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Organochlorine Pesticides Residues in the Breast Milk of Some Primiparae Mothers in La Community, Accra, Ghana

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Abstract: This study was conducted to determine the types and levels of Organochlorine pesticide residues in the breast milk of 21 primiparae mothers in La, a suburb of Accra an urban community in the Greater Accra region of Ghana. Liquid-liquid extraction procedure was employed and extract clean-up was done using silica gel solid phase extraction. Fourteen (14) different organochlorine pesticides residues namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were identified and quantified in the individual breast milk samples using a Gas Chromatograph (GC) with an Electron Capture detector. The GC recoveries of spiked samples were between 89 to 97%. P,p'- DDE recorded 100% incidence ratio. Also p,p'-DDT, delta-HCH, gamma-HCH, and endosulfan sulfate recorded incidence ratios of 76.79, 95.25, 80.95 and 85.71%, respectively for the breast milk samples. The concentrations of organochlorine pesticide residues in the human breast milk samples ranged from 1.839 to 99.05 μg/kg fats. With the exception of Endosulphan Sulphate whose mean concentration (99.052 μg/kg) was above the Australian Maximum Residue Limit (MRL) of 20 μg/kg for milk, the mean concentrations for all the other organochlorines detected were below their respective limits.

Key words: Breast milk, gas chromatograph, Ghana, maximum residue limit, organochlorine pesticide residues, primiparae

INTRODUCTION

The use of pesticides became very relevant in an attempt to control and eradicate crop pest and also to produce quality and bumper harvest to feed the ever growing population. Over the years, human population has been on the increase thus the territory of these pests became larger and larger hence the need to look for stronger and more effective alternatives to meet food security and to survive from disease vector organisms (Hodgson, 2003). It is estimated that as much as 45% of the world's crop is destroyed by plant pest and disease (Bhanti and Taneja, 2007). In Ghana, the merits of these pesticides cannot be disputed as they were massively used in the agriculture and public health sectors to curb crop pest and for disease control (Clarke *et al*., 1997). Organochlorine pesticides were extensively used by most Ghanaian farmers due to their low cost, high efficacy and its wide range suitability for plants (Osafo and Frempong, 1998). These pesticides were greatly used in most farming communities in the Western, Ashanti and Brong Ahafo regions of Ghana (Amoah *et al*., 2006) in vegetable production, cocoa farms, and mixed crop farms (Gerken *et al*., 2001, Ntow *et al*., 2006). Organochlorine pesticides such as DDT, Lindane and endosulfan were also employed to control ectoparasites of farm animals and pets in Ghana (Ntow *et al*., 2006). Pesticides have also been used to control black flies along the banks of the Tano and Pra Rivers (Ntow, 2001). Unfortunately, pesticides usage has been abused since most pesticide users are ignorant or have little knowledge about these chemicals. Some farmers are of the view that the more or as often as they apply pesticides the greater their chances of higher yield and also destroying crop pest (Ntow *et al*., 2006). They have no idea of the half lives of these chemicals nor the dangers they pose when misused. The environment is contaminated with pesticides because of their massive use in both the agriculture and public health sectors. The deleterious effects of these organochlorine pesticides on wildlife primarily led to their ban from routine use in the US and many other countries in 1970s and 1980s (Carson, 1962; Dunlap, 1981). With the exception of Endosulphan which was considered for restricted use in 2008, Ghana banned the use of most organochlorine pesticides since 1985 and Lindane in 2006, the persistent, long range transport, lipophilic and bioaccumulative nature has resulted in residual amount in the environment. There is evidence of organochlorine

