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Distribution of Organochlorine Pesticides in Human Breast Milk and Adipose Tissue from Two Locations in Côte d'Ivoire

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Abstract: The levels of organochlorine residues in 40 samples of milk and 20 human adipose tissues from two locations in the northern of Côte d'Ivoire were determined. A system of Gas Chromatography with an Electron Capture Detector (CG-ECD) was used for the qualitative and quantitative analysis of the samples. This study revealed in general that the level of pesticides in samples of adipose tissue was higher than that in samples of milk from any source (city or rural). Indeed, while the average of 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT), predominant specie and its isomers in adipose tissue was 10.02 mg kg^{-1} in countryside and 6.93 mg kg^{-1} in the city, it was respectively 0.013 and 0.019 mg kg^{-1} in milk. Qualitatively, the pesticide residues detected in milk samples were Lindane, hexachlorohexane and its isomers, DDT and its metabolites, heptachlor, heptachlorepoxide, aldrin, endrin, dieldrin and endosulfan α and β. Adipose tissue samples revealed the presence of polychlorinated biphenyl (PCB), Hexachlorobiphenyl (HCB) in addition to pesticides which are found in the milk Average concentrations of organochlorine pesticides in milk matrix were above the Maximum Residue Limits (MRL) of WHO. Regarding adipose tissue, this trend was also observed, except for DDT and its metabolites whose values were far below the MRL.

Key words: Breast milk, adipose tissues, organochlorine residues, pesticides contamination, human cumulative effect, gas chromatography, Côte d'Ivoire

INTRODUCTION

Organochlorine pesticides (OCPs) have been used all over the world (Africa, Asia, Latin America) since 1940 because of their efficiency in protecting crops and in the fight against the vectors of some endemic diseases such as malaria, typhoid fever and trypanosomiasis. These substances have not only a direct effect on the target organisms but they also have a short and long term impact on other vertebrates or non vertebrates and non-target. Despite the ban on the production and the use of OCPs in industrialized countries, in accordance with the Stockholm Convention in 2001 (Ennacer *et al*., 2008; Cruz *et al*., 2003), these persistent toxic substances continue to cause great damage to the environment and living organisms (Zawiyah *et al*., 2006; Hongtao *et al*., 2008). This situation is more pronounced in the Third World countries because of the low prices of theses substances. Organochlorine pesticides are very persistent liposoluble compounds with high half-life times in human organism. They are characterized by their bioaccumulation properties in the fatty parts of biological beings, such as blood,

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breast milk and fatty tissues via the food chain (Smith and Gangolli, 2002). Because of its position in the food chain (the end of the chain), man is exposed to the effect of these micropollutants by eating foods coming from contaminated water or earth (William *et al*., 2008). During the past forty years, special emphasis has therefore been placed on the toxic effects of these persistent organic products on humans and particularly on children (Ennacer *et al*., 2008). The woman, with a higher fat because of her eating habits after birth, undergoes greater accumulation than other mammals and can thus serve as a good bioindicator. Several works have been conducted all over the world for the determination of organochlorine pesticide residues, particularly in breast milk so as to prevent any risk of contamination of babies through breastfeeding, in the serum and in adipose tissues (Celeste and da Silveira, 2006; William *et al*., 2008; Ennacer *et al*., 2008; Lopez-Espinosa *et al*., 2007; Maria-Jose *et al*., 2008; Heck *et al*., 2007; Wang *et al*., 2008). The use of organochlorine pesticides has resulted in improved protection of cotton crops against unexpected losses and improving in the quantity and the quality of food. Food crops (millet, sorghum, rice and maize) which are grown on the same land like cotton have a high probability to accumulate the organochlorine pesticides, used for their protection. Thus, these foodstuffs can cause inconvenience to consumers if the rate of their residues are higher than the Maximum Residues Limits (MRL) of WHO/FAO (Tejada *et al*., 1993). This study was conducted in northern of Côte d'Ivoire in Korhogo and Sinématiali, in order to determine the degree of women and food contamination in these two localities.

