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In-vitro Analytical techniques for PCBs & OCPs in Plasma Employing Solid-phase Disk Extraction Technique.

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ABSTRACT

Solid phase extraction techniques are generally simple, economical and use less solvent, making them more appropriate for use in epidemiological studies. The Current study was focused on application of C-18 solid phase extraction coupled to sampli Q Alumina B cartridges in isolation of 7 polychlorinated biphenyls and 16 organochlorine pesticides from plasma samples donated by volunteers from population in Lagos Nigeria, followed by quantification with GC-ECD. Mean recoveries (n=4) vary from 90-92 % with a good reproducibility (RSD ≤ 8 %), while LOD range from 0.01-0.05 mg/L. Linearity range of calibration curves were 0.02-2.00 and 0.10-2.0 mg/L for PCBs and OCPs respectively while correlation coefficients (R^2) ranged from 0.997-0.999. Mean concentrations (mg/L) of PCBs and OCPs in samples were 0.0849-1.4785 and 0.3781-1.2473 respectively, while for low and high chlorinated PCBs were 0.0849-1.374 and ND-0.1709 respectively. Also, the mean concentrations (mg/L) of total DDTs and BHCs ranged from 0.02796-0.96491 and 0.01051- 0.05751 respectively. The most frequent detected were PCB 28 and permethrin, which were detected in all samples, while the least detected were PCB 180 and heptachlor epoxide, which were detected in 20 and 50% of samples respectively. The study shows that the analytical method gave good recoveries for the analytes, while the mean concentrations of OCPs were relatively higher than PCBs in 90% of samples investigated. The PCA result shows that the PCBs and OCPs were present in similar range in all the samples/variables investigated. The variables components for PCA 1 positive loading (PCB 52) remarkably correlated and suggesting that all the samples have similar origin of contamination.

Keywords: Solid phase extractions, plasma, mean concentrations, recoveries, analytical method, and contamination

Introduction

Organochlorines (OCs) are a diverse group of persistent synthetic compounds, many of which are endocrine disruptors, altering normal function of the endocrine and reproductive systems and posing serious health hazards to humans (El-Shahawi *et al*., 2010). Many experimental assays have shown that p,p'-DDE (dichlorodiphenyl-dichloroethene) and polychlorinated biphenyls (PCBs) comprise the

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bulk of organochlorine residues in human tissues (Margherita *et al*., 2005: Bradman *et al*., 2007). Organochlorine and organophosphate pesticides have been used in several countries including Africa for mosquito eradication and malaria control as well as in agricultural practices, while PCBs were widely used in the past for electrical materials, flame retardants as well as transformer fluids and could leach out from these equipments (Eriksson *et al*., 2006). The major routes of exposure of OCs to humans include inhalation, dermal contact and through contamination of animal feed (Schecter *et al*., 2010). The transfers of PCBs from mother to infant via breast milk have also been reported (Covaci *et al*., 2002) and high levels of OCs have been previously reported in adipose tissues and serum (James *et al*., 2002). The main factors responsible for persistence of OCs include high chemical stability, strong lipophilicity and low volatility (Brown *et al*., 2006).

Several animal studies have suggested that many OCs are neuro-developmental toxicants even at moderate doses (Akira and Kazuichi, 2007) and a high-level of exposure in humans appear to cause nervous system dysfunction, reproductive failures, liver dysfunction and cancers (Stapleton and Baker, 2005). The mechanism of toxicity posed to humans is widely attributed to stimulation of the central nervous system (Rudel and Perovich, 2008). For instance, endosulfan and lindane are gamma aminobenzoic acid antagonists and can also inhibit Ca^{2+} influx as well as Ca and Mg adenosine triphosphatase (Stewart and Carter, 2009). The resulting accumulation of Ca^{2+} at neuronal endplates causes sustained release of excitatory neurotransmitters. DDT affects potassium and voltage-dependent Na channels which could result into cell proliferation, immunodeficiency and several neurological effects such as agitation, confusion and seizures, while the cardiac effects have been attributed to sensitization of the myocardium to circulating catecholamines (Dallaire *et al*., 2004). Several evidences have also suggested insidious effects of background exposure to OCs and of particular concern are neonatal hypotonia or hyporeflexia and hepatic effects such as pophyria (Ennaceur *et al*., 2007). As a result of acute toxicity, several regulatory bodies have listed OCs among the priority compounds and have set allowable concentration limit for PCBs and OCPs in plasma samples (ATSDR, 2001; Meeker *et al*., 2007). Several studies have focused on pre-concentration techniques for determination of organochlorine residues in water, fish and sediment samples (Akan *et al*., 2013; Ize-Iyamu *et al*., 2007; Adeboyejo *et al*., 2013). SPE have gained popularity for isolation of PCBs and OCPs from liquid matrix and purified extracts (Blanco *et al*., 2002; Goncalves and Alpendurade, 2004), prior to quantification on GC coupled to ECD, MS or a high resolution MS (Adeyemi *et al*., 2010; Tran *et al*., 2009; Kang *et al*., 2008)**.** The SPE techniques are generally simple, economical and use less solvent making them more appropriate for use in most epidemiological research. In addition, SPE technique coupled to efficient clean-up procedure can significantly improve sample throughput and analyte recoveries with minimal interference. To

the best of our knowledge, there has been limited report on pre-concentration techniques for determination of OCs in plasma samples.

