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## Manisha

Department of Fisheries,  
Chhattisgarh, India

## NK Chandravanshi

Department of Fisheries,  
Chhattisgarh, India

## RK Trivedi

Department of Aquatic  
Environment Management,  
Faculty of Fishery Sciences,  
W.B.U.A.F.S., Kolkata, India

## SK Banjare

Department of Fisheries  
Extension, Central Institute of  
Fisheries Education, Mumbai,  
India

## VK Nirala

Department of Fisheries,  
Chhattisgarh, India

## Correspondence

### Manisha

Department of Fisheries,  
Chhattisgarh, India

## Availability of pesticide residues in *Labeo bata* (Hamilton, 1822) in selected markets of Kolkata, West Bengal, India

Manisha, NK Chandravanshi, RK Trivedi, SK Banjare and VK Nirala

### Abstract

The levels of Chlorpyrifos (organophosphate), P,P'-DDE (Para, Para'-Dichlorodiphenyldichloroethylene), Dieldrin, O,P'-DDT (Ortho, Para'-Dichlorodiphenyldichloroethane) (organochlorine) and Cypermethrin (synthetic pyrethroid) pesticides were measured in whole fish body of *Labeo bata* species from eight selective markets (Garia, Mukundpur, Gongajoyara, Sonarpur, Dhakuria, Sealdah, Jadavpur and Chindarihata) in Kolkata, West Bengal. This fish species are a significant part of the diet of residents of Kolkata. The fish *Labeo bata* were collected from the above markets for eight months. The analysis was done using Gas Chromatograph with Electron Capture Detector. The fish samples analyzed however did not show the presence of pesticide residues, and were found to be Below Detectable Limit (BDL) in all the samples. In all of the fish samples the pesticides were at Below the Food Safety Standard for pesticides.

**Keywords:** Pesticides, organophosphate, P, P'-DDE, cypermethrin

### 1. Introduction

Pesticides are the biocide substances meant for attracting and destroying or mitigating any pests [38]. The pesticide is mainly used for protection of crops from the damaging agents such as insects, fungi or weeds. The term pesticide includes the following categories, i.e., herbicide, insecticide, insect growth regulator, Nematocide, Termiticide, Molluscicide, Piscicide, Avicide, Rodenticide, Predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant and sanitizer [30].

Pesticides are also used in aquaculture operations such as pond preparation, eradication of aquatic insects as well as for harvest. The production of pesticides started in India in 1952 with the establishment of a plant for the production of Benzene Hexachloride (BHC) near Kolkata, and India is now the second largest manufacturer of pesticides in Asia after China and ranks 12<sup>th</sup> globally [27]. The pattern of pesticide usage in India is different from that for the world in general.

A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. Pesticides used to cover a wide range of chemicals used to control insect pests, plant diseases, weeds, rats or other unwanted organisms. Currently, more than 800 pesticide active ingredients in a wide range of commercial products are registered for use in agriculture to meet food supply demands [34].

The toxicity effects of pesticides on human and animal health depend on the toxicity of the chemical and the length and magnitude of exposure [24]. A number of pesticides i.e., Dichlorodiphenyltrichloroethane (DDT) and Hexachlorocyclohexane (HCH) compounds hyper accumulated in the biological system through the food chain and causing a serious threat to the biological system [13, 22]. The main objective of this study is to find out the availability of pesticide residues in the marketable finfish *L. bata* in markets of Kolkata, West Bengal.

### 2. Materials and Methods

#### 2.1 Sample collection

The samples were collected from different popular and important fish markets situated in and around Kolkata region of West Bengal. The selected locations were Garia, Mukundpur, Gongajoyara, Sonarpur, Dhakuria, Sealdah, Jadavpur and Chindarihata fish markets. This study was carried for a period of 8 months from August 2017 to March 2018.

Live fish samples *L. bata* (100-120 gm) were freshly bought from the retailer. The fresh fishes were kept in the ice box to avoid spoilage, contamination, damage and carried to the laboratory. Then the whole fish was dissected out separately. All the samples were kept in plastic pouch and preserved in a deep freezer at -18°C till the time of analysis, normally within 48 hours.