MATERIALS AND METHODS

Sampling and Collection

Human breast milk samples (40 samples) and adipose tissues (20 samples) were collected from mothers (17 to 56 years old, average age 30) living in two different locations in the north of Côte d'Ivoire (Korhogo and Sinématiali) from 2004 to 2007. The specificities of the studied areas were that organochlorine pesticides were wildly used for cotton culture and malaria control for a long period. Samples were collected in Korhogo and Sinématiali maternity. Breast milk was extracted manually (5-10 mL) into sterilized glass containers by lactating mothers. The adipose tissue donors were women giving birth to their first, second, third or fourth child by caesarean operation in Korhogo regional Hospital (CHR). Specimen's subcutaneous fat (about 1 to 5 g) was collected from the mother during the caesarean operation. The samples were frozen immediately at -20°C until analysis. All participants were healthy, non-smokers and no occupational contact with OCPs, with regard to the fact that some environmental and physiological factors (example age of mother, number of parity, dietary intake, area of residence) can influence the levels of OCPs in milk, a detailed questionnaire was given to obtain an informative record of the participant for subsequent data analysis.

Sample Extraction and Cleanup

Pesticide-grade anhydrous sodium sulfate, petroleum benzene, dichloromethane and hexane were obtained from Merck (Darmstadt, Germany). Fluorisil (PR Grade, 60-100 mesh) was purchased from Silica (Silica Co, USA). Organochlorine pesticides were extracted and purified according to the method used by Manda *et al*. (2003). Two to five grams of breast milk or adipose tissues were weighed and added to 20 g of anhydrous sodium sulfate (Na_2SO_4) and the whole was homogenized so as to obtain a powder. The sample was then extracted twice with 150 mL of 4:1 (v/v) petroleum benzene and dichloromethane in a glass column containing 20 g of fluorisil. The aliquots of the different extracts were collected in a 500 mL flask previously dried in an oven (100°C), cooled and weighed. The aliquots were then completely evaporated in a rotavapor at 40-50°C and the flask was dried in the oven, cooled and weighed again for the fat rate determination. The extracts were dissolved in hexane and filled up to an appropriate volume (10 mL) for GC analysis.

Gas chromatography Analysis

Analysis of OCPs was performed on a SHIMADZU GC-14A with ^{63}Ni electron capture detector, commonly used in organochlorine pesticide analysis (Gelsomino *et al*., 1997; Ennacer *et al*., 2008) for its efficiency (more sensitive, lower LOD than the FID or the TCD). The LOD was calculated by application of NF V03-120 (Norme française) or ISO for LOD. This LOD expressed on fat basis for all OCPs studied was estimated to be 5 ng kg^{-1}. A capillary column (DB-1 phase, 30 m×0,25 I.D×0,25 μm film thickness) was used with Nitrogen as carrier gas at a flow rate of 18 mL min^{-1}. Sample volumes of 2 μL were injected. Split injection of sample was undertaken at 250°C and the detector temperature was 300°C. The column oven was programmed from an initial temperature of 100°C (because of the low volatility of OCPs) and held at that temperature for 5 min, raised to 170°C (at a rate of 5°C min^{-1}) and held for 10 min, then raised to 250°C (at the rate of 10°C min^{-1}) and held for 20 min (with a total run time of 45 min). An external standard was used to perform quantitative and qualitative analysis. Moreover in order to determine the gas chromatography system precision and accuracy, a 0.5 mg L^{-1} concentrated multi-element solution containing every organochlorine pesticide was prepared (pesticide standards were purchased from Dr Ehrenstorfer, Ausberg, Germany and most of them were of over 99% certify purity). Then 2 mL of this solution had been injected 5 times consecutively. The results obtained were used to calculate the expected parameters.

RESULTS

Validation criteria: The GC-ECD is a device that gives good results even at very low levels concentrations because of the selectivity and sensitivity of its detector (Gelsomino *et al*., 1997). The results of the determination of the precision and the accuracy of the SHIMADZU GC-14A used for the analysis are given in Table 1.

For all pesticides analyzed, the values of the exactness and fidelity are all below 6%, a value beyond which the results can be consider as bad. So, the chromatograph SHIMADZU GC-14A is able to take the right measurements.

Organochlorine pesticides residues in milk: The mean concentrations of Lindane, ΣHCH, ΣDDT, heptachlor, heptachlorepoxide, aldrin, endrin, dieldrin and endosulfan (α + β) in milk samples in Korhogo and Sinématiali are gathered respectively in Table 2 and 3. These tables also contain the

Table 1: Calculation of criteria validation

Pesticides	X_m (mg kg^{-1})	σ	J (%)	F (%)
γ-HCH	0.5201	0.010	4.02	2.09
Op-DDD	0.4982	0.020	0.36	4.91
α-endosulfan	0.5060	0.007	1.21	1.25
Aldrin	0.5232	0.028	4.64	5.28
Dieldrin	0.5313	0.020	6.26	4.02
Endrin	0.4737	0.013	5.26	2.68
Hepta chloride	0.4718	0.028	5.63	5.82