MATERIALS AND METHOD

Method design

This study was focused on application of C-18 SPE coupled to sampli Q Alumina B cartridges in clean-up and determination of 7 PCBs :- 28 (2,4,4′-Trichlorobiphenyl); 52 (2,2′,5,5′-Tetrachlorobiphenyl); 101 (2,2′,4,5,5′-Pentachlorobiphenyl); 137 (2,2′,3,4,4′,5-hexachlorobiphenyl); 153 (2,2′,4,4′,5,5′-Hexachlorobiphenyl); 180 (2,2′,3,4,4′,5,5′-Heptachlorobiphenyl) and 194 (2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl) and 16 OCPs:- α-BHC, β-BHC, lindane, δ-BHC, chlorothalonil, heptachlor, aldrin, heptachlor epoxide, dieldrin, P,P'DDD, endrin, endosulfan, P,P'DDT, methoxychlor, lambda cyhalothrin and permethrin in plasma samples of volunteers from Lagos, Nigeria by quantification on GC-ECD.

Artificial plasma for recovery studies were obtained from Biopanda diagnostics (Newtownards, U.K). All solvents including methanol, hexane and acetonitrile, 99.9% (Sigma-Aldrich), potassium dihydrogen orthophosphate, 99% (BDH chemicals limited, Poole, England), sodium hydroxide pellet, 99.8% (Merck, Germany) were high pressure liquid chromatography grade standards. An empore ™ C-18 bonded extraction disk cartridge and a varian positive pressure manifold from Sigma-Aldrich (Buchs, St Gallen, Switzerland) were employed for pre-concentration. Empty cartridges (1ml) were purchased from Supelco (Belle-fonte, PA, USA). The equipment includes Mettler Toledo ® analytical weighing balance, while standard stock solutions of PCBs and OCPs were 100 mg/L dissolved in isooctane and stored at -5^{o}C until ready for use. The selected working concentrations for the calibration curves were within the range detected in past studies in human blood serum (Covaci *et al*., 2002). 6 Working concentrations (0.25 mg/L, 0.50 mg/L, 1.00 mg/L, 2.00 mg/L) were then prepared daily from the stock solution by dilution in isooctane. Calibration curve were prepared by spiking aliquots of blank plasma at five different concentrations.

Study Centre, Population and Design

The study was carried out at the Lagos University Teaching Hospital ldi-Araba South West of Nigeria after obtaining permission from the College of medicine, University of Lagos Health Research ethics committee with approval detail MUL/HREC/08/17/232. Only healthy adult volunteers certified free of chronic medical conditions such as cardiovascular, liver or renal defects were allowed to participate in the study, while habitual smokers, farmers, mechanics were excluded from the study. A total of 30 volunteers (age bracket, 23-27 years) were recruited by random sampling, each participant was briefed on the study goals, risks, inclusion and exclusion criteria and volunteers were asked to sign a written, informed consent form before participation. However, only 10 participants gave informed consent and completed a comprehensive questionnaire. Blood samples (5.0 mL) were drawn from individual subjects by venipuncture and

collected in heparin specimen tubes to prevent coagulation and plasma was immediately 'placed on ice in a cooler. The Plasmas obtained after centrifugation at 4500 rpm for 5 min were then stored at -20°C prior to analysis.

SPE technique

Prior to analysis, samples were liquefied at room temperature and extracted by SPE (Figure 1). Pre-concentration procedure was according to the modified method (Lu-Lu *et al*., 2012) . Recoveries were determined by spiking the artificial plasma samples with 0.05, 0.2 and 1.0 mg/L of analytes and estimated by using peak areas obtained from extracted samples containing known amounts of the compound compared to the peak areas of samples with corresponding concentration prepared in the solvent (iso-octane). Each plasma sample (0.5ml) containing 0.1ml spiking solution of each analyte (Figure 2) was sequentially added to 5 mls vials, sealed with Teflon screw caps, equilibrated in an ultrasonic bath for 20 min and then extracted by SPE. The C_{18} disk cartridge employed in analyte pre-concentration step is non-polar in nature, and also possesses a size exclusion property which helps in overcoming macromolecular interferences in biological extracts (Covaci and Schepens, 2001) and can effectively retained organochlorine and other non-polar compounds. Prior to extraction, C-18 cartridges were conditioned with 2 ml methanol and 1ml x 2 mixture of water-1 propanol (v: v, 85:15). The addition of water -1-propanol (v: v, 85:15) mixture prior to extraction serve as protein denaturant and prevent protein co-precipitation with analytes. As a result, the extraction cartridge was not clogged, as the spiked serum sample was able to pass through the C_{18} cartridge in a drop wise manner. The Sampli Q Alumina B cartridges were washed with a 2 ml mixture of hexane and dichloromethane (v: v 50:50) and were attached to the lower end of the C-18 cartridges and samples were then percolated through at a steady flow rate. The Al-B cartridge which was chosen for the clean-up step serves to remove other interfering compounds like lipids which are capable of adhering to the chromatographic column and the technique also helps to lower the LOD (Lu-Lu *et al*., 2012). Thereafter, the cartridges were then washed with 2ml de-ionized water. The sorbent bed was dried thoroughly under a nitrogen stream. The column was then eluted with 1ml mixture of hexane-dichloromethane (v: v, 80:20). The eluate was concentrated to dryness under a gentle stream of nitrogen, reconstituted in 1.0mL of isooctane and transferred in to a vial for analysis.

Chromatographic conditions

G-C analytical conditions for determination of mean concentrations of OCPs and PCBs were according to the modified method (Covaci and Schepens, 2001). Analysis were by using 6820 Agilent GC (Santa Clara, C.A, U.S.A), equipped with a split-splitless injector and a ^{63}Ni electron capture detector (ECD). A DB 1701 column (60 m x 0.25 mm x 0.25 μm) manufactured by Agilent Technologies (Wilmington, DE) was employed in the separation. The analytical conditions are as shown in Table 1. Helium was used as the carrier gas while nitrogen was used as the make-up gas. A 1.0 μl volume was injected in a splitless mode.

