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Organochlorine Pesticide Residues in Inland Wetland Fishes of Karnataka, India and Their Implications on Human Dietary Intake

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Abstract As part of a larger study assessing contamination status of inland wetlands of India, we evaluated the organochlorine pesticide (OCP) residues in fishes collected from different inland wetlands in Karnataka, India and their suitability for human consumption. Among the OCPs tested, isomers of hexachlorocyclohexane (HCH) were the most frequently detected with β- and γ-HCH as the main pollutants. Average concentration of $\sum$HCH and $\sum$DDT ranged from 2.1 to 51.7 µg/kg and below detection level to 12.3 µg/kg respectively. Other organochlorine pesticides such as heptachlor epoxide, dieldrin and endosulphan were found at lower levels. Among various fish species tested, the higher pesticide burden was recorded in *Anguilla bicolor bicolour* (77.9 µg/kg) and the lower was in *Heteropneustes fossilis* (2.1 µg/kg). OCPs detected in the present study were well below the tolerance limits recommended for fishes. The calculated daily dietary intake of OCPs in all the species examined was lower than the maximum acceptable dietary intake (ADI) limits prescribed for human consumption. However, continuous monitoring is recommended to facilitate the early identification of risks.

Keywords Fish · Human · Daily dietary intake · Organochlorine pesticides · India

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Organochlorine pesticides are still in use in developing countries despite their ban in many countries due to their associated problems of indiscriminate potency and persistency. The chemical stability of these compounds, their high lipid solubility and toxicity to human and animals (Aulakh et al. 2006), has led government and researchers to be concerned with their presence in the environment. Organochlorines continue to be the potential group of chemicals used in control of agricultural pests and vectors of diseases like malaria (David et al. 2003), even though many new broad spectrum pesticides have been developed in recent years. The pesticides applied on land eventually find their way to the aquatic environment, thus contaminating soil and water for several years and subsequently get accumulated in aquatic organisms (Singh et al. 2005). The aquatic organisms like fish are able to accumulate several fold higher concentration of pesticide residues than the surrounding water (Siddiqui et al. 2005). It has been found that greater than 80% of the total intake of pesticide residues in human beings is through the food chain (Martinez et al. 1997) and considerable amount of residues find their way into humans through consumption of contaminated fishes (Mwevura et al. 2002). Studies have also related the presence of organochlorine residues in breast milk and consumption of contaminated fish, and their role in alteration of thyroid function (Hagmar et al. 2001).

India is now both the largest manufacturer and consumer of pesticides in South Asia. Despite the proliferation of different types of pesticides, organochlorines such as hexachlorocyclohexane (HCH) and DDT still account for two-thirds of the total consumption in the country (Kumari et al. 2001) for agriculture and public health purposes respectively. In India as most of the rivers pass through agricultural fields, they are subjected to contamination with different pesticides used for crop protection. Detectable levels of organochlorine pesticides are being reported to occur in Indian environment and it is estimated that about 25 tonnes of organochlorines produced have been already transported to the sea (Mohapatra and Saha 2000).

Fish are valuable source of high-grade protein and occupy an important position in the socio-economic conditions of South Asian countries. India is the third largest producer of fish and second in inland fish production (Feroz and Panikkar 2006) with annual production of 7.75 million tonnes. In India there are many studies on the presence of organochlorine residues in aquatic system; water and fishes (Sarkar et al. 2003) freshwater fish (Kaur et al. 2008) and marine fish (Muralidharan et al. 2009). Studies done by Kole et al. (2001) reported the presence of endosulfan and HCH residues in fishes sold at Calcutta market. But information available on the dietary intake of persistent organochlorine pesticide residues through consumption of wetland fishes to human beings is scarce in literature. As part of a larger study assessing contamination status of inland wetlands of India, the present study was aimed at assessment of OCPs in select species of fishes in inland wetlands of Karnataka; and evaluation of the potential exposure to human through the consumption of these fishes. Karnataka is south western state of India which has great potential for integration of inland wetland fishes. Although more than 15 species reported in this state, nine species of fish which are commonly available in all the wetlands were chosen for the study.