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pesticide residues in sediments, water and biota, crops, meat and human fluids (Osafo and Frimpong, 1998; Ntow, 2001; Kalantari and Ebodi, 2006; Khalid *et al*., 2007; Darko and Acquaah, 2007). Increase accumulation of these chemicals in the food chain may pose serious health hazards in the general populace (Jayashree and Vasudevan, 2007). For example, exposure to organochlorine compounds has been reported to affect thyroid function in preschool children (Natural Health News, 2008). Low sperm count in males, birth defects, increased in testicular cancer and other reproductive and development effects (Weltman and Norback, 1983) have also been reported as a result of organochlorine contamination. In spite of the massive use of organochlorine pesticides in Ghana, there is paucity of information on their environmental levels. Consequently, there is much concern over the environmental quality of Ghana and the health of its inhabitants. Very little work has been done to measure body burden of organochlorines in Ghana especially on human breast milk. Samples of body fluids such as breast milk have been shown as an adequate indicator of body burden of organochlorine pesticide residues (Ntow, 2001). The breast, a lipid-rich tissue acts as a depot or reservoir of lipophilic pesticide by virtue of physiochemical interactions of the cellular component with the pesticide (Mussalo-Rauhamaa, 1991). Ntow (2001) worked on organochlorine pesticide residues in human breast milk of some women in Akomadan, a farming community in the Ashanti region of Ghana and recorded 40 μg/kg fats of Hexachloro Cyclo Benzene (HCB) and 490 μg/kg fats of p,p'-DDE . The current study however, is limited to only first birth mothers. Such mothers have never breast fed any children to release body burden of organochlorine through breast feeding and are more likely to have most of the organochlorine pesticide they have accumulated in their life time. This will give an idea on the levels of organochlorine pesticide residues in first birth mothers as compared to mothers of subsequent births.

MATERIALS AND METHODS

The study was conducted in the Ghana Standards Board Pesticides Residue Laboratory within August 2008 to June 2009. Ethical clearance was sought from the Ministry of Health before selecting potential donors for the study.

Chemicals and reagents: The reagents used for the analysis were analytical grade petroleum ether 40-60ºC (Scientific and Chemical Supplies Ltd); N-Hexane, 95% HPL grade (Sigma-Aldrich), acetone (Scientific and Chemical Supplies Ltd.), diethyl ether AnalaR (BDH Chemical Ltd.), concentrated sulphuric acid, >95-97% (Fluka), ethylacetate pestanal (Riedel Haen), individual pesticide reference standards (>95.0% purity) from Dr. Ehrenstofer GmbH, Germany and stored in a freezer at -20ºC to minimize degradation. Solid Phase Extraction (SPE) cartridges (strata sI-1 Silica) (55 μm, 70 A) of density, 500 mg/6 mL.

Glassware: Twenty one (21) Schott Duran 100ml glass bottles with protective caps used for sampling were imported from Germany.

Equipment: Centrifuge Cri multifunction (Thermo Electron Industries SAS, France), T 25 Basic Ultra turax macerator (IKA® Werke Germany), Metler Toledo PG 10035 weighing balance, rotary evaporator Buchi RE-200 equipped with Buchi B740 re-circulating water chiller and Buchi V700 vacuum pump (BÜchi Labortechnic AG Postfach Switzerland). A gas chromatograph, Varian CP-3800 (Varian Association Inc. USA) equipped with ^{63}Ni Electron Capture Detector (ECD), CTC Analytic Combi PAL autosampler, split-splitless injector, Programmed Pneumatic Control (PPC) and a computer running star workstation data processor. For separation, a 5% diphenyl 95% dimethyl siloxane capillary column (30m × 0.25(lid) × 0.25 μm, thickness plus 10m guard column.

Study area: The city Accra was selected for this study. Labadi General Hospital was chosen as the main site for the collection of the human milk samples. The study area is shown below in Fig. 1.

Cleaning of glassware: All glasswares used for this study were rigorously scrubbed with a brush in hot water and detergent. The glasswares and the sampling bottles were rinsed five times with tap water and twice with distilled water. They were further rinsed with acetone followed by hexane. They were placed over night in an oven at 300ºC. The glasswares were then sealed tightly with aluminum foil to prevent contamination and were then stored in a dust free cabinet when not in use.