Identification of PCBs and OCPs congeners in samples were identified by the retention time match with those of the standards.

Quality control

All chemicals and reagents were of HPLC grade. All sample bottles, measuring cylinders, standard flasks and beakers were thoroughly cleaned and dried prior to use. For every set of 10 samples, a procedural blank and spiked sample consisting of all reagents was run to check for interference and cross-contamination. Analysis for blank solvents gave no recoveries for OCPs and PCBs, indicating that the blank solvents were free of contamination.

RESULTS

Some of the GC chromatogram of PCBs and OCPs standard mixture and extracted samples are as shown in Figure 3-5 respectively. The LOD range from 0.01-0.05 mg/L for analytes. The linearity ranges were 0.02-2.00 and 0.10-2.0 mg/L for PCBs and OCPs respectively. The correlation coefficients (R^2) of the calibration curves were 0.997-0.999. Limit of detection (LOD) were evaluated from the ratio of signal to noise ratio, the concentration at which each of the analyte peak is at least three times higher than the noise peak.

The mean recoveries (n=4) of some investigated compounds (PCB 28, 180, DDTs, endosulfan and heptachlor) as determined after subtraction of levels found in the non spiked from spiked ones vary from 90-92 % with a good reproducibility (RSD > 8 %). However, the mean concentrations reported were not corrected for recoveries. The mean concentrations of low chlorinated biphenyls in samples were relatively higher than highly chlorinated ones (Figure 6). Mean concentrations (mg/L) of low chlorinated biphenyls in samples (Table 2) include PCBs 28 (0.04332-1.325), 52 (0.0039-0.1678) and 101 (0.0064-0.0511), while the mean concentrations (mg/L) of highly chlorinated biphenyls were: PCB 137 (ND-0.0329), 153 (ND-0.0665), 180 (ND-0.0397), 194 (ND-0.0730). The most frequent detected PCB was 28, which was detected in all samples, while the lowest was 180 which were detected in only 20% of the samples. The mean concentrations of highly chlorinated congeners (PCB 137, 153, 180 and 194) were relatively low in current study.

The mean concentrations of OCPs (mg/L) in plasma samples (Table 3) varied from 0.3781-1.2473. The mean concentrations (mg/L) of DDTs in samples (0.02796-0.96491) were generally higher than the BHCs (0.01051-0.05751) levels (Figure 7). The most frequent OCP congeners detected was permethrin, which was detected in all the samples, while the lowest was heptachlor epoxide which was detected in 50% of the samples.

Table 1: Gas Chromatography analytic conditions

Conditions	Values
Carrier gas flow	2 ml/min
Make up gas flow	20 ml/min
Oven temperature	150°C (5min) and increase to 300 °C at 5 °C/min.
Injector temperature	250°C
Detector temperature	300°C

Table 2: Mean concentrations of PCBs (mg/L) congeners in human plasma samples

PCBs	MB2	AJ2	DK1	DK2	KT1	KT2	RW1	RW2	DL1	DL2
PCB 28	0.1192	0.2380	0.0736	0.2326	0.0738	0.1415	0.0434	0.0449	1.3249	0.0432
PCB 52	0.0080	0.1213	0.0049	0.1678	0.0039	0.0592	0.1081	0.0942	0.0269	0.0939
PCB 101	0.0107	0.0272	0.0064	0.0391	0.0090	0.0511	0.0490	0.0362	0.0222	0.0306
Mean conc. of low chlorinated PCBs	**0.1379**	**0.3865**	**0.0849**	**0.4395**	**0.0867**	**0.2518**	**0.2005**	**0.1753**	**1.374**	**0.1677**
PCB 137	0.0178	0.0215	ND	0.0275	0.0011	0.0065	0.0026	0.0005	0.0329	0.0021
PCB 153	0.0178	0.0567	ND	0.0665	0.0160	ND	0.0288	0.0180	ND	0.0282
PCB 180	ND	0.0320	ND	0.0397	ND	ND	ND	ND	ND	ND
PCB 194	0.0730	0.0428	ND	0.0372	ND	0.0009	ND	ND	0.0717	0.0049
Mean conc. of highly chlorinated PCBs	**0.1086**	**0.153**	**ND**	**0.1709**	**0.0171**	**0.0074**	**0.0314**	**0.0185**	**0.1046**	**0.0352**
Mean conc. of PCBs in individual subjects (ng/µL)	**0.2465**	**0.5395**	**0.0849**	**0.6104**	**0.1038**	**0.2592**	**0.2319**	**0.1938**	**1.4785**	**0.2029**

