SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 2, pp. 363-371, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

EXPOSURE AND RECOVERY RESPONSE OF PESTICIDES ON TISSUE BIOCONCENTRATION AND PLASMA SEX STEROID HORMONES IN *HETEROPNEUSTES FOSSILIS*

*Pratap B Singh and Vandana Singh
Department of Zoology, Tilak Dhari College, Jaunpur-222002, India

ABSTRACT

Effects of 40 days exposure and 20 days recovery response at sublethal concentration of technical grades of gamma isomer of hexachlorocyclohexane (γ-HCH-0.025 ppm), dichlorodiphenyltrichloroethane (DDT-5.0 ppm) and chlorpyrifos (0.5 ppm) on the percentage rate of bioconcentrations in blood, brain, liver, muscles, ovary, gonadosomatic index (GSI) and plasma levels of testosterone and estradiol-17β were studied during prespawning phase in catfish, *Heteropneustes fossilis* (Bloch) during its annual breeding cycle. All pesticides caused maximum bioconcentration either in liver or ovary. Recoveries of these pesticides have also been recorded in aforesaid tissues except DDT in blood, brain, muscles and ovary when kept the fishes in pesticide free water. The percentage of bioconcentrations were maximum in all tissues but recoveries were maximum for blood (γ-HCH), muscles (DDT) and liver (chlorpyrifos). The GSI, testosterone and estradiol-17β were decreased at all doses of pesticides. The exposed catfish kept in pesticide free water caused recoveries in GSI, testosterone and estradiol-17β. Our results indicate that pesticides have preferential order of percentage of bioconcentration in different tissues and have very selective effects on sex hormones thereby affecting reproductive physiology. Restoration of normal reproductive physiology during recovery phase might be due to dissipations of pesticides.

Keywords: Pesticide residues in fish; recoveries; testosterone; estradiol-17β; *Heteropneustes fossilis*.

INTRODUCTION

Tissue concentrations of pesticides in wild captured fish have been reported by several workers (Letherland and Sonstegard, 1982; Von Westernhagen *et al.*, 1987; Kannan *et al*., 1995; Singh *et al*., 1997; Sahagun *et al*., 1998; Ferreira *et al*., 2004; Antunes and Gil, 2004; Saqib *et al*., 2005; Amado *et al*., 2006; Antunes *et al*., 2007a, b). It has been reported that pesticides even very low doses inhibits lipid metabolism (Singh, 1992) and steroidogenesis (Singh *et al*., 1994; Kime and Singh, 1996; Singh and Canario, 2004). Hilmy *et al.* (1983) have reported when exposure of DDT was done to *Anguilla vulgaris* and *Mugil cephalus* the bioconcentration of DDT in tissue recorded highest in liver than gonads, gills and muscles. Organochlorine pesticide show very low accumulation in the muscles, but concentrations in liver are in general 10-100 fold higher. Recovery response for different pesticides have also been reported (Aditya and Chattopadhyay, 2000; Kumari *et al*., 2001; Sarkar *et al*., 2005).

Very few literatures are available which indicate the exposure study of pesticides under laboratory condition and their bioconcentration in different tissues during pre-spawning phase to monitor percentage rate of bioconcentration in different tissues and recovery response in pesticide free water during reproductive growth. This prompted us to explore 1- Exposure for 40 days at sublethal concentration of γ-HCH, DDT and chlorpyrifos on percentage of tissues bioconcentration of total hexachlorocyclohexane (ΣHCH), dichlorodiphenyl-trichloroethane (ΣDDT), gonadosomatic index (GSI) and plasma levels of sex steroids (testosterone and estradiol-17β) during prespawning phase (when the vitellogenesis was continuing and have maximum steroidogenic activity) and 2. Percentage recovery studies by keeping the exposed fish in pesticide free water for 20 days on the above parameters.

MATERIALS AND METHODS

Experimental fish

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). The experimental fish, *Heteropneustes fossilis* (65-70 g and length 21-22 cm) were collected from a pond of the same brood stock during prespawning

*Corresponding author email: pratap_b_singh@rediffmail.com

Table 1. Details of treatments of *Heteropneustus fossilis* for 40 days exposure at sublethal concentrations of pesticides*.

Batches	Treatments of technical grade (γ-HCH- 99.8%, DDT-72.74%, chlorpyrifos-94%) of pesticides
1	γ-HCH treated with 0.025 ppm
2	DDT treated with 5.0 ppm
3	Chlorpyrifos treated with 0.5 ppm

* The dose 1/5th of LC (50) 96 hour was used (0.125, 25, 2.5 ppm for HCH, DDT and chlorpyrifos respectively).

Table 2. Details of treatments of exposed fish for 20 day recovery during pre-monsoon.

Batches	Treatments
	Control
1	HCH treated fish kept for 20 days in pesticide free water*
2	DDT treated fish kept for 20 days in pesticide free water
3	Chlorpyrifos treated fish kept for 20 days in pesticide free water

* dechlorinated water

phase season and maintained in cemented tank of size 2 x 1 x 1-m supplied with circulating constant flow of dechlorinated tap water and enjoyed natural photoperiod (13.0L : 11.OD) and temperature (30 ± 2^{o}C). They were fed *ad libitum* with minced goat liver comprising 20% protein, 5% lipid, 15% carbohydrate the remaining 60% being water, minerals and vitamins etc.

Chemicals

Analytical grade chemicals were obtained from E. Merck, Hi Media, India and Sigma Chemicals Co., USA. After a week of acclimation, experiments were performed in glass aquaria. In each glass aquaria, 15 fish were kept in 20 l water separately for individual pesticides. The total fish used in this study was 40.