Materials and Methods

During 2002–2003, 156 fishes were collected from ten different inland wetlands (1. Tumkur Ammani Kere, 2. Bannur Heggere, 3. Karigala Kere, 4. Cauvery river stretch, 5. Krishnaraj Sagar Reservoir, 6. Mandhakhalli Kere, 7. Marchalli Kere, 8. Ravanduru Kere, 9. Samunder Talab and 10. Tailur Tank) in Karnataka. All samples were cleaned off external dirt, labelled and packed in clean polythene bags, transported on ice to the laboratory at Sálim Ali Centre for Ornithology and Natural History, Coimbatore and stored in deep freezer at −20°C till the dissection was carried out. Morphometric measurements such as body length and weight were recorded at the collection spot. The common name, size and weight of species included in the study are given in Table 1. Although the vital organs such as gill, kidney and liver are sensitive to pesticide accumulation, muscle forms the major edible portion in the fish. Therefore, muscle tissue alone was analyzed as the objective is to determine the dietary intake to human body.

The fish samples were thawed, cleaned in ultra pure water, and scales sloughed off. Muscle tissue was dissected between the pectoral fin and vent of the fish, minced into smaller pieces and a subsample was taken from the homogenate. About 10 g of muscle tissue was weighed, ground with anhydrous sodium sulphate and the mixture was packed in a thimble (Whatman) and desiccated overnight prior to extraction. The desiccated thimble was loaded and extracted in Soxhlet apparatus with 250 mL of pesticide grade hexane (Merck) for 6 h. The extract was condensed in a rotary flask evaporator to a specific aliquot (5 mL). The aliquot was then subjected to acid treatment by adding 2 ml of concentrated sulphuric acid then neutralized with saturated sodium hydroxide solution. Further the sample was cleaned by placing on a glass column packed with silica gel (60–120 mesh) and eluted with 250 ml of hexane. The eluate was again condensed in a rotary flask evaporator to near dryness and residues of OCPs were reconstituted in 2 mL of hexane. Processed samples were stored in deep freezer at −20°C till the gas chromatography analysis.

The solvents (Merck) used were glass distilled before use and they did not yield any interfering gas chromatographic peak when concentrated from 10 mL to 100 µL. Samples were analysed with Hewlett-Packard 5890 Series II gas chromatograph equipped with Ni^{63} electron capture detector and DB-608 fused silica capillary column (30 m × 0.32 mm × 0.5 µm) coated with 35% phenylmethyl polysiloxane. Chromatographic conditions were as follow: detector 300°C; injector 250°C; oven temperature was programmed as 180°C 3 min; 4°C/min to 260°C 15 min. All the samples were analysed for alpha-hexachlorocyclohexane (α-HCH), *β*-HCH, *δ*-HCH, lindane (*γ*-HCH), heptachlor epoxide (HE), dieldrin, *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, α-endosulfan, *β*-endosulfan and endosulfan sulphate. An equivalent mixture provided by Dr. Ehrenstorfer Laboratories (Germany) was used as the standard. The concentrations of the individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (50 ppb) ranged from 94 to 103% and the results were not corrected for percent recovery. All the results were expressed in wet weight basis. Analyses were run in batches of 10 samples plus four quality controls (QCs) including one reagent blank, one matrix blank, one QC check sample and one random sample in duplicate. The minimum instrument detection limit for all the compounds analysed was 1 µg/kg.

Results and Discussion

The concentrations of OCPs and their frequency of detection in the nine species of wetland fishes analyzed are summarized in Table 2. Among the organochlorines analyzed, HCH was the most frequently detected (100%) and

Table 1 List of fish species included in the present study

Common name	Scientific name	N	Mean (range)	
			Size (cm)	Weight (g)
Short-finned eel	*Anguilla bicolor bicolor*	21	37.7 (30.0–50.5)	121.2 (70–215)
Catla	*Catla catla*	11	27.9 (15.0–35.0)	372.7 (160–580)
Stripped snakehead	*Channa striatus*	11	20.5 (13.9–31.0)	142.3 (40–390)
Indian major carp	*Cirrhinus mrigala*	6	29.5 (19–37.5)	294.2 (65–500)
Walking catfish	*Clarias batrachus*	9	25.7 (15.8–32)	175.3 (32–300)
Common carp	*Cyprinus carpio*	22	21.0 (13–33)	191.5 (48–520)
Stinging catfish	*Heteropneustes fossilis*	11	20.7 (16.8–26)	71.0 (29.5–230)
Rohu	*Labeo rohita*	19	27.5 (18–45.5)	319.5 (65–1000)
Tilapia	*Tilapia mossambica*	46	17.0 (12–24.5)	101.3 (20–215)
Total		156		

