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The Analysis Of Chlorinated Pesticides In Blood And Muscles Tissues Of Some Fresh Water Fishes

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Abstract: ***The residues of such pesticides even in trace quantities are biomagnified through food chain which causes unprecedented damage to the ecological system. Fishes being end consumers in aquatic food chain highly affected with these chemicals. These chemicals are also toxic to fishes itself as well as to consumers of fishes in food chain.***

Keywords: Pesticides, Blood, Muscles, Fresh Water Fishes, Disease.

I. INTRODUCTION

Pesticides are chemical weapons against agricultural pests and vector-borne diseases and are commonly called as ECONOMIC-POISONS. At present the total annual use of pesticides in India is 1, 20,000 M.T. (Narsimharao, 1995). There are three major groups of pesticides namely, ORGANOCHLORINE-COMPOUNDS (OCC), ORGANO-PHOSPHORUS COMPOUNDS (OPC) and CARABAMATE-COMPOUNDS (cc) (Dishit, 1991). DDT (dichloro diphenyl trichloroethane) and HCH (1,2,3,4,5,6, hexachlorocyclohexane) are two major organochlorine pesticides while account for two third of the total consumption because they are cheap, easy to handle and can control a wide range of pests, out of which HCH contributes about 55% (Due et al.,1994).

On releasing these chemical result toxic concentration to target organisms as well as on non-target organisms too. Due to the classical stability and long persistence nature of these chemicals in the environment, their residues through run off water from agricultural land enter the aquatic eco-system. Hence aquatic ecosystem is more sensitive to chlorinated pesticides pollution than terrestrial eco-system (Kenago, 1972). The residues of such pesticides even in trace quantities are biomagnified through food chain which causes unprecedented damage to the ecological system (Edwards, 1973). Fishes being end consumers in aquatic food chain highly affected with these chemicals. These chemicals are also toxic to fishes itself as well as to consumers of fishes in food chain. Krishnagopal (1993) noted haemato biochemical disorders including hyperglycemia, hyperlactemia, leaukopenia, reduced blood clotting time and impaired swimming performance in fishes, when exposed to pesticides. DDT and HCH (BHC) act on fishes as a neural poison (Metelev et al., 1971).

Through food chain these pesticides residues reach to human body where they produce neurological, reproductive, carcinogenic and genotoxic effects (Gahakar, 1992). Consumption of crabs and fishes from fields sprayed with pesticides resulted in congenital dwarfism in 300 persons in KARNATAKA (SHINGOA and CHIKMANGLUR DISTRICTS) in 1975 (Gahakar, 1992). Global concern on pesticides became more and more evident with the identification of pesticides residues in air, water and food products, even in the tissue of the unborn child (Bami, 1992).

Several studies from India have shown the occurrence of DDT and HCH residues in, marine fishes (Rajendran et al.,1992) soil (Pillai. 1986), water (Bakre et al., 1990; Srivastava, 1993A; and Due et al., 1996), air (Kaushik et al., 1987), whole blood (Bhatnagar et al., 1992) and bovine milk (Battu et al., 1989; and Verma, 1990). Reports are also available on DDT and HCH contents in cereals and cereal products, pulses, vegetables, fruits, edible oils, butter, Ghee, mother's milk and various animal products from various places of India (Narsimha rao, 1995).

Bio-monitoring forms an important step in ecotoxicological studies. Because of the regular use of these pesticides in India the biological monitoring of their residues offers criterion to assess the magnitude of potential risk if any to the general population (Naik et al., 1995). Fishes represent

the highest tropic level in aquatic food chain hence they can be used as best indicators of environmentally persistent organochlorine compound in the fresh water environment. Studies on chlorinated pesticide residues contents in fresh water fishes have been reported by Kaphalia et al., (1986), Nair and Pillai (1992), Sharma et al., (1995) and Sharma and Dhakad (1995) etc. in India and abroad by Armodio and Arnese (1988), Mason (1993), Srivastava and Kumar (1982), and Bharnagar et al., (1992) have reported on chlorinated pesticide residues levels in blood of different organisms. However it appears from the literature that no attempt has been made to study the level of chlorinated pesticides (DDT and HCH) residues in blood and muscle tissue (edible portion) of fresh water fishes of district Raebareli. Hence the present studies have been undertaken.

The main objective of the present investigation was to study the extent of contamination by organochlorine compounds in food fishes of fresh water origin.

II. MATERIALS AND METHODS

A. CHEMICAL AND REAGENTS

All chemical and reagents were of analytical grade, Glass distilled n-hexane and deionized water was used. Standards were obtained from polyscience corporation, Niles, Illinois (U.S.A.)

B. EQUIPMENTS

Centrifuge Remi T

Thin layer chromatographic apparatus (TLC). Gasliquid Chromatograph (GLC). Varian Aerograph, series 2400 equipped with electron capture detector (ECD) Ni^{63}.

