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Chlorinated Hydrocarbons in Marine Female Fishes of Lagos Lagoon

Akan Bassey Williams &
Winifred Uduak Anake

Abstract:
Three female fish species of Snapper (*Lutjanus goreensis)*, Herring (*Sardinella maderensis*) and Oarfish (*Regalecus glesne*) were sampled from Lagos Lagoon during the dry and wet seasons of 2008 and 2009 and subjected to cold extraction and clean-up procedure. Their muscle tissues were analysed for chlorinated hydrocarbons because they can concentrate pesticide residues from sediments and water. The identification and quantitation of the chlorinated hydrocarbon residues were performed using a gas chromatograph with a ^{63}Ni electron capture detector. The fishes had condition factor of more than 1 except *Regalecus glesne*. A higher concentration of the residues was observed during the dry season. The residue distribution pattern in muscle tissues of the fishes were: *Regalecus glesne* > *Sardinella maderensis* > *Lutjanus goreensis. Regalecus glesne* recorded the highest chlorinated hydrocarbon content: 6181.16 ng/g. Except for endrin and heptachlor, the estimated daily intakes of the organochlorines were within the acceptable daily intakes while the levels of residues in the fishes were within the permissible residue limits.

Keywords. Chlorinated hydrocarbons, *Lutjanus goreensis*, *Sardinella maderensis, Regalecus glesne,* Lagos Lagoon

Introduction

Chlorinated hydrocarbons, namely aldrin, dieldrin, endrin, chlordane, dichlorodiphenyltrichloroethane (DDT), heptachlor, mirex, toxaphene, hexachlorobenzene (HCB), alpha hexachlorocyclohexane, beta hexachlorocyclohexane, lindane, pentachlorobenzene, and industrial chemicals and byproducts, including PCBs, dioxins, furans, chlordecone, octabromodiphenyl ether, pentabromodiphenyl ether, perfluorooctane sulfonic acid and perfluorooctane sulfonyl fluoride constitute the twenty one chemical substances called the "dirty twenty one". The manufacture and use of chlorinated pesticides have been banned or restricted in many countries because of their persistence and bioaccumulative tendencies that lead to diseases. However, some developing countries are still using these compounds for agricultural and public health purposes [1].

The contamination of the environment and food by chlorinated hydrocarbons has become a topical issue of considerable concern in many parts of the world, and has led many researchers to investigate their occurrence, distribution and concentrations in several ecosystems [2, 3, 4]. The pesticides applied on land eventually find their way to the aquatic environment, thus contaminating it. Being lipophilic, chlorinated hydrocarbons can be concentrated to harmful levels in the aquatic environment through bioaccumulation and biomagnification [5]. The toxicity of pesticides could be acute and chronic. There is growing evidence of cancer, neurological damage, endocrine disruption and birth defects arising from exposure [6].

Nigeria is a rich fishery resource and fishes are major sources of proteins in the country. Finfishes constitute the major components of most aquatic habitats and are important biomarkers of residue levels in aquatic ecosystems. Industrial establishments in Lagos account for over 40% of all industries in Nigeria [7]. The proliferation of urban settlements and slums in Lagos has also led to increased human pressure and the generation of domestic effluents, which eventually find their way into the Lagos Lagoon. This study was undertaken to determine the occurrence and concentrations of chlorinated hydrocarbon residues in muscle tissues of female Snapper (*Lutjanus goreensis)*, Herring (*Sardinella maderensis*) and Oarfish from Lagos Lagoon.

Materials and Methods

Area of study

The area of study for the investigation is Lagos Lagoon. The lagoon lies between latitude 6^{o} 26' - 6^{o} 37' N and longitude 3^{o} 23' - 4^{o} 20' E on the South-Western part of Nigeria.

Sampling

Female *Lutjanus goreensis*, *Sardinella maderensis* and *Regalecus glesne* (Plates 1-3) were sampled between December 2008 and September 2009 during the dry and wet seasons. The sexes of the fishes were identified by examining their gonads. They were wrapped in aluminium foil, and stored in ice-packed coolers before they were transferred to the laboratory for biometric determination. They were subsequently frozen, thawed and cleaned in distilled water while their scales were sloughed off.

Measurement of length

The total and standard lengths of the fishes were measured using a ruler.

Plate 1: Snapper (*Lutjanus goreensis)*

Plate 2: Herring (*Sardinella maderensis*)

Plate 3: Oarfish (*Regalecus glesne*)

Calculation of percentage dry matter

1.0 - 2.0 g of muscle tissue of each fresh fish was weighed and dried in an oven maintained at 105 ^{0}C for 8 hours. The dried fishes were cooled and weighed to constant weight and the percentage dry matter was calculated.

