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Determination of Organochlorine Pesticides in Some Imported Frozen Fish Species Consumed within Kaduna Metropolis

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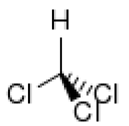
Abstract: *This study was carried out to determine the levels of Organochlorine pesticide residues in some commonly imported frozen fish. Three (3) species of fish namely Mackerel, Sardine and Shawa were obtained within Kaduna Metropolis. The fish parts such as liver, gills, flesh and stomach of the fish samples were separated, dried, grounded and extracted using diethyl ether and dichloromethane as solvent. Extracts obtained were further purified using hexane, dichloromethane, diethylether and analysed to ascertain the presence of Organochlorine Pesticicides. Four Organochlorine pesticides namely: Lindane, Aldrin, Endosulfan and Dichlorodiphenyltrichloroethane residues were detected in some of the fish samples in concentration ranges of (1.053±0.028 – 5.77±0.019 µg/g) for Lindane, (0.468±0.006 µg/g) for Aldrin , (ND −0.006±0.004µg/g) form Dichlorophenyltrichloroethane and (ND – 0.572±0.031 µg/g) for Endosulfan. All the pesticides residues determined have their mean concentrations above the permissible limit 0.01µg/g set by EPA. The higher concentrations of these pesticides above the permissible limit exposed the consumers to serious health problems.*
Keywords: *Extraction, Fish, Species, Organochlorine, Pesticides*

I. Introduction

Fish is a high-protein, low-fat food that provides a range of health benefits. Fish is lower in fat than any other source of animal protein, and oily fish are high in omega-3 fatty acids. As our body does not make significant amounts of these nutrients, fish are important source. However, most fish nowadays has lost their nutritional values due to environmental pollution. The impact of pollution on fish and the potential health implication of eating contaminated fish are areas of considerable concern for the government bodies and general public. Fish consumes much toxic wastes which are polluted materials discharged into their ambient environment. These toxic wastes are usually industrial wastes, sewage, pesticides and heavy metals such as mercury and cadmium. The pollutants especially mercury could possible end up in human bodies due to the consumption of affected fish[1]. Fish contribute about 55% of the protein intake in Nigeria, which is one of the largest importers of fish with per capita consumption of 7.52kg per annum and a total consumption of 1.2million metric tons with import making up about 2/3 of the total consumption [2]. The Nigerian government made a tariff reduction in year 2001 on all fishery products from 25% to 5% and since then, Nigeria has become a major destination for imported seafood [3].

Persistent organic pollutions (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological and photolytic process [4]. As a result of this, they have been observed to persist in the environment, to be capable of long-range transport, bio accumulate in human and animal tissue, bio accumulate in food chain and have to potential significant impacts on human health and the environment [4].

1.1 Organochlorine Pesticides



An Organochloride, Organochlorine compound, Chlorocarbon,or Chlorinated ydrocarbon is an organic compound containing at least one covalently bonded atom of chlorine that has an effect on the chemical behavior of the molecule. The Chloroalkane class (alkanes with one or more hydrogens substituted by chlorine) provides common examples. The wide structural variety and divergent chemical properties of organochlorides lead to a broad range of names and applications. Organochlorides are very useful compounds in many applications, but some are of profound environmental [5]. Pesticides are a group of chemicals made for the purpose of killing or otherwise deterring "pest" species. The word pesticides refer to insecticides, herbicides, fungicides, or other pest control formulations. Pesticides are inherently toxic and often pesticides may refer to insecticides, -associated with adverse health effects in non-target organisms [6]. Organochlorine pesticides are a large class of multipurpose Chlorinated hydrocarbon chemicals. Organochlorine pesticides break down slowly in the environment;accumulate in the fatty tissues of animals. Thus, they stay in the environment and food web long after they have been applied [7]. DDT(dichlorodiphenyltrichloroethane), now banned in the United States because of its harm to the health of wildlife and people, is a notable example of an Organochlorine pesticide. Many Organochlorine pesticides are endocrine disrupting chemicals, meaning they have subtle toxic effects on the body's hormonal systems. Endocrine disrupting chemicals often mimic the body's natural hormones, disrupting normal functions and contributing to adverse health effect.

