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Persistent Organochlorine Pesticide Levels in Maternal Blood Serum, Colostrum, and Mature Milk

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The presence of persistent organochlorine pesticide residues in the human body has originated from their use in plant protection and their contemporary widespread use in tropical areas in sanitation to combat vector-transmitted diseases, such as malaria (WHO 1984). They receive the most attention because of their persistence in the environment, ability to concentrate up the food chain, continued detection in breast milk, and ability to be stored in adipose tissue of animals and humans. Human exposure to these pesticides occurs as a consequence of their persistence in a variety of environmental elements, such as air, soil, plants and food (Bailar 1995; Schweizer et al. 2000). Thus, analysis of their residues is of great importance to reflect the existing situation and these efforts will continue in the future (Hodgson et al. 1996).

For carrying out the monitoring surveys of environmental and food samples in order to establish human exposure to pesticide residue levels, a study of the accumulation properties and a determination of the amount of exposure is required (Ott 1985). The monitoring studies of human tissues and fluids provide measurements which express their links to the human food chain and the accumulative doses and the permanence in the tissues throughout the entire period (Lee et al. 1995). As a consequence of the persistence, the organochlorine pesticides were determined as ubiquitous in lipids and lipoprotein-rich tissues of human beings (Ludwicki et al. 1994; Burgaz et al. 1995; Gallelli et al. 1995; Gómez-Catalán et al. 1995). Accumulated in tissues of the organisms, they come into a steady state and circulate in the body according to the equilibrium factors (Rogan et al. 1986; Travis et al. 1988). Thus, analysis of lipid-rich tissues and fluids could reflect the extent of human exposure.

Breastfeeding plays a vital role in the development and well-being of infants. One potential obstacle is presented when organochlorine pesticide residues are mobilized with endogenous fat in milk production (Jensen et al. 1991; Sonawane 1996) and then incorporated into the nursing infant's body (LaKind et al. 2000). Although the composition of human milk and its immunological properties provide many benefits as a principal nutrient, a

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wide range of lipophilic compounds present in it can have potential adverse health effects on a nursing infant.
The principal source of human exposure in tropical countries, including Mexico, is inhaled vapors (Jury et al. 1982; Mathies et al. 1991) followed by the consumption of contaminated foods of animal origin, such as milk and meat (Waliszewski et al. 1996; 1997; 1998). The city of Veracruz and its surrounding areas are endemic for malaria. To combat the propagation of vectors, the zone has been treated with DDT at a rate of $2g/m^2$ since 1956.

The aim of this study was to determine the organochlorine pesticide levels and to establish the correlation between their concentrations in human fluids: maternal blood serum, colostrum and mature milk during lactation of mothers living in Veracruz, in areas where the organochlorine pesticides are applied for malaria or ectoparasite control, implicating the sanitary actions as a principal source of human exposure.

MATERIALS AND METHODS

One hundred randomly selected volunteer mothers among hospital patients admitted for delivery in the period 1998 – 1999 were chosen to participate. Each had lived at least three years in Veracruz or its suburban zone and had not indicated professional exposure to pesticides. The volunteers who signed their consent to participate in the study, revealed a mean age of 27.8 ± 5.3 years (median 27 years) and a parity of 2.0 ± 0.7 babies (median 2.0 babies). Maternal blood samples of approximately 10 ml, taken by venipuncture for routine clinical analysis, were collected from participants two weeks before the delivery. The serum was separated by centrifugation and, after the clinical analysis for organochlorine pesticides determinations, the sample constituted the remaining portion of blood serum. The colostrum samples on the 5^{th} day postpartum and the mature milk samples on the 30^{th} day postpartum were taken in the domiciles of participants. The donors were asked to manually express about 30 ml of breast milk to a chemically clean glass bottle.