Experiment 1- Toxicity test of γ-HCH, DDT and chlorpyrifos

The toxicity tests have been done as per method described by Singh *et al.* (1994; Singh and Singh, 2007). The LC_{50} for 96 hr of technical grade for γ-HCH (99.8%), DDT (72-74%) and chlorpyrifos (94%) was 0.125, 25 and 2.5 ppm respectively. The dose 1/5th of LC_{50} 96 h was taken for each pesticide as sublethal concentration for 40 days exposure. The details of treatments for exposure and recoveries study have been given (Tables 1, 2).

Experiment 2- Estimation of percentage of bioconcentration of pesticide residue in fish after 40 days exposure during prespawning phase of the annual reproductive cycle

During experimentation fish were fed every fourth day when the aquarium water was changed with fresh water having similar concentration of pesticides. At the end of exposure fish were bled by caudal puncture and blood was collected to required volume in heparinized (1% heparin sodium salt activity 1,00,000 units 140.3 U/mg) glass tubes. After decapitation brain, liver, abdominal muscles and gonad were dissected out, washed in saline (0.6% NaCl) blotted and kept at -20^{o}C for extraction and analysis of percentage of bioconcentration of total individual pesticide residues. The half of the blood was centrifuged at 10,000 rpm for 15 minutes at 4^{o}C for plasma sex steroid hormone analysis by radioimmunoassay. The GSI was calculated (gonad weight x 100/ body weight).

Experiment 3- Recovery studies in pesticide free water of exposed fish tissue concentrations and sex steroid hormones

Five fish from exposed fish of each batch were kept in pesticide free water for 20 days to observe the recoveries of percentage of tissue concentration of pesticide, GSI and plasma sex steroid hormones.

Extraction procedure and cleanup of organochlorines insecticides from blood and tissues

Extraction and cleanup procedure was followed as per method described (Dale *et al*., 1965) with modifications. Briefly, the fatty tissues from 5 individual of different groups of each in duplicate (0.5 g-brain and ovary or 1.0 g non-fatty tissues-liver, muscles and 1ml blood in heparinized tubes) were homogenized in a homogenizer cup by using 7 ml of formic acid and 5ml *n*-hexane (glc grade only). The homogenate was transferred in the conical flask by rinsing the cup and teflan pestle twice with 10 ml of *n*-hexane each time. The homogenate was shaken on shaker for 1 h at 37^{o}C. The upper layer of *n*-hexane was separated in another conical flask and the aqueous layer was again extracted with 10 ml of *n*-hexane 2 times (20 ml) by similar fashion. The organic layer was collected together. The resididue (formic acid) from the extract was removed by shaking the organic phase with distilled water (50 ml) in separating funnel. This extract

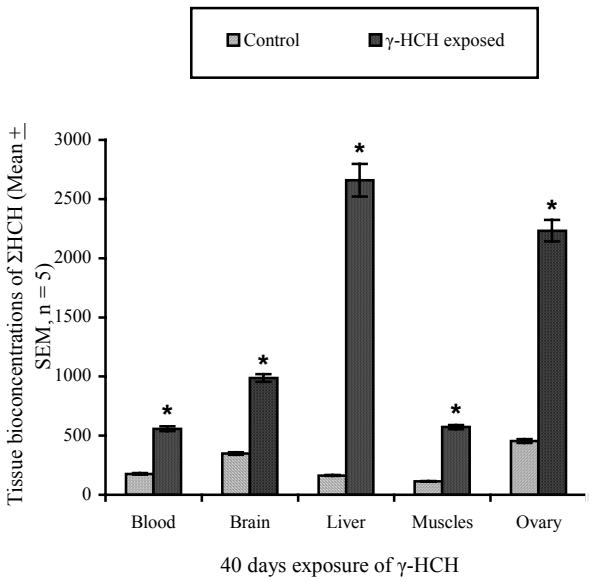


Fig. 1. Tissue bioconcentrations of γ-hexachlorocyclohexane (γ-HCH-0.025 ppm technical grade at sublethal concentration) in different tissues of the catfish, *H. fossilis* after 40 days exposure during prespawning phase of the annual reproductive cycle. Control vs γ-HCH exposed were compared by Students t -test. The level of significance (P)- *P< 0.001.

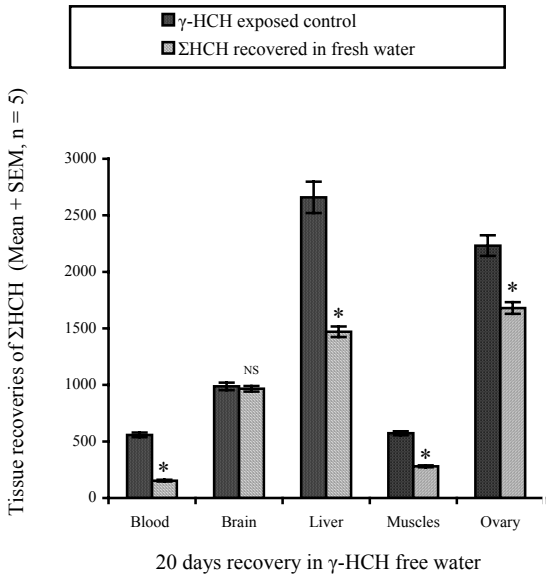


Fig. 2. Tissue recoveries of ΣHCH after 20 days treatments w γ-HCH free water of 40 days γ-HCH exposed catfish, *H. fossi* γ-HCH exposed control vs recovered values were compared Students t-test. The level of significance (P)- *P< 0.001. NS- n significant.