C. COLLECTION OF SAMPLES

To study DDT and HCH residues concentration in blood and muscle tissue (edible portion), two fishes of high food value were selected belonging to two chief fresh water fishes groups; 1- *Catla Catla* (herbivorous) from major carps, 2- *Mystus Tengara* (Omnivorous) from cat fishes. Fishes were trapped by various means from Hathinasa pond (Block-Sareni). Blood samples were collected directly from heart puncture by disposable syringe in heparinized vials.

D. EXTRACTION AND CLEAN UP PROCESS

Extraction and clean up processes were carried out according to the method followed by Mills(1961), Kaphalia & Seth (1981) and Stefen and Hrzegorz (1991).

E. EXTRACTION OF BLOOD

- ✓ Pipit 2 ml. of blood in 25 ml of graduated stoppered tube.
- ✓ Added 2 ml. of acetic acid and left for half an hour except for occasional shaking.
- ✓ Extracted with 10 ml portion of n-hexane (three times) in 125ml. separating funnel.
- ✓ The collected hexane layer was extracted with 2ml. of distl. water to remove acid. Collected the acid free hexane layer in 50ml. conical flask.

F. EXTRACTION OF MUSCLE TISSUE

- ✓ Weighted accurately 0.5gm of tissue on an aluminum foil and put it in a glass homogenizer. Added to this 7 ml. of formic acid and 5.0 ml. of n-hexane.
- ✓ Homogenized the tissue using the pestel in a rotatory motion by motorized homogenizer.
- ✓ Transferred the homogenized mixture in 50ml. conical flask, rinse homogenized tube and pestel twice with 20ml. of n-hexane.
- ✓ Shaked the content of conical flask on a water bath shaker for 1 hour at 40°C.
- ✓ Decanted the hexane layer in another 100ml. conical flask.
- ✓ Re-extracted twice by adding 20ml. of n-hexane followed by shaking for 45 minutes.
- ✓ Transferred the hexane layer to next conical flask.

G. DRYING AND DIMINISHING

During liquid extraction some traces of water or moisture goes along with organic solvent, which was removed by passing through the bed of anhydrous sodium sulphate.

H. CONCENTRATION

The dried hexane extract was evaporated on reduced pressure upto1 to 0.5ml. It was finally transferred to 5 or 10ml. volumetric flask and made up the volume with n-hexane.

I. CLEAN UP

In some cases particularly in case of tissue it requires clean up.

- ✓ Took 5ml. portion of n-hexane extract and added 2ml. of AR grade sulphuric acid (Conc.).
- ✓ Vortex for two minutes.
- ✓ Centrifuged the tube at 3000 rpm in cold centrifuge.
- ✓ Recovered the upper layer for direct application on GLC.

J. CONDITION FOR GLC

Column: 6'×48'' (S.D.) with 80-100 mesh gas chrome coated with a mixture 1.5%, OV-17 and 1.95%, OV-210 by weight.

Temp. : 180°

Injector: 250°C

Detector: ECD Ni^{63}, Temperature 250°

Carrier gas: IOL AR grade Nitrogen (60ml/min.)

This study was conducted in April 2013, and gas liquid chromatography was done at I.T.R.C., Lucknow.

III. OBSERVATIONS

Our finding for chlorinated pesticides residues in blood and muscle tissue in fresh water fishes in district Raebareli, (U.P.) have been presented in table-1 and 2.Table-1 defined the mean residue concentration of total DDT and their metabolites (P, P' DDF, P, P' DPD, P, P' DDT) in blood and muscle.Table-2 defines the mean residue concentration to total HCH and their metabolites (Alfa-HCH, Gama-HCH and Bita-HCH) in blood and muscles.

All the sample showed the presence of P' p' DDE, P, P' DDD (except in blood sample of *Catla Catla*), P, P' DDT, α-HCH, γ-HCH and β-HCH in varying quantities. In *Catla Catla*, P, P' DDD in blood did not appeared within detectable limits in sample analyzed.