ND: non-detectable

Table 3: Mean concentrations of OCPs (mg/L) congeners in human plasma samples

SAMPLE	MB2	AJ2	DK1	DK2	KT1	KT2	RW1	RW2	DLI	DL2
Endosulfan	0.17735	0.03844	0.03148	0.04095	0.02952	0.03044	0.03468	0.02584	0.03801	0.02719
Dieldrin	0.00296	0.00386	0.00716	0.00372	0.00251	0.00926	0.00104	0.00956	0.00345	0.00912
Endrin	0.01258	0.01256	0.01208	0.01198	0.01213	0.02283	0.02293	0.02293	0.01226	0.02315
p,p' DDD	0.1653	0.0366	0.1236	0.0069	0.9499	0.2270	0.1377	0.0116	0.5106	0.0128
p,p'DDT	0.02359	0.04711	0.01551	0.06697	0.01501	0.02936	0.01675	0.01636	0.06046	0.03728
Total DDTs	**0.18889**	**0.08371**	**0.1391**	**0.07387**	**0.96491**	**0.25636**	**0.15445**	**0.02796**	**0.57106**	**0.05008**
Methoxychlor	0.09181	0.05861	0.07521	0.06463	0.05750	0.07859	0.01016	0.07542	0.06983	0.07631
Lambda cyalothrin	0.04795	0.04611	0.04150	0.04902	0.04552	0.04380	0.04103	0.05877	0.06402	0.04387
Permethrin	0.11059	0.48793	0.05572	0.55856	0.09139	0.10716	0.19288	0.08326	0.24577	0.13082
α-BHC	0.00167	0.00159	0.00169	0.00151	0.00171	0.00208	0.00212	0.00208	0.00157	0.00208
β-BHC	0.0168	0.00195	0.000319	0.00310	0.0162	0.000534	0.00172	0.00047	0.00248	0.00041
Lindane	0.00481	0.00286	0.00481	0.00370	0.00481	0.0481	0.0481	0.0481	0.00481	0.0460
δ-BHC	0.00463	0.00411	0.00432	0.00417	0.00456	0.00554	0.00557	0.00557	0.00410	0.00561
Total BHCs	**0.02791**	**0.01051**	**0.01114**	**0.01248**	**0.02728**	**0.05625**	**0.05751**	**0.05622**	**0.01296**	**0.0541**
Chlorothalonil	0.0125	0.00948	0.00805	0.0146	0.0125	0.0144	0.0148	0.0143	0.0128	0.0141
Heptachlor	0.00318	0.00242	0.00301	0.00224	0.00322	0.00302	0.00311	0.00292	0.00276	0.00297
Aldrin	0.00099	0.00205	0.0821	0.00218	0.00084	0.00149	0.00099	0.00087	0.00229	0.000933
Heptachlor epoxide	0.000663	ND	0.000151	ND	ND	0.000066	ND	0.000053	ND	0.000048

Mean conc. Of OCPs	**0.6774**	**0.7557**	**0.4667**	**0.8342**	**1.2473**	**0.6237**	**0.5336**	**0.3781**	**1.0352**	**0.4327**

ND: non-detectable

Table 4: Results for PCBs USED Minitab.MTW Correlations: (MB2, AJ2, DK1, DK2, KT1, KT2, RW1, RW2, DL1, and DL2)

	MB2	AJ2	DK1	DK2	KT1	KT2	RW1	RW2	DL1
AJ2	0.729								
	0.063								
DK1	0.817	0.914							
	0.025	0.004							
DK2	0.583	0.976	0.822						
	0.170	0.000	0.023						
KT1	0.790	0.892	0.976	0.796					
	0.034	0.007	0.000	0.032					
KT2	0.643	0.906	0.918	0.886	0.868				
	0.119	0.005	0.004	0.008	0.011				
RW1	-0.101	0.467	0.192	0.634	0.175	0.526			
	0.829	0.290	0.680	0.126	0.708	0.225			
RW2	-0.000	0.564	0.292	0.720	0.257	0.606	0.990		
	1.000	0.187	0.525	0.068	0.577	0.149	0.000		
DL1	0.862	0.902	0.994	0.794	0.969	0.875	0.110	0.215	
	0.013	0.006	0.000	0.033	0.000	0.010	0.815	0.643	
DL2	-0.002	0.559	0.254	0.717	0.241	0.550	0.985	0.990	0.185
	0.996	0.192	0.582	0.070	0.603	0.201	0.000	0.000	0.692

Table 5: Results for: OCP USED Minitab.MTW (Correlations: MB2, AJ2, DK1, DK2, KT1, KT2, RW1, RW2, DLI, DL2)

	MB2	AJ2	DK1	DK2	KT1	KT2	RW1	RW2	DLI
AJ2	0.408								
	0.117								
DK1	0.663	0.279							
	0.005	0.296							
DK2	0.365	0.998	0.229						
	0.164	0.000	0.393						
KT1	0.607	0.067	0.725	0.002					
	0.013	0.805	0.001	0.993					
KT2	0.739	0.390	0.794	0.332	0.900				
	0.001	0.136	0.000	0.209	0.000				
RW1	0.644	0.810	0.555	0.773	0.581	0.797			
	0.007	0.000	0.026	0.000	0.018	0.000			
RW2	0.426	0.664	0.329	0.662	-0.021	0.402	0.554		
	0.100	0.005	0.213	0.005	0.937	0.123	0.026		
DLI	0.711	0.424	0.772	0.365	0.929	0.966	0.817	0.247	
	0.002	0.102	0.000	0.164	0.000	0.000	0.000	0.355	
DL2	0.437	0.858	0.311	0.861	-0.009	0.420	0.686	0.934	0.325
	0.090	0.000	0.241	0.000	0.973	0.105	0.003	0.000	0.219

