The Evaluation of Organochlorine Pesticide Residues in The Blood and Urine Samples of Cattles in Kaduna Metropolis

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Abstract**—** *The levels of Organochlorine pesticide residues in the blood and urine samples of Cattles in some selected areas within Kaduna metropolis in Nigeria were determined using standard methods. The results revealed the presence of alpha-lindane, delta-lindane, Dichlorodiethyldichloroethylene (DDE), Dichlorodiphenyldicloroethane (DDD)and endosulfan. In the blood, Alpha-lindane, delta-lindane, Dichlorodiethyldichloroethylene (DDE), Dichlorodiphenyldicloroethane (DDD) and endosulfan were detected at concentrations of 2.06 mg/kg, 2.23 mg/kg, 1.84 mg/kg, 1.94 mg/kg and 0.32 mg/kg respectively. In the urine samples, alpha-lindane, delta-lindane, DDD and endosulfan were detected at concentrations of 0.03 mg/kg, 0.03 mg/kg, 0.01 mg/kg and 0.38 mg/kg respectively. The levels of some of the detected organo-chlorine pesticides residues were above permissible limit of 0.01 mg/kg approved by European Union (EU) and therefore pose threats to the lives of human beings by causing some harmful diseases such as cancer, birth defect and respiratory problems.*

Keywords— Organochlorine pesticides (OCPs), Blood and Urine samples, European Union (EU), Gas –Chromatography – Mass Spectrometry (GC – MS).

I. INTRODUCTION

Pesticides are substances that protect plants against moulds, fungi and insects, and hence reduce the percentage of crop loss. Organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) have been used extensively in agriculture and various industries in the last century. The OCPs are highly resistant to degradation by biological, photochemical or chemical means. They are also toxic, hazardous and bioaccumulate in the environment (Darko and Acquaah, 2007). Their persistence in the environment still makes them to be detected in food items especially in different meat parts despite the fact that their use has been banned (Katsoyiannis and Samara, 2004). Also some developing countries still use them extensively on large farms (Matte*wet al*., 2013) as in Cameroon and for vector control purposes in Tanzania (Colombo *et al*., 1990;Mwevura*et al*., 2002). Organochlorine pesticides applied to livestock most frequently include those used to control disease vectors, such as tick and tsetse flies which may therefore have positive impacts on health. eg, improving animal productivity and curbingdisease transmission to humans. At the same time, chemicals applied to livestock or infiltrating livestock products from the broader environment can impact negatively on health. eg, through residues or tainting of food products, pollution of drinking water sources and bioaccumulation in the food chain (WHO/UNEP, 2008). In vertebrate animals and humans, pesticides are retained in the adipose tissues from where they are gradually released in the circulatory system (Erickson, 1997). OCPs may cause heritable changes in the genetic materials, DNA, cancer, mammary and uterine tumors, neurological effects such as numbness or weakness of arms, legs, feet or hands, memory loss, anxiety and loss of concentration, death may also occur. Hence, due to the above effects, Organochlorine pesticide residues in the blood and urine samples of some Cattles in selected areas within Kaduna metropolis was evaluated

II. EXPERIMENTAL

2.1 Sampling location and collection of samples

Blood and urine samples were collected randomly from Cattles in abattoirs located in Kaduna south, Kaduna north and Chikun local government areas respectively in Kaduna state, Nigeria.Figure 1 shows the geographical locations of sampling sites and the control. In the various abattoirs, blood samples were collected from adult Cattles in each of the selected local government areas and about 10 cm^3 of samples were collected in glass vials containing EDTA, to prevent the blood samples from clotting. About 10$cm^3$of urine samples were also collected in glass vials containing hydrochloric acid so as to prevent the urine samples from degrading. The samples altogether were kept in dry ice and transported to the laboratory where it was kept at 20 °C.