Calculation of condition factor (CF)

The condition factor of the fishes was calculated [8].

Determination of fat content

10 g of fish muscle tissue was homogenized with 10 g of anhydrous Na_2SO_4. Cold solvent extraction was carried out using 50 cm^3 petroleum ether/acetone (1:1 v/v) mixture. The mixture was well shaken, allowed to stand and filtered. The fat content of the muscle tissue was determined gravimetrically after evaporating the solvent extracts.

Extraction and pre-concentration

10 g of fish muscle was homogenized with 10 g of anhydrous granulated Na_2SO_4 and cold solvent extraction was performed. 50 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 [9]. The fish extracts were concentrated to 1 cm^3 and kept for clean-up procedure.

Clean-up of fish extracts

The clean-up of the fish extracts was carried out using column chromatography [10]. The glass separating column was packed with activated silica gel (90% < 45 µm) and washed down with n-hexane. The extracts were demoisturized over 1 g of anhydrous granulated Na_2SO_4 and separated into two eluted fractions using mixtures of dichloromethane, hexane and acetonitrile as eluting solvents. 30 cm^3 of dichloromethane/hexane (20/80) mixture was used for the first fraction, while 30 cm^3 of dichloromethane/hexane/acetonitrile (50/49.5/0.5) mixture was used for the second fraction to ensure that the polar acetonitrile eluted any remaining residue. The two fractions were combined, concentrated to 1 cm^3 using a rotary evaporator and subsequently analysed.

Identification and determination of chlorinated hydrocarbon residues

A gas chromatograph with a ^{63}Ni electron capture detector (GC-µECD Agilent Technology 7890A) equipped with the ChemStation software was used for the identification and determination of the chlorinated hydrocarbon residues. The cleaned-up extracts were dried and re-dissolved in 1.0 cm^3 analar grade isooctane before injecting 1 µL of the purified extract into the injection port of the gas chromatograph [11]. Organochlorine Pesticides II EPA Method 8081A was employed for the analyses [12]. The fish extracts were analysed for aldrin, dieldrin, endrin, DDT, heptachlor, HCH, endosulfan, chlordane and methoxychlor. The stock solution of the organochlorine pesticide (OCP) standards was purchased from Restek Corporation, USA and was serially diluted to obtain 10 ng/mL, 20 ng/mL and 40 ng/mL. Strict cleaning procedures, recovery of spiked standards and monitoring of detector response were some of the quality assurance measures that were adopted. The correlation coefficients of calibration curves were all higher than 0.998. The limits of detection and quantification of the organochlorine pesticide residues were determined by multiplying the standard deviation obtained from six replicates at lowest expected concentration by 3 and 10 respectively [13].

Recovery study

Recovery was performed by spiking the previously analysed samples with the pesticide standard.

$$\% \text{ Recovery} = \frac{CS_2 - CS_1 \times 100}{CS}$$

where, CS_1 = concentration of pesticide residues in the sample

CS_2 = concentration of pesticide residues in the spiked sample

CS = concentration of added pesticide standard

Estimation of daily intakes (EDI) of chlorinated hydrocarbons by humans

The dietary intake of chlorinated hydrocarbons by humans was estimated by multiplying concentrations in the muscle tissues of fish by the per capita consumption [14]. Interviews were conducted in 100 families where respondents were categorized into males and females.

Results

Concentrations of chlorinated hydrocarbon residues were calculated individually and as the sum of their isomeric

forms. The mean and standard deviation were calculated from the detectable values, and values below the detectable limit were considered not detected (ND). The mean was calculated from triplicate determinations. The mean biometric data of the fishes are as shown in Tables 1 - 2 while the mean concentrations of the chlorinated hydrocarbons in their muscle tissues are shown in Tables 3 and 4. The mean recoveries of the residues ranged from 88.45 to 98.42%. The estimated daily intakes (EDI) of the chlorinated hydrocarbons by humans are shown in Table 5. The appraisal of dietary intake was based on comparison of acceptable daily intakes established by the joint FAO/WHO expert committee, Health Canada and USEPA as shown in Table 6.

Table 1: Mean biometric data of female *Lutjanus goreensis*, *Sardinella maderensis* and *Regalecus glesne* during the dry season

Fish species	Feeding mode	Wet weight (g)	% Dry matter	% Fat	TL (cm)	SL (cm)	CF
L. goreensis	*Herbivorous*	33.6±0.4	20.7±0.4	0.2±0.1	13.5±0.4	11.0±0.4	1.4±0.3
S. maderensis	*Carnivorous*	90.9±0.2	19.9±0.2	0.3±0.1	20.5±0.2	16.5±0.2	1.1±0.2
R. glesne	*Carnivorous*	68.9±0.8	25.3±0.7	0.3±0.1	50.0±0.7	46.0±0.7	0.1±0.6

TL = total length of wet fish; SL = standard length of wet fish; CF = condition factor of fish
The mean value was calculated from 3 fishes of each species.

