

# PESTICIDE CONTAMINATION IN MUSCLE TISSUES OF CROAKER FISHES FROM LAGOS LAGOON, NIGERIA

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## Abstract:

Two croaker fish species, *Pseudotolithus senegalensis* and *Pseudotolithus typus* were sampled seasonally from Lagos Lagoon between December 2008 and September 2009 and analysed for pesticide residues. The muscle tissues of the fishes were subjected to cold extraction and subsequently clean-up. The detection and determination of the organochlorine residues were performed using gas chromatography. A higher concentration of the pesticide residues was observed during the dry season. There was no particular pattern in the accumulated organochlorine pesticides in the muscle tissues of the fishes. The residue distribution was more in the muscle tissues of *Pseudotolithus senegalensis* than in *Pseudotolithus typus*. The levels of OCP were all within permissible limits. The dietary surveys showed a mean fish consumption value of 40 g/day. Except for endrin and heptachlor, the estimated daily intakes of the pesticides were within the acceptable daily intakes.

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**Keywords:** Pesticide, muscle tissues, croaker fish, Lagos Lagoon

## Introduction:

Pesticides are chemicals used to kill or control pests. They play significant roles in increasing food production and eliminating diseases even though exposure to them can also be harmful to humans. Organochlorine pesticides (OCPs) are among the first set of pesticides to be used in Nigeria and are still in use. Fishes are suitable indicators for environmental pollution monitoring because they concentrate pollutants in their tissues directly from water and also through their diet (Lanfranchi *et al.*, 2006). The low activity of the mono-oxygenase enzymes in fishes limits their ability to metabolize organochlorines (Dearth and Hites, 1991) hence, fishes reflect the levels of organochlorine pollution in the aquatic environment (Muir *et*

*al.*, 1990). This offers the opportunity to study the influence of environmental and biological factors on the bioaccumulation of pollutants (Sarkar *et al.*, 2008).

Pollution by persistent chemicals is potentially harmful to the organisms at higher trophic levels in the food chain. Research efforts indicate that more than 80% of the total intake of pesticide residues in human beings is through the food chain (Trotter and Dickerson, 1993; Martinez *et al.*, 1997). It has been reported that the consumption of contaminated fishes is one of the important pathways of human exposure to OCPs (Zhou *et al.*, 2007; Muralidharan *et al.*, 2008). Data on the presence and distribution of OCPs in edible fishes are, therefore, important from the ecological and human health perspectives.

Organochlorine pesticides are insecticides that act by poisoning the nervous systems of target harmful insects. They have the ability to accumulate in biological tissues and to concentrate in organisms that occupy positions in the upper trophic levels. The aquatic organisms like fish are able to accumulate several fold higher concentration of pesticide residues than the surrounding water (Siddiquiet *al.*, 2005). OCPs contribute to many acute and chronic health effects including cancer, neurological damage, birth defects, tremors, headache, dermal irritation, respiratory problems and dizziness (IARC, 2001).

Literature data on the concentrations of OCP residues in the Nigerian environment are inadequate. There is need for continuous monitoring to identify the occurrence and the levels of OCPs in fishes in Nigeria. In order to obtain more information about the pathways along which bioaccumulation occurs, it is important to investigate the distribution of pesticide residues in muscle tissues of fishes. This study was undertaken to determine the levels of organochlorine residues in muscle tissues of *Pseudotolithus senegalensis* and *Pseudotolithus typus*, two species of croaker commonly consumed in Nigeria.

## **Materials and methods:**

### **Area of study**

The area of study for the investigation is Lagos Lagoon which lies between latitude 6° 26' - 6° 37' N and longitude 3° 23' - 4° 20' E and empties into the Atlantic Ocean.

### **Sampling**

The croaker fish species were sampled between December 2008 and September 2009 during the dry and wet seasons.

### Collection of croaker fish

Male and female croaker fish, *Pseudotolithus senegalensis* and *Pseudotolithus typus* were collected and wrapped in aluminium foil, and stored in ice-packed coolers before they were taken to the laboratory for biometric determination. They were preserved in the refrigerator, thawed and cleaned in distilled water.