## 2.2 Analysis of Chlorpyrifos, P, P'-DDE, Dieldrin, O, P'-DDT and Cypermethrin in Whole fish

Analysis of pesticides (Chlorpyrifos, P, P'-DDE, Dieldrin, O, P'-DDT and Cypermethrin) in whole body of *L. bata* fish was done by a multi residue technique [33]. Samples were taken from the whole body of *L. bata*. The whole fish body was taken, bones and scales were removed. After that the body was minced with scissor and grinded properly to make fine paste. From those samples, 2 gm sample was weighed into a conical flask. 10 ml distilled water, 10 ml Ethyl acetate (80% Ethyl acetate and 20% Cyclohexane) and 1 gm Sodium chloride and was added respectively. Sample was homogenized in Micro homogenizer, kept in Sonicator for 15 minutes. After that 3 gm Sodium sulfate was added. The mixture was transferred to 50 ml centrifuge tube and kept in shaker for 30 minutes. Sample was mixed thoroughly by vortex machine for 1 minute and kept in cooling centrifuge for 10 minutes for 7000 rpm. Then supernatant was collected. 10 ml ethyl acetate was added. Again sample was homogenized in Micro homogenizer, kept in shaker for 30 minutes. The sample was kept in a cooling centrifuge for 10 minutes in 7000 rpm. Then the total 20 ml sample was collected and dried in rotatory vacuum operator in 40°C and makeup to 5 ml. From the 5 ml, 1 ml was collected in 2 ml of the ependruff tube for clean-up.

Primary secondary amine (50 mg), Endcapper C18 (50 mg), Magnesium sulphate (250 mg) were added in the supernatant and shaken by vortex for 1 minutes and then centrifuged that for 10000 rpm for 5 minutes in centrifuge. The supernatant was taken and filtered through the 0.2 micron syringe filter and 1 µl was pushed through injector and analyzed by Gas Chromatography (GC).

The quantitative estimation of pesticide was performed by using a Varian CP 3800 Gas chromatography (Galaxy Software) equipped with a 63Ni electron capture detector (ECD).

## 2.2 The concentration of pesticides in whole fish was calculated using following equation

$$\text{Concentration of pesticide in sample tissues } (\mu\text{g/ml}) = \frac{a_2 \times v_2 \times C}{a_1 \times v_1}$$

$$\text{Residue of Pesticides in tissues } (\mu\text{g/gm}) = \frac{a_2 \times v_2 \times C}{a_1 \times g}$$

### Where as

$a_1$  = Area of standard chromatogram

$a_2$  = Area of sample chromatogram

$v_2$  = Final volume of sample after processing (ml)

$v_1$  = Volume of taken for processing (ml)

$C$  = Concentration of standard

$g$  = Amount of tissue taken for processing.

## 2.3 Recovery experiment

Recovery percentage of Chlorpyrifos, P,P'-DDE, Dieldrin, O,P'-DDT and Cypermethrin from different substrates was carried out to ascertain the reliability of the analytical method after fortifying three different substrates viz., whole fish with technical grade Chlorpyrifos, P,P'-DDE, Dieldrin, O,P'-DDT and Cypermethrin at different spike concentration of 0.1, 0.2, 0.3 (µg/ml). After necessary work up, the concentration of these five pesticides from different tissues/substrates was analyzed by Gas Chromatography (GC). Average recovery per cent of Chlorpyrifos, P, P'-DDE, Dieldrin, O,P'-DDT and Cypermethrin in *L. bata* is 86, 84.25, 84.89, 85.55 and 88.05% respectively.

## 3. Results and Discussion

Analysis of the fish samples collected from eight sub-urban fish markets of Kolkata was carried to look for the presence of any pesticides. Five numbers of *Labeo bata* fish species, were procured from Garia, Mukundpur, Gongajoyara, Sonarpur, Dhakuria, Sealdah, Jadavpur, and Chindarihata markets on a monthly basis for eight months and analyzed to find out the presence of residual levels of Chlorpyrifos, P,P'-DDE, Dieldrin, O,P'-DDT and Cypermethrin in their body.

### 3.1 Standardization and Recovery study of Chlorpyrifos, P,P'-DDE, Dieldrin, O,P'-DDT and Cypermethrin

Recovery experiments were carried out with the entire representative groups. The recoveries from fish samples fortified at the level of 0.05 to 0.3 ppm varied from 84.25% to 88.05% for organochlorine, organophosphate and synthetic pyrethroids pesticides for the electron captured detector.

