

## **Comparison of Organochlorine Pesticide Levels in Adipose Tissue and Human Milk of Mothers Living in Veracruz, Mexico**

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In tropical and subtropical countries, persistent organochlorine pesticides, such as DDT and HCH, up to now have been found to be significantly useful in agriculture, especially in public health actions, in the combat of vector borne diseases in humans (WHO 1984). The use in large quantities and the remarkable biological persistence in the environment cause their widespread presence in all elements of the food chain. The remarkable biological persistence combined with great lipophilicity leads to accumulation of these residues in food chains and in human adipose fat (Burgaz et al. 1995; Gallelli et al. 1995; Gómez-Catalan et al. 1995; Ludwicki et al. 1994). These properties of organochlorine pesticides led to their restriction and prohibition in many countries and caused concern about health risks (Lopez-Carrillo et al. 1996; Rivero-Rodriguez et al. 1997). Since the 1980's, DDT, which was previously banned, is in use again being recommended by WHO as insecticide of choice in the prevention and control of transmission and spread of malaria and other vector transmitting diseases in tropical and subtropical countries (Najera et al. 1998; WHO 1984). In Mexico, DDT has been used exclusively for the purposes designed by WHO in the combat of malaria vectors (DGE SSA 1996).

In recent years, there has been renewed interest in breast feeding. Psychological and medical studies have underlined the great benefits to nursing infants such as increased immunological defenses and a healthier development of the baby. In parallel, with an increased interest in nursing, there is an increased concern about the excretion of drugs and environmental contaminants into human milk, since human milk is considered the most important route of elimination of persistent organochlorine pesticides deposited in a mother's body (Jensen and Slorach 1991; Sonawane 1995). The aim of this research was to compare the organochlorine pesticide levels in adipose tissue and human milk of mothers, residents of Veracruz and suburban areas, where DDT is sprayed by the Secretary of Health to combat vector transmitting diseases.

## MATERIALS AND METHODS

The human adipose tissue samples and human milk samples were obtained on the 30th day after delivery during the period of October 1997 to June 1998 from sixty volunteer mothers admitted to IMSS Hospital "Benito Coquet Lagunes" for caesarean delivery. The volunteers selected had lived a minimum of one year in Veracruz or its suburban zone. The samples were taken to pre-treated glass jars. Human adipose tissue was immediately frozen and kept at  $-25^{\circ}\text{C}$  until analyzed and the human milk samples were centrifuged at 3000 rpm to separate the fat which was immediately frozen. The samples were labeled according to donor and type of sample.

The analyses of organochlorine pesticide residues were performed according to the method of Waliszewski and Szymczynski (1982) and detailed by Waliszewski et al. (1996; 1998). The quantification of organochlorine pesticides was carried out on gas chromatograph, Varian Model 3400CX equipped with a  $^{63}\text{Ni}$  electron capture detector, according to the US EPA 608 Method. A  $1\mu\text{L}$  of extract was injected in splitless mode into a PTE-5 QTM 15 m x 0.53 mm id,  $0.5\mu\text{m}$  film capillary column, using nitrogen carrier gas with a 6.7 mL/min flow rate and the following temperature program:  $140^{\circ}\text{C}$  (3 min) to  $250^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$ , hold 10 min. The temperatures of the detector and injector were  $320^{\circ}\text{C}$  and  $220^{\circ}\text{C}$  respectively.

The following pesticides were determined in all of the samples: HCB,  $\alpha,\beta,\gamma$ -HCH, aldrin, heptachlor and their epoxide, pp'DDT, op'DDT, pp'DDE, pp'DDD,  $\alpha,\beta$ -endosulfans and endosulfan sulfate. The minimum detection limits on fat basis for the residues analyzed were 1 ng/g for HCB and HCH isomers, 2 ng/g for aldrin, heptachlor and their epoxide and pp'DDE, and 3 ng/g for pp'DDT, op'DDT, pp'DDD and endosulfans. To determine the quality of the analytical method, a recovery study was performed on a blank cow fat sample overspiked in ten replicates. The fortification study performed at 10-30 ng/g on fat basis levels, depending on the pesticide, showed mean values from 89% to 97% of recovery (except  $\alpha$ - and  $\beta$ -Endosulfans, caused by the partial conversion of  $\alpha$ -Endosulfan to  $\beta$ -Endosulfan under the influence of concentrated sulfuric acid during the clean-up step). The standard deviation (4.8 to 9.5) and the coefficient of variation (5.0 to 9.8) were below 10 indicating excellent repeatability of the method.

Differences among the organochlorine pesticide residue values in adipose tissue and mothers milk at 30th day post partum were examined using multi-way analysis of variance. The paired *t* test compares the variability between adipose tissues and mothers milk organochlorine pesticide contents indicating differences among both groups of samples. Moreover, the correlation coefficient "*r*" was calculated in adipose tissue and mother milk samples of all pesticides by simple linear regression (Minitab 12).

## RESULTS AND DISCUSSION

The results of organochlorine pesticide residues determined in adipose tissue and mother milk are expressed on fat basis (mg/kg) and presented in Table 1. Of the sixty analyzed samples of adipose tissue and mother milk neither Heptachlor, Heptachlor epoxide, Aldrin,  $\alpha,\beta$ -Endosulfans nor Endosulfan sulfate were detected at the level above the limit of detectability. Table 1 presents the frequency of positive samples of adipose tissue and mother milk with quantifiable amounts of residues, the means (X) of sixty analyzed samples and their standard deviations(SD).