### Sex determination

The croaker species were separated into males and females.

### Measurement of length

The total and standard lengths of the croaker fish were measured.

### Calculation of percentage (%) dry matter

Between 1.0 - 2.0 g of muscle tissue of each fresh croaker fish was weighed and dried in an oven at 105<sup>0</sup>C for 8 hours. The dried croaker fish was cooled and weighed to constant weight. The percentage dry matter was calculated.

$$\% \text{ Dry matter} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100 \text{ ----- (1)}$$

### Evaluation of condition factor (CF)

The condition factor (CF) of the fishes was evaluated (Busacker *et al.*, 1990).

### Determination of fat content

10 g of fish muscle tissue was homogenized with 10 g of anhydrous Na<sub>2</sub>SO<sub>4</sub>. Cold solvent extraction was carried out using 50 cm<sup>3</sup> petroleum ether/acetone (1:1 v/v) mixture in a bottle. The mixture was shaken, allowed to stand and filtered. After evaporating the solvent extracts using a rotary evaporator, the fat content of the muscle tissue was gravimetrically determined.

$$\% \text{ Fat} = \frac{\text{weight of fat}}{\text{weight of tissue}} \times 100 \text{ ----- (2)}$$

### **Extraction of croaker fish**

10 g of muscle tissue of the croaker fish was homogenized with 10 g of anhydrous  $\text{Na}_2\text{SO}_4$ . Cold solvent extraction was carried out. 50  $\text{cm}^3$  of the petroleum ether/acetone (1:1 v/v) mixture was introduced into a bottle containing the homogenized fish sample. The mixture was shaken, allowed to stand and filtered into a glass container (US EPA Method 3570C, 2002).

### **Pre-concentration of croaker fish extracts**

The croaker fish extracts were concentrated to 1  $\text{cm}^3$  using a rotary evaporator and kept for clean-up procedure.

### **Clean-up of croaker fish extracts**

The clean-up of the fish extracts was carried out using column chromatography (US EPA method 3630B, 1996). The glass separating column was packed with activated silica gel (90% < 45  $\mu\text{m}$ ) and washed down with n-hexane. The extracts were demineralized over 1 g of anhydrous granulated  $\text{Na}_2\text{SO}_4$  and separated into two eluted fractions using mixtures of dichloromethane, hexane and acetonitrile as eluting solvents. 30  $\text{cm}^3$  of dichloromethane/hexane (20/80) mixture was used for the first fraction, while 30  $\text{cm}^3$  of dichloromethane/hexane/acetonitrile (50/49.5/0.5) mixture was used for the second fraction. The two fractions were combined, concentrated to 1  $\text{cm}^3$  using a rotary evaporator.

### **Determination of organochlorine pesticide residues**

The cleaned-up extracts were dried and re-dissolved in 1.0  $\text{cm}^3$  isooctane (Pandit *et al.*, 2002). Organochlorine Pesticides II EPA Method 8081A was used for the analyses. The detection and determination of the residues were carried out by injecting 1  $\mu\text{L}$  of the 1.0  $\text{cm}^3$  purified extract into the injection port of a gas chromatograph with a  $^{63}\text{Ni}$  electron capture detector (GC- $\mu\text{ECD}$  Agilent Technology 7890A). The column consisted of a DB-5 fused silica capillary column (30 m length  $\times$  0.32 mm i.d.  $\times$  0.25  $\mu\text{m}$  film thickness). The column temperature was programmed from 50 $^\circ\text{C}$  at a rate of 25 $^\circ\text{C}/\text{min}$  to 100 $^\circ\text{C}$ , held for 1 min, and then at a rate of 5 $^\circ\text{C}/\text{min}$  to 300 $^\circ\text{C}$ , held for 5 mins. The temperatures of the injector and detector were 250 $^\circ\text{C}$  and 300 $^\circ\text{C}$ , respectively. The injection was carried out on a splitless injector at 250 $^\circ\text{C}$  and the purge activation time was 30 s. The carrier gas was helium while nitrogen was used as the makeup gas. The run time was 17 mins. Identification of pesticide

residues was accomplished using reference standards and relative retention time techniques while the residues were determined by comparing the peak heights of the samples with the corresponding peak heights of the reference standards of known concentrations. The concentrations (ww) of aldrin, dieldrin, endrin, DDT, heptachlor, HCH, endosulfan, chlordane and methoxychlor were calculated directly by the gas chromatograph.

