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Comparison of Organochlorine Pesticide Levels in Adipose Tissue and Blood Serum from Mothers Living in Veracruz, Mexico

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For decades, organochlorine pesticides have provided great benefits in agriculture for farm protection and in the combat of vectors that transmit serious diseases to humans. The resistance of organochlorine pesticides to environmental degradation has caused widespread contamination of the environment and their accumulation in all elements of food chains. The recognition of their persistence has led to restricted use in favor of less persistent alternative pesticides (PAHO 1995). The organochlorine pesticides being lipophilic are sequestered by lipid rich tissues in all organisms (Franke *et al.* 1994). From adipose tissue, they are distributed to all parts of the organism according to the equilibrium mediated by blood transport (Gómez-Catalán *et al.* 1991). The partitioning of organochlorine pesticides from adipose tissue to blood serum is related to the lipid content of the serum (Brown and Lawton 1984, Burse *et al.* 1989) because they are bound to lipids, phospholipids, albumin and macromolecular components of blood serum (Bach and Sela 1984, Maliwal and Guthrie 1981). Thus, the blood serum can constitute a good indicator of body burden, especially when the pesticide residue levels are expressed at the lipid base (Pfaffenberger *et at.* 1980, Mes 1992). For the monitoring surveys, human tissues in-vivo are difficult to access but the blood samples provide easy resolution.

MATERIALS AND METHODS

Adipose tissue and blood serum samples from sixty-four volunteer mothers were obtained during the period from October 1997 to June 1998, among patients admitted to IMSS Hospital "Benito Coquet Lagunes" for cesarean delivery. The volunteers were selected from those who lived a minimum of one year in Veracruz or its suburban zone. The blood samples, approximately 10 ml, were collected from fasting participants by venipuncture two days before the cesarean and the serum was separated by centrifugation. For organochlorine pesticides analysis, the sample constituted the remaining portion of blood serum taken for routine clinical analysis. The adipose tissue samples, approximately 5 g, were obtained from abdominal cavities during the cesarean before the suture.

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The adipose tissue and blood serum samples were analyzed according to previously described methods (Waliszewski and Szymczynski 1982, 1991). 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 1 µl was injected in splitless mode into a PTE-5 QTM 15 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: HCB, α,β,γ-HCH, aldrin, heptachlor, heptachlor epoxide, pp'DDT, op'DDT, pp'DDE, pp'DDD, α,β-endosulfans and endosulfan sulfate. Chlordane and its isomers, dieldrin and methoxychlor are degraded during clean-up of extracts with concentrated sulfuric acid. The minimum detection limits expressed on fat basis for the organochlorine pesticides studied were: 0.001 mg/kg for HCB and HCH isomers, 0.002 mg/kg for aldrin, heptachlor, heptachlor epoxide and pp'DDE, and 0.003 mg/kg for pp'DDT, op'DDT, pp'DDD and endosulfans. 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 fat sample, which revealed contamination levels below the detection limits. The fortification study done at 0.005 to 0.020 mg/kg levels, depending on the pesticide, showed mean values from 89% to 95% of recovery (except α– and β-endosulfans, caused by the partial conversion of a-endosulfan to P-endosulfan under the influence of concentrated sulfuric acid during the clean-up step). The standard deviation and coefficient of variation were below 10 indicating excellent repeatability of the method.

The total serum lipids were determined colorimetrically with phosphovanillin according to the method recommended by Wiener Lab for clinical laboratories.

Differences among the organochlorine pesticide residue values in blood serum and adipose tissue were examined using multi-way analysis of variance. The paired Pearson correlation coefficients *(r)* and linear regression were calculated between both samples by the statistical software Minitab 12.

RESULTS AND DISCUSSION

The results of gas chromatographic analysis of organochlorine pesticide residues in adipose tissue and blood serum expressed on fat basis (mg/kg) are presented in Table 1. In all of the analyzed samples neither heptachlor, heptachlor epoxide, aldrin, α,β-endosulfans nor endosulfan

Table 1. Comparison of organochlorine pesticide levels (mg/kg on fat basis) in adipose tissue and human blood serum from 64 mothers living in Veracruz.