From samples standard concentration are taken to make the recoveries which justify the method that the method is appropriate or not. After standardization of the samples were above 90%. It means this method is appropriate for GC analysis (Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5).

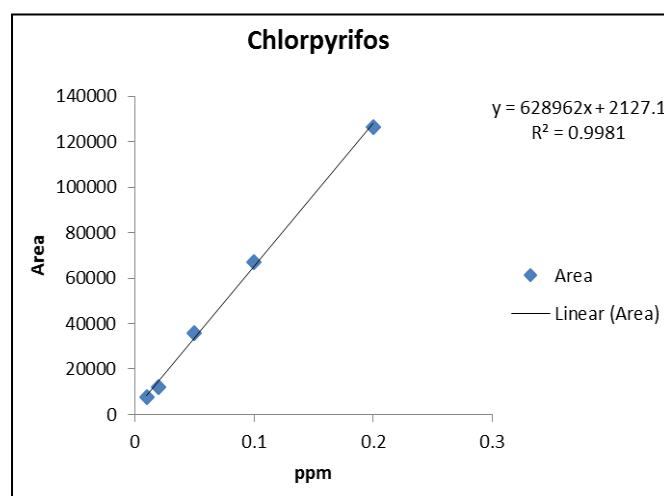


Fig 1: Peak area of Chlorpyrifos in different concentration

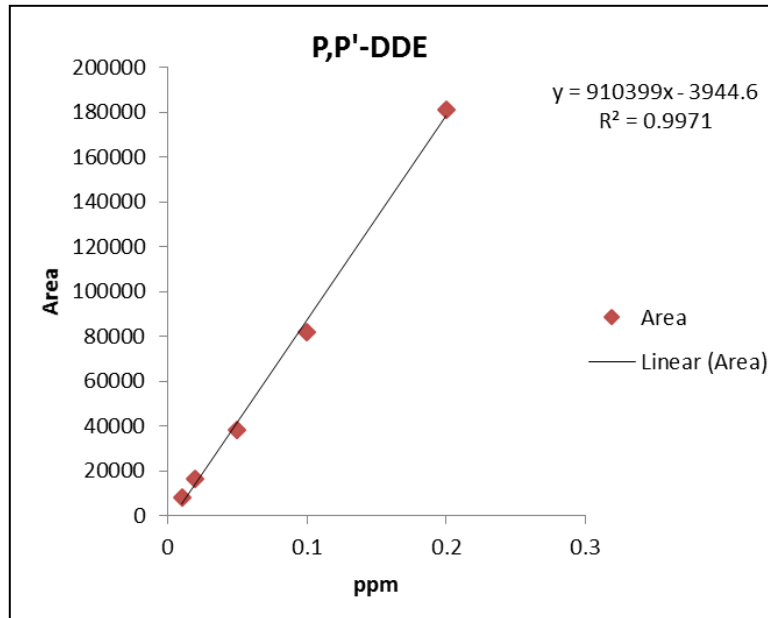


Fig 2: Peak area of P,P'-DDE in different concentration

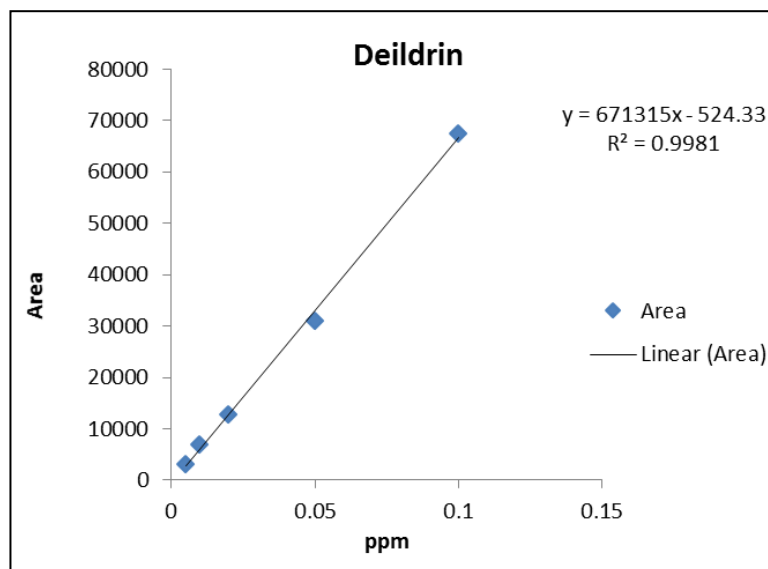


Fig 3: Peak area of Dieldrin in different concentration