Table 2: Mean biometric data of female *Lutjanus goreensis*, *Sardinella maderensis* and *Regalecus glesne* during the wet season

Fish species	Feeding mode	Wet weight (g)	% Dry matter	% Fat	TL (cm)	SL (cm)	CF
L. goreensis	*Herbivorous*	35.5±0.3	20.8±0.2	0.2±0.2	13.6±0.3	11.2±0.3	1.4±0.1
S. maderensis	*Carnivorous*	90.7±0.6	19.8±0.5	0.3±0.2	20.4±0.5	16.4±0.6	1.1±0.2
R. glesne	*Carnivorous*	78.9±0.5	24.2±0.5	0.3±0.2	50.0±0.4	46.0±0.5	0.1±0.3

Table 3: Mean concentrations (ng/g) of chlorinated hydrocarbons in the muscle tissues of female *Lutjanus goreensis*, *Sardinella maderensis* and *Regalecus glesne* during the dry season

OCPs	*Lutjanus goreensis*	*Sardinella maderensis*	*Regalecus glesne*
Alpha-BHC	ND	21.2±6.42	67.86±6.65
Beta-BHC	0.32±0.30	94.26±4.06	269.45±9.34
Lindane	ND	20.04±8.35	29.95±5.62
Delta-BHC	0.64±0.29	59.24±6.31	41.11±7.51
ΣBHC	0.96±0.59	194.75±25.14	408.36±29.12
Heptachlor	1.55±1.93	47.31±5.38	86.50±4.13
Heptachlor-epoxide (B)	0.74±0.17	19.44±8.42	106.15±3.52
Aldrin	1.31±1.56	25.83±3.03	39.97±4.15
Dieldrin	0.59±0.22	52.02±5.14	ND
Endrin	0.99±0.27	114.71±12.12	127.33±8.13
Endrin aldehyde	0.51±0.30	263.09±7.92	ND
Endrin ketone	ND	558.83±5.04	4635.05±3.75
Cis-Chlordane	0.6±0.54	41.68±4.13	130.79±8.49
Trans-Chlordane	1.16±1.04	67.12±0.22	99.02±4.53

Endosulfan 1	1.06±0.37	55.40±0.15	99.13±5.16
Endosulfan 11	0.71±0.12	25.84±0.69	82.14±3.42
Endosulfan sulphate	0.35±0.19	71.99±0.24	ND
Methoxychlor	ND	64.08±0.25	73.95±6.25
p,p´-DDE	0.67±0.23	31.64±0.63	292.76±7.19
p,p´-DDD	ND	57.12±0.82	ND
p,p´-DDT	ND	138.33±0.16	ND
ΣDDT	0.67±0.23	227.09±1.61	292.76±7.19
ΣOCPs	11.19±7.53	1829.18 ±70.48	6181.16±87.84

Table 4: Mean concentrations (ng/g) of chlorinated hydrocarbons in the muscle tissues of female *Lutjanus goreensis*, *Sardinella maderensis* and *Regalecus glesne* during the wet season

OCPs	*Lutjanus goreensis*	*Sardinella maderensis*	*Regalecus glesne*
Alpha-BHC	ND	1.30±0.20	60.77±2.23
Beta-BHC	0.36±0.29	0.99±0.59	226.07±5.49
Lindane	0.65±0.33	0.52±0.38	23.44±8.02
Delta-BHC	0.70±0.17	0.66±0.19	35.41±6.17
ΣBHC	1.72±0.79	3.47±1.36	345.69±21.91
Heptachlor	0.96±0.35	1.40±1.74	73.46±4.64
Heptachlor-epoxide (B)	ND	0.61±0.36	95.54±6.32
Aldrin	0.87±0.28	1.80±1.62	32.76±4.43
Dieldrin	ND	ND	ND
Endrin	10.68±6.55	0.70±0.20	119.75±7.17
Endrin aldehyde	ND	ND	ND
Endrin ketone	ND	30.97±15.53	3796.53±18.03
Cis-Chlordane	ND	0.55±0.29	123.88±6.28
Trans-Chlordane	ND	0.55±0.40	85.66±8.81
Endosulfan 1	2.09±1.06	0.87±0.25	86.66±4.62
Endosulfan 11	ND	ND	74.43±6.83
Endosulfan sulphate	ND	ND	ND
Methoxychlor	ND	ND	69.88±4.29
p,p´-DDE	ND	0.67±0.30	281.79±7.93
p,p´-DDD	ND	1.76±1.04	ND
p,p´-DDT	ND	ND	ND
ΣDDT	ND	2.42±1.34	281.79±7.93
ΣOCPs	16.32±9.03	43.36±23.09	5186.04±101.26