### **Quality assurance**

Cleaning procedures, recovery of spiked standards and monitoring of detector response were carried out. Blank runs were made for background correction. The correlation coefficients of calibration curves of the pesticides were higher than 0.998. The stock solution of the pesticide standards was purchased from Restek Corporation, USA and contained 1000 ppm in n-hexane. It was serially diluted to obtain desired concentrations of 10, 20 and 40 ng/mL.

### **Recovery study**

The recovery of organochlorine pesticide was carried out in replicate and was determined by spiking the previously analysed samples with the pesticide standard at concentrations similar to those expected in the samples. The recovery percentages were calculated from the chromatograms.

### **Estimation of daily intakes (EDI) of organochlorine pesticides by humans**

The daily intake of organochlorine pesticides by humans was estimated based on questionnaires and interviews conducted in 100 families. Respondents were categorized into males and females. Information on preference of the fish species, age and weight of the respondents and frequency of consumption was obtained and the dietary intake of the pesticides was calculated by multiplying concentrations measured in the muscle tissues of fish by the per capita consumption. The estimated daily intake of the organochlorine pesticides was calculated (IPCS, [2006](#)).

**Results and discussion:****Table 1. Mean biometric data of *Pseudotolithus senegalensis* and *Pseudotolithus typus* in Lagos Lagoon during the dry season**

Fish species	Feeding mode	Sex	Wet weight (g)	% Dry matter	% Fat	TL (cm)	SL (cm)	CF
<i>P. senegalensis</i>	Carnivorous	Male	45.6±0.4	21.5±0.4	1.0±0.1	15.5±0.3	13.0±0.3	1.2±0.4
		Female	47.6±0.1	21.8±0.1	1.1±0.2	17.0±0.2	15.0±0.2	0.9±0.2
<i>P. typus</i>	Carnivorous	Male	56.6±0.3	23.2±0.3	0.5±0.1	19.5±0.3	16.5±0.3	0.8±0.2
		Female	100.7±0.9	22.0±0.8	2.0±0.2	22.0±0.8	19.5±0.8	0.9±0.7

**Table 2. Mean biometric data of *Pseudotolithus senegalensis* and *Pseudotolithus typus* in Lagos Lagoon during the wet season**

Fish species	Feeding mode	Sex	Wet weight (g)	% Dry matter	% Fat	TL (cm)	SL (cm)	CF
<i>P. senegalensis</i>	Carnivorous	Male	45.0±0.1	21.5±0.1	1.0±0.2	15.5±0.2	13.0±0.2	1.2±0.1
		Female	47.2±0.2	21.8±0.2	1.0±0.1	17.0±0.2	15.0±0.2	0.9±0.2
<i>P. typus</i>	Carnivorous	Male	57.0±0.3	23.3±0.3	0.5±0.1	19.6±0.2	16.5±0.3	1.0±0.1
		Female	90.6±0.4	21.7±0.4	2.0±0.1	20.0±0.3	17.5±0.3	1.1±0.2

**Table 3. Mean concentrations (ng/g) of organochlorine pesticide residues in the muscle tissues of male and female *Pseudotolithus senegalensis* during the dry and wet seasons in Lagos Lagoon**