The organochlorine pesticide residues detected in 100% of mother adipose tissue and mother milk were the most persistent pesticides HCB,  $\beta$ -HCH, pp'DDE and, in mother adipose tissue, pp'DDT. Quantifying the most ubiquitous pesticides, HCB reveals from all pesticides studied the lowest level which reached 0.058 mg/kg in adipose tissue and 0.025 mg/kg in mother milk followed by  $\beta$ -HCH at 0.143 mg/kg in adipose tissue and 0.061 mg/kg in mother milk. Higher levels were determined for pp'DDE at 4.355 mg/kg in adipose tissue and 3.997 mg/kg in mother milk, followed by pp'DDT in adipose tissue at 1.224 mg/kg. The pp'DDT in mother milk was determined in 90% of analyzed samples at a minor level of 0.651 mg/kg. The HCB and  $\beta$ -HCH reveal minor levels of contamination and a ubiquitous presence in the environment. The pp'DDE, a metabolite of pp'DDT, used by the Secretary of Health as insecticide of choice to combat vectors of malaria, reveals widespread presence in the Veracruz inhabitants reaching 4.355 mg/kg in the adipose tissue samples and 3.997 mg/kg in the mother milk. The frequency and levels of  $\alpha$ -HCH, op'DDT and pp'DDD indicate minor values, frequency and contamination.

In the production of milk, the human body employs approximately 75% of endogenous fats and the remainder proceed from the diet (Jensen 1989; Perez and Perez 1984). Comparing the quantities of the most persistent pesticides determined in both types of samples, the ratio of adipose tissue to mother milk, indicates values of: 43.1% for HCB, 42.7% for  $\beta$ -HCH, 91.8% for pp'DDE, and 53.2% for pp'DDT which do not correlate to the above-mentioned order of 75% of endogenous fat utilized by the human body in milk production. It seems, that the excretion order of persistent organochlorine pesticides from the human body has a major correlation to the chemical-physical property of each pesticide and to their specific affinity to human fat tissue and retention by the body. These differences are clearly shown in the variation of pesticide residue contents in adipose tissue and mothers milk. The observation is supported by the additional statistical evaluation presented in Table 2. The comparison of sixty mother adipose tissue and milk organochlorine pesticide levels was performed through paired *t* test, obtaining significant to extremely significant differences among all pesticides studied. The pairing and correlation coefficients calculated for both types of samples indicate the

Table 1. Comparison of organochlorine pesticide levels (mg/kg on fat basis) in adipose tissue and human milk from 60 mothers living in Veracruz.

Pesticide	Adipose tissue			Human milk		
	Frequency	X $\pm$ SD	Ranges	Frequency	X $\pm$ SD	Ranges
HCB	100 %	0.058 $\pm$ 0.029	0.010 - 0.189	100 %	0.025 $\pm$ 0.018	0.004 - 0.097
$\alpha$ -HCH	37 %	0.005 $\pm$ 0.010	0 - 0.047	20 %	0.001 $\pm$ 0.001	0 - 0.015
$\beta$ -HCH	100%	0.143 $\pm$ 0.113	0.019 - 0.652	100%	0.061 $\pm$ 0.044	0.003 - 0.181
$\gamma$ -HCH	63%	0.008 $\pm$ 0.012	0 - 0.068	32%	0.002 $\pm$ 0.003	0 - 0.013
$\Sigma$ -HCH		0.156 $\pm$ 0.118	0.021 - 0.652		0.063 $\pm$ 0.045	0.003 - 0.183
pp'DDE	100%	4.355 $\pm$ 3.463	0.305 -16.042	100%	3.997 $\pm$ 5.163	0.182 -34.281
pp'DDD	27 %	0.020 $\pm$ 0.048	0 - 0.246	5%	0.002 $\pm$ 0.010	0 - 0.049
op'DDT	88%	0.063 $\pm$ 0.070	0 - 0.286	27%	0.017 $\pm$ 0.038	0 - 0.198
pp'DDT	100%	1.224 $\pm$ 2.124	0.013 - 9.034	90%	0.651 $\pm$ 0.943	0 - 4.267
$\Sigma$ -DDT		5.661 $\pm$ 5.020	0.341 - 24.980		4.696 $\pm$ 5.871	0.324 -38.701

Table 2. Results of statistical analysis, paired *t* test, two-tailed *p* value and correlation coefficient (*r*) and pairing between mother adipose tissue and human milk at 30th day post partum.

Pesticide	Two-tailed <i>p</i> value of pairing	Correlation coefficient ( <i>r</i> ) of pairing
HCB	0.0030 very significant	0.3512 effective
$\alpha$ -HCH	0.0038 very significant	0.3409 effective
$\beta$ -HCH	0.0001 extremely significant	0.5885 effective
$\gamma$ -HCH	0.0438 significant	0.2224 not effective
$\Sigma$ -HCH	0.0001 extremely significant	0.5495 effective
pp'DDE	0.0001 extremely significant	0.8849 effective
pp'DDD	0.0001 extremely significant	0.7460 effective
op'DDT	0.0001 extremely significant	0.4950 effective
pp'DDT	0.0001 extremely significant	0.9469 effective
$\Sigma$ -DDT	0.0001 extremely significant	0.9088 effective

effective correlation of obtained results and high degree of coherence of these pesticides in the human body, indicating the specific equilibrium between accumulated pesticides, degree of exposure and excretion through human milk.

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