



Original Research Article

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## Studies on Levels of Pesticides Residues in Market Fish of Punjab (India)

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### ABSTRACT

In this study, the pesticide residues of organochlorine, organophosphate, and synthetic pyrethroid were monitored in market fish samples of different regions of Punjab. The pesticide residues were estimated by multiple residue analytical technique using gas chromatography equipped with GC-ECD and GC-FTD. The confirmation of residues was done on gas chromatography mass spectrometry. The results showed the presence of hexachlorocyclohexane (HCH) as predominant contaminant in market fish samples. Residues of HCH were detected in 33 % samples, whereas endosulfan sulphate, DDT, chlorpyrifos, aldrin, cyhalothrin and ethion residues comprised 31, 21, 5, 4, 3, and 3 per cent, respectively. In market fish in different districts of Punjab, the highest levels of HCH were found in Ludhiana district. The highest levels of endosulfan were found in Patiala district. The highest levels of DDT were found in Bhatinda district. The highest levels of cyhalothrin, aldrin and chlorpyrifos were found in Ferozepur/Moga district. The highest levels of endosulfan sulphate and ethion were found in Bhatinda district. Mean levels of residues in market fish were HCH, endosulfan sulphate, DDT, chlorpyrifos, aldrin, cyhalothrin, endosulfan and ethion: 0.018, 0.0164, 0.011, 0.00020, 0.0023, 0.0014, 0.00014 and 0.0004 µg/g, respectively.

#### Keywords

Pesticides, Market fish, Organochlorine

#### Article Info

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### Introduction

Pesticides are divided into many classes, of which the most important are organochlorine and organophosphorous compounds. Organochlorine pesticides are known to resist biodegradation and therefore they can be recycled through food chains and produce a significant magnification of the original concentration at the end of the chain (Milidas, 1994). The organochlorine pesticides (OCPs) are compounds are persistent and remain stable under most of the environmental

conditions. Being fat-soluble, OCPs lead in bioaccumulation through food chain. The residues of them cause environmental pollution and toxic effects have been observed in humans and animals. The long persistence and tendency to accumulate in the human tissues, they have concern for human health.

Since time immemorial, man has been a victim of pest and diseases, which have destroyed his food, fiber and threatened his very existence. Hence a large variety of synthetic pesticides intended for destroying, repelling or inducing the harmful effects of

pests is in use for agriculture and public health today (Hassan, 2002). The indiscriminate and uncontrolled use of pesticides in agriculture leads to high level of pesticides in water bodies near to lands, rivers and estuarine bodies (Sarkar and Gupta, 1988) resulting in high concentration in aquatic life especially fish, prawns, otter, eels and shrimps present in water bodies either by direct ingestion or by maintenance of solubility equilibrium with water. Pesticide residues also reach the aquatic environment through direct run off, leaching, careless disposal of empty containers, equipment washings etc. Pesticide enters a biological system mainly by three routes: aerial, terrestrial and aquatic. The rate of accumulation of pesticides is higher through aquatic route than through other routes because of chemical nature of pesticide, higher lipid solubility and lower water solubility. Pesticides can accumulate in the fish tissues either as a result of contaminated feed intake or through contact with pesticides in water, posing a hazard to public health through the consumption of fish.

## **Materials and Methods**

### **Chemicals**

Analytical grade reagents viz. Acetone, hexane, acetonitrile were glass distilled before use. Sodium sulphate was heated for 2 hr in a muffle furnace at 400°C. Florisil (60-100 mesh) 200g treated with dichloromethane and acetone. After this it dried in hot air oven at 130°C for 2h. Florisil was used as such without any treatment. Analytical technical grade standards with 93- 99% purity for OCPs; organochlorine pesticides (HCH and its isomers, heptachlor, aldrin, fipronil, butachlor, dieldrin, DDT and its metabolites, endrin,  $\beta$ -endosulfan and endosulfan sulphate), OPs; Organophosphorus pesticides (chlorpyrifos, monocrotophos, dimethoate, phorate, fenitrothion, parathion-methyl, malathion,

fenamiphos, profenphos, ethion, triazophos and phosalone) and SPs; synthetic pyretheroids (cypermethrin, permethrin, cyfluthrin, cyhalothrin, deltamethrin and fenvalerate) were used to detect the residues of these pesticides in the samples.

### **Sampling**

The one hundred and seven fish samples were collected from the various markets of five districts of Punjab, representing the different zones of Punjab. All the samples were placed in plastic bags and transported in styrofoam boxes with recyclable ice and stored at -20°C until processing. All the samples were processed in duplicate. Three samples of each species of fish weighing 400-500g were collected from different organized fish farms and two hundred and fifty gram meat of each fish species was collected from market and representative sample of 50g was taken for further processing.