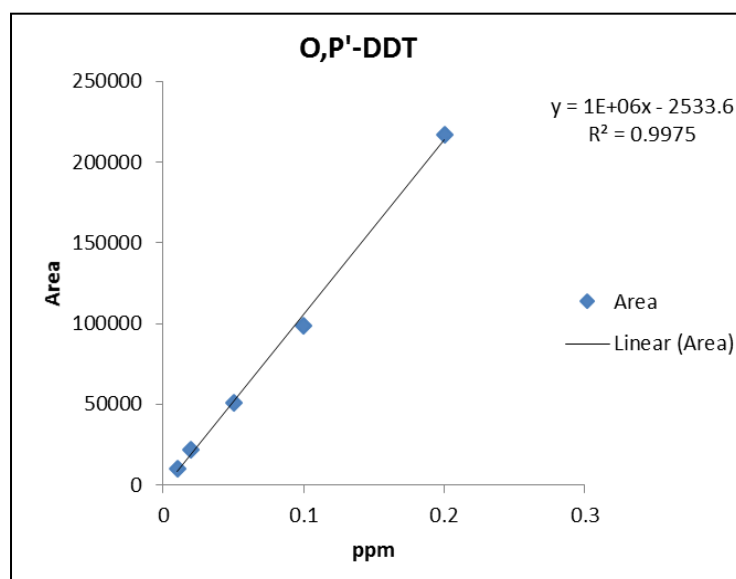


Fig 4: Peak area of O, P'-DDT in different concentration

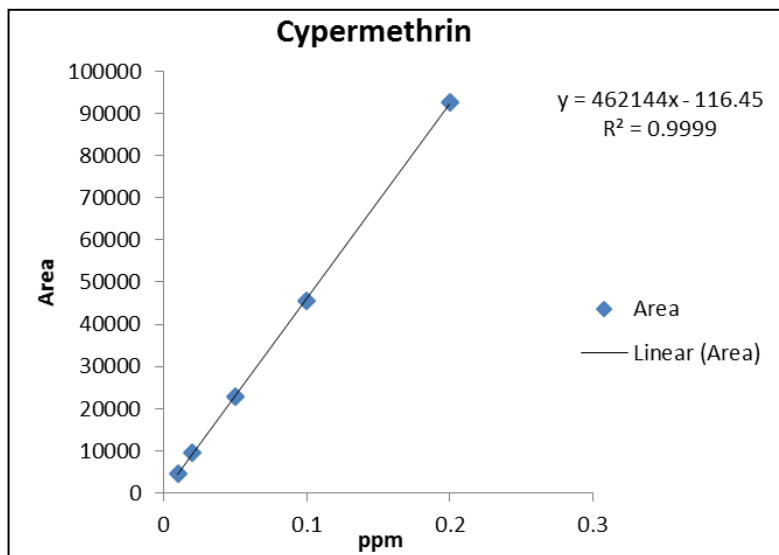


Fig 5: Peak area of Cypermethrin in different concentration

### 3.2. Retention time of the pesticides

The standard and sample (1µl) were injected by using a 10µl SGE microsyringe. Pesticides were estimated by using Varian-3800 gas chromatography (GC). The chromatograph

showed well-resolved peak of the pesticides. The retention time of Chlorpyrifos, P,P'-DDE, Dieldrin, O,P'-DDT and Cypermethrin were 9.40, 12.32, 12.52, 14.09 and 21.07 minutes respectively (Figure 6 and Figure 7).