Sample collection: A total of twenty one (21) nursing mothers satisfied the criteria and were selected for the study. Mothers who expressed their willingness to partake in the study were made to fill a questionnaire which was basically about their personal information and diet. Selected mothers were made to sign a Prior Informed Consent Form before samples were taken. After the signing of the Consent Form, mothers were given the already cleaned glass jar with a very tight and well protected lid labelled with their individual identification code, A1 to A21 for the human breast milk samples. The samples were collected manually and directly into the individual glass jars. Babies whose mothers donated some of their milk samples were giving a baby Tee shirt donated by the World Health Organization as a sign of appreciation to the mothers.

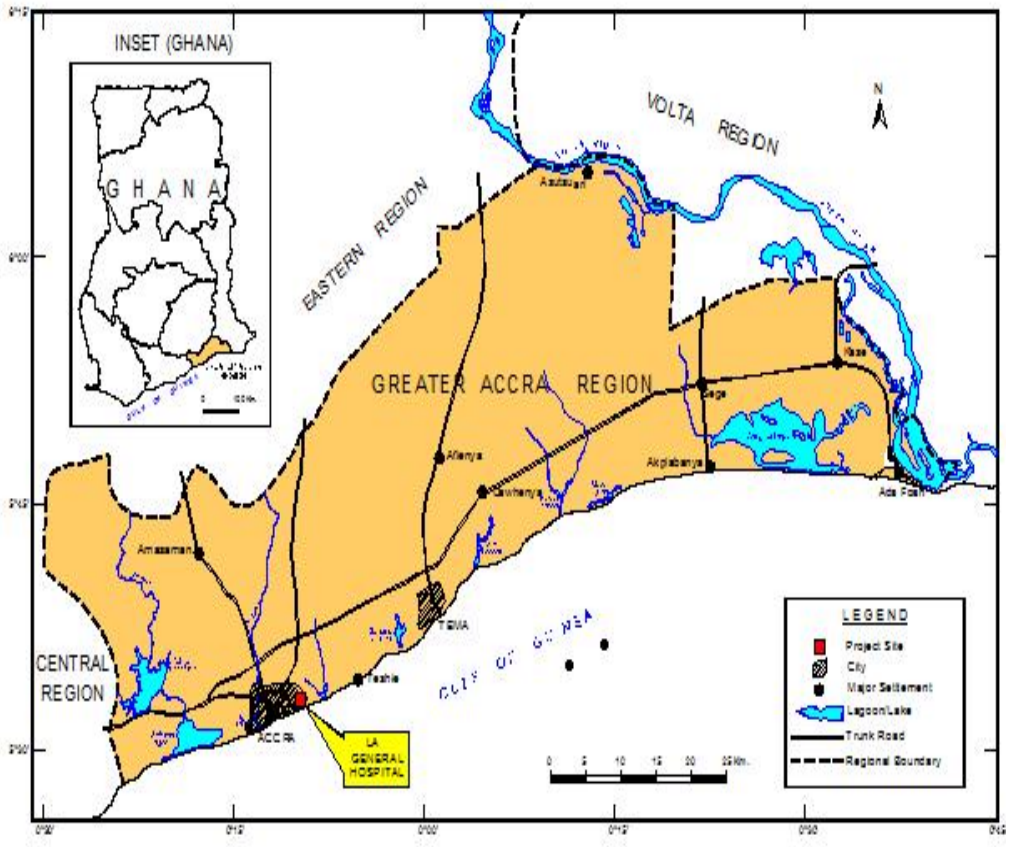

Fig. 1: A map showing the sampling area

The samples were stored in an ice chest with dried ice at -4ºC. The samples were later transported to the Ghana Standard Board Pesticide Residues Laboratory and stored at -20ºC in a freezer prior to analyses. This is the recommended temperature at which all microbial actions in biological samples are ceased (Kiriluk *et al*., 1996).

