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RESEARCH ARTICLE

CONTAMINATION OF BREAST MILK AND SEBUM WITH ORGANOCHLORINE PESTICIDES : A CORRELATIVE STUDY.

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Abstract

The objective of this work is to study the correlations between organochlorine pesticide (POC) residues detected in two human biological fluids, namely breast milk and facial sebum. 15 sebum and breast milk samples were therefore collected from volunteer mothers in the Yamoussoukro district of Côte d'Ivoire. The processed samples were analyzed on a GC (SHIMMADZU GC-14A split split splitless) equipped with a 63Ni electron capture detector and a SHIMADZU C-R6A CHROMATOPAC integrator. The results obtained show a positive correlation between the concentrations of HCH in milk (R^2 > 0.80 with P= 0.0001) and sebum (R^2 > 0.80 with P= 0.0001) and between DDT and its metabolite DDE in milk (R^2 > 0.80 with P= 0.0001) and sebum (R^2 > 0.80 with P= 0.0001).

Introduction:-

In Côte d'Ivoire, the agricultural sector that was at the origin of the strong growth of the 1960s and 1970s occupies an essential place in its economy. Thus, one of the country's main assets is its soils. Agriculture contributes 27% to GDP (gross domestic product), employs 2/3 of the working population and provides 40% of export earnings with the agro-industrial sector [1]. This performance was achieved largely due to the excessive use of chemical fertilizers and pesticides. Unfortunately, the use of plant protection products has had harmful effects on the environment (humans, animals, birds, soil, air, water, etc.) [2,3]. This problem is becoming considerable in a country where the majority of the peasant population is illiterate and ignores the dangers of improper handling of these products due to the total lack of safety precautions. In addition to pesticide suicides, poisoning accidents are observed across the country [4]. Among these substances, organochlorine pesticides from the dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) groups, although banned for several years because of their stability in the environment, are found in various biological media such as breast milk. Indeed, due to their lipophilic nature and persistence, these compounds accumulate in different biological environments and breast milk, due to its fat content, is therefore the preferred route for their elimination in humans [5]. Organochlorine pesticide residues have been detected in human milk collected from the populations of certain agricultural regions in Côte d'Ivoire [6]. Due to their accumulation in the fat tissue, body fat and fat of breast milk, facial sebum could therefore be one of the ways

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in which these compounds are eliminated in humans, just like breast milk. Thus, this study aims to establish correlations between contaminants in the two human biological fluids (breast milk and facial sebum).

Material And Methods:-

Biological material

It consists of samples of breast milk and sebum taken from nannies.

Sampling

Number of subjects sampled

The study was carried out in the Yamoussoukro region (Côte d'Ivoire). Fifteen volunteer mothers from the Yamoussoukro district, aged 18 to 47 years old and considered clinically healthy, participated in this study. Thus, 15 samples of breast milk and sebum each were collected. A questionnaire containing information on place of residence, age, body weight, birth weight, parity and eating habits was completed for each donor.

Sampling of matrices

Breast milk is taken by direct manual pressure from the breast by midwives on the 15th day after delivery. A minimum of 25 mL of milk collected is collected in 50 mL glass vials with a Teflon cap. The sebum samples are taken with cotton wool soaked in ethyl alcohol by applying this set to the face for 10 minutes. The donor's face is first cleaned with soapy water and thoroughly rinsed with water. The sample was taken after 2 hours of waiting, a period necessary for maximum sebum excretion. The samples are wrapped in aluminium foil before being packed in glass tubes. All samples were stored in a freezer at -20°C until analysis.

Extraction and purification

The fresh milk samples were treated with a mixture of acetone/hexane (2:1, V/V) for the extraction of organochlorine pesticides. Part of the organic solvent (10%) from this treatment was used to determine the percentage of fat. The remaining fraction is treated with concentrated H_2SO_4. It is then washed with distilled water and a diluted aqueous solution of KOH (10% ; v/v) to remove all traces of acid before being concentrated by evaporation. The concentrated extracts were successively purified on columns of acidified silica gel and 3% deactivated florisil. The purified extracts were concentrated and used for the analysis.