### **Extraction and analysis**

The extraction and clean-up of fish samples were carried out by the method as described by Abdullah and Hassan (1990) with modification. The samples were thawed before extraction and only edible portion was taken for analysis. Samples thoroughly homogenize in a homogeniser. Thoroughly grounded sample take in a mortar and add 100g anhydrous sodium sulphate to combine with water present and to disintegrate sample. Extraction was done with petroleum ether, acetonitrile and dichloromethane. For organophosphorus pesticide residues, extraction was done with hexane by using the same procedure. The collected extracts were evaporated completely and reconstituted with n-hexane. This extract (2  $\mu$ l) was injected into gas chromatograph (GC) equipped with electron capture detector (ECD) for OCPs and SPs detection and flame thermionic detector

(FTD) for OPs detection. Analyte was identified by comparing the retention times and peak height/area with the reference standard which was run under similar operating conditions. The confirmation of pesticide residues was done by GC-Mass Spectrometer, in which a characteristic mass spectrum was obtained based on mass-charge ratio of a compound. The reagent and sample blank were extracted and analyzed to negate the false peaks in common. The trueness of the method used for extraction and estimation was validated by the processing of spiked fish, samples with standard pesticides at concentrations of 0.5 and 1 ppm. Average recoveries were between 80-92 %. The results are expressed in arithmetic mean value of pesticide residues with unit of  $\text{ng g}^{-1}$  on wet weight basis.

The formula used for the quantification of residues was:

$$\text{Residues (mg/kg)} = \frac{\text{Peak area of the sample} \times \text{ng of insecticide standard injected} \times \text{final volume of the sample extract (ml)}}{\text{Peak area of the standard} \times \text{volume of the sample } (\mu\text{l}) \text{ injected} \times \text{weight of the sample (g)}}$$

## Results and Discussion

The fish samples were collected from different markets and analysed for the presence of pesticide residues. The results of analysis of samples collected from different markets showed the presence of HCH isomers, DDT metabolites, aldrin, endosulfan, endosulfan sulphate, cyhalothrin, chlorpyrifos and ethion residues. HCH was found to be the most predominant pesticide comprising on an average 33 per cent of total pesticide residues in market fish; endosulfan sulphate, DDT, chlorpyrifos, aldrin, cyhalothrin and ethion residues comprised 31, 21, 5, 4, 3, and 3 per

cent, respectively. Levels (mean  $\pm$ S.E) of pesticide residues ( $\mu\text{g/g}$ ) in market fish in various districts of Punjab have been shown in Table 1 and 2.

The mean and range of  $\beta$ -HCH in fish samples were 0.0149  $\mu\text{g/g}$  and 0.0111-0.022  $\mu\text{g/g}$ , respectively. Whereas, mean and range of  $\gamma$ -HCH were 0.003  $\mu\text{g/g}$  and 0.0008 - 0.0047  $\mu\text{g/g}$ , respectively. The predominance of occurrence of  $\beta$ -HCH, this is the most persistent form due to the isomerisation of  $\alpha$ -HCH and  $\gamma$ -HCH into  $\beta$ -HCH. Also, due to its high stability and also resistant to enzymatic and metabolic degradation (Dhananjayan and Muralidharan 2010). Presence of high concentrations of  $\beta$ -HCH has been reported in various biological components (Kumari *et al.*, 2001; Kole *et al.*, 2001). Begum *et al.*, (2009) reported higher levels of  $\beta$ -HCH i.e. 0.98  $\mu\text{g/kg}$  in silver carp of Cauvery river, India. 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). The lowest levels of  $\beta$ -HCH and  $\gamma$ -HCH residues were present in fish samples collected from Amritsar/Taran Taran district (0.0111  $\mu\text{g/g}$  and 0.0008  $\mu\text{g/g}$ ) and highest levels were present in Ludhiana district samples (0.022  $\mu\text{g/g}$  and 0.0047  $\mu\text{g/g}$ ).

Among the DDT metabolites, p,p'-DDD and p,p'-DDE had significantly higher burden in fishes. Sethajintanin *et al.*, (2004) and Yang *et al.*, (2007) have also reported dominance of p,p'-DDE in the fish samples. p,p'-DDE is a highly persistent metabolite in the environment and organisms. The predominance of occurrence of p,p'-DDE residue can be due to the conversion of p,p'-DDT to p,p'-DDE by mixed function

oxygenase enzyme through metabolic functions. DDT and its metabolites undergo strong biomagnifications along trophic transfer. Metabolism of DDT in fish is generally accomplished through dechlorination to DDE but generally not to DDD (Schmitt *et al.*, 1999).