1.2 Health Implication as a Result of Exposure to Organochlorine Pesticides

As a result of the multiple types and uses of Organochlorine pesticides, there are many ways people can be exposed to these chemicals. Wind and rain may move pesticides away from where they were used, causing contamination of surface waters, groundwater and/or soil [8]. The use of pesticides in homes or on lands may increase exposure to these chemicals, and living or working close to where pesticides are used is also a risk factor [9]. Organochlorine pesticides are not often used in personal products because of their toxicity, but some products, such as lice shampoos, may be a source of exposure. Exposure may also occur through consumption of contaminated foods. Many studies have linked Organochlorine pesticide exposure with consumption of contaminated animal products, mostly meat, dairy, fish, and marine mammals [10].

The presence of environmental chemicals in the human body does not necessarily imply that they are causing adverse health effects; however, environmental chemical exposures can and do affect human health. It is important to note that both the dosage and the timing of exposure have significant effect on any potential health outcome. The following information is intended to inform the reader about the current state of knowledge on the health effects of Organochlorine pesticides, including both human and animals studies. The health effects of Organochlorine pesticide exposure depend on the specific pesticide, the level of exposure, the timing of exposure and the individual. Different pesticides result in a range of health symptoms [10].

1.3 Cancer

Numerous studies have linked organochlorine pesticide exposures with cancers and other health effects. Exposure to DDT has been linked to pancreatic cancer [11]. Exposure to DDT early in life is associated with an increased breast cancer risk later in life [12]. Many other organochlorine pesticides, such as mirex, chlordane and toxaphene, are known to be carcinogenic as well [13]. A study of women from an agricultural area in India showed that women with breast cancer had much higher total organochlorine pesticide concentrations in their blood. The women had average total pesticide concentrations of 7,468 ± 771 ppb (ng/mL) in their blood [14].

1.3 Aim

The aim of this work is to determine the levels of Organochlorine Pesticide residues in some commonly imported frozen fish.

1.4 Justification

Fish consumption is recommended because it is a good source of high quality protein, minerals, vitamins and omega – 3 polyunsaturated fatty acids. The later composite protects consumers against coronary heart disease, reducing arrhythmias and thrombosis and risk of fatal heart attack and sudden death, lowers plasma triglyceride levels. However, the presence of Organochlorine Pesticides in fish makes it difficult to establish clearly the roles of fish consumption on a healthy diet.

1.5 Scope of Study

Frozen fish samples were purchased from two (2) local markets namely (Railway Station market and Central market) within Kaduna metropolis.

II. Sampling

2.1 Collection of fish samples

15 samples each of Shawa, Sardine and Mackerel were obtained from the Central Market and Railway Station within Kaduna Metropolis in Kaduna state Nigeria. Each sample was kept in sterile plastic bag and then taken to the laboratory as quickly as possible. The samples were dissected to remove the flesh, liver, stomach and gills of each species of fish and placed in the pre-marked sample bag and stored in the refrigerator till the next day. The various parts of the fish samples were placed in an oven to dry at 105^{oc} and later grinded into powdered form using mortar and pestle, before extraction was carried out.

III. Methods Of Analysis

3.1 Extraction

10g of the various part of the fish samples was weighed into 150ml conical flask followed by the addition of 10g and 2.5g of anhydrous sodium sulphate and sodium hydrogen carbonate, respectively. 50ml of 1:1(v/v) ethyl acetate and dichloromethane mixture was transferred into the 10g fish samples and thorough mixed by shaking the conical flask while corked. Another 10g of sodium sulphate was added to the content of the conical flask followed by 10g of sodium hydrogen carbonate. The conical flask was corked and the mixture was shaken thoroughly.

The content was allowed to stand for 3hrs. The upper layer was decanted using a separatory funnel into a 50ml beaker and evaporated using a hot water bath. The pesticides were dissolved and collected with 2ml of ethyl acetate and transferred into a 2ml vial and ready for the clean – up [15].

3.2 Silica-Gel Cleanup of Sample Extract

Silica Gel clean-up was carried out by measuring 10g portion of activated silica gel into 10mm glass chromatographic column followed by addition of 3g of anhydrous sodium sulphate. 10ml of n-hexane was used to wet and rinse the column.The elution was carried out to assess the ability of different solvents to fractionate the OCPs from the silica gel column. The column flow rate was adjusted to 5ml/min. The following schemes were attempted.