Sample preparation and extraction of the human breast milk samples: The extraction procedure carried out was that described by Weisenberg *et al. (*1985) and cited by Ntow (2001) with slight modifications. The human breast milk samples frozen at -20ºC were allowed to thaw and then stirred thoroughly. 10 mL of the milk samples were then pipetted and homogenised with 40 mL of 1:1 petroleum ether/acetone mixture by macerating the mixture with the aid of an Ultra-Turrax T 25 basic at a speed of 9,500 rpm for 2 min to enhance extraction. The homogenate were then centrifuged at 2500 rpm for 2 min. After centrifuging the organic layer was collected into an already weighed round bottom flask. The milk phase was re-extracted twice with two separate aliquots of 30 mL petroleum ether. The combined organic phase collected was evaporated to dryness by the rotary evaporator with water bath at 40ºC. The dried organic phase was weighed and dissolved in 5 mL hexane and then subjected to clean-up procedure below. The same extraction procedure was followed for the other individual milk samples. Spiked samples were treated in a similar manner Sample extracts clean-up: The silica solid phase extraction column (500 mg / 6 mL) cartridges were conditioned with 10 mL petroleum ether. The organic layer dissolved in 5 mL hexane was cleaned up by shaking for 1min in 2 mL concentrated sulphuric acid. The sample extracts were loaded onto the columns and eluted with 1: 9 diethyl ether/petroleum ether mixture. Spiked samples were treated similarly.

The cleaned extracts were concentrated to dryness by rotary evaporation. The dried residues were for each sample were dissolved in 1 mL ethyl acetate and then picked into a 2 mL vial for analysis by the gas chromatograph.

Analysis of milk extract for organochlorine pesticide residues: The sample extracts as well as spiked extracts were analyzed by a Varian gas chromatograph CP-3800 equipped with ^{63}Ni electron capture detector which is very sensitive for detecting halogens. The GC conditions used for the analysis included a capillary column coated with

Table 1: Organochlorine pesticide residue (µg/kg) in human breast milk samples from Accra

	Milk fat		*Whole milk		
Organochlorine pesticide	Mean	SD	Mean	SD	**Incidence ratio (%)
Gamma-HCH	4.207	0.608	0.298	0.068	80.95
Delta-HCH	13.855	2.003	0.686	0.071	95.24
Heptachlor	11.791	1.223	0.514	0.033	76.19
Aldrin	2.962	0.210	0.156	0.017	85.71
Gamma-chlordane	1.839	0.182	0.101	0.007	33.33
Alpha-endosulfan	4.704	0.477	0.211	0.015	80.95
p,p'- DDE	23.367	3.233	1.124	0.117	100
Dieldrin	2.407	0.316	0.115	0.010	71.43
p,p'- DDT	3.085	0.398	0.371	0.029	76.19
Endrin	7.669	1.004	0.125	0.015	80.95
Endrin aldehyde	7.769	2.735	0.224	0.040	42.86
Endosulfan sulfate	99.052	10.693	4.907	0.503	85.71
Endrin ketone	63.846	33.097	0.153	0.024	42.86
Methoxychlor	20.116	4.149	0.716	0.115	42.86

SD = Standard Deviation; **: Incidence ratio = Number of samples that tested positive; *: => Whole milk is the total composition of milk expressed for analysis of which fat is a part

RB-5 (30×0.25 mm, 0.25 µm film thickness), a carrier gas at a flow rate of 1.0 mL/min and a make-up gas of Nitrogen also at a flow rate of 29 mL/min. The temperature of injector operating in splitless mode was held at 225ºC, oven temperature was set at 225ºC and electron capture detector was also set at 300ºC. The column oven temperature was programmed as follows; 60ºC for 2 min, 180ºC/min up to 300ºC held for 31.80 min. The injection volume of the Gas Chromatograph (GC) was 1.0 µL. The residues detected by the GC analysis were confirmed by the analysis of the extract on two other columns of different polarities. The first column was coated with ZB-1 (methyl polysiloxane) connected to ECD and the second column was coated with ZB-17 (58% phenyl, methyl polysiloxane) and ECD was also used as detector.

Quantification: The quantities of residues in the samples were determined using an external standard method. An organochlorine standard mixture with known concentrations was run and the detector response for each compound was determined. The areas of the corresponding peak in the samples were compared with that of the known standards.