OCPs	Lagos Lagoon			
	Dry season		Wet season	
	Male	Female	Male	Female
Alpha-BHC	18.63±10.45	14.04±0.23	1.70±0.02	1.46±1.17
Beta-BHC	32.88±8.19	103.69±0.84	1.83±0.93	2.40±1.14
Lindane	51.46±5.53	36.10±0.12	0.75±0.16	2.08±1.63
Delta-BHC	64.46±9.08	27.05±0.43	0.85±0.24	ND
ΣBHC	167.42±33.25	180.87±1.62	5.13±1.35	5.94±3.94
Heptachlor	63.25±12.23	42.07±2.84	2.27±2.63	1.43±1.03
Heptachlor-epoxide (B)	20.02±8.12	15.39±3.43	2.83±2.02	0.53±0.11
Aldrin	37.58±9.65	29.90±4.62	3.44±2.24	2.05±1.62
Dieldrin	73.93±6.14	39.42±6.29	ND	ND
Endrin	150.75±34.74	88.64±6.37	22.21±9.32	0.42±0.19
Endrin aldehyde	298.74±34.43	96.66±8.73	104.94±10.43	0.53±0.26
Endrin ketone	891.95±56.46	337.89±23.16	10.54±8.54	0
Cis-Chlordane	73.56±7.24	47.41±6.30	3.39±2.14	0.57±0.35
Trans-Chlordane	110.47±9.94	61.49±5.12	7.03±4.81	ND
Endosulfan I	100.89±8.12	56.92±5.42	6.66±2.20	0.79±0.40
Endosulfan II	81.46±3.65	55.73±8.53	ND	ND
Endosulfan sulphate	170.28±6.45	74.61±6.76	ND	ND
Methoxychlor	90.86±4.34	23.75±4.34	77.24±9.39	0
p,p'-DDE	55.92±9.92	40.89±7.08	ND	ND
p,p'-DDD	119.03±8.95	78.37±8.26	ND	ND
p,p'-DDT	86.52±7.23	130.71±16.64	ND	ND
ΣDDT	261.47±26.10	249.97±31.98	ND	ND
ΣOCPs	2592.61±0.04	1400.73±125.51	245.67±53.07	12.26±7.90

**Table 4. Mean concentrations (ng/g) of organochlorine pesticide residues in the muscle tissues of male and female *Pseudotolithus typus* during the dry and wet seasons in Lagos Lagoon**

OCPs	Lagos Lagoon			
	Dry season		Wet season	
	Male	Female	Male	Female
Alpha-BHC	12.59±5.63	30.07±9.95	1.11±1.03	1.19±1.62
Beta-BHC	23.82±8.92	60.79±5.32	0.60±0.62	0.61±0.10
Lindane	18.53±3.03	27.76±8.64	0.82±0.57	1.14±1.42
Delta-BHC	43.41±4.54	97.31±22.23	ND	ND
ΣBHC	98.35±22.12	215.93±46.14	2.53±2.22	2.94±3.14
Heptachlor	37.41±9.26	69.83±6.52	1.42±1.62	1.80±1.83
Heptachlor-epoxide (B)	54.64±5.94	88.84±8.63	0.60±0.86	0.64±0.26
Aldrin	24.07±8.13	53.54±6.71	2.32±2.12	1.04±1.34
Dieldrin	12.99±5.28	95.05±5.29	0.68±0.18	0.69±0.28
Endrin	61.83±8.74	132.69±31.83	2.05±2.38	0.58±0.12
Endrin aldehyde	106.17±7.13	555.58±8.24	ND	0.35±0.32
Endrin ketone	145.52±9.44	641.25±9.49	16.52±8.25	0
Cis-Chlordane	34.72±5.04	56.97±4.22	0.58±0.10	0.54±0.48
Trans-Chlordane	106.99±8.96	79.29±8.43	0.81±0.29	0.82±0.02
Endosulfan 1	43.68±6.27	75.06 ±4.26	0.90±0.43	1.40±0.19
Endosulfan 11	15.41±8.16	40.38±5.92	ND	ND
Endosulfan sulphate	38.94±4.63	135.55±6.13	ND	0.42±0.25
Methoxychlor	4.76±2.43	69.89±5.42	44.66±34.32	0
p,p'-DDE	46.28±2.14	26.31±6.13	0.70±0.17	0.79±0.13
p,p'-DDD	32.47±3.32	65.75±8.84	ND	2.01±1.27
p,p'-DDT	45.71±16.83	62.13±5.43	ND	ND
ΣDDT	124.47±22.29	154.19±20.40	0.70±0.17	2.80±1.40
ΣOCPs	909.93±133.82	2464.06±177.63	73.78±52.94	14.03±9.63