Human milk and blood serum samples were analyzed according to previously described methods (Waliszewski et al. 1982; 1991). For clean-up of extracts, concentrated sulfuric acid was used to degrade the ubiquitous phthalate esters that interfere in gas chromatographic identification of organochlorine pesticides. The qualitative and quantitative determinations were done by gas chromatography on a Varian 3400 CX apparatus equipped with a ^{63}Ni electron capture detector. A volume of 1µl was injected in splitless mode into a SPB-608 30 m x 0.53 mm id. 0.5 µm film capillary column, using nitrogen as carrier gas with a 6.7 ml/min flow rate and the following temperature program: 140°C (3 min) to 250°C at 10°C/min, hold 10 min. The temperatures of the detector and injector were 320°C and 220°C, respectively.

All of the samples were analyzed for determination of the most persistent pesticides: HCB, β–HCH, pp'DDT and pp'DDE. The minimum detection limit expressed on fat basis for all organochlorine pesticides studied was 0.01 mg/kg. To determine the quality of the method, the recovery study was performed on ten overspiked replicates of a blank cow blood serum sample and a blank cow milk fat sample, which revealed contamination levels below the detection limit. The study, done at 0.03 mg/kg levels, showed mean values from 89% to 95%. The standard deviation and coefficient of variation were below 10, indicating excellent repeatability of the method.

Total serum lipids were determined colorimetrically with phosphovanillin according to the method recommended by Wiener Lab (Mexico) for clinical laboratories and milk fat content was determined gravimetrically.

The organochlorine pesticide residue mean values in blood serum, colostrum and mature milk were calculated by basic statistics. To compare variability among matrices studied, the Pearson correlation coefficients (r), squared Pearson correlation factor - coefficients of determination (r^2) and coefficients of regression (β) were calculated using the statistical software Minitab 12.

RESULTS AND DISCUSSION

The collected samples from 100 participants were studied to observe fluctuation of persistent organochlorine pesticide concentrations among maternal blood serum, excreted colostrum and mature milk. The results of determined levels expressed on fat basis (mg/kg) as means, standard deviations of means, geometric means and 95% of confidential intervals (CI) in maternal blood serum, colostrum and mature milk samples are presented in Table 1. Due to the prolonged use of DDT in Mexico in the combat of vector-transmitting diseases, pp'DDE forms the principal contaminant determined in analyzed samples that manifested widespread environmental contamination. The mean value of pp'DDE reached 3.78 mg/kg in maternal blood serum, 4.21 mg/kg in colostrum and 3.24 mg/kg in mature milk. The same tendency reveals pp'DDT content reaching 0.82 mg/kg in maternal blood serum, 0.83 mg/kg in colostrum and 0.58 mg/kg in mature milk. The DDT-total presented 4.50 mg/kg mean value in maternal blood serum, 4.96 mg/kg in colostrum and 3.79 mg/kg in mature milk. This phenomena can be explained by great lipophility of these pesticides due to their physical – chemical properties and inclination towards body-rich lipid fraction. Thus, the phenomena govern its behavior in the human body and bind to polyunsaturated fatty acids which are excreted in a greater quantity in colostrum than in mature milk (Harzer et al. 1986).

Table 1. Comparison of mean values (mg/kg on fat basis), standard deviations (SD), geometric means (GM) and confidential intervals (CI) of persistent organochlorine pesticides among maternal blood serum, colostrum and mature milk

Pesticide	Mean	SD	GM	95% CI
HCB serum	0.09	0.04	0.08	0.08 – 0.09
HCB colostrum	0.04	0.02	0.04	0.04 – 0.05
HCB mature milk	0.03	0.02	0.02	0.02 – 0.03
β-HCH serum	0.18	0.13	0.14	0.15 – 0.22
β-HCH colostrum	0.11	0.10	0.08	0.08 – 0.13
β-HCH mature milk	0.07	0.07	0.05	0.02 – 0.09
pp'DDE serum	3.78	2.69	2.77	3.11 – 5.45
pp'DDE colostrum	4.21	3.17	3.22	3.42 – 5.01
pp'DDE mature milk	3.24	2.78	2.35	2.55 – 3.94
pp'DDT serum	0.82	1.02	0.49	0.55 – 1.10
pp'DDT colostrum	0.83	0.98	0.53	0.57 – 1.09
pp'DDT mature milk	0.58	0.75	0.29	0.38 – 0.78
Σ-DDT serum	4.50	3.38	3.21	3.65 – 5.34
Σ-DDT colostrum	4.96	3.68	3.75	4.04 – 5.87
Σ-DDT mature milk	3.79	3.10	2.75	3.02 – 4.57