For sebum samples, pesticides were extracted with dichloromethane. The sebum-dichloromethane mixture is placed in a water bath between 40°C and 50°C. As with milk, part of the organic solvent from this treatment is used to determine the percentage of fat. The other fraction has undergone the same treatment as the remaining milk fraction. The recovery rates of the method in the case of sebum vary from 93 to 98% for the pesticides sought except for hexachlorobenzene (89%) and from 85 to 95% in the case of breast milk.

Instrumental analysis

Breast milk and sebum extracts were analyzed on a GC (SHIMMADZU GC-14A split splitless) equipped with a 63Ni electron capture detector and a SHIMADZU C-R6A CHROMATOPAC integrator. The characteristics of the capillary analysis column are as follows: liquid phase DB-1; diameter 0.25µm; film thickness = 3 x 0.25mm. Limiting temperature: 60°C to 325/350°C in program. A compact column with 1.95% QF-1 and 1.5% OV-17 was used to confirm the analyses. The operating conditions are : carrier gas, high purity nitrogen (99.9%) at 2 bar ; injector at 220°C and detector at 350°C. The furnace temperature program : 160°C (0 min) to 180°C at 2°C/min, then 180°C (0 min) to 245°C at 1.5°C/min, 245°C (16 min) to 285°C at 20°C/min and 285°C (7 min). The volume of the injected sample is 3µL.

Statistical analysis

The statistical study and those of correlations between the levels of organochlorine pesticide residues evaluated in milk and sebum, respectively, were examined with Statview 5 software**.**

Results And Discussion:-

Contamination of different human biological fluids

Table 1 presents the results of organochlorine pesticide residues tested in human milk and facial sebum. This table shows by residue the average (mean), minimum (min) and maximum (max) concentrations measured as well as the

deviation standards (STD) and the detection frequency (% det) or the percentage of samples with the pesticide residues measured.

Table 1:-Average residue concentrations in milk (mg/kg) and sebum (g/Kg)

Biological fluids		α-HCH	γ-HCH	β-HCH	δ-HCH	pp'-DDE	pp'-DDD	pp'-DDT
Breast milk	moy	0.329	0.343	0.414	0.436	0.636	0.201	0.717
	min	0.103	0.029	0.012	0.005	0.005	0.016	0.024
	max	1.020	1.230	1.204	1.194	3.372	0.578	2.453
	STD	0.272	0.332	0.323	0.387	0.945	0.194	0.798
	% detection	86.87	86.67	100	73.33	80	66.67	80
Sebum	moy	7.865	11.862	20.508	12.451	15.245	9.815	8.036
	min	2.512	0.868	4.523	1.985	0.233	0.004	0.017
	max	25.510	50.420	59.918	36.365	89.354	29.640	44.543
	STD	6.314	12.755	15.925	9.745	23.717	10.871	11.863
% detection		73.33	100	86.67	86.67	93.33	66.66	93.33

The results in this table indicate that most milk and sebum samples are contaminated with the same pesticide residues. Only the levels of contamination vary according to the compounds. A total of 7 organochlorine pesticide residues were detected in each of the carriers : pp'-DDT, pp'-DDDE, pp'-DDD, -HCH (Lindane), γ-HCH, α-HCH, β-HCH and δ-HCH. These residues belong to the DDT and HCH groups.

Table 2 below shows the presence of the DDT group at 57.78% in milk and 46% in sebum and the HCH group at 42.22% in milk and 54% in sebum.

