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# Levels of Organochlorine pesticide residues found in the breast milk of some first-birth mothers from a rural community (Ada) in Ghana

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# ABSTRACT

The aim of this study was to determine the types and levels of organochlorine pesticide residues in the breast milk of some first birth mothers in Ada, a rural 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. Thirteen different organochlorine pesticides residues namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, endrin, 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%. Gamma-HCH recorded the highest incident ratio of 95.2% and p, p'-DDE, endosulphan sulphate, delta-HCH and dieldrin also recorded incidence ratios of 90.5%, 81.0%, 66.7% and 57.1% respectively in the twentyone individual human breast milk samples. The mean concentrations of organochlorine pesticide residues in the human breast milk samples ranged from 0.682 to 63.803 µg/kg fats. Endosulphan-sulphate recorded the highest concentration of  $63.803 \mu g/kg$  fats which is about three times greater than 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 maximum residue limits.

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# Introduction

Organochlorine pesticides are a class of chemicals that came into widespread use in the late1940s. Until the early 1980s, many chlorinated insecticides, mainly aldrin, dieldrin, DDT, and lindane had been used in controlling pests of crops, vectors of some diseases and other aspects of public health in Ghana (UNEP, 2002). Some of these pesticides are still widely used by farmers because of their effectiveness and their broadspectrum activity (Amoah *et al.*, 2006). Despite being banned in industrialized countries since the 1970s, or subjected to restrictions in use in many others, they persist to this day in the environment. Apart from occupationally

exposed individuals, most exposure to these chemicals occurs via dietary intake (DeVoto *et al.*, 1998; Ahlborg *et al.*, 1995), especially food of animal origin, but also through water, ambient and indoor air, dust and soil (Covaci *et al.*, 2002; Dua *et al.*, 2001; Manirakiza *et al.*, 2002). These lipophilic compounds accumulate and even biomagnify their concentrations along the food chain, especially in fatty foods (Manirakiza *et al.*, 2002). Some published reports (Ahlborg *et al.*, 1995; DeVoto *et al.*, 1998; Hanaoka *et al.*, 2002; Manirakiza *et al.*, 2002) suggest that serum levels of organochlorines are related to the consumption of various foods. In Ghana, analytical investigations of a number of organochlorine pesticides in human organs, body fluids and other reported incidents suggest that some of these chemicals are still in use illegally despite their ban or considered strictly under restrictive use. (Ghana NIP, 2007). Organochlorine pesticides

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(Hunter et al., 1997). Adverse health effects including reproductive failures, tumor induction, endocrine disruption and cancers can occur once living organisms are exposed to organochlorine pesticides (Makris and Rowe, 1998). These chemicals pose a serious risk to health, especially for infants in whom enzymatic and metabolic systems are not fully active (Garry, 2004). Information from research indicates that exposure to organochlorine compounds affect thyroid function in preschool children (Natural Health News, 2008). Low sperm count in males, birth defects, increase in testicular cancer and other reproductive and development effects (Weltman, 1983) have also been reported as a result of organochlorine contamination. With regard to exposure to organochlorine pesticides in early pregnancy, several epidemiological studies suggest that maternal employment in agriculture may be a risk factor for birth defects (Nurminen, 1995; Weidner et al., 1998; Engel *et al.*, 2000). In spite of the massive use of organochlorine pesticides in Ghana, there is paucity of information on their environmental levels and their levels in humans. 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 et al., 2001). The breast, a lipid-rich tissue acts as a depot or reservoir of lipophilic

are very harmful to both humans and the environment at large

pesticide by virtue of physiochemical interactions of the cellular component with the pesticide (Mussalo-Rauhamaa, 1991). The current study however, is limited to only first birth mothers in a rural community. Such mothers have never breast fed any children to release their 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 study will give vital information on the levels of these 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 40-60<sup>o</sup>C (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 -20<sup>o</sup>c to minimize degradation. Solid phase extraction (SPE) cartridges (strata sI-1 Silica) (55um, 70 A) of density, 500mg/6ml.

**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 <sup>63</sup>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 ( $30m \times 0.25$ (lid)  $\times$ 0.25µm, thickness plus 10m guard column.

#### Study area:

A rural community (Ada) was considered for this study. Ada is a rural community in the Greater Accra region of Ghana. Ada Foah Health Centre was selected for the collection of the human milk sample. Ada is farming and fishing community. Most of the farmers are engaged in vegetable production along the Volta River. The study area is shown in figure 1.

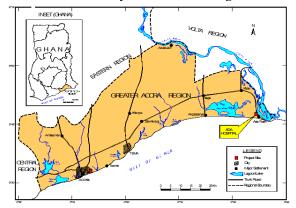


Figure 1.0: Map showing the sampling sites

**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  $300^{\circ}$ c. 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, A22 to A42 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.

