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# LEVELS OF ENDOSULFAN AND O,P'-DICHLORODIPHENYLTRICHLOROETHANE RESIDUES IN *CLARIAS GARIEPINUS,* WATER AND SEDIMENT FROM SOME CREEKS IN AKWA IBOM STATE, NIGERIA

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## ABSTRACT

Fish samples (*Clarias gariepinus*) from five fishery sites in Akwa Ibom State, Nigeria were studied. Extraction of the pesticides from the fish was carried out followed by purification in a column conditioned with n-hexane and dichloromethane as mobile phase. The extract was then analyzed with GC-MS. The result displayed that the highest mean residual concentration of endosulfan occurred in fresh fish obtained from Ifiayong Esuk (0.021± 0.071 x $10^{-2}$ mg/kg), smoked-dried fish from Ayadehe (0.016 ± 0.000 x $10^{-2}$ mg/kg), water from Ifiayong Esuk (0.530± 0.000 x $10^{-2}$ mg/kg) and sediment from Issiet Creek (0.922 ± 0.000 x $10^{-2}$ mg/kg). All the values were statistically significant (p-value <0.05) compared to the reference value. The mean residual concentration of o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT) detected in sediment from Ibeno Creek was 0.005± 0.007 x $10^{-2}$ mg/kg. The health risk estimation (hazard quotient) of endosulfan residues in fresh and smoked-dried fish samples were lower than the US Environmental Protection Agency's chronic reference concentration of 0.006 mg/kg day and the toxicity threshold limit of 1.00. From the results of this work, the aquatic and terrestrial environments under study contained endosulfan and o,p'-DDT and these chemicals may induce lethal or sublethal effects on *Clarias gariepinus*.

**KEYWORDS**: Pesticides, Toxicity, Pollution, Catfish, Water, Sediment, GC-MS.

## INTRODUCTION

The behaviour, biochemistry, and physiology of fauna such as fish, are altered by interaction of the chemical composition of the natural marine environment with harmful substances like pesticides.[1,2] Pesticides have the tendency to accumulate in the soil and body organs, Organochlorine compounds can cause histopathological alterations in fish kidney, liver, gills, muscles and other organs.[3,4] A variety of histopathological toxic effects and disorders are caused by long term effect of water contamination by pesticides.[2,5] Fish absorbs these toxic compounds directly from water or by ingesting contaminated food. The aftermath of this bio-accumulation in fish poses potential threat to fish and human when the fish is consumed.[5]

Endosulfan is an organochlorine insecticide, acaricide and environmental contaminant used by farmers to protect crops like coffee, cotton, tea, soy and vegetables.[3] o,p'-Dichlorodiphenyltrichloroethane (o,p'-DDT) was used in public health for the control of mosquito.[6,7] The biotransformation processes of both chemicals correlate with their nature of semi-volatility and persistence in an environment.[6,7] The aim of this study is to determine the levels of endosulfan and o,p'-DDT residues in *Clarias gariepinus,* water and sediment from some creeks in Akwa Ibom State, Nigeria.

## MATERIALS AND METHODS

### Equipment, Apparatus and Reagents

GC-MS (Shimadzu, Japan), Analytical balance (Merck Company, Germany) and Fish net. All reagents were sourced from Sigma Aldrich Chemicals (USA), Merck Company (Germany), Central Research Laboratory in the Faculty of Pharmacy, University of Uyo.

### Study Area

In this study, five different creeks and adjoining local fish markets (Ayadehe, Ibeno, Ifiayong Esuk, Issiet, Itu) in Akwa Ibom State, Nigeria were considered.