HCB indicates a level of 0.09 mg/kg in maternal blood serum, which decreases in colostrum to 0.04 mg/kg and to 0.03 mg/kg in mature milk. The same tendency is present in β-HCH with mean levels reaching 0.18 mg/kg in maternal blood serum, 0.11 mg/kg in colostrum and 0.07 mg/kg in mature milk.

Analyzing the geometric mean values of the determined pesticides, the obtained results indicate a similar tendency, such as arithmetic mean values, pointing out specific binding forces of these pesticides to lipoproteins, a factor that influences excretion patterns of organochlorine pesticides in colostrum and human mature milk. The assumption of equilibrium of persistent organochlorine pesticides in the maternal body during lactation involve their toxicokinetic processes and transport them from adipose tissue to blood serum and posteriorly to mammary glands. This equilibrium permits internal distribution of stored organochlorine pesticides, their transport and excretion through produced milk (Waliszewski et al. 1999). Due to the lipophilic properties of persistent organochlorine pesticides that largely reside in lipids of the human body, the equilibrium between adipose tissue and maternal blood serum (Waliszewski et al. 2000) and their rate of excretion can be defined as a factor of their concentration in serum and milk expressed on fat basis.

Table 2. Pearson correlation factors (r), squared Pearson correlation factors (r^2) and regression coefficients (β) calculated among serum, colostrum and mature milk.

Serum	Colostrum			mature milk		
	r	r^2	β	r	r^2	β
HCB	0.73	0.53	1.12	0.65	0.43	1.31
β-HCH	0.72	0.51	0.95	0.69	0.48	1.25
pp'DDE	0.87	0.76	0.74	0.84	0.70	0.81
pp'DDT	0.92	0.85	0.96	0.89	0.79	1.21
Σ-DDT	0.90	0.82	0.83	0.88	0.77	0.95

In order to compare the relationships of organochlorine pesticide concentrations in maternal blood serum, colostrum and mature milk, the results were paired and calculated using the Pearson correlation coefficient *(r)* and the squared Pearson correlation factor *(r^2)* (Table 2). Results express a great correlation of organochlorine pesticide contents in maternal blood serum, colostrum and mature milk. The serum – colostrum correlation factors are greater than 0.72 and for serum – mature milk they are 0.65 and above. Further diversity of results between paired sample groups depends principally on the physical-chemical properties of each pesticide and sample composition, which form a specific equilibrium between body compartments and excretion patterns (Haddad et al. 2000). Generally, the greater correlation can be observed between maternal

blood serum and colostrum with larger concentrations of DDTs excreted through colostrum.

The regression coefficient (β), which indicates the magnitude of correlation, expresses good agreement of all DDTs between maternal blood serum and colostrum and of DDE and Σ-DDT between serum and mature milk.

In conclusion, it can be pointed out that there still exists significant contamination and exposure to DDT and DDE in Veracruz inhabitants, expressed by elevated levels of these pesticides in samples studied. Breast milk offers infants unparalleled nutrition to support their growth and development, providing immune factors that increase their resistance to various infections and prevent risks of developing chronic diseases such as diabetes, allergies and asthma. The xenobiotics in adipose tissue, which were accumulated in body fat over the course of a person's lifetime are removed from exposed mothers to the blood serum and are excreted during lactation. Depending on the milk constitution and differences in chemical composition between colostrum and mature milk, both sample groups present specific differences in organochlorine pesticide concentrations. DDT's presence in human milk and posterior newborn exposure during lactation, can contribute to the health status of a developing infant (Barrett 2001). Thus, a program of breast milk monitoring is recommended to provide information to assess exposure and risks of nursing infants in areas where organochlorine pesticides have been used in sanitary actions.

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