Cell Contents: Pearson correlation
P-Value

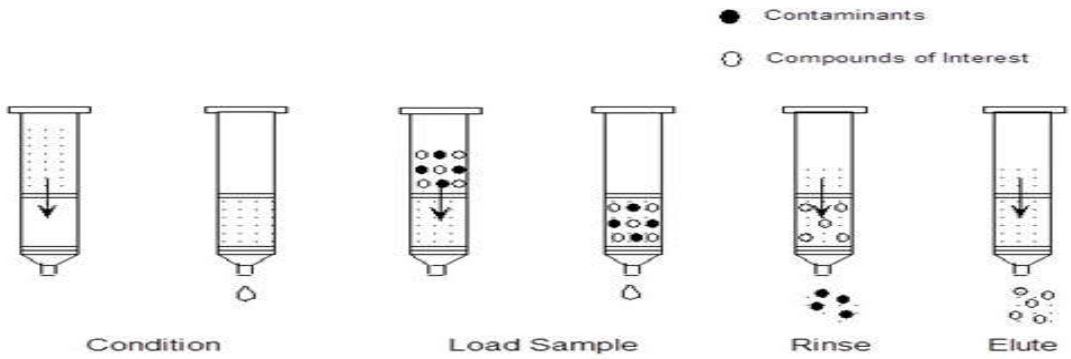

Fig 1: Principles of Solid-Phase Extraction

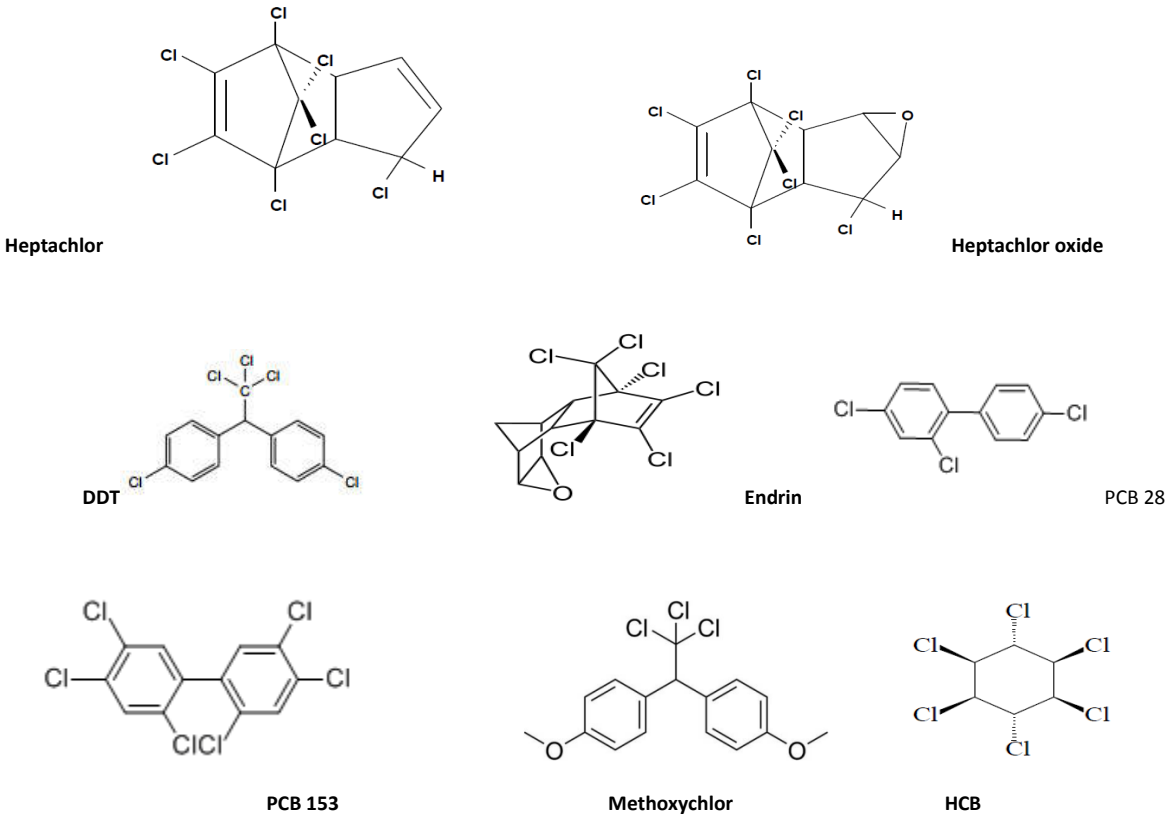

Fig 2: Chemical structures of representative PCBs and OCPs analyzed

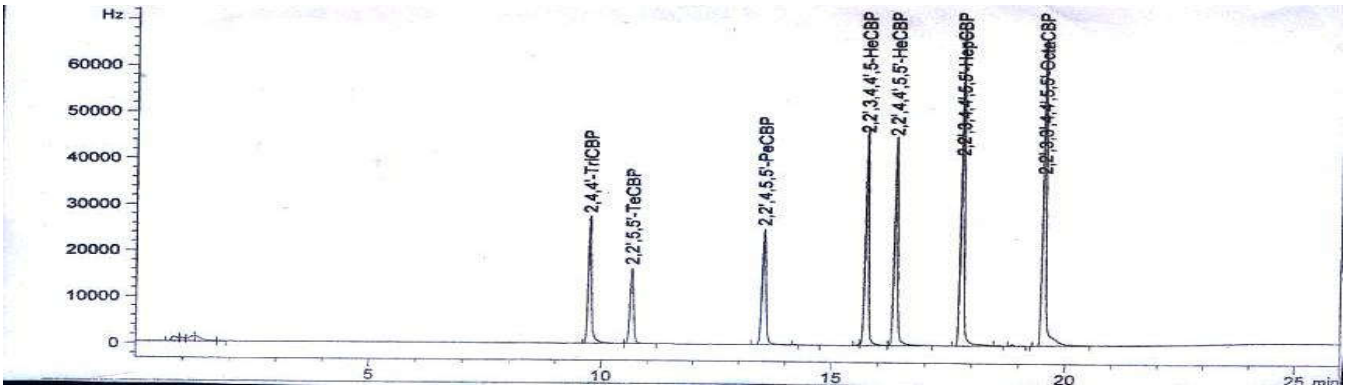

Fig 3: GC Chromatogram of PCBs standards mixture

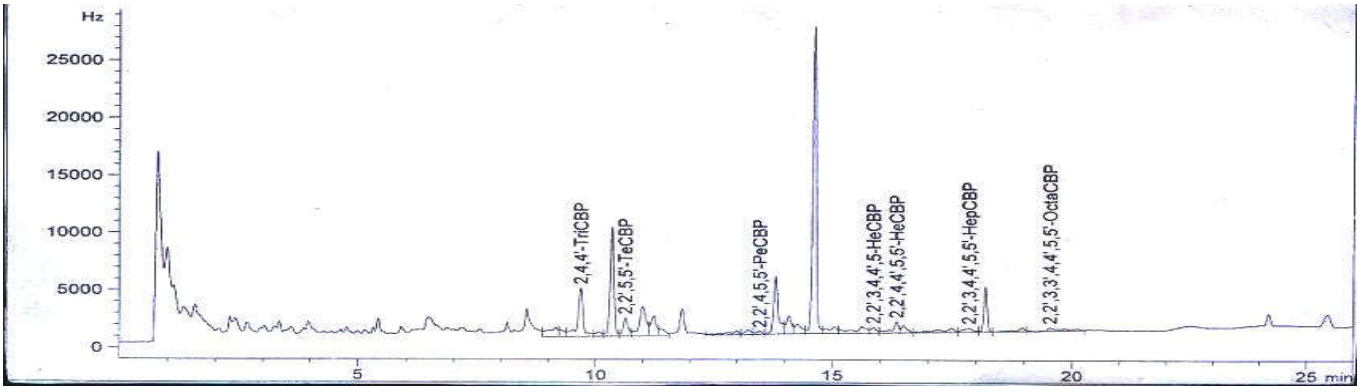

Fig 4: GC Chromatogram of PCBs in plasma samples