Pesticide	Adipose tissue			Human blood serum			Pearson correlation coefficient
	Frequency	X ± SD	Ranges	Frequency	X ± SD	Ranges	
HCB	100 %	0.065 ± 0.052	0.010 - 0.401	100 %	0.183 ± 0.146	0.016 - 1.116	0.9698 effective
α-HCH	36 %	0.005 ± 0.009	0 - 0.047	16 %	0.007 ± 0.022	0 - 0.152	0.5142 effective
β-HCH	100%	0.160 ± 0.229	0.019 - 1.780	72%	0.205 ± 0.204	0 - 0.857	0.5635 effective
γ-HCH	63%	0.007 ± 0.010	0 - 0.068	25%	0.011 ± 0.022	0 - 0.084	0.3280 effective
Σ-HCH		0.173 ± 0.230	0.021 - 1.800		0.223 ± 0.218	0 - 0.857	0.5492 effective
pp'DDE	100%	4.550 ± 3.604	0.305 -16.042	100%	4.454 ± 3.951	0.242 –20.856	0.9249 effective
pp'DDD	27 %	0.020 ± 0.047	0 - 0.246	3%	0.007 ± 0.046	0 - 0.360	0.3899 effective
op'DDT	88%	0.061 ± 0.068	0 - 0.286	13%	0.038 ± 0.139	0 - 0.918	0.1887 not effective
pp'DDT	100%	1.209 ± 2.061	0.013 - 9.034	42%	0.731 ± 1.805	0 -11.264	0.7184 effective
Σ-DDT		5.851 ± 5.057	0.339 - 24.980		5.226 ± 5.521	0.242 -32.632	0.8974 effective

sulfate were detected above their respective detection limits. At the same time, Table 1 reveals the frequency of pesticide presence in the samples, the means (x) and their standard deviations (SD), ranges of determined levels and Pearson correlation coefficients.

The most ubiquitous pesticides determined in the samples studied were HCB, β-HCH, pp'DDE and pp'DDT showing 100% of presence in adipose tissue whereas in the blood serum samples the 100% frequency is revealed only by HCB and pp'DDE. The β-HCH indicates only 72% and pp'DDT only 42% frequency caused by the strongest affinity of these pesticides to human adipose tissue. On the other hand, comparing the frequency values of pesticides determined in adipose tissue and blood serum taken from the same mothers, it seems that the organochlorine pesticides are sequestered rapidly from the blood to the adipose tissue.

Table 1 reveals the greater concentrations of HCB and HCH isomers in blood serum compared to the adipose tissue that probably are caused by a greater affinity of these pesticides to lipoproteins presented in the blood serum and greater coefficient of partition between adipose fat and blood serum lipids. The DDTs determined reveal differences in their behavior caused by the shorter lifetime in the blood serum. Some DDTs determined in the adipose tissue of mothers that were recently exposed to them due to use by the Secretary of Health to combat mosquitos, reveal higher levels of pp'DDT and presence of pp'DDD, which can be determined exclusively as the result of the recent exposure to DDT vapors. The DDT metabolite disappears rapidly from the bloodstream being sequestered by a more lipophilic phase of adipose tissue. The phenomena of shorter lifetime of DDTs in blood serum observed during the study period was expressed by the minor levels of DDTs determined. This observation indicates the careful attention to the evaluation of DDT levels that can be correlated with diseases of any exposed person, particularly when the person lives in a zone where DDT is sprayed to combat vectors and where exposure to DDT vapors can easily occur. The metabolite pp'DDE reveals the same frequency and similar levels in the adipose tissue and blood serum of paired samples.

The pairing of 64 samples of adipose tissue and 64 samples of blood serum expressed on fat basis and provided by mothers living in Veracruz and its suburban zone indicates extremely significant results arising from two different sample groups and the Pearson linear regression coefficient *r* indicates the effective correlation of both sample groups that is in agreement with the previous observations of Mussalo-Rauhamaa (1991) Sasaki *et al.* (1991) and López-Carrillo *et al.* (1999). These results indicate great utility of blood serum samples in the evaluation of DDT contamination levels. However, they call special attention to the fact, that some DDTs remain for a shorter time in the bloodstream and are quickly sequestered by the adipose tissue which can lead to erroneous conclusions regarding the interpretation of the total body burden of persons recently exposed to DDT (Gómez-Catalán *et al.* 1991, Mes 1992, Archibeque-Engle *et al.* 1997).