Table 2:-Proportion of different organochlorine groups present in the samples as a percentage

	%HCH	%DDT	DDE/ΣDDT	βHCH/ΣHCH
Milk	42.22	57.78	0.41	0.27
Sebum	54	46	0.46	0.37

These results are unduly justified in view of FAO's warning [7] to sub-Saharan countries. Indeed, more than 100.000 tonnes of particularly toxic and persistent substances such as DDT, dieldrin and HCH are still in use in developing countries. These results also confirm the conclusions of some studies [6,8,9] that reveal the clandestine use of these banned plant protection products. The various values obtained, all below 0.50, show that pp'-DDT and -HCH are of recent use in the Yamoussoukro Region. Indeed, according to Marchand and Martin [10], it is accepted that a high percentage of DDT in relation to the two metabolites reflects a recent origin of this product in the surrounding receiving environment. On the other hand, a small percentage of DDT will be significant, either from old inputs or from already metabolized products. The same is true for lindane.

Correlation between Sebum contaminants and milk from Yamoussoukro populations

Statistical analyses of the results of the analysis of milk and sebum samples taken from the same patients have made it possible to determine correlations between the residue concentrations (HCH and DDT) measured in these matrices. Table 3 presents the regression equations established between the concentrations of the different residues detected in milk and sebum respectively, as well as the regression coefficients (R^2) and the precision of the measurement (P).

Table 3:-Relationship between the different residues of (HCH and DDT) detected in milk and those detected in sebum

	Equation	(R^2)	(P)
$[\gamma\text{-HCH}]_S = f\,[\gamma\text{-HCH}]_L$	$Y = -1.016 + 32.75\ X$	0.9	0.0001
$[\beta\text{-HCH}]_S = f\,[\beta\text{-HCH}]_L$	$Y = 35.581\ X^{(0.989)}$	0.922	0.0001
$[\alpha\text{-HCH}]_S = f\,[\alpha\text{-HCH}]_L$	$Y = 0.19 + 45.063\ X$	0.889	0.0001
$[pp'\text{-DDT}]_S = f[pp'\text{-DDT}]L$	$Y = 13.214\ X^{(1.065)}$	0.953	0.0001
$[pp'\text{-DDE}]_S = f[pp'\text{-DDE}]L$	$Y = 1.092 + 26.055\ X$	0.978	0.0001

The equations in Table 3 above are translated by the graphs fa, fb, fc, fc, fd and fe respectively in the figure below.