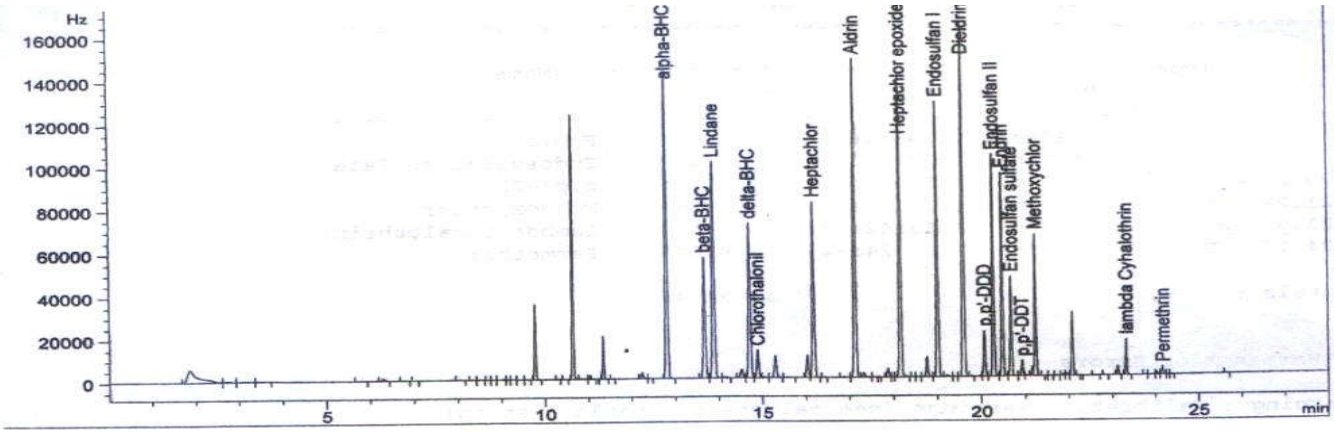

Fig. 5: GC Chromatogram of OCPs Standards mixture

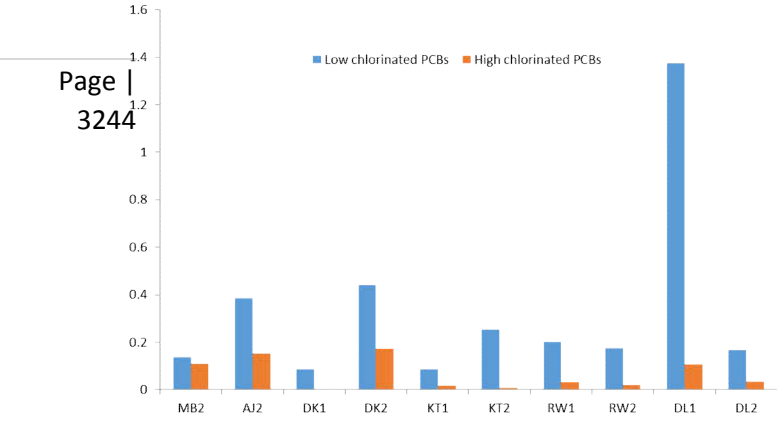

Fig. 6: Graphical illustration of sum of Mean concentrations of low and highly chlorinated biphenyls (mg/L) in plasma samples.

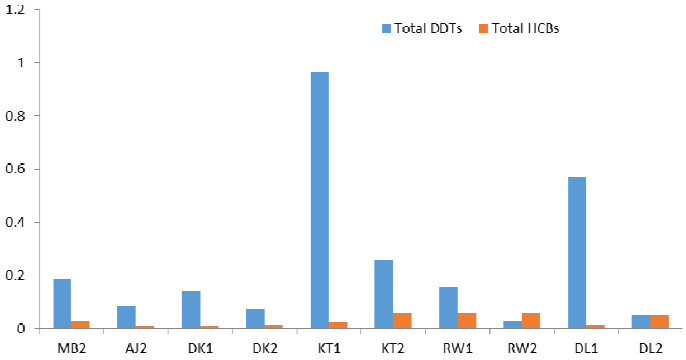

Fig. 7: Graphical illustration of mean concentrations of Total DDTs and HCBs (mg/L) in plasma samples.