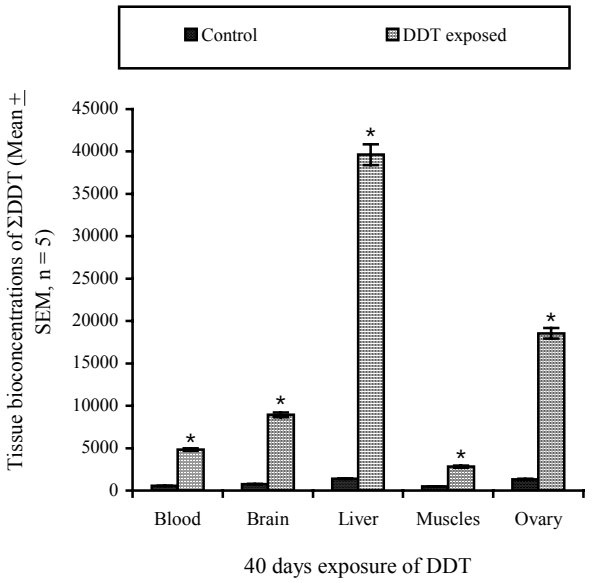
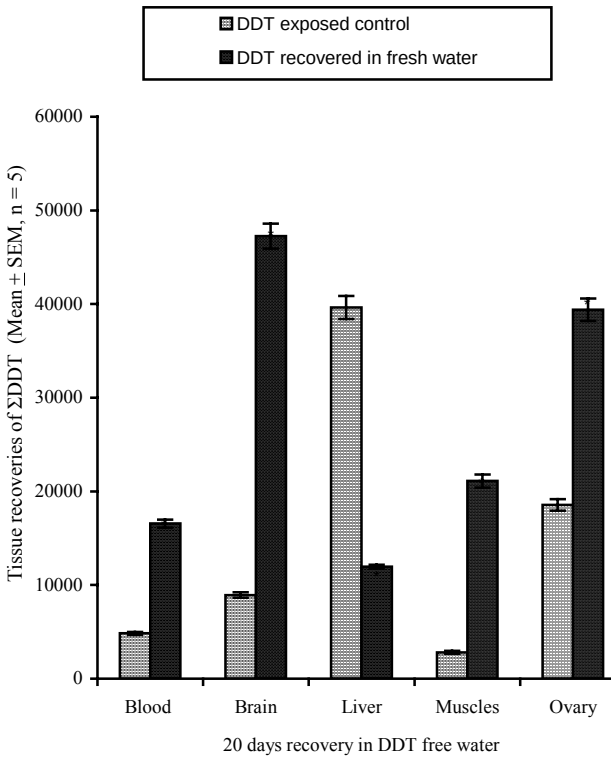


Fig. 3. Tissue bioconcentrations of dichlorodiphenyl-trichloroethane (DDT-5.0 ppm technical grade at sublethal concentration) in different tissues of the catfish, *H. fossilis* after 40 days exposure during prespawning phase of the annual reproductive cycle. Control vs DDT exposed were compared by Students t-test. The level of significance (P)- *P< 0.001.



was demoisturised by passing through anhydrous sodium sulfate bed. The demoisturised extract was concentrated on rota evaporator up to 1 ml and finally volume was made up to 2 ml in volumetric flask with help of *n*-hexane by rinsing the rota evaporator flask. The cleanup procedure for required pesticide was done by adding 2 ml concentrate sulfuric acid in the above extracted 2 ml extract. This mixture was vortexed and centrifuzed to separate the aqueous and non-aqueous layer. The upper *n*-hexane layer was separated in 2 ml clean volumetric flask with the help of Pasteur pipette individual samples. This concentrated sample was applied on Gas Liquid

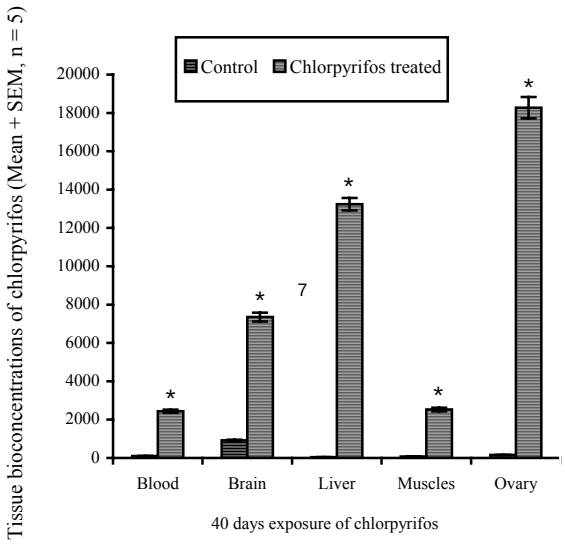


Fig. 5. Tissue bioconcentrations of chlorpyrifos (0.5 ppm technical grade at sublethal concentration) in different tissues of the catfish, *H. fossilis* after 40 days exposure during prespawning phase of the annual reproductive cycle. Control vs treated were compared by Students t-test. The level of significance (P)- *P< 0.001.

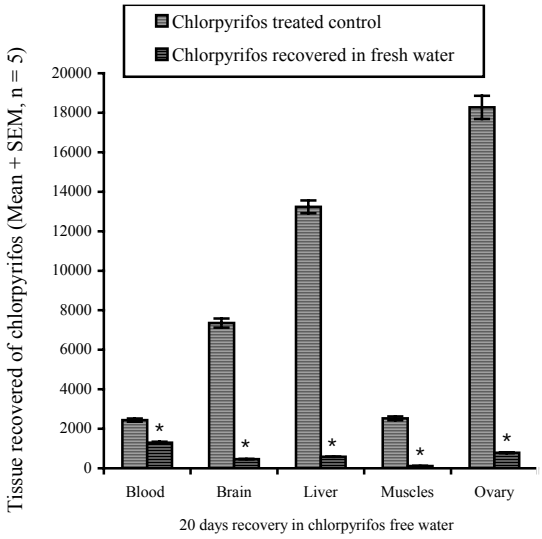


Fig. 6. Tissue recoveries of chlorpyrifos after 20 days treatments with pesticide free water of 40 days chlorpyrifos exposed catfish, *H. fossilis*. Chlorpyrifos exposed control vs recovered values were compared by Students t-test. The level of significance (P)- *P< 0.001.