### Sample Collection

### Collection of African Catfish (*Clarias gariepinus*) at Fishery Sites

Fresh and smoked African catfish (*Clarias gariepinus*) were collected from local fish markets and fishermen at the five creeks. Manually eviscerated fish including the head and flab parts were minced before being kept at -20°C until used.[8,9]

### Sampling of Coastal Water at the Fishery Site

Muddy water was collected in a 750 mL sterile Polyethylene terephthalate (PET) bottle in duplicate. The color, volume, date and time of collection of the water samples were documented and used as soon as possible.[10,11]

### Collection of Soil Samples at the Fishery Site

Mud soil sample (sediment) was collected in a white transparent sanitary plate in duplicate. The weight and color of the sediment were examined while the date and time of collection were noted and recorded as well.[11,12]

**Extraction of Pesticides in Samples:** Pesticides extraction was carried out in fresh and smoked fish samples, creek water and sediment according to known procedures.[12,13,14]

### Extraction of Pesticides in Smoked-dried and Fresh Fish samples

Eviscerated fresh fish sample (2 g) comprising gills, fins, skin and liver, was weighed, ground and transferred into 100 mL beaker. Extraction was done successively in duplicate using dichloromethane (3 x 10 mL). Each extract was filtered using a filter paper packed with anhydrous sodium sulphate ($Na_2SO_4$) and stirred for 15 minutes. The same procedures were repeated for smoked-dried fish from all the creeks.

### Extraction of Pesticides in Creek Water Samples

Dichloromethane (3 x 10 mL) was used to extract water sample (50 mL), dried with anhydrous $Na_2SO_4$, stripped of dichloromethane with nitrogen gas and later kept in a desiccator for further use.

### Extraction of Pesticides in Soil Samples (Sediment)

Soil sample (2.0 g) in 10 mL of distilled water was extracted with $CH_2Cl_2$ (3 x 10 mL). The same procedures stated above for drying of water samples were repeated and extracts were kept in the desiccator until when needed.

**Purification of Sample (Clean-Up):** The clean-up of the extract was executed by solid-phase extraction (SPE) using a short column (15 x 1 cm) packed with 2 g silica gel (50 - 200 mesh), and 0.65 g of anhydrous $Na_2SO_4$ with dichloromethane (10 mL) as mobile phase. The eluates were concentrated using nitrogen sent for GC-MS analysis.

**GC-MS Analysis:** The identities, screening and determination of endosulfan and o,p´-DDT in fish samples was carried out using GC-MS, QP2010 SE (Shimadzu, Japan). Separations were carried out on a Restek fused silica capillary column with injector port temperature of 250 ºC. The injection volume was 8.0 µL, the gas flow rate of helium carrier was 1.0 mL $min^{-1}$, the electron-impact ionization (70 eV) with the electron multiplier voltage of 2000 V. Similarly, dried eluate of fish samples free from endosulfan and o,p´-DDT pesticides (Control) were also analyzed by GC-MS.

### Statistical Analysis

Data were analyzed using SPSS Version 16, using analysis of variance (ANOVA) inferential analysis to determine the differences in the concentration of each of the pesticide residue in each sample analyzed as the level of statistical significance was set at p-value < 0.05.

### i. Estimation of Amount of Insecticide Residues Present in the Sample

Sample results were quantified in mg/mL automatically by the GC-MS software, which is represented by the concentration of the final volume injected and from the value, the actual amount of insecticide residues present in the sample was determined using the following formula:

$$\frac{\text{Concentration obtained in injected volume} \times \text{Final volume}}{\text{Amount of sample used}}$$

### ii. Estimation of Average Daily Intake (EADI) of Insecticide Residues

The estimated average daily intake (EADI) of insecticide residue according to these methods[15,16,17] using the formula below:

$$\frac{\text{Average mean residual pesticide concentration (mg/kg)} \times \text{Food consumption (kg)}}{\text{Body weight (kg)}}$$

### iii. Estimation of Hazard Quotient (HQ) or Potential Health Risk

Hazard quotient (HQ) according to the literature methods[18] was obtained by using the formula below:

$$\frac{\text{Estimated average daily intake (EADI) of insecticide residues (mg/kg/day)}}{\text{Reference dose (mg/kg/day)}}$$

The food and agricultural organization (FAO) quotes the per capita consumption of fishes in Nigeria as 9 kg while body weight was set at 70 kg for adult population group.[17,18]

## RESULTS

In this study endosulfan was detected in the five different creeks: Ayadehe, Ibeno, Ifiayong Esuk, Issiet and Itu. o,p'-DDT was detected only at Ibeno Creek (0.005± 0.007 x $10^{-2}$ mg $kg^{-1}$) as seen in Table 1.

