Bull. Environ. Contam. Toxicol. (2003) 70:920–926

DOI: 10.1007/s00128-003-0070-9

Bulletin of Environmental Contamination and Toxicology

Differences in Persistent Organochlorine Pesticides Concentration Between Breast Adipose Tissue and Blood Serum

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Received: 31 May 2002/Accepted: 1 February 2003

The persistent organochlorine pesticides, introduced to the human environment, produce desirable and undesirable effects (Pimentel 1996; Charlier and Plomteux 2002). DDT and Lindane (γ-hexachlorocyclohexane) used in tropical countries have provided great benefits to inhabitants, controlling the spread of vector-borne diseases and ectoparasites in livestock. Due to their persistence and lipophility, these compounds tend to accumulate in lipid-rich tissues of organisms (Travis et al. 1988). After entering the organism, the residues come into a steady state and bioconcentrate in the lipid-rich compartment of tissues according to an equilibrium pattern (Mussalo-Rauhamaa 1991; Waliszewski et al. 2001; 2002). The lipid-rich tissues, such as adipose tissue, act as depots or reservoirs of persistent chemical substances by virtue of physiochemical interactions of cellular components (Mussalo-Rauhamaa 1991). The analyses of human lipid-rich compartments, such as adipose tissue and blood serum lipids, reflect the rate of environmental exposure and provide data that permit the quantitative evaluation of organochlorine pesticide hazards associated with health risk for humans (Waliszewski et al. 2000).

Recently, discussions have concerned the link of organochlorine pesticides to breast cancer (Aronson et al. 2000; Safe 2000; Woolcott et al. 2001). To establish the role of these compounds in the development of breast cancer, the study was done analyzing human serum levels and the extent of their accumulation in the adipose tissue (Lopez-Carrillo et al 1997; Archibeque-Engle et al. 1997; Aronson et al. 2000). Human serum has been considered as an easier sample to obtain than adipose tissue in epidemiological studies. But the doubt has been raised, if the serum sample brings comparable results to those of breast adipose and if it should be the appropriate medium to establish the relationships between organochlorine pesticide accumulation status and breast cancer.

The purposes of the present study were to determine the levels of organochlorine pesticides in breast adipose tissue and blood serum

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expressed on fat basis and to compare these concentrations to examine variations that can lead to erroneous conclusions.

MATERIALS AND METHODS

In the study, from thirty randomly selected adult female cadavers, submitted to autopsy at the Institute of Forensic Medicine of the University of Veracruz, samples of breast adipose tissue, approximately 5 g, and blood from the ventricular heart cavity were taken. The adipose samples were stored in pre-treated glass jars, immediately frozen and kept at – 25ºC until analyzed. The blood samples (approximately 10 ml) were centrifuged to separate the serum from blood cells. Total serum lipid contents were determined. The rest of the serum was weighed to determine weight of sample and the total lipid content in the sample.

Breast 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. For pesticides separation, according to the US EPA Method 608, a fused silica column SPB 608 30 m X 0.32 mm ID, 0.5 µm film was used at the following temperature program: 193ºC (for 7 min) to 250ºC at 6ºC/min, hold 20 min. The carrier gas was nitrogen at 6.3 ml/min, and split/splitless sample injection of 1 µl was employed.

All samples were analyzed for: HCB, β-HCH, pp'DDE, op'DDT and pp'DDT. The minimum detection limits expressed on fat basis for the organochlorine pesticides studied were: 0.001 mg/kg for HCB and β-HCH, 0.002 mg/kg for pp'DDE, op'DDT and pp'DDT. To determine the quality of the method, the recovery study was performed on ten overspiked replicates of a blank cow blood sample and a blank cow fat sample, which revealed contamination levels below detection limits. The fortification study, done at 0.010 to 0.020 mg/kg levels, depending on the pesticide, showed mean values from 88 to 94% of recovery. The standard deviation and coefficients of variation were below 8, indicating excellent repeatability of the method. The concentrated sulfuric acid, used in the clean-up step of serum and adipose tissue extracts, permits quantitative fat precipitation and degrades the ubiquitous phthalate esters that interfere in the gas chromatographic identification of organochlorine pesticides (Waliszewski y Szymczynski 1990).

Total serum lipids were determined colorimetrically with phosphovanillin according to the method recommended by Wiener Lab for clinical laboratories (Anonymous 1996).

To compare variability between breast adipose tissue and blood serum, paired T-Test, the Pearson correlation coefficients (r), linear regression

coefficients (β) and analysis of covariance to determine tail probability were calculated using the statistical software Minitab version 12.