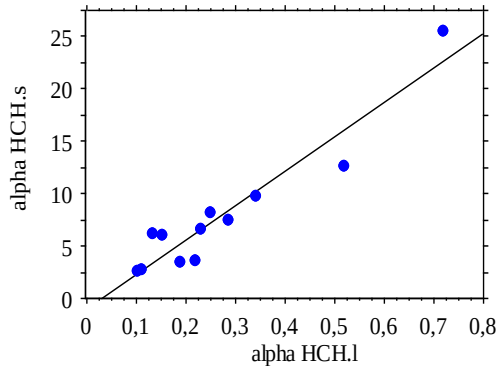

Figure a: Correlation between HCH concentrations in milk and sebum

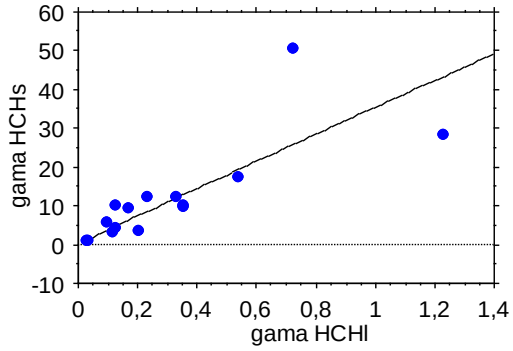

Figure b: Correlation between γ-HCH concentrations in milk and sebum

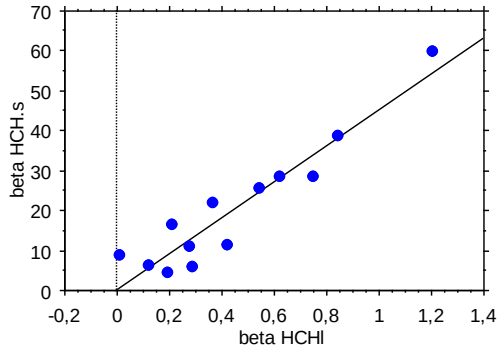

Figure c: Correlation between concentrations β- HCH of milk and sebum

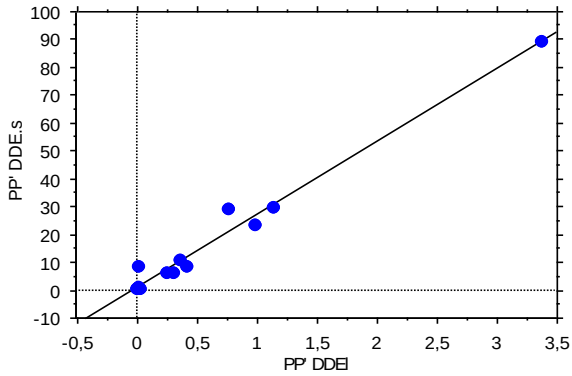

Figure d: Correlation between PP' DDE concentrations in milk and sebum

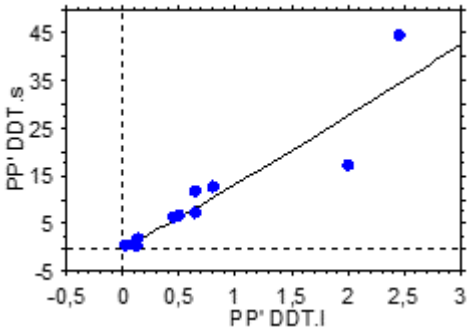

Figure e: Correlation between PP' DDT from milk and sebum

Figure :-Representation of correlations between pesticide residue concentrations measured in milk and sebum respectively

Concentrations are in mg/kg for milk (abscissa) and in µg/kg for sebum (ordinate)

Thus, the figure and Table 3 highlight the character of the strong global links between the determined concentrations of residues in facial sebum and those measured in breast milk. These positive and strong correlations are explained by the existence of a dynamic equilibrium between the concentrations of organochlorine pesticides stored in tissues

[6] and those circulating in biological fluids. According to the work of Robinson et al. [11], lipid-rich tissues act as reservoirs of lipophilic pesticides through physico-chemical interactions between cellular components and pesticides. According to Gulati et al. [12], the lipophilic nature of these pesticides allows them to cross cell membranes and accumulate in the body's fatty tissues. As needed, they are transported and distributed throughout the body by blood and lymph.

The results of the studies conducted here and elsewhere, presented in Table 3, show the same trends as before: qualitatively, the analysis of milk and sebum give similar results. From a quantitative point of view, the excretion of residues is more important in milk than in sebum. DDT is more mobilized than HCH in the "milk" matrix while sebum has the opposite.

The dynamic equilibrium appears to exist between the concentrations of HCH and DDT residues identified in sebum and milk respectively. It would therefore be possible, in view of all the above, to use facial sebum as a bioindicator matrix for monitoring human exposure to organochlorine pesticides. However, this technique will undoubtedly have limitations when assessing the contamination status of highly exposed populations, if the skin is a preferred route of penetration for the active ingredient sought, as in the case of arochlor 1016 [13,14].

Conclusion:-

The analysis of biological fluids (breast milk and facial sebum) collected from Ivorian populations in agricultural regions showed the exposure and contamination of populations by organochlorine pesticides. This is the case for DDT and its metabolites as well as HCH and its isomers which have been detected in both breast milk and sebum. The results obtained indicate that the concentrations of DDT and its metabolites on the one hand and HCH and its isomers on the other hand, are higher in milk than in sebum. The presence of these organochlorine compounds banned from use in Côte d'Ivoire is undoubtedly the consequence of the use of these active ingredients in agriculture and public health in the fight against disease vectors on the one hand and fraudulent use on the other hand because of their long-term efficacy and relatively low cost compared to the new molecules proposed. These results show a strong correlation between the determined concentrations of residues in facial sebum and those measured in breast milk. This study showed that in tropical environments, facial sebum can constitute, like human milk, a good indicator of the body burden of organochlorine pesticides.

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