Table 5: Estimated daily intake (EDI) of chlorinated hydrocarbons (ng/g) by humans

Organochlorines	*Lutjanus goreensis*	*Sardinella maderensis*	*Regalecus glesne*
BHC	8.33	18.08	37.92
Heptachlor	2.04	6.21	17.89
Aldrin	1.47	2.84	3.71
Dieldrin	3.86	4.83	ND
Endrin	27.02	86.97	442.22
Chlordane	3.85	10.11	21.34
Endosulfan	20.92	14.52	16.83

Methoxychlor	ND	6.19	6.87
DDT	54.42	26.72	27.18

Table 6: Acceptable daily intake (ng/g body weight/day) of chlorinated hydrocarbons in fish

Pesticides	FAO/WHO	Health Canada	USEPA (R_fD)
BHC	42	18	18
Heptachlor	5	-	-
Aldrin	7	-	-
Dieldrin	-	-	-
Endrin	6	-	-
Chlordane	-	3	30
Endosulfan	-	-	-
Methoxychlor	-	-	-
DDT	1200	1200	30

Discussion

The distribution profile of the chlorinated hydrocarbons in the muscles of the fishes indicate that different fishes have varied concentrations of residues. Except for *Regalecus glesne,* the fishes had a condition factor of more than 1. The condition factor describes the physiological condition of fishes [15] and usually increases when sexual maturation approaches. An undernourished or thin fish has a condition factor less than 1 while an adequately fed or fat fish has a condition factor greater than 1. The carnivorous fishes accumulated more of the residues probably due to their position in the food chain. A higher concentration of the chlorinated hydrocarbon residues was observed during the dry season. This observation may be due to dilution effect that characterizes the wet season. The residue distribution pattern in muscle tissues of the fishes were: *Regalecus glesne* > *Sardinella maderensis* > *Lutjanus goreensis. Regalecus glesne* recorded the highest chlorinated hydrocarbon residue of 6181.16 ng/g. The values obtained were higher when compared to studies earlier carried out in Ogun and Edo Rivers [16-17]. In previous studies, the mean concentration of chlorinated hydrocarbons in fish samples from rivers in Edo State, Nigeria ranged from 0.36 to 0.71 ng/g. In Ogun River, the residues ranged from 0.06 to 19 ng/g while a range of 0.01 to 8.92 mg/kg was obtained in the studies by Adeyemi et al. [18]. However, the concentrations of chlorinated hydrocarbons in the fish species in this study were within the permissible limits [19-21], confirming that the consumption of the fishes was safe.

Fish consumption represents an important pathway for exposure to chlorinated hydrocarbons and the assessments of risks to human health have been undertaken in various environmental media [22]. The highest EDI for the fishes was observed in endrin for *Regalecus glesne* (442.22 ng/kg body weight/day). The dietary surveys conducted in 100 families showed that the amount of fish consumed ranged from 20 to 200 g/day, with a mean value of 40 g/day. The mean consumption of fish in this study compared with the dietary surveys earlier conducted in China [23]. In a survey conducted in 325 families in Coimbatore city, India, Muralidharan et al. [24] also reported fish mean consumption of 47 g/day. Muscle tissue was used in determining the dietary intakes to human body as it is the edible portion in a fish. ΣBHC, Σaldrin, Σendrin, Σchlordane, Σheptachlor and total dichlorodiphenyltrichloroethane (ΣDDT) 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. The appraisal of dietary intake was based on comparison of acceptable daily intakes established by the joint FAO/WHO expert committee, Health Canada and USEPA (Table 6) with the estimated daily intakes in this study.

Conclusion

A total of 23 chlorinated hydrocarbons were detected and quantitated in the muscle tissues of the fishes sampled in this study. A higher concentration of the residues was observed during the dry season. The concentrations of chlorinated hydrocarbons in the fishes and the estimated daily intakes of the pesticides in them were below the maximum permissive residue limits. The present study could serve as a reference for future work in comparing the chlorinated hydrocarbons in male and female species of these fishes. We plan to undertake studies on their polychlorinated biphenyl accumulation.

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About the Authors:

Akan Bassey Williams and Winifred Uduak Anake: Department of Chemistry, Covenant University, Ota, Ogun State, Nigeria.
akan.williams@covenantuniversity.edu.ng, +234-8036706086