Chromatography (GLC) for identification and quantification of isomers of HCH and metabolites of DDT from which ΣHCH and ΣDDT calculated.

Extraction procedure and cleanup of chlorpyrifos from blood and tissues

The method for extraction was used with modification as have been described earlier (Lino *et al.,* 1994). Known amount of each tissue from 5 individual of different groups in duplicate (0.5 g fatty tissue- brain and ovary, 1 g non-fatty tissue- liver and 1 ml blood in heparinized tubes) were collected separately and then homogenized with 5 ml of *n*-hexane. The content was then transferred into conical flask by adding the wash (10 ml) of teflon pestle twice with *n*-hexane (20 ml). Now, each flask was shaken 1 h for the extraction of pesticides at 37°C. Decanted the *n*-hexane layer and residue was re-extracted with 10 ml of *n*-hexane twice after 30 min shaking each. Filtered the extracted content with Whatman filter paper No. 1. Now contents were passed through the anhydrous sodium sulfate bed and concentrated on rota evaporator up to 5 ml. This concentrated extract was passed through the bed of anhydrous sodium sulfate (1g), activated Florisil (4g) and anhydrous sodium sulfate (1g) prepared in column 400 mm x 20 mm i.d. for pesticides cleanup. The contents was eluted with 200 ml of diethylether : *n*-hexane mixture (6 : 94) for the extraction of chlorpyrifos. Now concentrated the contents into 1 ml on evaporator and finally made up the volume to 2 ml by rinsing the evaporatory flask with *n*-hexane for the analysis and quantification of chlorpyrifos by GLC.

Quantitative analysis

The quantitative analysis of organochlorines (isomers of HCH and metabolites of DDT) and organophosphorus (chlorpyrifos) were performed by Gas Chromatography (Nucon 5765) equipped with ^{63}Ni electran capture detecter (ECD). The GC 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°C. The injector and detector temperature were kept at 250°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 2-5µl depending upon concentration of pesticides in samples. Pesticides were estimated from individually resolved peak of samples with corresponding peaks of standards. The confirmation of chlorpyrifos was done on NPD detector in same column and conditions.

Recovery studies for pesticide extraction and elution

Representative samples of each matrix were spiked with known concentrations of the isomers of HCH and DDT metabolites as well as chloripyrifos and kept at least for 3 h. The samples were extracted and cleaned up for analysis using GLC equipped with ECD system. The commodities are calculated and the recovery was approximate 94% and recovery factor 1.0638. Detection limit was 0.1ng/g (ppb) for HCH, DDT and chlorpyrifos.

Extraction and radioimmunoassay (RIA) of sex steroids

Sex steroids were extracted twice from plasma (400 µl or less as appropriate) with 5 ml of distilled dichloro-

methane. The details of RIA have been described elsewhere (Singh and Singh, 1992). The precision of the method as expressed in the percentage coefficient of variation were 4.2 and 3.9% intra-assay and 5.1 and 6.1% inter-assay for testosterone (T) and estradiol-17β (E2), respectively. Detection limits in the present assay were 6 pg for T and E2. Steroid antiserum had a cross reactivity of 100% with the respective hormones. The % recovery of double extraction of T and E2 were 97.7 and 97.3% respectively. The concentration coefficient between observed and expected values was 0.99 for each of sex steroids.

Statistical analysis

Values were expressed as ng / ml or ng / g of tissue (ppb) for pesticides and ng / ml plasma for sex steroid hormones (Mean ± SEM, n = 5). Data were analyzed by Students *t*-test for the level of significance (Bruning and Kintz, 1977).

RESULTS

Effect of γ-HCH exposure for 40 days on tissue bioconcentrations at sublethal concentrations and recoveries in γ-HCH free water for 20 days during prespawning phase in *H. fossilis* under laboratory conditions

The effect of γ-HCH exposure for 40 days have indicated that the bioconcentrations was maximum in liver and minimum in blood. The preferential order of bioconcentration for total HCH were recorded in tissues (liver > ovary > brain > muscles > blood) (Fig. 1). After 40 days of γ-HCH exposure when fish were treated with γ-HCH free water for 20 days, the bioconcentrations were dissipated in preferential order of tissues (ovary > liver > brain > muscles > blood) (Fig. 2).

Effect of DDT exposure for 40 days on tissue bioconcentrations at sublethal concentration and depuration in DDT free water for 20 days during prespawning phase in *H. fossilis*

The effect of DDT exposure for 40 days has indicated that the bioconcentrations was maximum in liver and minimum in muscles. The preferential order of bioconcentrations for ΣDDT were recorded in tissues (liver > ovary > brain > blood > muscles) (Fig. 3). After 40 days of DDT exposed fish when treated with 20 days of DDT free water the bioconcentration of DDT was raised in preferential order of tissues (brain > ovary > muscles > blood) whereas declined in liver (Fig. 4).

Effect of chlorpyrifos exposure for 40 days on tissue bioconcentrations at sublethal concentration and depuration in chlorpyrifos free water for 20 days during prespawning phase in *H. fossilis*

The effect of chlorpyrifos exposure for 40 days has showed that the bioconcentrations was maximum in ovary and minimum in blood. The preferential order of bioconcentrations for chlorpyrifos were recorded in tissues (ovary > liver > brain > muscles > blood) (Fig. 5). After 40 days of chlorpyrifos exposed fish when treated with 20 days of chlorpyrifos free water the bioconcentration were dissipated in the preferential order (blood > ovary > liver > brain > muscles) of tissues (Fig. 6).