PISCES SPS.	***SAMPLE***	***P.P' DDE***	***P,P' DDD***	***P,P' DDT***	***Σ DDT***
Labeo rohita	Blood Muscle	0.004 ± 0.0002 0.006 ± 0.0001	BDL 0.002 ± 0.001	0.012 ± 0.004 0.026 ± 0.003	0.019 ± 0.003 0.039 ± 0.005
Clarias batrachus	Blood Muscle	0.006 ± 0.0006 0.024 ± 0.003	0.004 ± 0.001 0.029 ± 0.0005	0.034 ± 0.004 0.109 ± 0.012	0.043 ± 0.002 0.166 ± 0.010
Channa striatus	Blood Muscle	0.009 ± 0.002 0.033 ± 0.006	0.002 ± 0.0004 0.023 ± 0.004	0.046 ± 0.005 0.131 ± 0.016	0.059 ± 0.003 0.191 ± 0.014
Grand Mean	Blood Muscle	0.0063 0.0210	0.0020 0.0180	0.0306 0.0886	0.040 0.132

* All values are in PPM at wet weight basis and are mean ± S.E. of 3 observation.
* BDL = Below detectable limit

Table 1: Mean residue levels of DDT and its isomers in the blood and muscle tissue of fresh water fishes of District Rae Bareli

PISCES SPS.	***SAMPLE***	***α - HCH***	***γ - HCH***	***β - HCH***	***ΣHCH***
Labeo rohita	Blood Muscle	0.058 ± 0.007 0.104 ± 0.012	0.014 ± 0.004 0.039 ± 0.013	0.006 ± 0.001 0.044 ± 0.009	0.083 ± 0.014 0.193 ± 0.020
Clarias batrachus	Blood Muscle	0.043 ± 0.010 0.093 ± 0.011	0.014 ± 0.004 0.028 ± 0.005	0.017 ± 0.003 0.021 ± 0.006	0.073 ± 0.007 0.144 ± 0.018
Channa striatus	Blood Muscle	0.017 ± 0.004 0.039 ± 0.006	0.008 ± 0.002 0.016 ± 0.005	0.009 ± 0.001 0.017 ± 0.004	0.035 ± 0.004 0.077 ± 0.008
Grand Mean	Blood Muscle	0.0393 0.0786	0.0120 0.0277	0.0106 0.0273	0.636 0.1380

* All values are in PPM at wet weight basis and are mean ± S.E. of 3 observation.

Table 2: Mean residue levels of HCH and its isomers in the blood and muscle tissue of fresh water fishes of District Rae Bareli

A. TOTAL DDT

The range of total DDT in examined fishes was observed (table-1) 0.019 ± 0.003 ppm in blood and 0.039 ± 0.005 ppm in muscles. The estimated mean residue concentration of total DDT in *Catla Catla*, and *Mystus Tengara*, were 0.019 ± 0.003, 0.043 ± 0.002 ppm in blood while these were 0.039 ± 0.005, and 0.166 ± 0.010 ppm in muscle respectively. Grand mean of total DDT was 0.040 ppm in blood and 0.132 ppm in muscle, of all samples. The trend of accumulation of total DDT in fishes was *Mystus Tengara* (Omnivorous > *Catla Catla* (herbivorous).

B. P'P'DDE

During present study it was recorded (table-1) between, 0.004 ±0.002 ppm and 0.006±0.0006 in blood samples and 0.006 + 0.001 ppm and 0.024± 0.003 to recorded in muscle of *Mustus Tengara*. Grand mean of P'P'DDE was 0.0063 ppm in blood and 0.0210 ppm in muscles, of all samples. It's observed grand mean concentration in present study was lower than P'P'DDT isomer but was high than P'P'DDD isomer.

C. P'P DDD

Estimated values of this isomer (table-1) ranged between BDL to0.002±0.001 in blood and 0.002 ± 0.001 ppm to 0.029 ± 0.0005 ppm in muscle. Highest concentration (0.029 + 0.0005 ppm) was noted in muscle of *Mustus Tengara*. Grand mean of P'P DDT was 0.0020 ppm in blood and 0.0180 ppm in muscles of all sample fishes. It's observed grand mean concentration in present study was lower than rest both studied isomers, in blood and muscle both.

D. P'P DDT

It was recorded (table-1) between, 0.012 ± 0.004 ppm to 0.034±0.004 in blood and 0.026 ± 0.003 to 0.109±0.012 ppm in muscle. Highest concentration (0.109 ± 0.012 ppm) was observed in muscle of *Mustus Tengara*. Grand mean of P'P'DDT in blood and muscle was 0.0306 ppm 0.0886 ppm respectively. This isomer was greater in quantity among DDT blood and muscles both. The trend accumulation of studied

DDT isomers was P'P' DDT> P'P' DDE > P'P' DDD in all samples.

E. TOTAL HCH

Total HCH was recorded (Table-2) in between, 0.083 ± 0.014 to 0.073±0.007 ppm in blood and 0.193 ± 0.020 to 0.144±0.018 ppm in muscle samples. Peak level of 0.193 ± 0.020 ppm was noted residue concentration in *Catla Catla*. The estimated values of HCH residues concentration in *Catla Catla*, and *Mustus Tengara* were 0.083 ± 0.14, and 0.073 ± 0.007 ppm in blood and 0.193 ± 0.020, and 0.144 ± 0.018 ppm in muscles respectively. The grand mean of total HCH was 0.0636 ppm in blood and 0.1380 ppm in muscles. The trend of accumulation of total HCH residues in fishes was *Catla Catla>Mustus Tengara*. However grand mean of total HCH was greater than those of total DDT in blood and muscles both.