Water sample from Ifiayong Esuk Creek recorded the highest level of endosulfan (0.530± 0.000 x $10^{-2}$ mg $L^{-1}$) and lowest (0.120± 0.000 x $10^{-2}$ mg $L^{-1}$) from Itu Creek.

**Table 1: Levels of o,p'-DDT and endosulfan in *Clarias gariepinus* (fresh and smoked-dried catfish), sediment and water collected at five Creeks in Akwa Ibom State, Nigeria.**

| Creek/Pesticides | Fresh Fish (mg/kg) | Smoked-dried fish (mg/kg) | Sediment (mg/kg) | Water (mg/L) |
|---|---|---|---|---|
| Ayadehe | | | | |
| D | - | ND | - | - |
| E | - | 0.016 ± 0.000 [a] | - | - |
| Ibeno | | | | |
| D | ND | ND | 0.005 ± 0.007 | ND |
| E | 0.003 ± 0.000 [a] | ND | 0.058 ± 0.078 [a] | 0.389 ± 0.000 [a] |
| Ifiayong Esuk | | | | |
| D | ND | ND | ND | ND |
| E | 0.021 ± 0.071 [a] | 0.010 ± 0.000 [a] | 0.020 ± 0.021[a] | 0.530 ± 0.000 [a] |
| Issiet | | | | |
| D | ND | ND | ND | ND |
| E | 0.005 ± 0.000 [a] | ND | 0.922 ± 0.000 [a] | 0.477 ± 0.000 [a] |
| Itu | | | | |
| D | - | ND | ND | ND |
| E | - | ND | 0.083 ± 0.092[a] | 0.120 ± 0.000 [a] |

ND = Not detected, D = o,p'-DDT, E= Endosulfan, values are presented as mean ± SE(Standard error). Comparison of values with different superscript shows statistically different (p-value < 0.05) levels of pesticide residues in sample.

**Table 2: Health Risk Assessment of Endosulfan in Fresh (F) and Smoked-dried (S) *Clarias gariepinus* (Catfish) sampled at five Creeks in Akwa Ibom State, Nigeria.**

| Creek/Fish | Mean Residual Conc. of Endosulfan (mg/kg) | EAD1 (mg/kg/day) | HQ | US EPA's cRfD (mg/kg/day) |
|---|---|---|---|---|
| Ayadehe | | | | |
| F | ND | ND | ND | 0.006 |
| S | 0.016 ±0.000 [a] | 0.206 | 0.343 | 0.006 |
| Ibeno | | | | |
| F | 0.003±0.000 [a] | 0.039 | 0.064 | 0.006 |
| S | ND | ND | ND | 0.006 |
| Ifiayong Esuk | | | | |
| F | 0.021±0.071 [a] | 0.270 | 0.450 | 0.006 |
| S | 0.010±0.000 [a] | 0.129 | 0.214 | 0.006 |
| Issiet | | | | |
| F | 0.005 ± 0.000 [a] | 0.064 | 0.107 | 0.006 |
| S | ND | ND | ND | 0.006 |
| Itu | | | | |
| F | ND | ND | ND | 0.006 |
| S | ND | ND | ND | 0.006 |

ND: Not detected. US EPA's cRfD: US EPA's chronic reference dose. EADI: Estimated average daily intake of insecticide residue. HQ: Hazard quotient**.** a: x $10^{-2}$. Comparison of EADI and HQ values from different locations and Mean ± SE (Standard error) values with different superscript shows statistically different (p-value < 0.05) levels of pesticides in sample. Pesticide residue with HQ values greater than 1.00 is said to exceed the reference dose and toxicity threshold limit.