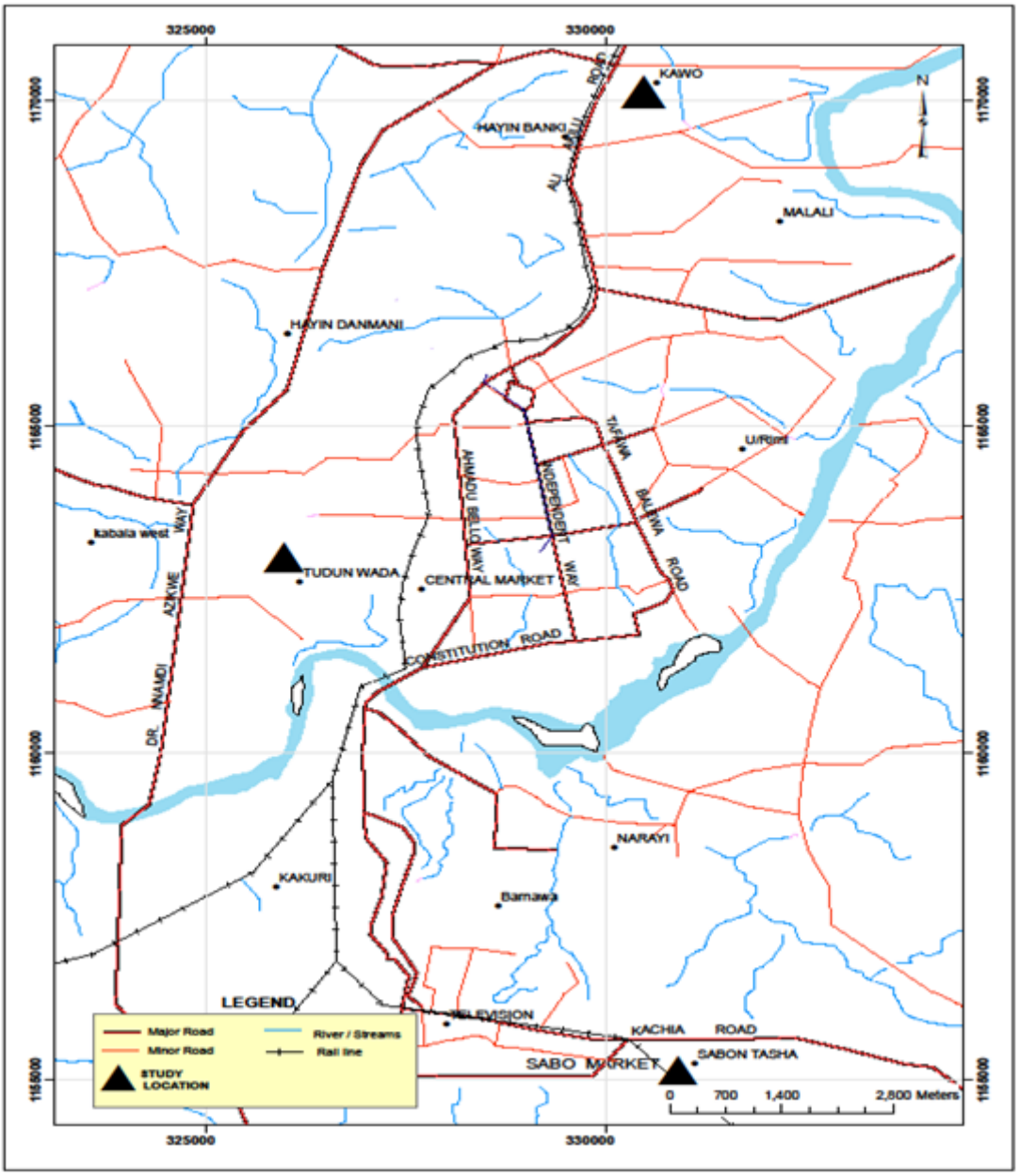

FIG 1 GEOGRAPHICAL LOCATIONS OF SAMPLING SITES AND THE CONTROL

2.2 Sample Extraction and Clean-Up

2.2.1 Extraction of Blood samples

Extraction was based on the method by (Agarwal*et al.*, 1996) with some modifications. Blood (5 cm^3) was diluted with 25 cm^3distilled water and a 2 cm^3 of saturated brine solution was added,then it was transferred to a 125 cm^3capacity separatory funnel. The sample was extracted with hexane: acetone (1:1) (20 cm^3) thrice by shaking the separatory funnel vigorously for 3 minutes therebyreleasing the pressure intermittently. As a result, the layers wereallowed to separate. The three combined extracts were passedthrough anhydrous sodium sulfate and concentrated to about 2 cm^3 using a rotary vacuum evaporator.