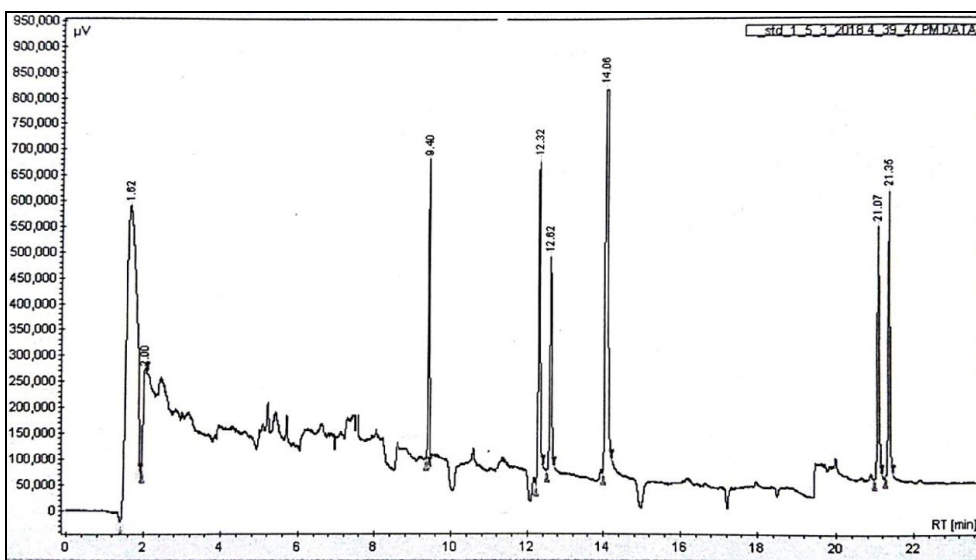


Fig 6: Peak showed by different pesticides in different time

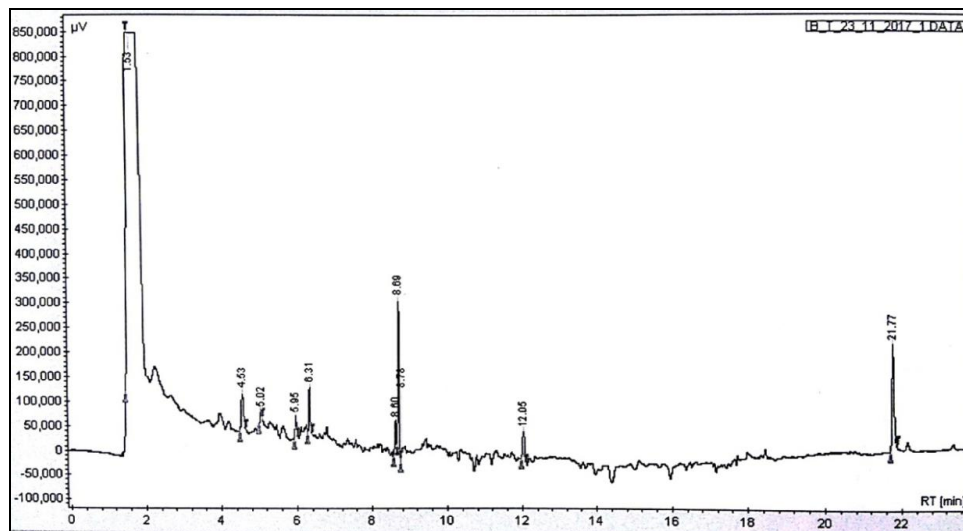


Fig 7: Chromatography of pesticides in the whole fish body of *L. bata*

The residual levels of various pesticides in whole fish are presented in Table 1. The results revealed that the degree of contamination of organochlorine (P,P'-DDE, O,P'-DDT, Dieldrin) organophosphate (Chlorpyrifos) and synthetic pyrethroids (Cypermethrin) pesticides were Below Detection Limit (BDL) in the whole body samples of *L. bata* fish species. This was in accordance with the findings [18], where they found that the residue level of Dieldrin in rohu (*L. rohita*) of Punjab region was 0.001 ppm which was below the MRL value (0.1 ppm). The present findings also corroborated with that of [5], where they estimated different pesticide residues in fish tissues from various markets of Assam which was also at BDL value. On the other hand, [3] had conducted a study on fish from Kolleru Lake, India and had taken twelve farms for his study purpose. No residue level of Dieldrin and Chlorpyrifos could be detected in fish muscles.

According to United States Environmental Protection Agency [38], the MRL value of Dieldrin, Cypermethrin and Chlorpyrifos is 0.1 ppm [39]. The WHO and FAO (Codex Alimentarius Commission, 2009) set Maximum Residue

Limits (MRLs) of 0.30 ppb for Chlorpyrifos, 1.0 ppb for DDT and 0.2 ppb for Dieldrin. The Acceptable Daily Intake values (ADIs) of Chlorpyrifos for fish samples is 0.01 ppb and 0.0001 ppb for Dieldrin. However, as of 1992, the EPA had not established criteria or standards for chlorpyrifos as related to human consumption of fish [8]. The tolerance limit of 7000 ppb recommended by Food Safety and Standards Authority of India for DDT, DDE and DDD, singly or in any combination, for fish on a whole product basis [10].