F. α -HCH

It was recorded (table-2) in between 0.058±0.007 to 0.043±0.010 ppm in blood and 0.104 ±0.012 to 0.093±0.011 ppm in muscles; highest concentration (0.104 ± 0.012 ppm) was noted in muscles of *Catla Catla*. Grand mean of α-HCH was 0.0393 ppm in blood and 0.0786 ppm in muscles. α- HCH was greater among HCH in blood and muscles both in all sample fishes. The mean trend of accumulation of studied HCH isomers was α– HCH > γ- HCH > β - HCH in all samples.

G. γ-HCH (LINDANE)

It was recorded (table-2) in range from 0.014 ± 0.004 to 0.014±0.004 ppm in blood and from 0.039 ± 0.013 to 0.028±0.005 ppm in muscles. Peak level of 0.039 ± 0.013 ppm was noted in muscle of *Catla Catla*. Grand mean of γ-HCH was 0.023 ppm in blood and 0.046 ppm in muscle of all studied fishes.

H. β- HCH

It was estimated (table-2) in range from 0.006 ± 0.001 ppm to 0.017 ± 0.003 ppm in blood samples and from 0.044 ± 0.009 ppm to 0.021±0.006 ppm in muscle samples. Highest concentration (0.044 ± 0.009 ppm) was observed in the muscles of *Catla Catla* Grand mean of β-HCH was 0.0106 ppm in blood and 0.0273 ppm in muscles.

IV. DISCUSSION

The gas liquid chromatographic investigation revealed significant residual concentrations of DDT and HCH with their examined metabolites in fresh water fishes of district Raebareli. However total HCH concentration was greater than those of total DDT. This might be due to that DDT spraying by district health department for disease vector control in this area has been not done for last several years but the use of HCH on fruits, vegetables crops is continue indiscriminately which is resulting in the increasing level of HCH residues than those of DDT. According to Bami (1992), "use of DDT in agriculture and HCH use on fruits, vegetables, food grains have been banned in India" but in this area HCH (BHC/Gammexene) is commonly said and used as one medicine for all plant diseases. The values of total DDT and total HCH levels observed in the blood samples of present study were when compared with those of terrestrial animals; Uromastix (herbivorous, ∑DDT-BDL, ∑HCH-0.004 ppm) and Varanus (carnivorous, ∑DDT-0.034 ppm, ∑HCH-0.031 ppm) as reported by Srivastava and Kumar (1982) than it was concluded that fishes (aquatic fauna) having higher values for the same are more sensitive to chlorinated pesticides residues than reptiles (terrestrial fauna). This conclusion accord fairly well with those of Kenago (1972) who were of the same opinion.

Concentration of total DDT in the muscle of fishes of the present study are lower than those of the fishes of district Indore (M.P.) as recorded by Sharma et al. (1995) (*Channa* sps. -0.531ppm, *C. batrachus* -0.375 ppm, *L. rohita*-0.098 ppm) but are higher than those of fishes of river GOMTI (Lucknow, U.P.), *Channa* sps. -0.085 ppm, C. batrachus -0.048 ppm, L. rohita -0.022 ppm) as reported by Kaphalia et al. (1986).

The trend of accumulation of total DDT in fishes in present study was *C. striatus (Carnivorous)> C. batrachus (Omnivorous) > L. rohita (herbivorous*) which followed the same pattern as reported by Kaphalia et.al. (1986) and Sharma et.al. (1995), Joshi P. (2002) .This might be due to that carnivorous fish represent the highest trophic level in aquatic food chain. The food of carnivorous organism might have contained significant amount of pesticides residual concentration which may be a main source of chlorinated pesticides residues in them (Srivastava, 1983, Joshi Namita,2007).

Total HCH concentration in the muscles in the muscles of the fishes of present study are higher than those of fishes of river GOMATI as reported by Kaphalia et.al.(1986) Khalaf-Allaha, S. S., (1999) , (Channa sps. -0.019ppm, C.batrachus -0.042ppm, L. rohita-0.04ppm).However DDT and HCH concentration of present study are below the tolerance limit in food as mentioned by Chadha (1992).

Under Indian conditions there is need for continuous national surveys for pesticides residues in food and dietary intake survey in various regions of India Chandrasekhara Rao A and Krishnan L**.** (2011) , Khalaf-Allaha, S. S., (1999) Koteswara Rao D (2003): . It is generally accepted that higher pesticides residues in food are more often due to excessive use of pesticides and /or their application without following the prescribed norms. It is therefore imperative that choice of pesticide and their use pattern must receive equal importance (Bami, 1992).

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