2.2.2 Extraction of urine samples

Urine Samples was extracted in hexane using the method described by Tewari and sharma (2005), 20$cm^3$of urine was measured and filtered and 15$cm^3$of distilled water was added. 10g of anhydrous sodium sulfate was also added and the volume filled up to 100 cm^3 of distilled water. The mixture was transferred to a separating funnel and 40 cm^3 of n-hexane was added and shaken well for 5 minutes. After separation of layers, extract was filtered and transferred again to a separating

funnel and 10cm^3 of hexane was added and shaken well for another 5 minutes. After separation of layers, the hexane layer was collected and taken for clean-up so as to eliminate any form of contamination.

2.2.3 Clean-up of samples

Cleaning up of the extracted samples was performed by transferring the extract into a glass chromatographic column containing 15g activated florisil (60/100 mesh) topped with 15g layer of anhydrous sodium sulfate. This column was rinsed with 100 cm^{3}petroleum ether and the extracted samples were applied to the column. The column was eluted with 300 cm^{3}eluent (20 percent dichoromethane and 80 percent petroleum ether). Then the collected eluate was concentrated to dryness on a rotary vacuum evaporator and re-dissolved in n-hexane to a volume of 5 cm3 (Salem *et al*., 2009). Finally, an aliquot of each extract were transferred to a 2 cm^{3}injection vials to be ready for analysis using a GC-MS machine. Hence, the gas chromatography oven temperature was initiated at 80 °C for 1 minute, raised to 175 °C (at a rate of 30 °C/ min), then raised to 290 °C (at a rate of 10 °C) and held for 2 minutes. Injection port temperature and detector temperature was maintained at 250 °C and 320 °C respectively. The sample volume injected was 1µL.

2.3 Gas Chromatographic Analysis

Organochlorines were analyzed by Gas Chromatography with the ^{63}Ni selective electron-capture detector. The column used was DB-17021. The carrier gas and makeup gas was helium with a 1.0 mL /Min and 40 mL /Min flow rate respectively employing the split less mode. 1.0µL of the final extract (2mL) was injected at a temperature of 250 °C. The oven temperature was kept at 85 °C with a hold time of 1 minute, then from 85 °C to 195 °C at a rate of 30 °C/min with a hold time of 1 minute and finally from 195 °C to 240 °C at a rate of 5 °C/ minute with a hold time of 6 minutes. The total run length was 50 minutes. The detector was maintained at 250 °C, peak identification was performed by the GC software (MSD Model Agilent system 5975).

2.4 Pesticide Residues Confirmation on Mass Spectrometer (MS)

Pesticide residues were confirmed by using Agilent-5775 with mass spectrometry detector (GC-MS), Quadrupole on electron ionization (EI) mode equipped with split less auto injector. The non-polar stationary phase used was a fused silica capillary column of length 30 m, 0.32 mm I.D and 0.25 µm film Thickness.

III. RESULTS AND DISCUSSIONS

Tables 1 and 2 shows the concentrations, retention time and response factors of the detected organochlorine pesticide residues from the blood and urine samples obtained from selected Cattles in abattoirs located at Kaduna south, Kaduna north and Chikun local government areas respectively.

Among the ten organochlorines and other analyzed in the whole blood and urine samples of Cattles in selected local government areas in Kaduna metropolis, the concentration of alpha-lindane detected from the blood samples were 2.06 mg/kg and 0.01 mg/kg from Kaduna north and Chikun local government areas respectively. Alpha-lindane was not detected from samples obtained from Kaduna south local government. Alpha-lindane was also detected in urine samples at concentrations of 0.03 mg/kg in Kaduna south local government and 0.01 mg/kg in both Kaduna north and Chikun local government areas.