A. F1: n-hexane (50ml)
B. F2: n-hexane and ethyl acetate (1:1v/v)
C. F3: Dichloromethane (50ml)
D. F4: Dichloromethane and n-hexane 1:1v/v)
E. F5: Ethyl acetate (50ml)
F. F6: Ethyl acetate and Dichloromethane (1:1v/v)

All the fraction of each sample was concentrated to dryness using a rotary evaporator at 40^0C [16].The various fractions of each sample were later subjected to further analysis using TLC plates and viewed with a UV-Lamp at short-UV 2554nm and long-UV 365nm and also the fractions was suspended in iodine tank, to detect the presence of any possible contaminant. Each fraction were dissolved and collected in 2ml ethyl acetate for GC-MS analysis.

3.3 Gas Chromatography/ Mass Spectroscopy Analysis

The extracts were taken to the laboratory for GC/MS analysis. The instrumentation laboratory at Shimadzu Training Center for Analytical Instrument (STC) Lagos was used for this analysis. The GC-MS instrument separate chemical mixtures (the GC component) and identifies the components at a molecular level. The GC works on the principle that a mixture will separate into individual substance when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substance emerge from the column opening, the flow into the MS. The MS identifies the component by mass of the analyte molecules. A “library” of mass spectra covering several thousand compounds is stored on computer attached to the MS. The identified components are compared with the compound in the library and various option of possible compound are given with their percentage confidence having the first option as the most probable.

IV. Figures And Tables

The Result of concentration (ug/g) of Organochlorine Pesticides residues in various fish samples.

TABLE 4.1; Concentrations (ug/g) of Organochlorine pesticide residues in Mackerel fish sample

Part	LND	ALDRIN	DDT	END
Flesh	ND	8.354±0.250	ND	ND
Liver	5.770±0.019	8.110±0.239	ND	ND

ND; Not detect,LND;Lindane,ALDRN;Aldrin,DDT;Dichlorodiphenyltrichloroethane, END; Endosulfan.

Discussion

The mean concentrations of some Organochlorine pesticides (Lindane, Aldrin, DDT, Endosulfan) residues in the flesh and liver of Mackerel fish are presented in table 4.1, DDT, END were not detected in both the flesh and liver sample while for those present, the concentration range from 8.354±0.250 flesh and 5.770±0.019 and 8.110 ± 0.239 liver. The Lindane mean content (5.77 ± 0.019(µg/g) in mackerel liver was very high and was above the (0.01ug/g) maximum permissible. The Lindane contents of the fish sample obtained is higher than (0.013 ± 0.064(µg/g) range of Lindane reported by [17]for fish samples and the permissible limit (0.01µg/g) set by [18]. These high levels of Lindane in the fish samples may result to severe health problems such as immune system and dysfunction, disruption of endocrine and birth defect [19].

Aldrin mean concentration (8.354 ± 0.250µglg) of flesh and (8.110 ± 0.239µglg) of Liver, with the mackerel liver recording the highest concentration. The Aldrin concentration in both flesh and liver of the mackerel fish in this study was much higher than the (2.72 ± 20.57nglg) range reported by [20] for fish samples. This study showed that the Aldrin concentrations of the fish parts determined were higher than the permissible limits of 0.01µglg set by [18].

TABLE 4.2: Concentrations (µg/g) of Organochlorine pesticide residues in Sardine fish sample

Part	LND	ALDRIN	DDT	END
Flesh	1.952±0.041	3.540±0.077	0.006±0.004	ND
Gill	1.990±0.026	3.721±0.074	ND	ND
Stomach	3.751±0.245	0.468±0.006	ND	ND

ND; Not detect, LND; Lindane, ALDRN;Aldrin,DDT;Dichlorodiphenyltrichloroethane,END; Endosulfan.

V. Discussion

The mean concentration of some Organochlorine pesticides (Lindane, Aldrin, DDT, Endosulfan) residues in the flesh, gill and stomach of the sardine fish are presented in table 4.2. Endosulfan, were not detected in the three samples. Dichlorodiphenyltrichloroethane (DDT) residue was only detected in the sardine flesh sample with low mean concentration. Dichlorodiphenyltrichloroethane (DDT) pesticides residues in these fish samples may have resulted due to the ban on the use of these substances in farming and fishing. More so, the absence or relatively low mean contents of DDT in the fish samples could also be as a result of metabolism of DDT to DDE, this is because DDT can be biodegraded DDE and DDD under both aerobic and anaerobic conditions respectively. Meanwhile, the level (0.006 ± 0.004µglg) of DDT in the flesh of sardine fish sample is within the range (0.002 ± 0.062µglg) in fish samples as reported by [17].