In Table 2 the comparison of differences between arithmetic and geometric means derived from 64 samples of adipose tissue and blood serum can be appreciated, as well as the adipose tissue / blood serum ratio expressed on fat basis for HCB, b-HCH, DDE and DDT total. The ratio values, especially of geometric means, are different for each pesticide due to their different affinity to lipoproteins and to their octanol/water partitioning coefficients. The obtained values are in agreement with those obtained by Needham *et al.* 1990, Mussalo-Rauhamaa 1991, Kanja *et al.* 1992, Archibeque-Engle *et al.* 1997.

Table 2. Adipose tissue/serum HCB, β-HCH, DDE and DDT total ratios of 64 paired samples from mothers living in Veracruz

Pesticide	Arithmetic means Ranges			Geometric means Ranges		
	Adipose Tissue	Blood serum	Ratio IC95%	Adipose Tissue	Blood serum	Ratio IC95%
HCB	0.06 0.01-0.40	0.18 0.02-1.12	0.39 0.32-0.46	0.05 0.01-0.40	0.15 0.02-1.12	1.44 1.12-1.76
β-HCH	0.16 0.19-1.78	0.20 0 - 0.86				
DDE	4.55 0.30-16.0	4.45 0.24-20.85	1.10 1.05-1.11	3.19 0.30-16.00	2.90 0.24-20.90	0.90 0.78-1.20
DDT total	5.85 0.34-24.98	5.22 0.24-23.70	1.27 1.16-1.38	3.90 0.33-25.00	3.22 0.24-32.46	0.81 0.17-1.44

Table 3 reveals the normalized values of variance of geometric means obtained for HCB, DDE and DDT total in adipose tissue. The age factor indicates an increase of DDE and DDT total with age, which correlate with the previous observations of Ludwicki and Góralczyk 1994, Gallelli and Mangini 1994, Waliszewski *et al.* 1998. Moreover, the factor of parity indicates a slight decrease in DDE and DDT values, which is not statistically significant. The principal factor that influenced the rate of contamination of mothers living in Veracruz was determined be the place of residence. The suburban area, which is more intensively sprayed by the Secretary of Health to the combat of malaria vectors, reveals their adipose tissue deposits and is the more influential factor in the contamination of the Veracruz community (Waliszewski *et al.* 1998).

Table 3. Selected statistical characteristics in relation to geometric means of HCB, DDE and DDT total in adipose tissue of mothers living in Veracruz .

Variables	HCB		DDE		DDT		N
	Mean	CI95%	Mean	CI95%	Mean	CI95%	
Age (years)							
<26	0.06	0.05-0.08	2.40	1.55-3.70	3.29	1.97-5.42	23
26-30	0.05	0.04-0.06	3.10	2.10-4.60	3.60	2.39-5.42	22
>30	0.05	0.03-0.06	3.10	3.06-6.96	5.20	3.42-7.92	19
Parity							
1	0.04	0.03-0.06	3.45	1.88-6.36	3.90	2.16-7.06	11
2	0.05	0.05-0.07	3.06	2.22-4.22	3.90	2.74-5.54	40
>3	0.04	0.03-0.06	3.10	1.97-5.47	3.82	2.25-6.49	13
Residence							
Urbane	0.05	0.04-0.06	2.70	1.97-3.70	3.20	2.28-4.47	31
Suburban	0.06	0.05-0.08	3.71	2.60-5.31	4.67	3.20-6.82	33

It can be concluded from this study, that the evaluation of body burden with organochlorine pesticide done through blood serum determination on fat basis can serve as a rapid epidemiological method. However, a careful interpretation of these results is necessary in respect to the different equilibrium pattern of these pesticides among adipose tissue and blood serum caused by their characteristic affinity to fat, albumin and lipoproteins and their characteristic lifetime in the blood serum.

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