India banned DDT for agricultural purposes in 1989, but continues to use for malaria vector control and also used to control the Sand fly (*Phlebotomus argentipes* and *P. papatasi*) the vector of Kala-azar disease (Singh *et al.*, 1997). Therefore, the presence of p,p'-DDD in fish tissue can be from direct input of p,p'-DDD from the environment.

The mean and range of  $\Sigma$ -DDT in fish samples were 0.011 $\mu$ g/g and 0.0022-0.0268  $\mu$ g/g, respectively. p,p'-DDE levels in present study were higher than the levels reported by Dhananjayan and Muralidharan (2010) i.e. 5.9  $\mu$ g/kg in wetland fishes of Karnataka and Malik *et al.*, (2007) i.e. 0.16 ng/g, in fish samples from the river Gomti, Uttar Pradesh but levels of  $\Sigma$ -DDT were lower than reported by Kumari *et al.*, (2001) i.e. 750.2 ng/g, in fish samples from the river Ganges, Bihar. The lowest levels of  $\Sigma$ -DDT residues were present in fish samples collected from Amritsar/Taran Taran district (0.0022  $\mu$ g/g) and highest levels of  $\Sigma$ -DDT residues were present in fish samples collected from Bathinda district (0.0228 $\mu$ g/g).

The cyclodiene pesticides (endosulfan, endosulfan sulphate and aldrin) were also found in the market fish samples. Aldrin residues were detected in 37 out of 107 samples of market fish analysed. The mean and range of aldrin in fish samples were 0.0023 $\mu$ g/g and 0.0005-0.0053  $\mu$ g/g, respectively. Aldrin levels in present study were lower than the levels reported by Malik *et al.*, (2007) i.e. 2.53 ng/g in fish from Gomti river and Kumari *et al.*, (2001) i.e. 112.4ng/g

in Bihar. The lowest levels of aldrin residues were present in fish samples collected from Bathinda (0.0005  $\mu$ g/g) district and highest levels of aldrin residues were present in fish samples collected from Ferozepur/Moga (0.0053  $\mu$ g/g) district.

In the environment, the endosulfan can be oxidized to the corresponding sulfate (endosulfan sulphate) (Kathpal *et al.*, 1997), which is persistent than its parents (Guerin 2001). The residues of endosulfan were found minimum in all the fish but the residues of endosulfan sulphate were most dominant in the fish samples. Eighteen out of 107 samples of market fish showed the presence of endosulfan residues, while endosulfan sulphate was found in 70 out of 107 samples. Pesticides like endosulfan are commonly used by fish cultivators to harvest their produce, probably for minimizing the cost of harvesting. Thus, there is likelihood of accumulation of pesticide residues in the fish tissue (Chakravarty *et al.*, 1996).

Moreover, after the restriction imposed on the use of aldrin, endosulfan is used widely in the management of termites and other insect pests in agriculture and can be the potential source of residues in animal feed and water (Ghosh *et al.*, 1999). The mean and range of endosulfan and endosulfan sulphate residues in fish samples were 0.00014 $\mu$ g/g and BDL-0.0005  $\mu$ g/g, 0.0164 $\mu$ g/g and 0.0023-0.0251  $\mu$ g/g, respectively. Endosulfan levels in present study were higher than the levels reported by Kaur *et al.*, (2008) i.e. 0.033 $\mu$ g/g in freshwater fishes of Punjab and Dhananjayan and Muralidharan (2010) i.e. 3.7  $\mu$ g/kg in fishes of Karnataka but levels of endosulfan were lower than the levels reported by Kole *et al.*, 2001 i.e. 1.41 $\mu$ g/g in Calcutta. Bhattacharya *et al.*, (2003) also reported levels of endosulfan sulphate i.e. 0.29  $\mu$ g/g in sediments of tropical mangrove estuary, India.