Delta-lindane was not detected in the blood samples obtained from Kaduna south and Chikun local government areas but was present at 2.23 mg/kg in Kaduna north local government. Delta-lindane was also detected in the urine samples obtained from Kaduna south and Chikun local government areas at concentrations of 0.03 mg/kg and 0.01 mg/kg respectively. Alpha and delta lindane are used against sucking and biting pests and as smoke for control of pests in grain stores. It is also used to control various crop pests such as flea beetles and mushroom flies. It is in the list of banned pesticides in Nigeria. Lindanes are also known as gamma hexachlorocyclohexane or gammallin. In humans, lindanes affect the nervous system, liver and kidneys, and may as well be a carcinogen (El Beit*et al*., 1981). In this study, alpha and delta lindanes detected in the blood and urine samples might be because it is more resistant to biological and chemical degradation under aerobic condition (Ejobi*et al*., 1996)

A major metabolite of DDT, 2, 2-bis (chlorophenyl)-1, 1-dichloroethylene (DDE), was detected in blood samples only from Kaduna north local government area at concentration of 1.84 mg/kg but was not detected in urine samples gotten from any of the local government areas.

Another metabolite of DDT, 2, 2-bis (chlorophenyl)-1, 1-dichloroethane (DDD) was also detected in blood and urine samples at concentrations of 1.94 mg/kg and 0.01 mg/kg from Kaduna north and Kaduna south local government respectively. DDT is known to undergo metabolic conversion and dehydrochlorination. Presence of metabolites of DDT which is DDD and DDE encountered in this study is due to such metabolic processes (Dua*et al*., 1996)

Endosulfan was detected in all samples at relatively high concentrations. Endosulfan was detected in blood samples at 0.02 mg/kg, 2.65 mg/kg and 0.04 mg/kg from Kaduna south, Kaduna north and Chikun local government areas respectively. Endosulfan was also present in urine samples at concentrations of 0.38 mg/kg, 0.01 mg/kg and 0.32 mg/kg from Kaduna south, Kaduna north, and Chikun local government areas respectively. Endosulfan, an organochlorine insecticide of the cyclodiene subgroup acts as a poison to a wide variety of insects and mites on contact, and also acts as a stomach acaricide, according to this study, endosulfan appeared to be the most persistent organochlorine pesticide used on farmlands within Kaduna metropolis as a result of its high residue concentration.

Residues of organochlorines were detected in most of the samples as they are persistent in nature due to their slow decomposition ratio, long half-life and high stability in the environment (WHO/UNEP, 2008)

TABLE 1
RESULT OF LEVELS OF ORGAN CHLORINE PESTICIDE RESIDUES IN THE BLOOD SAMPLES

Local Govt	Organochlorine Compound	Concentrations (µg/g)
Kaduna south	Alpha lindane	ND
	Delta lindane	ND
	Hetachlor	ND
	Hepa-epoxide	ND
	Endosulfan	0.02
	DDE	ND
	Endorine	ND
	DDD	ND
	Hept-2-epoxide	ND
Kaduna north	Alpha lindane	2.04
	Delta lindane	2.23
	Hetachlor	ND
	Hepa-epoxide	ND
	Endosulfan	2.65
	DDE	1.84
	Endorine	ND
	DDD	1.94
	Hept-2-epoxide	ND
Chukun	Alpha lindane	0.01
	Delta lindane	ND
	Hetachlor	ND
	Hepa-epoxide	ND
	Endosulfan	0.04
	DDE	ND
	Endorine	ND
	DDD	ND
	Hept-2-epoxide	ND

TABLE 2
RESULT OF LEVELS OF ORGAN CHLORINE PESTICIDE RESIDUES IN THE URINE SAMPLES.

Local Govt	Organochlorine Compound	Concentrations (µg/g)
Kaduna south	Alpha lindane	0.03
	Delta lindane	0.03
	Hetachlor	ND
	Hepa-epoxide	ND
	Endosulfan	0.38
	DDE	ND
	Endorine	ND
	DDD	0.01
	Hept-2-epoxide	ND
Kaduna north	Alpha lindane	0.01
	Delta lindane	ND
	Hetachlor	ND
	Hepa-epoxide	ND
	Endosulfan	0.01
	DDE	ND
	Endorine	ND
	DDD	ND
	Hept-2-epoxide	ND
Chukun	Alpha lindane	0.01
	Delta lindane	0.01
	Hetachlor	ND
	Hepa-epoxide	ND
	Endosulfan	0.03
	DDE	ND
	Endorine	ND
	DDD	ND
	Hept-2-epoxide	ND