Recovery test: One sample in each batch of analysis was spiked with 0.1 mL/kg of a mixed standard. The spiked samples were extracted and analyzed under the same conditions as the samples. The percentage recovery was calculated as:

$$\text{Recovery (\%)} = \frac{\text{(Amount of analyte received)}}{\text{(Amount of analyte spiked)}} \times 100$$

The recovery for the different organochlorine pesticides in the milk samples were between 89 to 97%.

RESULTS AND DISCUSSION

Table 1 presents the mean concentrations of organochlorine pesticide residues analysed in the fat and whole milk of the samples. It is evident that the concentrations of the organochlorine pesticide residues in the milk fat were higher than respective concentrations in the whole milk. This is due to the fact that organochlorines are lipophilic and thus accumulate more in the fatty medium. Fourteen organochlorine pesticides namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and Methoxychlor were detected in the 21 samples analysed.

Figure 2 shows the percentage incidence of the various organochlorine pesticide residues that were analysed in the samples. All the samples tested positive to p,p'-DDE thus 100% incidence ratio and 76.19% tested positive to p,p'-DDT. Although DDT has long been banned in Ghana; in 1985, its residues can still be detected in the environment after a decade and halve of its ban. This is due to the high persistent nature. About 95.24 and 80.95% of the breast milk sampled tested positive to Delta-HCH and Gamma-HCH, respectively. Alpha-Endosulphan was recorded in 80.95% of the samples from Ada and 85.71% recorded Endosulphan-Sulphate.

From Table 1, the concentrations of p,p'-DDT and p,p'-DDE in the milk fat were 3.085 and 23.367 µg/kg respectively in the human breast milk samples. The presence of both DDT and its metabolite DDE in the human milk fat samples even though they have been banned since 1985 (EPA Ghana, 2008), may be due to the persistence and long range transport nature of DDT and its metabolite DDE (Ritter *et al*., 1995) and also their ability to bioaccumulate and biomagnify in the food chain (Travis and Arms, 1988). The body burden of DDT and DDE might be through food since residual levels of both

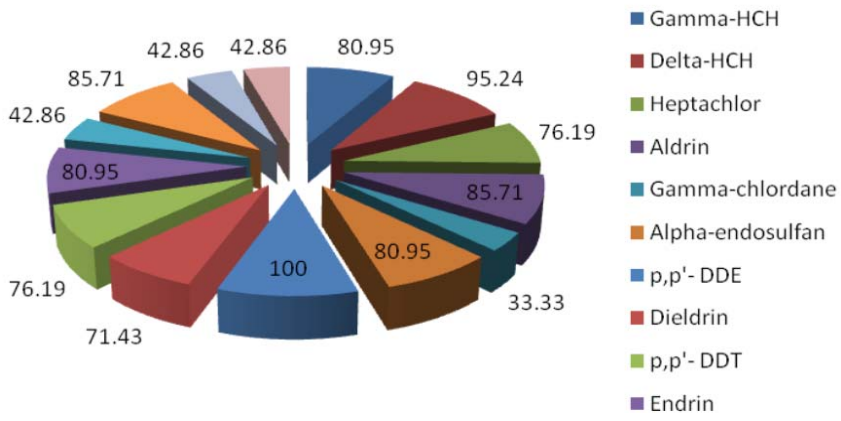

Fig. 2: incidence ratio of organochlorine pesticides in samples from Accra

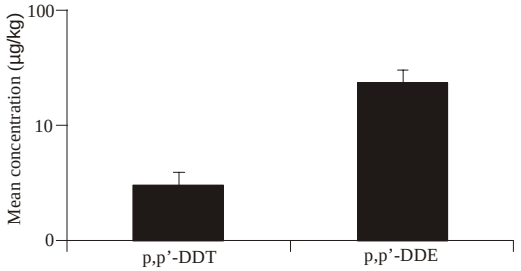

Fig. 3: Mean concentration of DDT and DDE in milk fat samples from Accra. The error bars represent standard deviation

