin levels and *in vitro* stimulation of gonadal steroid production by carp hypophyseal hemogonate in *Carassius auratus*. *J. Fish. Biol.,* **44**, 195- 204.

Singh, P.P., Battu, R.S. and Kalra, R.L. (1988c) Insecticide residue in wheat grains and straw arising from their storage in premises treated with BHC and DDT under malaria control Programme. *Bull. Environ. Contam. Toxicol.,* **44**, 696-702.

Singh, N., Shukla, M.M., Chand, S.K. and Sharma, V.P. (1997) Outbreak of falciparum malaria in submerged villages of Narayanganj PHC, district Mandla due to Narmada irrigation Project, Central India (Madhya Pradesh). *Curr. Sci.,* **73**, 689-691.

Singh, K.P., Malik, A., Mohan, D. and Takroo, R. (2005) Distribution of persistant organochlorine pesticide residues in Gomti River, India. *Bull. Environ. Contam. Toxicol.,* **74**, 146-154.

Tanabe, S., K. Kannan, M.S. Tabucanon, C. Siriwong, Y. Ambe and R. Tatsukawa (1991) Organochlorine pesticide and polychlorinated biphenyl residues in foodstuffs from, Thailand*. Environ. Pollut.,* **72**, 191-203.

Tomizawa, C. (1977) Past and present status of residues of pesticides marketed in agriculture commodities in Japan. *Japan Pest. Inf.,* **30**, 5-42.

Von Westernhagen, H., Dethlefsen, V., Cameron, P. and Janssen, D. (1987) Chlorinated hydrocarbon residues in gonads of marine fish and effects on reproduction. *Sarsia,* **72**, 419-422.

Verma, D.K. (1991) Gas liquid Chromatographic analysis of DDT residue in the kidney of *Labeo rohita*. *Acta. Hydrochim. Hydrobiol.,* **19**, 327-329.

Wester, P.W., Canton, J.H. and Bisschop, A. (1985) Histopathological study of *Poecilia reticulata* (guppy) after long term â hexachlorocyclohexane exposure. *Aquat. Toxicol.,* **6**, 271-296.