**Table.1** Levels (mean ±S.E) of pesticide residues (µg/g) in market fish in various districts of Punjab

Pesticides	Districts				
	Bathinda	Patiala	Amritsar/ Taran Taran	Ferozepur/ Moga	Ludhiana
β-HCH	0.0123 <sup>a</sup> ±0.003	0.0128 <sup>a</sup> ±0.013	0.0111 <sup>a</sup> ±0.004	0.0164 <sup>a</sup> ±0.014	0.022 <sup>a</sup> ±0.005
γ-HCH	0.0028 <sup>a</sup> ±0.008	0.0036 <sup>a</sup> ±0.007	0.0008 <sup>a</sup> ±0.002	0.0031 <sup>a</sup> ±0.019	0.0047 <sup>b</sup> ±0.004
p,p'-DDE	0.0179 <sup>ab</sup> ±0.007	0.0032 <sup>ab</sup> ±0.014	0.0022 <sup>a</sup> ±0.002	0.0111 <sup>ab</sup> ±0.020	0.0149 <sup>b</sup> ±0.011
p,p'-DDD	0.0049 <sup>b</sup> ±0.032	BDL±0.000	BDL±0.000	0.0003 <sup>a</sup> ±0.005	0.0027 <sup>a</sup> ±0.000
Aldrin	0.0005 <sup>a</sup> ±0.000	0.0013 <sup>ab</sup> ±0.001	0.0022 <sup>ab</sup> ±0.001	0.0053 <sup>b</sup> ±0.001	0.0024 <sup>ab</sup> ±0.001
Endosulfan	BDL±0.000	0.0005±0.001	BDL±0.000	0.0002±0.001	BDL±0.000
Endosulfan sulfate	0.0251 <sup>b</sup> ±0.011	0.0154 <sup>ab</sup> ±0.021	0.0023 <sup>a</sup> ±0.003	0.0177 <sup>a</sup> ±0.033	0.0217 <sup>b</sup> ±0.010
Cyhalothrin	BDL±0.000	0.0003±0.001	0.002±0.000	0.0048±0.013	BDL±0.000
Chlorpyrifos	0.0003 <sup>a</sup> ±0.000	BDL±0.000	0.0002 <sup>a</sup> ±0.000	0.0008 <sup>b</sup> ±0.000	BDL±0.000
Ethion	0.0011 <sup>b</sup> ±0.000	BDL±0.000	0.00056 <sup>a</sup> ±0.001	BDL±0.000	0.0003 <sup>a</sup> ±0.000

BDL –Below Detectable limit (0.0001); values represent Mean±S.E. of different pesticides and values with different superscript within a row differ significantly from each other.

**Table.2** Levels (mean and range) of pesticide residues (µg/g) in market fish in Punjab

Pesticides	Mean	Range
β-HCH	0.0149	0.0111-0.022
γ-HCH	0.003	0.0008-0.0047
p,p'-DDE	0.0099	0.0022-0.0179
p,p'-DDD	0.0016	BDL-0.0049
Aldrin	0.0023	0.0005-0.0053
Endosulfan	0.00014	BDL-0.005
Endosulfan sulfate	0.0164	0.0023-0.0251
Cyhalothrin	0.0014	BDL-0.0048
Chlorpyrifos	0.0002	BDL-0.0008
Ethion	0.0004	BDL-0.0011

BDL –Below Detectable limit (0.0001)

The lowest levels of endosulfan sulphate residue were present in fish samples collected from Amritsar/Taran Taran district (0.0023 µg/g) and highest levels of endosulfan sulphate residue were present in fish samples collected from Bathinda district (0.0251 µg/g).

Among the synthetic pyrethroids, cyhalothrin residues were found in 17 out of 107 samples of market fish analyzed for pesticide residues. The mean and range of cyhalothrin residues in fish samples were 0.0014µg/g and BDL-0.0048 µg/g, respectively. The lowest levels of cyhalothrin residues were present in fish samples collected from Bathinda district (BDL) and highest levels of cyhalothrin residues were present in fish samples collected from Ferozepur/Moga district (0.0048 µg/g).

The presence of cyhalothrin in fish samples may be due to the reason that most of organochlorine pesticides have been banned due to their highly environmental persistence and their adverse effects. The synthetic pyrethroids are less toxic than organochlorine pesticides, but still pyrethroids residues were found in samples of fish because pyrethroids residues are extremely sensitive to fish.

Among the organophosphate pesticides, chlorpyrifos and ethion were found in the samples of fish analysed for pesticide residues. Eleven out 107 samples of market fish showed the presence of ethion residues, while chlorpyrifos were found in 7 out of 107 samples.

The mean and range of ethion and chlorpyrifos in fish samples were 0.0004 µg/g and BDL-0.0011 µg/g, 0.00020 µg/g and BDL-0.0008µg/g, respectively. Chlorpyrifos levels in present study were lower than the levels reported by Amaraneni (2002) i.e. 12.6µg/g in fish of Andhra Pradesh. The lowest levels of chlorpyrifos residues were present in fish samples collected from Patiala district (BDL) and highest levels of chlorpyrifos residues were present in fish samples collected from Ferozepur/Moga district (0.008 µg/g). Exchange

of pesticides in aquatic world also progresses very rapidly, even in pond water.

The persistent and ubiquitous nature of pesticides combined with tendency to concentrate in organisms as they move up the food chain, may increase their toxicity to fish, birds and other forms of life including man

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