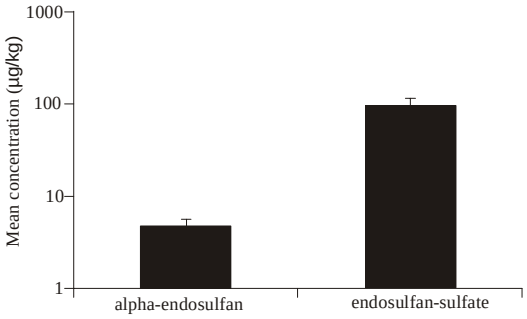

Fig. 4: Mean concentration of endosulfan and endosulfan sulfate in human milk (fat) samples from Accra. The error bars represent standard deviation

DDT and DDE have been detected in crops and the tissues of animals (Ntow, 2001).

Figure 3 compares the mean concentrations of p,p'-DDT and p,p'-DDE in the samples. The mean concentration of p,p'-DDT is lower than that of its metabolite p,p'-DDE. This may be that most of the DDT massively used in the past is in the metabolite state and fresh input of DDT in the environment is minimal.

The mean concentrations of alpha-endosulfan and endosulfan-sulphate are shown in Fig. 4. Endosulfan

Table 2: Comparison of organochlorine pesticides residue in human milk (fat) samples from selected nursing mothers in Accra with Australia maximum residue limits (µg/kg)

Name of Pesticides	Mean (fat)	Australian MRL(fat)
Gamma HCH	4.207	200
Delta-HCH	13.855	200
Heptachlor	11.791	150
Aldrin	2.962	150
Dieldrin	2.407	150
Gamma-chlordane	1.839	50
Alpha-Endosulphan	4.704	20
Endosulfan Sulphate	99.052	20
p,p'-DDT	3.085	1250
p,p'-DDE	23.367	1250
Endrin	7.669	-
Endrin aldehyde	7.769	-
Endrin Ketone	63.846	-
Methoxychlor	20.116	-

Sulphate, a metabolite of alpha-endosulfan recorded the highest mean concentration of 99.052 µg/kg (fat) and alpha endosulfan also recorded a mean concentration of 4.704 µg/kg (fat).

Endosulfan, just like other organochlorine pesticides are known to persist in the environment even years after their use. Darko and Acquaah (2007) detected endosulfan- sulphate mean concentration of 21.35 µg/kg in meat. Ntow (2001) recorded 30.8 µg/kg mean concentration of Endosulfan sulphate residues in water (Ntow, 2001). Alpha-endosulfan residues have also been recorded in crops and in fish (Ntow, 2001; Osafo and Frimpong, 1998). An appreciable concentration of alpha-endosulfan was measured in the breast milk samples and this might be due to the fact that it was recently considered for restrictive use in Ghana, precisely 2008. The relatively higher level of endosulfan-sulphate compared to alpha-endosulfan may also be due to the fact that previous inputs of alpha-endosulfan has metabolized to endosulfan- sulphate or there is minimal inputs of alpha-endosulfan at present.

Table 2 gives the mean concentrations in µg/kg of the various organochlorine pesticides residues detected in the human breast milk sample analyzed compared to that of the Australia maximum residue limit.

It is clear from Table 2 that with the exception of endosulfan Sulphate whose mean concentration, 99.052 µg/kg was found to be higher, the mean concentrations of the organochlorine pesticides detected were lower than that of the Australian Maximum Residue Limit.

CONCLUSION

The results obtained from this research work revealed that there are still residues of some organochlorine pesticides in the environment. Fourteen different organochlorine pesticides namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were detected. Seven out these fourteen organochlorine pesticide residues; aldrin, chlordane, DDT, dieldrin, Endrin, Lindane and heptachlor are on the list of banned pesticides by the Environmental Protection Agency of Ghana. The mean concentrations of the organochlorine pesticides detected were between the ranges of 1.839 -99.052 µg/kg (fat). P, p'- DDE recorded 100% incidence ratio. Endosulfan Sulphate recorded the highest mean concentration of 99.052 µg/kg (fat) which was about five times greater than the Australian Maximum Residue Limit (MRL) value for milk fat.

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