The samples were stored in an ice chest with dried ice at  $4^{0}$ C. The samples were later transported to the Ghana Standard Board Pesticide Residues Laboratory and stored at  $-20^{0}$ C 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 -20°c were allowed to thaw and then stirred thoroughly. 10 ml of the milk samples were then pipetted and homogenised with 40ml 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 aliquots of 30 ml petroleum ether. The combined separate organic phase collected was evaporated to dryness by the rotary evaporator with water bath at 40°c. 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 (500mg/6ml) cartridges were conditioned with 10 ml petroleum ether. The organic layer dissolved in 5ml 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 1ml 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 <sup>63</sup>Ni electron capture detector which is very sensitive for detecting halogens. The GC conditions used for the analysis included a capillary column coated with RB-5 (30×0.25mm, 0.25µm 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 225°C, oven temperature was set at 225°C and electron capture detector was also set at 300°C. The column oven temperature was programmed as follows; 60°C for 2min, 180°C/min up to 300°C held for 31.80 min. The injection volume of the Gas Chromatograph (GC) was 1.0µL. 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 0.1ml/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:

% of Recovery = (Amount of analyte received) \* 100

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

#### **Results And Discussion**

Based on the analysis carried out, thirteen different types of organochlorine pesticides were detected in the twenty-one individual human breast milk sample analysed. They include p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-ketone, alpha-Endosulphan, Endosulphan-sulphate, gamma-chlordane, dieldrin, and Methoxychlor. The mean concentrations of the various analyte were recorded in µg/kg fat.

Figure 2 gives detailed description of incidence ratios of the various organochlorine pesticides in the human breast milk samples.

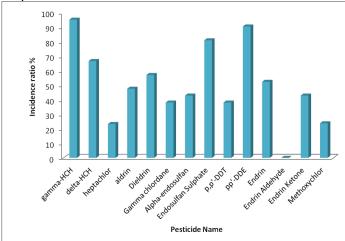


Fig 2: incidence ratio of organochlorine pesticides in samples from Ada

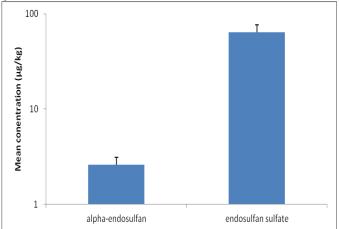
Gamma-HCH recorded the highest incidence ratio of 95.2%. This indicates that majority of the first birth mothers selected for the study has gamma-HCH in their breast milk. About 90.5% of the breast milk samples tested positive to p, p'-DDE. Endosulphan Sulphate recorded an incidence ratio of 81%. Delta-HCH, Dieldrin, and Endrin recorded incidence ratios

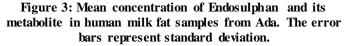
of 66.7%, 57.1, and 52.4% respectively. The incidence ratios of the other organochlorine pesticides detected in the milk samples were below 50% and their individual incidence ratios can be seen from figure 2.

Table 1 shows the mean concentrations in  $\mu g/kg$  of the various organochlorine pesticide residues in the human breast milk samples.

Endosulphan Sulphate recorded the highest mean concentration of 63.803  $\mu$ g/kg fat. Endosulphan sulphate is the main degradation product of Endosulphan; it is equally toxic as the parent compound and perhaps even more persistent (U.S. EPA, 2002). Endosulphan, marketed as thiodan, was widely used in cotton growing areas, on vegetable farms, and on coffee plantations (Gerken, *et al.*, 2001). Endosulphan was also employed to control ectoparasites of farm animals and pets in Ghana (Ntow *et al.*, 2006). The massive use of Endosulphan, its persistent nature, long range transport as well as its ability to bioaccumulate has resulted in it getting into the food chain (U.S. EPA, 2002).

The use of Endosulphan was only recently considered for restricted use in Ghana in 2008. This might have resulted in its residues still being recorded in the human milk fat samples analysed. From figure 3, Alpha Endosulphan which is a major component of the technical Endosulphan, recorded a lower mean concentration as compared to the metabolite Endosulphan sulphate, thus 63.803  $\mu$ g/kg for Endosulphan sulphate as against 2.588  $\mu$ g/kg for alpha Endosulphan. This means that most of the Endosulphan used in the past is in the metabolite state. Exposure in humans might have resulted through contaminated food or through direct contact during its application in agriculture and household use.





The mean concentration for p, p,-DDE recorded in the human milk samples from Ada is  $24.165\mu g/kg$  fat and that of p, p'-DDT is  $6.339\mu g/kg$  fat. Before the ban of DDT in most parts of the world, DDT was used in Ghana in the agriculture sector to control crop pest and in public health for disease vector control. It was also used to control ecto-parasites on household animals (Ntow *et al.*, 2001; Ntow *et al.*, 2006). Even though DDT is under restrictive use in Ghana, it is no more imported or used, not even in the public health sector, this is to prevent misapplication. p, p'-DDE is the main metabolite of DDT, and it is more persistent in the environment than the parent DDT. DDE levels in the breast milk may reflect previous exposure to DDT which has degraded to DDE or exposure to DDE itself through food or other means.