Table 2: OCPs residues in milk samples (mg kg^{-1}) in the area of Korhogo

Pesticides	Max (mg kg^{-1})	Min (mg kg^{-1})	Mean (mg kg^{-1})	MRL (mg kg^{-1})	Percentage
Lindane	0.068	<LOD	0.005	0.01	2.8
ΣHCH	0.246	<LOD	0.019	0.02-2	14.5
ΣDDT	0.684	<LOD	0.061	1-5	45.7
Heptachlor	0.013	<LOD	0.002	0.05-0.2	1.5
Heptachor epoxide	0.114	<LOD	0.006	0.05-0.2	4.5
Aldrin, dieldrin, endrin	0.204	<LOD	0.043	0.02	32.3
Endosulfan (α + β)	0.044	<LOD	0.002	0.02	1.5

Max: Maximum, Min: Minimum, LOD: Limit of detection, MLR: Maximum residue limit

Table 3: OCPs residues in milk samples (mg kg^{-1}) in the area of Sinématiali

Pesticides	Max	Min	Mean	MRL	Percentage
	---------- (mg kg^{-1}) ----------				
Lindane	0.006	<LOD	<LOD	0.01	0.0
ΣHCH	0.013	<LOD	<LOD	0.02-2	0.0
ΣDDT	0.252	<LOD	0.013	1-5	21.3
Heptachlor	0.007	<LOD	<LOD	0.05-0.2	0.0
Heptachor epoxide	<LOD	<LOD	<LOD	0.05-0.2	0.0
Aldrin, dieldrin, endrin	0.265	<LOD	0.048	0.02	78.7
Endosulfan (α + β)	<LOD	<LOD	<LOD	0.02	0.0

Max: Maximum, Min: Minimum, LOD: Limit of detection, MLR: Maximum residue limit

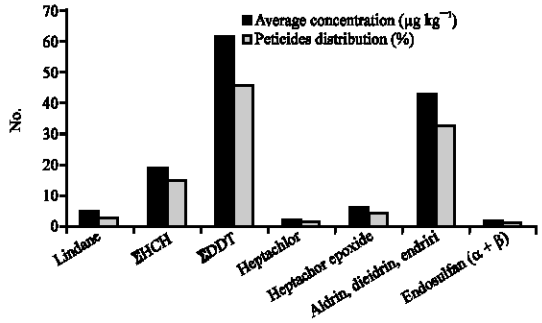

Fig. 1: OCPs residues distribution and average concentration in milk samples in the area of Korhogo

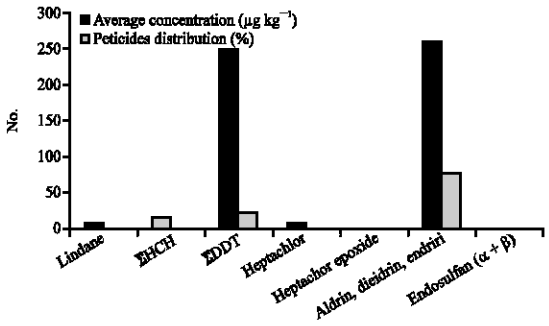

Fig. 2: OCPs residues distribution and average concentration in milk samples in the area of Sinématiali

maximum and minimum average concentrations in pesticides, the maximum limits of residues of WHO and the percentages of contamination for each group of pesticides. For a better perception the histograms for the rates of the distribution of pesticides and the average concentrations for each survey area was depicted by Fig. 1 and 2. From Fig. 1, it appears that in the area of Korhogo, almost all the groups of organochlorine pesticides are detected in each sample. However, the culminated point is represented by DDT and its derivatives, followed by the group of cyclodienes (Dieldrin and Aldrin) for which Dieldrin predominate. This situation could be explained on the one hand by the stability of DDT (higher half-life time) and on the other hand by the fact that Dieldrin results from Aldrin oxidation. Figure 1 in which the distribution of pesticides is shown, better explains the situation. DDT constituting the majority represents 45% of the total content of pesticides, followed by the group of HCH 31% and the Group of cyclodienes, 14%. It should be noticed that the rates of all the pesticides or group of pesticides detected in the area of Korhogo are lower than the MRL of WHO.