Percentage of bioconcentrations of ΣHCH, ΣDDT and chlorpyrifos exposure for 40 days under laboratory conditions in different tissues during prespawning phase in *H. fossilis*

After 40 days of γ-HCH and chlorpyrifos exposure the tissue bioconcentrations for ΣHCH were maximum for liver and minimum in brain whereas ΣDDT showed high percentage of incorporation in liver but minimum in muscles (Fig. 7).

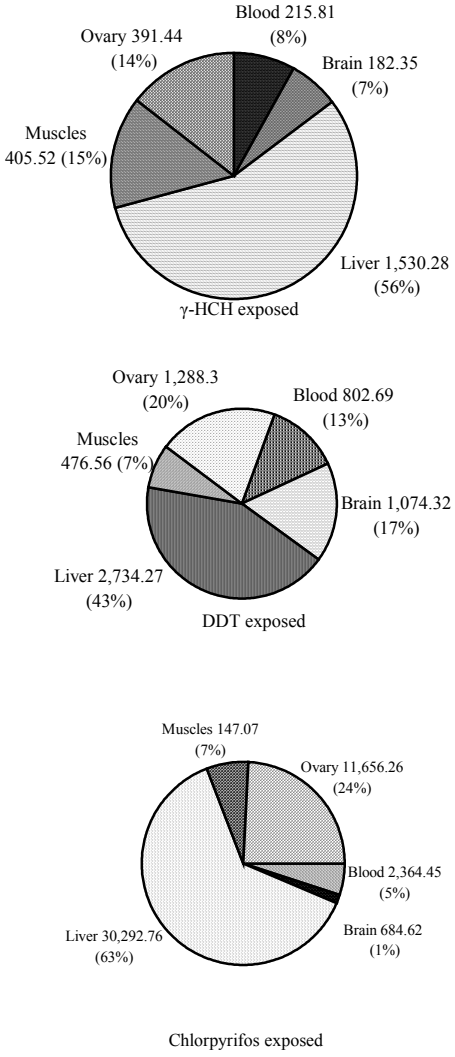


Fig. 7. Pie diagram showing percentage of bioconcentrations of ΣHCH, ΣDDT and chlorpyrifos exposure for 40 days under laboratory conditions in different tissues during prespawning phase in *H. fossilis.*

Percentage of depuration in tissue bioconcentrations of γ-HCH, DDT and chlorpyrifos treated fish when kept for 20 days in pesticide free water

Treatments of exposed fish with 20 days in pesticide free water the tissues dissipations of ΣHCH were maximum in blood and minimum in brain. The preferential order of depuration were recorded (blood > muscles > liver > ovary > brain) in tissues. The ΣDDT has shown the increased bioconcentrations (muscles > brain > blood > ovary) in tissues whereas only liver showed dissipations by 69.78%. The chlorpyrifos exposed fish when kept in chlorpyrifos free water for 20 days showed depuration maximum in ovary and minimum in blood and were recorded in preferential order of depuration in tissues (ovary > liver > muscles > brain > blood) (Fig. 8).

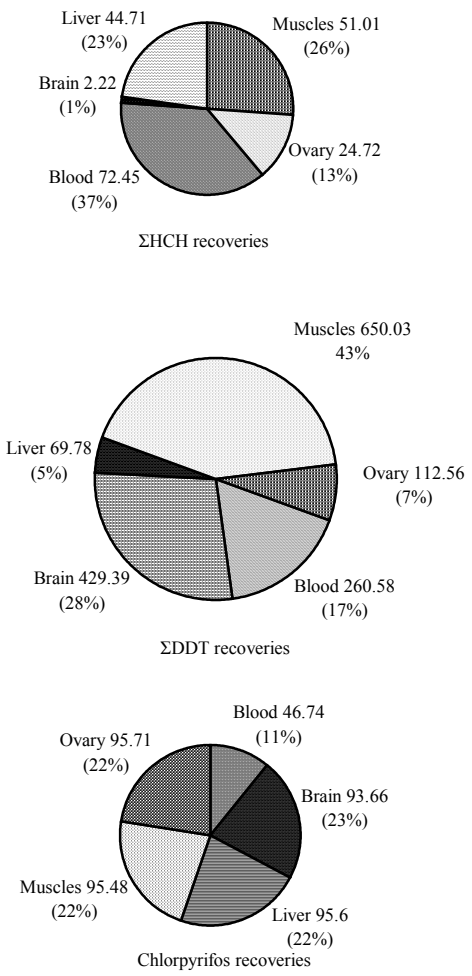


Fig. 8. Pie diagram showing percentage of recoveries of ΣHCH, ΣDDT and chlorpyrifos exposed fish when treated with pesticide free water for 20 days in *H. fossilis*.

Effect of γ-HCH, DDT and chlorpyrifos exposure for 40 days on gonadosomatic index (GSI) and plasma levels of testosterone (T) and estradiol-17β (E2) in *H. fossilis* during prespawning phase of the annual reproductive cycle

Exposure of pesticide caused significant decline in GSI and was maximum by γ-HCH than DDT and chlorpyrifos tested. The plasma levels of T was maximum in chlorpyrifos than the γ-HCH and DDT whereas the levels of E2 was suppressed maximum by DDT than the γ-HCH and chlorpyrifos exposure (Fig. 9, 10).