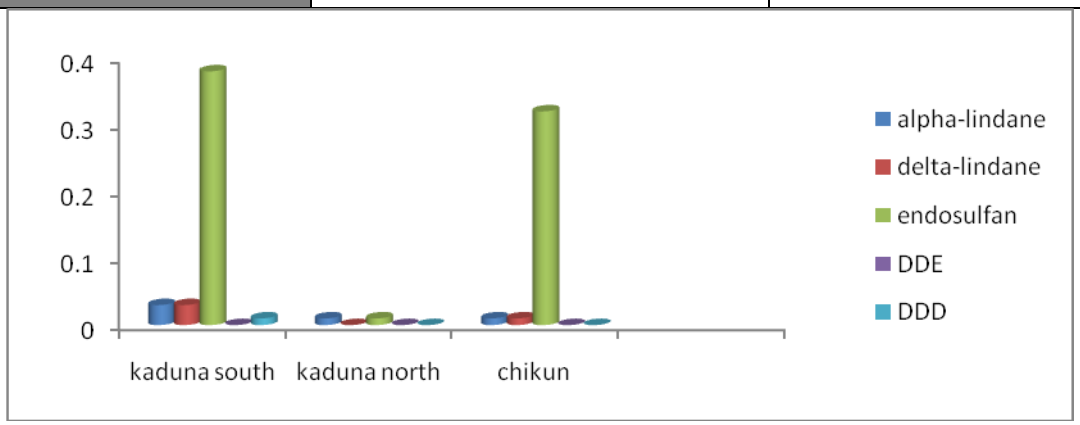

FIGURE 2 - COMPARING THE CONCENTRATION LEVELS OF SOME PESTICIDE RESIDUES IN THE URINE SAMPLES OF CATTLES IN SELECTED LOCAL GOVERNMENT AREAS IN KADUNA METROPOLIS

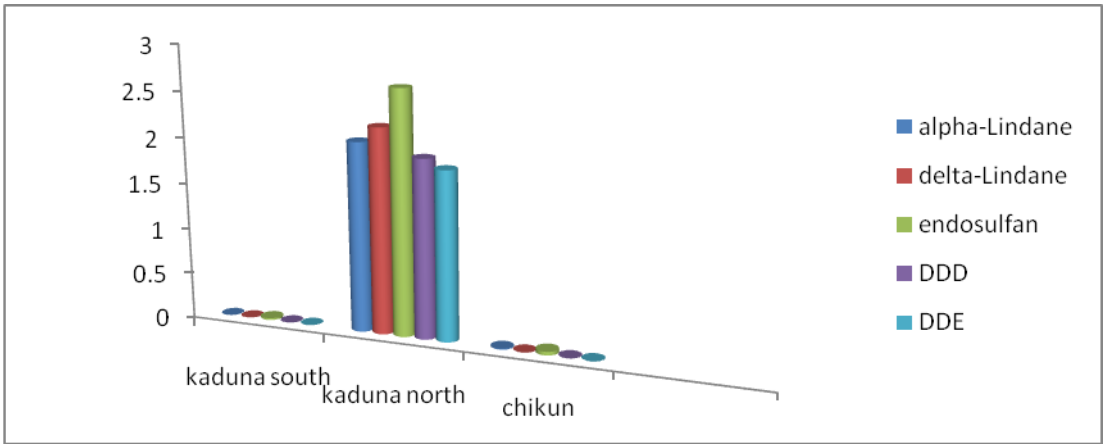

FIGURE 3 - COMPARING THE CONCENTRATION LEVELS OF SOME PESTICIDE RESIDUES PRESENT IN THE BLOOD SAMPLES OF SOME CATTLES IN SELECTED AREAS IN KADUNA METROPOLIS

IV. CONCLUSION

In this study, the presence of organochlorine pesticide residues such as alpha-lindane, delta lindane, DDD, DDE and endosulfan was confirmed.This study also shows that the level of alpha lindane, delta lindane, DDD, DDE and endosulfan in the blood and urine samples were higher than the permissible limit approved by the European Union. This study therefore shows that people consuming these Cattles within Kaduna south, Kaduna north and Chikun local government areas as studied stand the risk of contacting some of the diseases associated with the consumption of meat containing organochlorine pesticide residues.

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