Table 1: Organochlorine pesticide residue (µg/kg) in human breast milk samples from Ada.					
Name of Organochlorine Pesticide	Mean (fat)	SD	*Mean (whole milk)	SD	**Incidence ratio (%)
gamma-HCH	5.438	1.573	0.372	0.099	95.2
delta-HCH	6.728	3.489	0.206	0.029	66.7
heptachlor	0.682	0.148	0.054	0.021	23.3
aldrin	2.387	0.605	0.172	0.035	47.6
Dieldrin	2.222	0.542	0.18	0.047	57.1
Gamma chlordane	1.304	0.372	0.101	0.031	38.1
Alpha-Endosulphan	2.588	0.704	0.18	0.046	42.9
Endosulphan sulphate	63.803	11.167	4.241	0.635	81
p,p'-DDT	6.339	1.987	0.283	0.115	38.1
p,p'-DDE	24.165	7.597	1.618	0.623	90.5
Endrin	3.468	1.287	0.474	0.159	52.4
Endrin Aldehyde	ND	-	ND	-	-
Endrin Ketone	1.441	0.348	0.127	0.031	42.9
Methoxychlor	4.896	0.703	0.424	0.06	23.8

Table 1: Organochlorine pesticide residue (ug/kg) in human breast milk samples from Ada.

SD = Standard DeviationND = Not Detected

ND = Not Detected

\*\*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.

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

Ada with Australia maximum residue limits (µg/					
Name of	Ada Mean	Australian			
pesticides	(fat)	MRL (fat)			
Gamma HCH	5.438	200			
Delta-HCH	6.728	200			
Heptachlor	0.682	150			
Aldrin	2.387	150			
Dieldrin	2.222	150			
Gamma-chlordane	1.304	50			
Alpha-Endosulphan	2.588	20			
Endosulphan Sulphate	63.803	20			
p,p'-DDT	6.339	1250			
p,p'-DDE	24.165	1250			
Endrin	3.468	-			
Endrin aldehyde	ND	-			
Endrin Ketone	1.441	-			
Methoxychlor	4.896	-			
ND – Not Detected					

ND = Not Detected

Although DDE is more toxic and persistent in the environment than the DDT, the results give a positive signal that fresh input of DDT has minimized if not halted. This also means that the ban on DDT has been effective in Ghana.

The mean concentration for aldrin is 2.387  $\mu$ g/kg fat and that of dieldrin is 2.222  $\mu$ g/kg fat. Dieldrin is a metabolite of aldrin and from the result from Ada, the mean concentration for dieldrin is slightly lower than the mean concentration for aldrin.

This means there is more Aldrin in the environment than the metabolite Dieldrin (Figure 5).

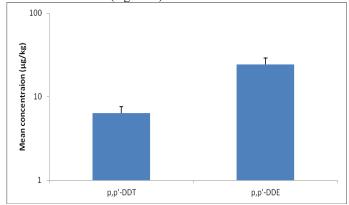


Figure 4: Mean concentration of DDT and DDE in human milk fat samples from Ada. The error bars represent standard deviation.

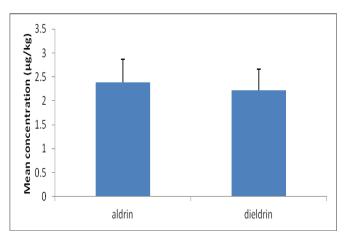


Figure 5: Mean concentration of aldrin and dieldrin in human milk fat samples from Ada. The error bars represent standard deviation.

Even though aldrin and dieldrin are the most widely banned and restricted class of pesticide in the world (Siedenburg, 1991) its persistent nature allows levels of its residues to be detected in the environment. Dieldrin has been detected in more than 99% of breast milk sample tested in most countries (WHO, 1989).

Table 2 gives the mean concentrations in  $\mu g/kg$  fat of the various organochlorine pesticides residues detected in the human breast milk sample analyzed compared to that of the Australia maximum residue limit. The mean concentration of

Endosulphan sulphate was about three times greater than the recommended Australia Maximum Residue Limit for milk. The mean concentration recorded in the milk sample was found to be 63.803  $\mu$ g/kg as against the recommended MRL value of 20  $\mu$ g/kg. The mean concentrations for all the other organochlorine pesticides detected were below their recommended MRLs for milk. This is clearly shown in the table 2.

# Conclusion

The results obtained from this research work revealed that there are still residues of some organochlorine pesticides in the environment. Thirteen different organochlorine pesticides p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, namelv heptachlor, aldrin, Endrin, 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 0.682 -62.803 µg/kg (fat). Gamma –HCH recorded the highest incidence ratio of 95.2%. Endosulphan Sulphate recorded the highest mean concentration of 63.803µg/kg (fat); which was about five times greater than the Australian Maximum Residue Limit (MRL) value for milk fat.

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