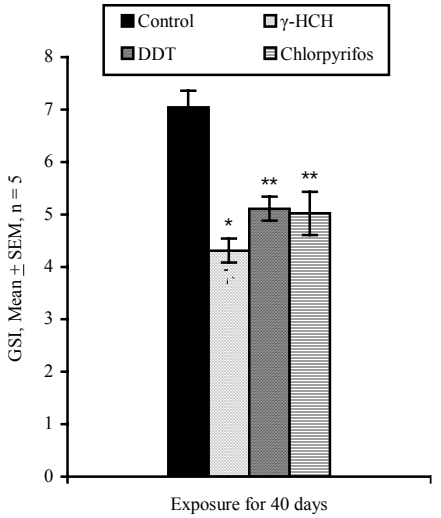


Fig. 9. Gonadosomatic index (GSI) after 40 days exposure at sublethal concentration of technical grade of γ-HCH, DDT and chlorpyrifos during pre-spawning phase (May and June) of the annual reproductive cycle in the female catfish, *H. fossilis*. Control vs pesticide treated were compared by Students t-test. The levels of significance (P)- *P< 0.001; **P< 0.005.

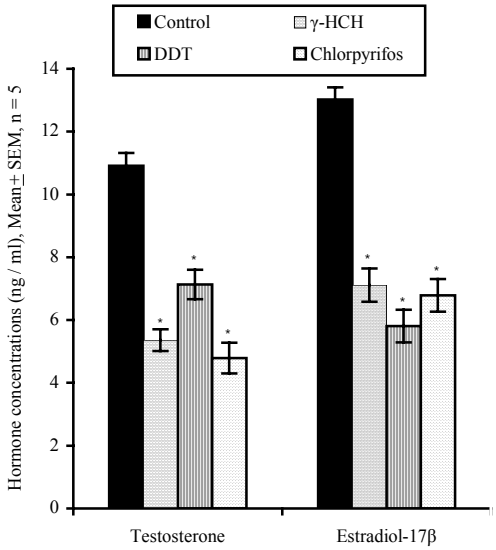


Fig.10. Plasma levels of testosterone and estradiol-17β after 40 days exposure at sublethal concentration of technical grade of γ-HCH, DDT and chlorpyrifos during pre-spawning phase of the annual reproductive cycle in the female catfish, *H. fossilis*. Control vs pesticide treated were compared by Students t-test. The level of significance (P) *P< 0.001.

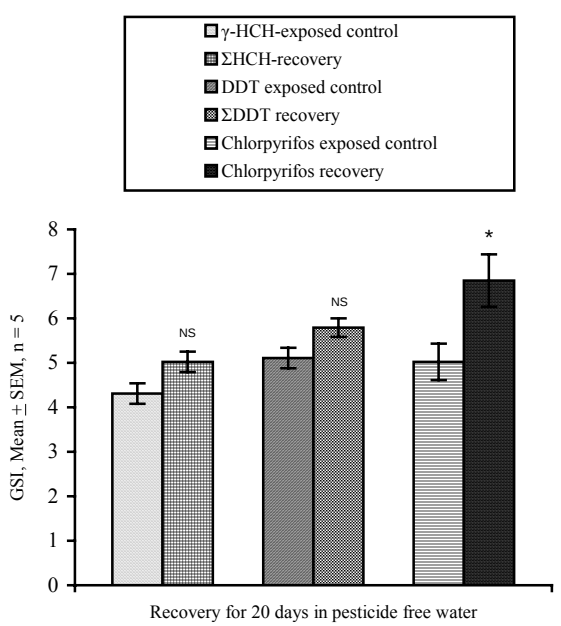


Fig. 11. Recoveries of gonadosomatic index (GSI) in pesticide free water for 20 days of exposed fish in the *H. fossilis*. Pesticide exposed (Control) vs recovered values were compared by Students t-test. Level of significance (P)- *P< 0.05. NS- not significant.

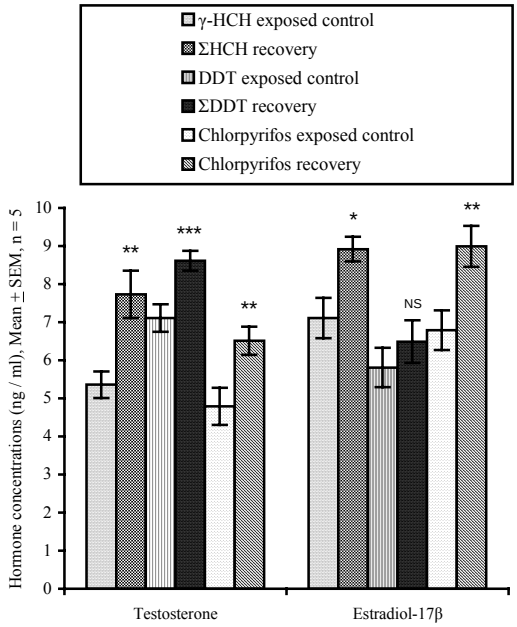


Fig. 12. Recovery of plasma levels of testosterone and estradiol-17β hormones in pesticide free water for 20 days of exposed fish in the *H. fossilis*. Pesticide exposed (control) vs recovered values were compared by Students t-test. The levels of significance (P)- *P< 0.01; **P< 0.02; ***P< 0.05. NS- not significant.

Recoveries of GSI, plasma levels of T and E2 in pesticide free water for 20 days

The γ-HCH, DDT and chlorpyrifos exposed fish when kept in pesticide free water showed varied effects. In γ-HCH and DDT groups the GSI has tendency to recover but it was not significant up to 20 days of the recovery in pesticide free water whereas chlorpyrifos treated fish recovered significantly. Interestingly, the plasma levels of T recovered significantly but more recovery was noticed by DDT. The recoveries for E2 was recorded only by γ-HCH and chlorpyrifos but remained insignificant by DDT treated fish kept in DDT free water (Fig. 11, 12).