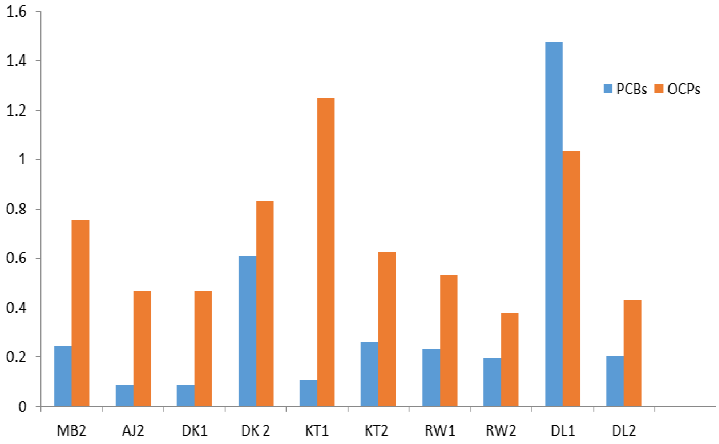

Fig. 8: Graphical illustration of sum of Mean concentrations of PCBs and OCPs (mg/L) in plasma samples

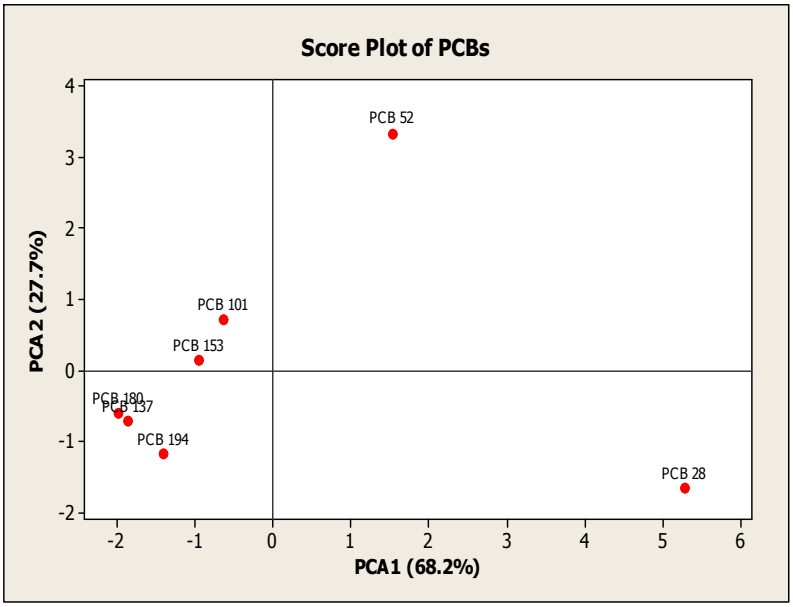

Fig. 9: Principal component analysis of PCBs

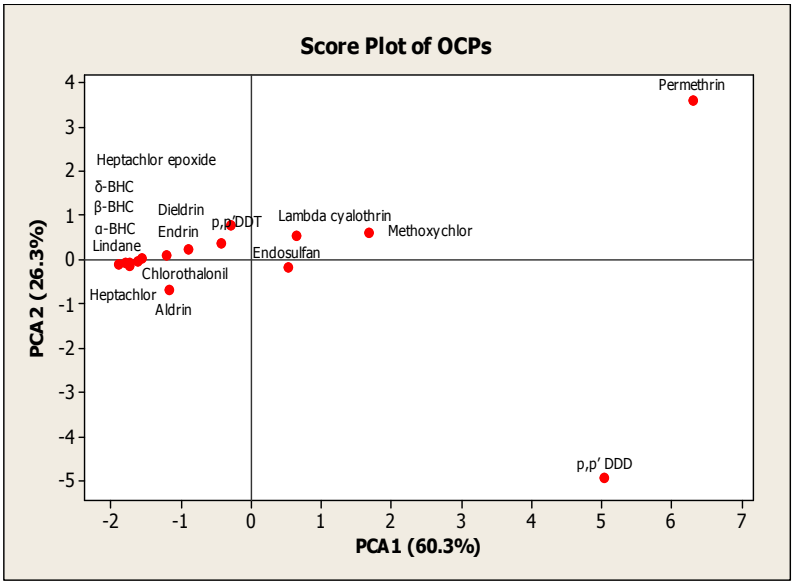

Fig. 10: Principal component analysis of OCPs

Discussion

PCBs have been produced commercially and widely used in all countries of the world as dielectrics in transformers, large capacitors, and ink carriers in carbonless copy papers, hydraulic fluids and in flame retardants. As a result of their deleterious effects on human health, the production and industrial uses of PCBs have been reduced since mid-70s. PCBs have tendency for bio-accumulation due to their high persistence and lipophilic nature. The lower chlorinated biphenyls (PCB 28, 52 and 101) are more rapidly metabolised and eliminated, and as a result, may not pose significant health hazards. Hence, their detection in all samples in this study (Figure 6) probably indicates that the subject participants were recently exposed to the chemicals (Yu *et al*., 2001). Past study had shown that highly chlorinated PCBs are environmentally prevalent as they are not readily metabolised (Yanez *et al*., 2002). The congeners are hormone interferon, posing hazard on procreation of humans and animals, and hence are most toxic congeners (Nie, 2003). However, the mean concentrations of highly chlorinated congeners detected in this study were lesser than lower chlorinated ones. Previous author have reported a higher PCBs accumulation in men when compared to women and fish intake being the dietary factor with the greatest correlation with serum PCBs and also that body mass index was inversely related to mean concentrations of PCBs (Agudo *et al*., 2009; Longnecker *et al*., 2005). Our future work in the country should be focused on ascertaining the aforementioned correlations for proper evaluation of the health risk posed by the PCBs. In addition, although there has been a law concerning registration of OCs that can be used, however, there are still reports on use of the chemicals in some developing countries most especially for agricultural practices and mosquito control (Akan, 2013).