The pesticides in the fish muscles from the river Ganga and Gomati near Varanasi and some ponds from Gujarat and Jaunpur as a reference site [32]. In the reference site, the fish muscle contained 0.002 ppm of Aldrin whereas fish from Ganga River contained 0.04 ppm. The residue reading showed that in between *Labeo rohita* and *Cyprinus carpio*, the latter had more pesticides residue in their body. The Chlorpyrifos value recorded from the reference site was 0.009 ppm and from Ganga River was 0.03 ppm which were much less than the MRL value suggested by EPA.

**Table 1:** The level (in mg/L) of different pesticides found in the whole body of different fishes collected from suburban markets of Kolkata

Month	Market	Chlorpyrifos	P,P'-DDE	Dieldrin	O,P'-DDT	Cypermethrin
August	Garia	BDL	BDL	BDL	BDL	BDL
	Mukundpur	BDL	BDL	BDL	BDL	BDL
September	Gongajoyara	BDL	BDL	BDL	BDL	BDL
	Sonarpur	BDL	BDL	BDL	BDL	BDL
October	Dhakuria,	BDL	BDL	BDL	BDL	BDL
	Sealdah	BDL	BDL	BDL	BDL	BDL
November	Jadavpur	BDL	BDL	BDL	BDL	BDL
	Chindarihata	BDL	BDL	BDL	BDL	BDL
December	Garia	BDL	BDL	BDL	BDL	BDL
	Mukundpur	BDL	BDL	BDL	BDL	BDL
January	Gongajoyara	BDL	BDL	BDL	BDL	BDL
	Sonarpur	BDL	BDL	BDL	BDL	BDL
February	Dhakuria	BDL	BDL	BDL	BDL	BDL
	Sealdah	BDL	BDL	BDL	BDL	BDL
March	Jadavpur	BDL	BDL	BDL	BDL	BDL
	Chindarihata	BDL	BDL	BDL	BDL	BDL

The surface and groundwater in the agriculture integrated area like Bhandara, Amravati and Yavatmal where they reported the Chlorpyrifos concentration was BDL [23]. The surface water and groundwater quality of Kolkata in the West Bengal did not contain any Dieldrin concentration [12]. The concentration of Dieldrin was BDL in water bodies of the Haryana region [19]. Tamiraparani river basin, South India for organochlorine pesticides contamination, they found that Dieldrin on the surface water was BDL [20]. The organochlorine pesticides O,P'-DDT, were not detected (ND) in water from the Peacock River [4]. The surface water bodies of the Pampa Ondulada, Argentina to know the Cypermethrin and Chlorpyrifos and Chlorpyrifos contamination level [26]. It had been studied in the river and stream which showed the reading of Chlorpyrifos and Cypermethrin to be BDL. The Cypermethrin residue level in the fish muscle present in Ravi River was below the detection limit. However, several studies did not correlate with our findings [25]. The average concentration of organophosphorus pesticide, Chlorpyrifos was high in rohu than the tilapia. The highest concentration of this pesticide was recorded in the gut, while liver recorded the lowest value according to all parts [2].

The public health hazards arising from excessive consumption of pesticide-contaminated fish can be exemplified as follows. According to [35], Chlorpyrifos is the second highest selling

organophosphate insecticide and is more toxic to fish than organochlorine compounds. Chlorpyrifos is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation [36]. Although pyrethroids have low mammalian and avian toxicity, they are extremely toxic to fish and aquatic invertebrates [6, 7].

An earlier study conducted by [21] reported a 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 Hexachlorocyclohexane, altered thyroid function was reported in women [14]. Among the metabolites, P,P'-DDT, P,P'-DDE had a 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.

Organochlorines have been implicated in a broad range of adverse human health effects including reproductive failures and birth defects, immune system malfunction, endocrine disruptions, and cancers [11]. The presence of organochlorine residues in breast milk and consumption of contaminated fish and their role in alteration of thyroid function [14].