Unlike the case of Korhogo, where several groups of pesticides were detected, in Sinématiali (Fig. 2), is denoted only the presence of two groups: DDT group (o, p-DDT and p, p DDT:with an

Table 4: OCPs residues in adipose tissues samples (mg kg^{-1}) in Korhogo countryside

Pesticides	Max	Min	Mean	MRL	Percentage
	---------- (mg kg^{-1}) ----------				
Oxamyl	47.451	<LOD	15.719	-	51.2
ΣHCH	0.265	<LOD	0.06	0.2-2	0.0
PCB and derivatives	21.275	<LOD	3.755	0.2-2	12.2
ΣDDT	35.287	<LOD	6.932	1-5	22.6
HCB and derivatives	0.086	<LOD	0.012	0.2-1	0.0
Endosulfan(α + β)	13.792	<LOD	3.598	0.2	11.7
Trans-heptachloride epoxide and chlordan	0.037	<LOD	0.011	0.05-0.2	0.0
Isodrin, aldrin,dieldrin	1.643	<LOD	0.655	0.2	2.3

Max: Maximum, Min: Minimum, LOD: Limit of detection, MLR: Maximum residue limit

Table 5: OCPs residues in adipose tissue samples (mg kg^{-1}) in Korhogo city

Pesticides	Max	Min	Mean	MRL	Percentage
	---------- (mg kg^{-1}) ----------				
Oxamyl	17.33	<LOD	7.474	-	32.0
ΣHCH	0.064	<LOD	0.013	0.2-2	0.0
PCB and derivatives	0.363	<LOD	0.107	-	0.5
ΣDDT	22.373	<LOD	10.017	1-5	43.0
HCB and derivatives	0.547	<LOD	0.256	0.2-1	1.1
Endosulfan(α + β)	8.695	<LOD	3.858	0.2	16.6
Trans-heptachloride epoxide and chlordan	<LOD	<LOD	<LOD	0.05-0.2	0.0
Isodrin, aldrin,dieldrin	4.195	<LOD	1.586	0.2	6.8

Max: Maximum, Min: Minimum, LOD: Limit of detection; MRL: Maximum residue limit average concentration

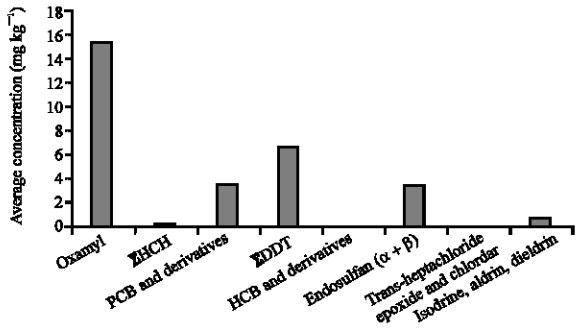

Fig. 3: OCPs residues in adipose tissues in the area of Korhogo City

average concentration of 19 μg kg^{-1} each) representing 21% and cyclodienes (79%) with the predominance of Dieldrin (67 μg kg^{-1}). In the case of Sinématiali, the average concentrations of DDT are normal while those of Dieldrin and its congeners are higher than the residue limits (MRL). The values found in this study are significantly lower than those found in Ghana and Tunisia (William *et al*., 2008; Ennacer *et al*., 2008).

Because of their physico-chemical properties, OCPs can accumulate in the fatty parts of animal organisms, particularly in adipose tissues. The results shown in Table 4 and 5, concerns people living in Korhogo (samples of Korhogo City area) and on subjects from the surrounding villages (samples of Korhogo countyside). In fact, complications during childbirth are treated in CHR of Korhogo. For the chromatographic analysis of these samples, were looked for, traces of polychlorinated biphenyl (PCB), hexachlorobiphenyl (HCB) and carbamate (oxamyl) in addition to pesticides which are commonly sought. The results are given in Table 4 and 5. As in the case of maternal milk, these tables show the minimum and maximum average concentrations, the maximum residue limits and the rate of repartition for each pesticide or group of pesticides. The average concentrations of these pesticides were depicted in Fig. 3 and 4 for each study area.

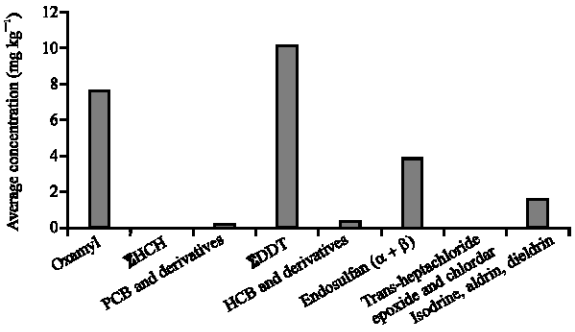

Fig. 4: OCPs residues in adipose tissues in the area of Korhogo Countryside

Qualitatively, the same results were obtained whether in the area of Korhogo city or in that of Korhogo countryside. Indeed, in the countryside as in the city, it can be noticed in almost all the samples, the presence of HCH, HCB and its derivatives, trans-heptachlor epoxide and chlordane, oxamyl, DDT and its isomers, endosulfan, isodrin , aldrin and dieldrin.