DISCUSSION

Our results have clearly demonstrated that the exposure of technical grades of γ-HCH, DDT and chlorpyrifos at sublethal concentrations during pre-monsoon caused bioconcentration of these pesticides in different tissues (Fig 1,3,5) and also diminished the plasma levels of sex steroids (Fig.10) thereby affected the reproductive physiology of this species. Tissue bioconcentrations of pesticide in different tissues has varied effects in preferential order of bioconcentration. Although ΣHCH and ΣDDT has similar trends of bioconcentrations in tissues (liver > ovary > brain) and gonad has intermediate in position. The chlorpyrifos bioconcentration in liver was in the intermediate (ovary > liver > brain) position in *H. fossils*. It is suggested that prespawning ovary appears to be the important organs of γ-HCH, DDT and chlorpyrifos bioconcentrations. Aditya and Chattopadhyaya (2000) have reported the accumulation of pesticide residue occurred in the order muscle > testes > ovary when adult *Labeo rohita* were sub lethally ($1/5^{th}$ 96h LC 50) exposed to methyl parathion during prespawning, spawning and post-spawning phase. Among the pesticide DDT was maximum in its percentage bioconcentration in liver compared to γ-HCH and chlorpyrifos which may be due to more persistent than the other insecticides. The decreased levels of sex steroids have been reported by Singh and Singh (1992) in this species for malathion and γ-BHC exposure.

Results have shown that when exposed fish kept in 20 days in pesticide free water pesticides dissipation occurred for ΣHCH and chlorpyrifos in different tissues. The DDT level was increased in all the studied tissues but declined in liver when exposed fish were kept in DDT free water for 20 days. Here it may be interpreted that the loss or dissipation in liver may be due to metabolism of these pesticides by microsomal enzymes involved in detoxification but reason for increase in bioconcentrations by DDT only is unclear at this stage. The percentage of bioconcentrations was highest in the liver by all pesticide tested. However, the percentage of bioconcentrations varied for γ-HCH, DDT and chlorpyrifos exposure in

different tissues among which chlorpyrifos was maximal. This result is supported by the work of Hilmy *et al*. (1983) who have demonstrated 10-100 folds higher for organochlorines in freshwater fishes. The recovery percentages have also been recorded for above pesticides when kept in free water for 20 days. The recovery responses have also been reported by Chandra *et al*. (2004) in C*yprinus carpio* after exposure to carbofuran which also supports result obtained in our observations. Another report of Begum (2005) has also demonstrated the recovery response in *Clarias batrachus* when transferred to cypermethrin free water for protein, glycogen and enzymes.

In the present observations, GSI was found to be depressed after pesticide exposure but recovery was noticed in GSI by chlorpyrifos. The decrease in GSI, T and E2 in exposed fish when recovered in pesticide free water indicates the significant correlation between GSI and steroidogenesis. Here it can also be suggested that withdrawal of pesticide may be due to recovery in aromatase enzyme responsible for steroidogenesis in this species. These results get support from the result of Sarkar *et al*. (2005) who has also recorded recovery response in histological changes in liver of *Labeo rohita* when kept in carbofuran and cypermethrin free water. Smita *et al*. (2003) has reported gonadal recrudescence and recovery response in *Cyprins carpio* after long term exposure to carbamate pesticide. Above findings supports our result. Interestingly in *H. fossilis* on exposure of three pesticide caused decline in plasma T and E2, and exposed fish when kept in pesticide free water for 20 days there was recovery in plasma T levels. The levels of E2 was elevated only by γ-HCH and chlorpyrifos free water but not by DDT which suggested that enzyme involved for the conversion of T into E2 is disrupted by DDT during prespawning phase. These findings get support form the results of Mills et al. (2001) who have reported that flounder treated with DDT showed in reduction in T and E2.

In conclusion, pesticide on exposure get bioconcentrated in tissues in the preferential order in which gonad is an important organ for its bioconcentrations causing disturbance in metabolism and inhibits steroidogenesis thereby affecting reproductive physiology of this species. Recovery studies have indicated that exposed fish has tendency to restore the hormonal profile in due course required for normal reproductive physiology, information which may be of use to pisciculturists.

ACKNOWLEDGEMENTS

A grant-in-aid from Department of Science and Technology (DST Ref. No. SR/SO/AS-07/2004 dated 12.7.05), Government of India, New Delhi-110 016 to PBS is greatly appreciated. The authors are grateful to Dr. B. Jayaraman, Hindustan Insecticide Ltd., Gurgaon (Haryana) for the gift of technical grades of DDT (70-72%), γ-HCH (lindane-99.8%) and chlorpyrifos (94.0%) for my experiments. We are also grateful to Industrial Toxicology Research Centre (ITRC), Lucknow for GLC facilities.

REFERENCES

Aditya, AK. and Chattopadhyaya, S. 2000. Accumulation of methyl parathion in the muscle and gonad of *Labeo rohita.* Journal of Environmental Biology. 21(1): 55-57.

Amado, J., Antunes, P., Gil, O. and Vale, C. 2006. Mobility of organochlorines in muscles of sardine (*Sardina pilchardus*) during spawning in the Portugues coast. Ciencias Marinus. 32 (2B): 369-377.

Antunes, P. and Gil, O. 2004. PCB and DDT contamination in cultivated and wild sea bass from Ria de Aveiro, Portugal. Chemosphere. 54 (10): 1503-1507.

Antunes, P., Gil, O., Ferreira, M., Vale, C. and Reis-Henriques, MA. 2007a. Depuration of PCBs and DDTs in mullet under captivity clean conditions. Chemosphere. 67 (9): 558-564.

Antunes, P., Amado, J., Vale, C. and Gil, O. 2007b. Influence of the chemical structure on mobility of PCB cogeners in female and male sardine (*Sardina pilchardus*) from Portuguese coast. Chemosphere 69: 395-402.

Anonymous, 1998. Guidelines for the treatment of animals in behavioral research and teaching. Animal Behavior. 55: 251-257.

Begum, G. 2005. In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure. Pesticide Biochemistry and Physiology. 82(3): 185-196.

Bruning, JL. and Kintz, BL. 1977. Computational Handbook of Statistics, Second Edition, Freeman, Chicago, IL, USA.