Studies have shown that Dichlorodiphenyltrichloroethane (DDT), Dieldrin and Polychlorinated biphenyls (PCBs) have endocrine disrupting capacities [29]. Similarly, epidemiological

studies have suggested an etiological relationship between exposure to organochlorines and Parkinson's diseases [9]. Children are more susceptible and sensitive to pesticides than adults. Pesticide can affect the reproductive system and early stages of fetus development [15].

According to Central Insecticides Board and Registration Committee, as on 20<sup>th</sup> October 2015, the pesticide banned for manufacture, import, and use includes Dieldrin and others; and the pesticide having restricted use in the country includes Cypermethrin, DDT and others.

India banned DDT for agricultural purposes in 1989 but continues to use for malaria control. Organochlorine pesticides have been banned in the world but still, it has been used in agriculture in an illegal manner which is very common for the developing countries [37]. Dieldrin is a banned pesticide because the persistence of this pesticide posed an

imminent danger to human health. So the National Agency for Food and Drug Administration and Control (NAFDAC) banned the major uses of Dieldrin in 2008. The presence of DDT in fish, in spite of its banning in the country. In China, DDT was banned 20 years ago; in spite of this Organochlorine pesticides were detected in soil, water, fish and sediment samples [17]. In Pakistan, [31] also detected DDE, Aldrin and Dieldrin residues in fish tissues while in Bangladesh reported the levels of DDT, Aldrin, Dieldrin, Lindane and heptachlor in different organs of fish in spite of its ban [17].

According to [28], the production of pesticides started in India in 1952. As depicted in Table 2, West Bengal witnessed 0.7 thousand tonnes in 1966/67, and later increased to 7.2 thousand tonnes in 1991/92. This is equal to 4.35% in 1966/67 to 8.49% in 1991/92 respectively.

**Table 2:** Pesticide consumption in Indian agriculture from 1966/67 to 1991/92 (Thousand Tonnes)

State	1966/67	1971/72	1976/77	1981/82	1986/87	1991/92
Andhra Pradesh	2.3 (14.29)	7.6 (29.45)	10.4 (30.14)	15.5 (32.98)	9.1 (13.525)	16.7 (19.695)
Bihar	0.6 (3.73)	1.7 (6.595)	1.8 (5.22)	2.6 (3.31)	2.0 (2.975)	3.9 (4.00)
Punjab	1.5 (9.32)	2.3 (8.91)	3.5 (10.14)	4.4 (9.365)	5.8 (8.625)	8.9 (10.505)
Tamil Nadu	1.8 (11.18)	3.0 (11.83)	5.3 (15.36)	8.5 (18.095)	11.0 (18.345)	13.3 (15.74)
Uttar Pradesh	1.2 (7.455)	3.4 (13.185)	5.1 (14.785)	8.2 (17.45)	6.6 (9.815)	9.5 (11.20)
West Bengal	0.7 (4.355)	1.2 (4.655)	1.9 (5.515)	2.7 (5.745)	5.1 (7.585)	9.5 (8.495)
All India	16.1 (100.0)	25.8 (100.0)	34.5 (100.05)	47.0 (100.0)	67.3 (100.0)	84.8 (100.05)

Source: [16].

The decreased limit (BDL) of Organochlorine pesticides residues may be due to a change in consumption pattern of pesticides from persistent Organochlorine pesticides to organophosphate pesticides and Organocarbamate pesticides [1].

#### 4. Conclusion

From the result obtained it can be concluded that level of all selected pesticide in fish samples were found to be at BDL. So it can be said that it was safe to consume these fishes. Though the level of pesticides is at BDL, still precautionary measures should be taken and continuous monitoring in water as well as fish should be carried. As fish is vital food ingredient for poor people, hence, proper care should be taken during its culture so that fishes remain safe without any contamination from any kind of toxicants.

Though the residual levels observed were below the Environmental Protection Agency (EPA) prescribed levels, however if human beings consume these fishes for long there is chance of getting exposed to health risks in near future. Further separate studies may be required to come to any conclusion about the human health hazard by consuming fish with very low pesticide contamination. From this research, it's seen that the whole fish body was not affected by pesticides so it's safe to consume fish. Though the level of pesticides is at BDL, still precautionary measures should be taken. There is need to continuously carry the comprehensive monitoring of pesticides residues in the fishes marketing in and around Kolkata.

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