Quantitatively, while HCH, HCB and its derivatives, trans-heptachlor epoxide and chlordane are less visible, the main peaks are oxamyl, DDT and its isomers, endosulfan, isodrin, aldrin and dieldrin. Meanwhile the maximum of the average concentrations is oxamyl in Korhogo City, with a value of 15.72 mg kg^{-1} the DDT and its isomers in Korhogo countryside were obtained with an average concentration of 10.02 mg kg^{-1}. This observation is normal insofar as people living in the campaign are more exposed to the OCPs effects. In fact, it is in the countryside that agriculture is more intense and that the rate of pesticide used should therefore be the highest in the different compartments of the ecosystem.

DISCUSSION

Human impregnation by persistent toxic substances like organochlorine pesticides is universal and they can be detected in any biological sample. Lipophilic OCs are highly resistant to both abiotic and biotic degradation. They can be transported by air and water and trace amounts are found all over the world, even where PCBs have never been produced or used (Ana *et al*., 2007). It was reported recently, that organochlorine pesticide are highly bioaccumultive in food chain and they are absorbed and accumulated into the fatty tissue of living bodies (Wang *et al*., 2008). Because humans occupy the top position in the trophic levels, they are obviously exposed to a higher level of these contaminants from aquatic and terrestrial food chain and become vulnerable to the toxic effects (William *et al*., 2008).

All over the world, similar studies have been conducted but in Côte d'Ivoire, this study is the first one on human fatty tissues. The findings in this study are consistent qualitatively with other studies conducted in Kenya by Kanja *et al*. (1992), in Tunisia by Ennacer *et al*. (2008) and in Germany by Schade and Heinzow (1998) but with lower values than ours (the average concentrations of ΣDDT were respectively, 0.98×10^{-3}, 1.9×10^{-3}, 0.24×10^{-3} mg kg^{-1}, while 6.1×10^{-3} mg kg^{-1} was obtained). Shannon *et al*. (1997) have got higher values of residues for organochlorine pesticides and PCBs in a study conducted in a Laboratory of Analytical Chemistry at the University of Colorado.

The higher values in this study may be explained by the non-compliance with the organochlorine pesticide prohibition in the north of Côte d'Ivoire. There are still entries by unauthorized fraudulent circuits which is exacerbated by illiteracy among the population. The second reason may be the high rate of birth among women (2, 3, 4 and sometimes 5). The diet which women are traditionally subjected to in Côte d'Ivoire increases the rate of fat and thereby the probability of accumulation of

organochlorine pesticides. Exposure of pregnant women to organochlorine pesticides (OCs) largely derives from ingestion of contaminated food, although environmental, occupational and other domestic exposures cannot be excluded (Maria-Jose *et al*., 2008). The levels of ΣDDT, aldrin, dieldrin, endrin in milk and adipose tissue samples confirmed what is said above. Biomarkers (e.g natural serum, umbilical blood cord, dairy products) have been applied in measurement of organochlorine exposure in many studies (Godfred and Acquaat, 2008; Heck *et al*., 2007; Maria-Jose *et al*., 2008). Organochlorine pesticides have been shown to elicit a wide range of toxic and biochemical effects in both and wild animals. They pose a serious risk to health, especially for infants, since their enzymatic and metabolic systems are not fully active (Heck *et al*., 2007).

CONCLUSION

The contamination of the environment and human via food chain by Organochlorine pesticides used heavily in Côte d'Ivoire (especially in the north) has been shown in this study. Even if the concentrations observed were generally not alarming, some pesticides derivatives concentration such as Dieldrin and its congeners are higher than the residue limits (MRL). This work seeks to provide informations on levels of pesticide residues in human milk and adipose tissues of the studied area that will assist in a scientific assessment of the impact of these persistent toxic substances on public health and agriculture in Côte d'Ivoire (Korhogo and Sinématiali). To our knowledge, this is the most recent and intensive study on human breast milk and adipose tissue contamination by organochlorine pesticides in Côte d'Ivoire. The results of the study indicated that no sample exceeded the maximum residue limits for the organochlorine compounds evaluated.

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