**Table 3: Health Risk Assessment of o, p'-DDT in fresh (F) and smoked dried (S) *Clarias gariepinus* (Catfish) sampled at five Creeks in Akwa Ibom State, Nigeria.**

| Creek/Fish | Mean Residual Conc. of o,p'-DDT (mg/kg) | EADI (mg/kg/day) | HQ | US EPA's cRfD (mg/kg/day) |
|---|---|---|---|---|
| Ayadehe | | | | |
| F | ND | ND | ND | 0.0005 |
| S | ND | ND | ND | 0.0005 |
| Ibeno | | | | |
| F | ND | ND | ND | 0.0005 |
| S | ND | ND | ND | 0.0005 |
| Ifiayong Esuk | | | | |
| F | ND | ND | ND | 0.0005 |
| S | ND | ND | ND | 0.0005 |
| Issiet | | | | |
| F | ND | ND | ND | 0.0005 |
| S | ND | ND | ND | 0.0005 |
| Itu | | | | |
| F | ND | ND | ND | 0.0005 |
| S | ND | ND | ND | 0.0005 |

ND: Not detected. US EPA's cRfD: US EPA's chronic reference dose. EADI: Estimated average daily intake of insecticide residue. HQ: Hazard quotient**.** a: x $10^{-2}$. Comparism of EADI and HQ values from different locations. Pesticide residue with HQ values greater than 1.00 is said to exceed the reference dose and toxicity threshold limit.

## DISCUSSION

Fish is a major route through which humans are exposed to organochlorine contaminants.[8,19] In this study endosulfan was detected in the five different creeks: Ayadehe, Ibeno, Ifiayong Esuk, Issiet and Itu. o,p'-DDT was detected only at Ibeno creek as seen in Table 1.

Water sample from Ifiayong Esuk Creek recorded the highest level of endosulfan and lowest from Itu creek. The variation in the level of endosulfan in the water sample between creeks was statistically significant (p-value < 0.05). Endosulfan was the predominant pollutant in all the samples tested. Endosulfan is readily absorbed in sediment and water and is toxic to fish and marine invertebrates.[14]

The EADI values of endosulfan residues were lower than the US EPA's chronic reference doses (cRfD = 0.0006 mg/kg/daily). The health risk estimation (hazard quotient) of endosulfan residue in the fresh and smoked-dried fish samples did not exceed the toxicity threshold limit of 1.00.[16,17,18]

Endosulfan is toxic to juvenile fish causing changes in sodium and potassium concentrations, inhibits brain adenosine triphosphatase (ATPase) and acetylcholinesterase, as well as decreased blood magnesium and calcium levels. Lack of coordination, hyperactivity, staggering, convulsions, skin irritation, tremors, difficulty in breathing, rashes and permanent brain damage are some symptoms of acute endosulfan poisoning. A dose of 35mg/kg resulted in death in humans.[19,20]

Endosulfan decreases the immune system, the levels of thyroid and sex hormones in animals. It also delays the synthesis of sex hormone and sexual maturity in male children.[21,22,23,24] The mean residual concentration of o,p'-DDT detected in soil sample from Ibeno creek was 0.005± 0.007 x $10^{-2}$ mg $kg^{-1}$. This value is significantly higher (p-value < 0.05) than the Federal Environmental Protection Agency (EPA) allowable tolerance limit of 0.0001 mg/kg/day and the US EPA's chronic reference dose (US EPA's cRfD) of 0.0005 mg/kg/day.[15,18]

The high concentration of o,p'-DDT in Ibeno creek may be due to increased use of this chemical by inhabitants of this community to repel mosquitoes. It may also be due to waste discharge from oil production company and from other neighbouring towns in the peninsular.

## CONCLUSION

This study revealed the presence of endosulfan and o,p'-DDT in the sampled fish, sediment and water. The mean residual concentrations of endosulfan detected in sediment and water and that of o,p'-DDT in sediment alone are indicators of potential hazards to aquatic environment.

The health risk estimation (hazard quotient) of endosulfan residue in fresh and smoked-dried fish could be attributed to the public health concern.

The presence of these organochlorine compound could be attributed to the people who use them to control agricultural pests or municipal waste from drainage and run off that flows into the creeks.

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