Chandra, S., Ram, RN. and Singh, IJ. 2004. First ovarian maturity and recovery response in common carp *Cyprinus carpio* after exposure to carbofuran. Journal of Environmental Biology. 25(3): 239-249.

Dale, WE., Copeland, MF. and Hayes, WJ Jr. 1965. Chlorinated insecticides in the bodies of peoples in India. Bulletin of World Health Organisation. 33: 471-477.

Ferreira, M., Antunes, P., Gil, O., Vale, C. and Reis-Henriques, MA. 2004. Organochlorine contaminants in flounder (*Platichthys flexux*) and mullet (*Mugil cephalus*) from Douro estuary, and their use as sentinel species for environmental monitoring. Aquatic Toxicology. 69 (4): 347-357.

Hilmy, AM., Badawi, HK. and Shabana, MB. 1983. Organochlorine pesticide residues in 12 freshwater Egyptian fish species with special emphasis on *Anguilla vulgaris* and *Mugil cephalus*. Comparative Biochemistry and Physiology. 76C: 163-171.

Kannan, K., Tanabe, S. and Tatsukawa, K. 1995. Geographical distribution and accumulation features of Organochlorine residues in fish in tropical Asia and Oceania. Environmental Science and Technology. 29: 2673-2683.

Kime, DE. and Singh, PB. 1996. *In vitro* effects of γ-hexachlorocyclohexane on *in vitro* biosynthesis and metabolism of steroids in goldfish, *Carassius auratus.* Ecotoxicology and Environmental Safety. 34(2): 165-173.

Kumari, A., Sinha, RK. and Gopal, K. 2001. Quantitative estimation of DDT residues in some freshwater fishes of river Ganga at Patna, Bihar. Environment and Ecology. 19(2): 396-399.

Letherland, JF. and Sonstegard, RA. 1982. Bioaccumulation of organochlorines by yearling coho salmon (*Oncorhychus kisutch* Walbaum) fed diets containing Great Lakes coho salmon, and the pathophysiological responses of the recipients. Comparative Biochemistry and Physiology. 72C: 91-99.

Mills, LJ., Gutjahr-Gobell, RE., Haebler, RA., Horowitz, DJB., Jayaraman, S., Pruell, RJ., McKinney, RA., Gardner, GR. and Zaroogian, GE. 2001. Effect of estrogenic (*o,p′*-DDT; octylphenol) and antiandrogenic (*p,p′*-DDE) chemicals on indicators of endocrine status in juvenile male summer flounder (*Paralichthys dentatus*). Aquatic Toxicology. 52 (2): 157-176.

Lino, CM. and Noronha da Silveira, MI. 1994. Chlorpyriphos, ethion, fenitrothion and methidathion residues in chicken, Bulletin of Environtal Contamination and Toxicology. 52: 425-431.

Saqib, TA., Naqvi, SNH., Siddiqui, PA. and Azmi, MA. 2005. Detection of pesticide residues in muscles, liver and fat of 3 species of *Labeo* found in Kalri and Haleji lakes. Journal of Environmental Biology. 26 (2): 433-438.

Sahagun, AM. Tern, MT., Garcia, JJ., Sierra, M., Fernandes, N. and Fiz, MS. 1998. Organochlorines pesticide residues in muscles tissues of rainbow trout *Onchorhynchus nukiss* taken from four fish farms in Leon, Spain. Food Additive and Contamination. 15 (5): 501-505.

Sarkar, B., Chatterjee, A., Adhikari, S. and Ayyappan, S. 2005. Carbofuran-and cypermethrin-induced histo-pathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. Journal of Applied Ichthyology. 21(2): 131-135.

Singh, PB**.** 1992. Impact of malathion and γ-BHC on lipid metabolism in the freshwater female catfish, *Heteropneustes fossilis***,** Ecotoxicology and Environmental Safety. 23: 22-32.

Singh, PB. and Singh, TP. 1992. Impact of malathion and γ-BHC on steroidogenesis in the freshwater catfish, *Heteropneustes fossilis*. Aquatic Toxicology. 22: 69-80.

Singh, PB. and Singh V. 2007. Exposure and recovery response of isomers of HCH, metabolites of DDT and estradiol-17β in the female catfish, *Heteropneustes fossilis*. Environmental Toxicology and Pharmacology, 24: 245-251

Singh, PB., Kime, DE., Epler, P. and Chyb, J. 1994. Impact of γ-hexachlorocyclohexane exposure on plasma gonadotropin levels and *in vitro* stimulation of gonadal steroid production by carp hypophyseal hemogonate in *Carassius auratus*. Journal of Fish Biology. 44(2): 195-204.

Singh, N., Shukla, MM., Chand, SK. and Sharma, VP. 1997. Outbreak of falciparum malaria in submerged villages of Narayanganj PHC, district Mandla due to Narmada irrigation Project, Central India (Madhya Pradesh). Current Science. 73: 689-691.

Singh, PB. and Canario, AVM. 2004. Reproductive endocrine disruption in the freshwater catfish, *Heteropneustes fossilis*, in response to the pesticide γ–hexachlorocyclohexane. Ecotoxicology and Environmental Safety. 58(1): 77-83.

Smita, C., Ram, RN. and Singh, IJ. 2003. Testicular recrudescence and recovery response in *Cyprinus carpio* after long term exposure to carbamate pesticide. Journal of Ecophysiology and Occupational Health. 3(1&2): 15-36.

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.

Singh, PB., Kime, DE. Epler, P. and Chyb, J. 1994. Impact of γ-hexachlorocyclohexane exposure on plasma gonadotropin levels and *in vitro* stimulation of gonadal steroid production by carp hypophyseal homogenate in *Carassius auratus*. Journal of Fish Biology. 44(2): 195-204.