OCPs have been used in many developing countries, for instance, technical HCH (mixtures of 60-70% α, 5-12% β, and 10-15% γ HCH) were widely used as pesticides and commercialized as lindane (Rusiecki *et al*., 2008), while methoxychlors and cyhalothrin were used in eradicating ticks, beetles and cockroaches (Smith and Gangoli, 2002). Chlorothalonil were used for treatment of mildew and rots in plants, while heptachlor, chlordane, permethrin and endosulfan were widely used as synthetic insecticide in cereals and sorghum (Halidin, 2005). Also, Aldrin and dieldrin were widely used as broad spectrum insecticides in protection of wooden structures from termites as well as in seed dressing, while DDT was widely used in mosquito eradication. To mention some transformation pathway, about 20% of heptachlor and chlordane can be oxidized readily into heptachlor epoxide in the environment by bacterial action (Hongtao, 2005). Aldrin can be converted readily to dieldrin by epoxidation under normal environmental conditions (Nakata et al., 2005), while DDT is capable of breaking down to DDE and DDD (Turusov *et al*., 2002). As a result, the possibility of detecting the aforementioned metabolites in the environment, despite discontinue in the use. In this study, mean concentrations of OCPs detected were greater than PCBs in 90% of samples (Figure 8) and this may be attributed to more agricultural than industrial applications in the past coupled with the possibility of spread via atmospheric transportation.

Previous studies have reported mean concentration of OCPs and PCBs in fish and water samples from Lagos lagoon (Adeyemi et al., 2010) and current study also contributes to the database on environmental monitoring. The mean concentrations of OCPs (mg/L) in this study (0.3781-1.2473) were higher when compared with values (0.0021-0.0271) obtained for similar studies on human subjects from Mexico (Waliszewski *et al*., 2004), but however lower than detected in human subjects from Portugal (Cruz and Lino, 2003). Generally, regulations on the use of various insecticides (DDT, aldrin, dieldrin, heptachlor and chlordane) in most countries of the world including Nigeria have resulted in general reduction in bio-concentrations in environmental samples

Principal component analysis of OCPs and PCBs

The Principal Component analysis (PCA) is a method to describe the variable with a minimum loss of information and commonly used to analyze the source of pollutants (Chu *et al*., 1999; Zhao *et al*., 2006a; Zhao *et al*., 2006b).

The PCA of PCBs and OCPs are as shown in Table 4 and 5 respectively**.** For this study, the principal components that accounted for more than 85% of all the variance were extracted. Figure 9 and 10 summarized the factor loading of 7 PCBs and 16 OCPs in the PCA respectively. The first two components accounted for 68.2%, 27.7% respectively and the remaining components each contribute less than 4% of the total PCB pollutants. The highly-chlorinated (PCB 137, 180 & 194) had negative loading in the second component except for PCB 153 which was on PCA 2 positive loading. The first component was dominated by low chlorinated congeners including PCB 52 in positive loading and PCB 28 in PC1 negative loading. Only PCB 101 (low-chlorinated biphenyl) was loaded in positive PCA 2. The variables components for PCA 1 positive loading (PCB 52) remarkably correlated and suggested that all the samples have the same origin or pathway to the targeted organs. Also variables MB2, AJ2, DK1, KT1 KT2 and DL1 components were suspected to have the same origin from highly-chlorinated biphenyl.

The PCA for the 16 compounds of OCPs is as shown in figure 10. The first principal component factor accounted for 60.3%, PCA-2 accounted for 26.3% and the remaining components each contribute less than 13% of the total OCPs variances (compounds). Permithrin and p, p' DDD were the predominant components in PCA-1. All the BHCs compounds were the predominant components of factor 2 in positive loading.

The PCA result shows that the PCBs and OCPs were present in similar range in all the samples/variables investigated. For example, all the variables gave a positive loading to PCA-1 suggesting similar origin of contamination. As a result, these two analyses confirmed that the contaminants in the investigated samples came from the same pathway/origin (or were injected/contacted through the same pathway either food or air). Notably, variables KT1, KT2, DK1, DL1, MB2 and AJ2 have negative values in PCA-2 factors of both score plots PCBs and OCPs. It is suspected that they all have same extraction method

and source of contamination for the variables. These were also observed and confirmed in the correlation (*p* value 0.05) results for the PCBs and OCPs variables. Variables KT1, KT2, DK1, DL1, MB2 and AJ2 were strongly correlated among each other both in PCBs and OCPs.

CONCLUSION

The techniques of SPE and clean-up for the pre-concentration of OCPs and PCBs from plasma samples were demonstrated. Mean concentrations of OCPs were relatively higher than PCBs in 90% of samples investigated. The most frequent detected were PCB 28 and permethrin, while the least detected were PCB 180 and heptachlor epoxide. The PCA result shows that the PCBs and OCPs were present in similar range in all the samples/variables investigated.

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Declaration of Interest statement

The authors declare no conflict of interest concerning this research studies.

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