**Table 5. Estimated daily intake (EDI) of organochlorine pesticides (ng/g) by humans**

Organochlorine Pesticides	<i>Pseudotolithus senegalensis</i>	<i>Pseudotolithus typus</i>
BHC	16.79	20.05
Heptachlor	7.73	14.73
Aldrin	3.49	4.97
Dieldrin	6.87	8.83
Endrin	124.56	123.46
Chlordane	17.09	13.16
Endosulfan	32.74	23.31
Methoxychlor	8.44	6.49
DDT	24.28	14.32

The mean biometric data of *Pseudotolithus senegalensis* and *Pseudotolithus typus* are shown in Tables 1 and 2 while the mean concentrations of organochlorine pesticides in their muscle tissues are presented in Tables 3 and 4. The mean recoveries of the pesticide residues ranged from 88.45 to 98.42%. A higher concentration of the residues was observed during the dry season. This could be as a result of dilution effect that characterizes the wet season. There was no particular pattern in the accumulated organochlorine pesticides in the muscle tissues of the fishes. However, the levels were higher than the concentrations obtained in earlier studies carried out in Nigeria (Unyimadu and Udochu, 2002; Ize-Iyamu *et al.*, 2007) but with lower levels with respect to another investigation (Adeyemi *et al.*, 2008). The residue distribution was more in the muscle tissues of *Pseudotolithus senegalensis* than in *Pseudotolithus typus*. The dominant BHC was beta-BHC. BHCs followed the order beta-BHC > lindane > delta-BHC > alpha-BHC. The total DDT concentration followed the pattern: p,p'-DDT > p,p'-DDD > p,p'-DDE. The high p,p'-DDT levels detected in this study was in contrast with previous studies (Naso *et al.*, 2005) which showed that p,p'-DDE was the major DDT residue in aquatic species. The levels of OCP were all within permissible limits (Oostdan *et al.*, 1999; FAO/WHO, 2005; USEPA, 2006).

In fishes, organic pollutants preferentially accumulate in lipids relative to other compartments. Pesticides gain entrance into fishes by ingestion, dermal absorption and respiration. Accumulation of contaminants in fish lipids can occur by diffusion from the water across the gills and by transfer from the gut into the body after consumption of contaminated food. When these organic pollutants are taken in by the fish, they bioaccumulate, biomagnify and remain in the fish until they are eventually consumed by man. The processes of bioaccumulation and biomagnification of persistent contaminants may be affected by the fish's physiology, age, trophic levels, habitat, structure of food web and contaminant physicochemical properties (McIntyre and Beauchamp, 2007).

The dietary surveys conducted in 100 families showed a mean fish consumption value of 40 g/day. The mean consumption of fish in this study compared with the dietary surveys earlier conducted in China and India (Yang *et al.*, 2006; Muralidharan *et al.*, 2008). Muscle tissue was used in determining the dietary intakes to human body as it is the edible portion in a fish. The estimated daily intakes (EDI) of organochlorine pesticides by humans are shown in Table 5.  $\Sigma$ BHC,  $\Sigma$ aldrin,  $\Sigma$ endrin,  $\Sigma$ chlordanes,  $\Sigma$ heptachlor and  $\Sigma$ DDT were used in estimating the daily intakes. Except for endrin and heptachlor, the estimated daily intakes of the pesticides were within the acceptable daily intakes.

### **Conclusion:**

A higher concentration of the pesticide residues was observed during the dry season. There was no particular pattern in the accumulated organochlorine pesticides in the muscle tissues of the fishes. The residue distribution was more in the muscle tissues of *Pseudotolithus senegalensis* than in *Pseudotolithus typus*. The levels of OCP were all within permissible limits. The dietary surveys showed a mean fish consumption value of 40 g/day. Except for endrin and heptachlor, the estimated daily intakes of the pesticides were within the acceptable daily intakes.

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