The Lindane mean concentration (3.751 ± 0.245µglg) in the sardine stomach was the highest and was the above maximum permissible value while the Sardine Gill as the lowest lindane mean content of (1.970±0.02µg/g).The Lindane concentration of fish samples obtained in this study (3.751 ± 0.24uglg) – (1.970 ± 0.026µglg) was higher than (0.013 ± 0.064µglg) range of Lindane reported by [17] for fish samples and the permissible limit (0.01µglg) set by [18]. These high levels of Lindane in the fish samples may result to severe health problems such as immune system dysfunction, disruption of endocrine and birth defect [19].

The level of Aldrin in the sardine fish samples assessed varied from (3.721 ± 0.074uglg) in the sardine stomach. The Aldrin contents of the sardine fish sample obtained in the work was much higher than the (2.72 ± 20.57nglg) range reported by [20] and the (0.009 ± 0.064uglg) recorded by [17] for fish samples. This study showed that the Aldrin contents of all the parts of the fish samples assessed were higher than the permissible limit of (0.01uglg) set by [18].

TABLE 4.3; Concentrations (ug/g) of Organochlorine pesticide residues in Shawa fish sample

Part	LND	ALDRIN	DDT	END
Flesh	ND	2.500±0.281	ND	ND
Liver	3.906±0.210	6.507±0.161	ND	ND
Gill	4.103±0.247	9.016±0.451	ND	ND
Stomach	ND	9.929±0.351	ND	0.572±0.031

ND; Not detect, LND; Lindane, ALDRN; Aldrin, DDT; Dichlorodiphenyltrichloroethane, END; Endosulfan.

VI. Discussion

Table 4.3, represent the mean concentrations of some Organoclorine Pesticides residues (Lindane, Aldrin, DDT and Endosulfan) residues in flesh, liver, gill and stomach. DDT was not detected in all the parts while Endosulfan, was only detected in the stomach with the range of (0.572±0.031µg/g). Aldrin was detected in all the parts and the level of the pesticides ranges from (2.500±0.281µg/g) in Shawa flesh which is the lowest and (9.929 ± 0.351µglg) in Shawa stomach which is the highest. Lindane was only detected in the Liver and Gill of the shawa fish sample.From the results of the Organochlorine Pesticides residues analyzed, it was observed that Endosulfan residues was only detected in the stomach of Shawa fish sample where (0572 ± 0.031µglg) of it was found. These maybe due to poor metabolism of the substance in the stomach of the fish.

Lindane was detected only in the Liver and Gill of the Shawa fish sample with concentration range of (3.906 ± 0.210µg/g) in Shawa Liver and (4.103 ± 0.247µg/g) in Shawa Gill. The gill and liver samples of Shawa fish had relatively high Lindane contents. Also, Shawa fish has poorly developed liver and kidney, this result to low secretion of salts and production of urine which makes the substance to be of high concentration in gill and liver[21]. The Lindane concentration of the fish samples obtained are higher than (0.013 ± 0.064µg/g) range of Lindane reported by [17] for fish samples and the permissible limit (0.01µg/g) set by [18].

The level of Aldrin determined varied from (2.500 ± 0.281µg/g) Shawa stomach. The Aldrin contents of all the fish sampled parts assessed in this study was much higher than (2.72 ± 20.57nglg) range reported by [20] and the (0.009 ± 0.064ug/g) recorded by [17] for fish samples. This study showed that the Aldrin contents of all the parts of the fish samples assessed were higher than the permissible limits of (0.01µg/g) set by [18]. These higher amounts of Aldrin in the fish samples can cause health disorders such as reproductive failure, neonatal damage and cancer [22].

VII. Conclusion

From the results obtained, the levels of Organochlorine pesticide (lindane, aldrin, dichlorodiphenyltrichloroethane and endosulfan) residues in some commonly imported frozen fish (mackerel, sardine and shawa) had been determined and information provided on the amount of the pesticides as well as the danger they may pose to consumers that depend on these imported fish as diet. Lindane and aldrin were identified as the main organochlorine in the fish samples assessed. The mean concentrations of the lindane and aldrin studied in all the fish samples were found to be very high compared to their EPA maximum permissible limit, while DDT and endosulfan were not detected in most of the fish samples except only in sardine flesh and shawa stomach. It was discovered that the mean concentrations of the pesticides in all the frozen fish samples analysed were above permissible limits and this exposed the consumers to serious health risk such as cancer.

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