one or more derivatives of DDT were detected in 67.9% of fishes. Among the cyclodiene pesticides, HE, total endosulfan and dieldrin were detected in 41, 22.4 and 21.8% of the samples analyzed respectively. The maximum levels of total HCH (51.7 µg/kg) and total DDT (12.3 µg/kg) were detected in *Anguilla bicolor bicolor*. The present study indicates that contamination of all fish species by one or metabolites of HCH or DDT. As far as the total pesticide residue burden is considered in different species, *Anguilla bicolor bicolor* had the maximum levels of OCPs (77.9 µg/kg) followed by *Labeo rohita* (44.5 µg/kg) and *Clarias batrachus* (42.1 µg/kg) (Fig. 1). The lowest pesticide burden was detected in *Heteropneustes fossilis* (2.1 µg/kg). Species-specific differences and lipid content of the fishes also influence the variation in accumulation pattern of organochlorine residues among the species.

The concentrations of β- and γ-HCH were the most dominated among isomers of HCHs. The predominance of occurrence of β-HCH, the most persistent form is due to the isomerisation of α- and γ-HCH into β-HCH and also due to its high stability. Presence of high concentrations of β-HCH has been reported in various biological components (Kumari et al. 2001; Kole et al. 2001). Although the production of technical HCH is being phased out, the annual production of γ-HCH in India increased from 40 metric tonnes in 1996–1997 to 250 metric tonnes in 1997–1998. The HCH levels in this study higher than the concentrations reported in freshwater fishes of Andhra Pradesh (2 µg/kg; Amaraneni and Pillala 2001) and Punjab (7 µg/kg; Kaur et al. 2008). Earlier study conducted by Kumari et al. (2001) also reported high concentration of HCH in fish and food products and possibilities of higher dietary intake of pesticides through fishes. Although no major ill effects in man have been correlated with the levels of HCH, altered thyroid function was reported in women (Hagmar et al. 2001).

Among the metabolites p,p'-DDT, p,p'-DDE had significantly higher burden in fishes. The high frequency of occurrence of p,p'-DDE can be due to its past use and the residues brought from surrounding areas. India banned DDT for agricultural purposes in 1989, but continues to use for malaria control. The presence of p,p'-DDT and its metabolites in fish species indicates its recent exposure at the minimum levels. Moreover, the conversion of p,p'-DDT to p,p'-DDE could be due to the activity of the mixed function oxygenase enzyme through metabolic functions. Concentrations of p,p'-DDE found in our study are higher with the levels reported in fishes of Ganges Estuary, Bangladesh (1–2 µg/kg) (Jabber et al. 2001). Further, the total DDT was lower than the levels reported in fishes (72.6–1666 µg/kg) from Ganga River, India (Anupma et al. 2001) and comparable with the levels reported in freshwater fishes of Punjab (Kaur et al. 2008).

The cyclodiene pesticides (endosulfan, HE and dieldrin) were found to be the minimum in all the fish. The presence of chlorinated cyclodiene pesticide in fish is partly due to their past use. It may be noted that endosulfan is being widely used in India. The concentrations of endosulfan were lower than the levels reported in Calcutta (Kole et al. 2001). These levels are also lesser than the levels that could produce histopathological changes in exposed gills (Khare et al. 2002). Similarly, concentrations of HE in the present study were lower than the concentrations reported (110 ppb) in the fishes of the Ganges Estuary, Bangladesh (Jabber et al. 2001).

The dietary intake of organochlorine pesticides was calculated by multiplying the pesticide concentrations measured in each species of fish by the per capita consumption. The previous study by Muralidharan et al. (2009) reported the average per capita consumption of 47 g fishes per day at Coimbatore, South India. In the present study, the daily dietary intake of all the organochlorine pesticides through consumption of these species are less than the acceptable dietary intake (ADI) recommended by various agencies [IARC (International Agency for Research on

Table 2 Average residue levels (µg/kg, wet wt.) of OCPs in different inland wetland fishes of Karnataka, India

Scientific name	N	α-HCH	β-HCH	γ-HCH	δ-HCH	∑HCH	HE	∑Endosulfan	Dieldrin	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	∑DDT
Anguilla bicolor bicolor	21	10.6 (52.4)	23.1 (90.5)	11.1 (85.7)	6.9 (28.6)	51.7 (100)	10.8 (57.1)	3.1 (47.6)	BDL	4.9 (80.9)	4.8 (52.4)	2.6 (47.6)	12.3 (90.5)
Catla catla	11	BDL	2.5 (72.7)	3.7 (63.6)	BDL	6.2 (81.8)	2.6 (54.5)	BDL	BDL	5.9 (72.7)	BDL	BDL	5.9 (72.7)
Channa striatus	11	2.8 (54.5)	1.9 (81.8)	2.7 (72.7)	1.9 (36.4)	9.3 (100)	2.8 (36.4)	4.3 (54.5)	BDL	4.1 (54.5)	BDL	BDL	4.1 (54.4)
Cirrhinus mrigala	6	1.6 (50)	2.8 (83.3)	1.5 (66.7)	BDL	5.9 (83.3)	BDL	2.3 (50)	1.6 (16.7)	3.4 (83.3)	BDL	BDL	3.4 (83.3)
Clarias batrachus	9	4.1 (44.4)	11.7 (77.8)	8.7 (77.8)	2.7 (33.3)	27.2 (100)	6.4 (55.6)	4.1 (44.4)	4.4 (33.3)	BDL	BDL	BDL	BDL
Cyprinus carpio	22	BDL	3.9 (72.7)	1.2 (54.5)	BDL	5.1 (86.4)	2.2 (40.9)	1.8 (36.4)	BDL	3.8 (72.7)	2.9 (27.3)	1.4 (22.7)	8.1 (81.8)
Heteropneustes fossilis	11	BDL	2.1 (54.5)	BDL	BDL	2.1 (54.5)	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Labeo rohita	19	4.8 (57.9)	14.1 (78.9)	5.9 (31.6)	BDL	24.8 (94.7)	6.7 (57.9)	3.7 (21.1)	2.7 (52.6)	3.9 (78.9)	BDL	2.7 (47.4)	6.6 (78.9)
Tilapia mossambica	46	3.7 (39.1)	7.5 (82.6)	2.4 (73.9)	2.5 (32.6)	16.1 (100)	4.6 (38.8)	BDL	2.7 (43.5)	4.4 (76.1)	BDL	2.5 (43.5)	6.9 (76.1)

Figure in parentheses indicates frequency of detection

BDL below detection limit

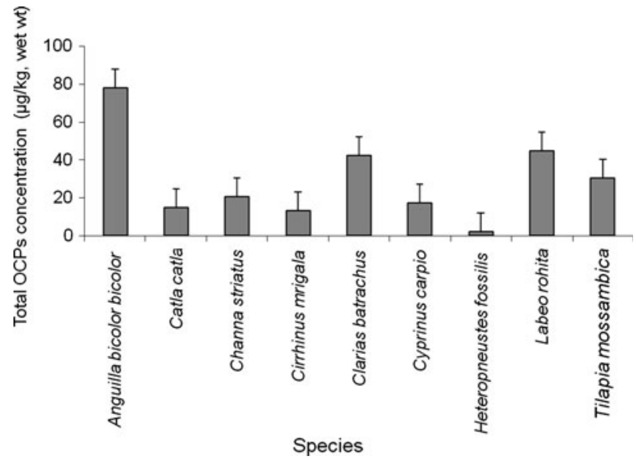

Fig. 1 Total organochlorine pesticide residues (mean and SE µg/kg, wet wt.) in fishes collected from different inland wetlands in Karnataka, India

Table 3 Comparison of calculated dietary intake concentration of organochlorine pesticides with the acceptable dietary intake (ADI) levels stipulated by various statutory agencies

Organochlorine pesticides	The average calculated dietary intake concentration through consumption of fishes in the present study (µg/person/day)	Allowable limits by various statutory agencies (µg/person/day)
∑HCH	0.61	18[a]
HE	0.21	–
∑Endosulfan	0.15	450[b]
Dieldrin	0.13	6[a]
∑DDT	0.27	300[b]

[a] Health Canada (1996)

[b] IARC (International Agency for Research on Cancer 1989)

Cancer) 1989; Health Canada 1996] (Table 3). The results obtained in this study fishes were also lower than the recommended tolerance limits (HCHs 1 mg/kg; total DDT (*p,p*′-DDT, *p,p*′-DDD and *p,p*′-DDE) 1 mg/kg) of the Ministry of Health, Government of India (Mohindru 2005). More importantly, in comparison to earlier reports in India the present levels of the contaminants are lower which indicate the gradual phase out of these compounds.

Although the varying levels of organochlorine pesticide residues detected in fishes in the current investigation are indicative of widespread occurrence in the environment, they do not appear to be harmful to human. In India, although studies on the levels of organic contaminants in fishes are carried out they are sporadic and not oriented towards assessing the impact on human health. Unfortunately, chemicals like DDT are still being used in India for specific purposes although banned for agriculture. Hence, regular monitoring of persistent chemicals is recommended. This study indicates that a large-scale investigation of OCP residues in different organisms in the food chain should be launched in order to adequately evaluate the potential health risk to humans.

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References

Amaraneni SR, Pillala RR (2001) Concentration of pesticide residues in tissues of fish from Kolleru Lake in India. Environ Toxicol 16:550–556

Anupma K, Sinha RK, Krishna Gopal Kumar Gopal K (2001) Quantitative estimation of DDT residues in some freshwater fish of river Ganga at Patna, Bihar. Environ Ecol 19:396–399

Aulakh RS, Gill JP, Bedi JS, Sharma JK, Joia BS, Ockerman HW (2006) Organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a poultry farm in Punjab, India. J Science Food Agric 86(5):741–744

Canada Health (1996) Provisional tolerable dietary intake (PTDI). values presently used by contaminants toxicology section. food directorate. Ottawa, Ontario

David M, Mushigeri SB, Philip GH (2003) Alterations in the levels of ions in tissues of fresh water fish *Labeo rohita* exposed to fenvalerate. Pollut Res 223:359–363

Feroz MK, Panikkar P (2006) Indian fisheries scenario, a peep into constraints/needs for its future development. Fishing Chimes 26(9):13–20

Hagmar L, Rylander L, Dyremark E, Klasson-Wehler E, Erfurth EM (2001) Plasma concentration of persistent organochlorine in relation of thyrotropin and thyroid hormone levels in women. Int Arch Occup Environ Health 74:184–188

IARC (International Agency for Research on Cancer) (1989) Overall evaluation of carcinogenicity: an updating of IARC monograph. Monogr Eval Carcinog Risks Hum1-42

Jabber SMA, Khan YSA, Rahman MS (2001) Organochlorine pesticide residues in the muscles of three fishes from the Ganges Estuary, Bangladesh. Int J Ecol Environ Sci 27:111–116

Kaur M, Sharma JK, Gill JP, Aulakh RS (2008) Determination of organochlorine pesticide residues in freshwater fish species in Punjab. India. 80:154–157

Khare S, Singh S, Mehrotra A (2002) Histophathological changes in the gills of *Nandus nandus* induced by endosulfan and carbaryl. Nat Environ Pollut Technol 11:1–4

Kole RK, Banerjee H, Bhattacharya H (2001) Monitoring of market fish samples for endosulfan and hexachlorcyclohexane residues in and around Calcutta. Bull Environ Contam Toxicol 67: 554–559

Kumari A, Sinha RK, Gopal K, Prasad K (2001) Dietary intake of persistent organochlorine residues through Gangetic fishes in India. Int J Ecol Environ Sci 27:117–120

Martinez MP, Angulo R, Pozo R, Jodral M (1997) Organochlorine pesticides in pasteurized milk and associated health risks. Food Chem Toxicol 35:621

Mohapatra BC, Saha C (2000) Pesticides in aquatic environment. In: Aquatic pollution and management, 1st edn. Central Institute of Fresh water Aquaculture, pp 29–53

Mohindru SM (2005) Food contaminants-origins. propagation and analysis. Analysis of pesticides/insecticides in/on foods. APH Publishing Corporation, New Delhi, India, p 93

Muralidharan S, Dhananjayan V, Jayanthi P (2009) Organochlorine pesticides in commercial marine fishes of Coimbatore, India and their suitability for human consumption. Environ Res 109:15–21

Mwevura H, Othman OC, Mehe GL (2002) Organochlorine pesticide residues in edible biota from the coastal area of Dares Salaam city. J Mar Sci 1:91–96

Sarkar UK, Basheer VS, Singh AK, Srivastava SM (2003) Organochlorine pesticide residues in water and fish samples: first report from rivers and streams of Kumaon Himalayan region, India. Bull Environ Contam Toxicol 70:485–493

Siddiqui MKJ, Anand M, Mehrotra PK, Sarangi R, Mathur N (2005) Biomonitoring of organochlorines in women with benign and malignant breast disease. Environ Res 98:250–257

Singh KP, Malik A, Mohan D, Takroo R (2005) Distribution of persistent organochlorine pesticide residues in Gomti River, India. Bull Environ Contam Toxicol 74(1):146–154