RESULTS AND DISCUSSION

The chemical equilibrium for deposition of persistent organochlorine pesticides in the human body considers the internal transport and equilibrium pattern between the pesticide concentrations in adipose tissue and blood serum. This model describes the internal distribution of lipophilic compounds, when this is expressed on fat basis (Parham et al. 1997). The equilibrium of contents between both compartments can be defined as chemical fugacities between adipose tissue and blood serum (Noren et al. 1999). The movement of persistent organochlorine pesticides across biological membranes occurs bidirectionally and the mode of membrane passage is applicable in both directions (Waliszewski et al. 2001). The liposolubility rate is a major factor, influenced by rate of accumulation and elimination from tissues and organs (Brown and Lawton 1984). Thus, it can be supposed that apparently equal concentrations exist in both compartments of the organism and the existing differences depend principally on lipid content of the tissues (Henriksen et al. 1998, Russell et al. 1999).

During the study, breast adipose tissue and blood from the ventricular cavity taken during autopsies were analyzed to determine the organochlorine pesticide residue concentrations in both paired samples. The results obtained expressed on fat basis (mg/kg) are presented in Table 1 as mean values (X), standard deviation of means (SD), standard error of means (SE Means) and 95% of confidence intervals (CI). The comparison of mean and standard deviation values for all organochlorine pesticides between both sample groups indicates the significantly highest values for serum lipids vs breast lipids (HCB 0.237 vs 0.058, β-HCH 0.710 vs 0.377, pp'DDE 3.196 vs 1.183, op'DDT 0.160 vs 0.078 and Σ-DDT 3.799 vs 1.883). The differences indicate the existence of real differences between both study samples. Only pp'DDT reveals 0.443 mg/kg in serum lipids compared to 0.619 mg/kg in breast lipids. The inverse levels of pp'DDT probably are due to the prolonged past use of DDT in the combat of vector-transmitting diseases that cause permanent exposure of the inhabitants, who inhaled the DDT volatilized from contaminated areas. The great differences between breast adipose and blood serum are expressed by standard deviation values, which approximate to the means and are significantly higher for β-HCH, op'DDT and pp'DDT in serum lipids group. The higher levels of pesticides determined in blood serum lipids indicate that these compounds incline to this body compartment and that the equilibrium pattern favor blood serum lipids. The analyses of standard error of mean (SE Mean) indicate the variability of observations and significantly higher values for serum lipids of all pesticides studied.

Moreover, this points out the major incompatibility of serum blood lipids to indicate breast adipose tissue accumulation.

Table 1. Mean (X), standard deviation (SD), standard error of mean (SE Mean) and 95% of confidential intervals (95% CI) of 30 breast adipose tissue and blood serum samples.

Pesticide	X ± SD	SE Mean	95% CI
HCB – breast lipids	0.058 ± 0.052	0.010	0.038, 0.078
HCB – serum lipids	0.237 ± 0.219	0.040	0.155, 0.319
β-HCH – breast lipids	0.377 ± 0.281	0.051	0.272, 0.482
β-HCH – serum lipids	0.710 ± 0.890	0.163	0.377, 1.042
pp'DDE – breast lipids	1.183 ± 0.540	0.099	0.981, 1.384
pp'DDE – serum lipids	3.196 ± 1.517	0.277	2.630, 3.763
op'DDT – breast lipids	0.078 ± 0.091	0.017	0.044, 0.112
op'DDT – serum lipids	0.160 ± 0.261	0.048	0.062, 0.257
pp'DDT – breast lipids	0.619 ± 0.410	0.075	0.466, 0.772
pp'DDT – serum lipids	0.443 ± 0.586	0.107	0.224, 0.662
Σ-DDT – breast lipids	1.883 ± 0.879	0.160	1.555, 2.211
Σ-DDT – serum lipids	3.799 ± 1.852	0.338	3.107, 4.490

The analyses of paired groups of breast and serum lipids (Table 2), show great diversity between paired compartments. The Pearson correlation coefficients (*r*) are low indicating a lack of correlation between these samples. The regression coefficients (β) that show the magnitude of correlation are low, also confirming the lack of correlation for organochlorine pesticide concentrations between breast and serum lipids. Table 2 presents tailed probability as a result of the one-factorial analysis of variance, indicating low levels and a lack of correlation between breast adipose tissue and blood serum lipid compartments. Thus, the results never come near values of 1.

Table 2. Pearson correlation coefficients (*r*), regression coefficient (β), and covariance levels among organochlorine pesticide levels in breast adipose tissue and serum lipids.

Pesticide	correlation coefficient (*r*)	regression coefficient (β)	covariance
HCB-breast and HCB serum	0.118	0.028	0.001
β-HCH breast and β-HCH serum	0.461	0.146	0.115
pp'DDE breast and pp'DDE serum	0.381	0.136	0.312
op'DDT breast and op'DDT serum	0.593	0.208	0.014
pp'DDT breast and pp'DDT serum	0.378	0.265	0.091
Σ-DDT breast and Σ-DDT serum	0.459	0.218	0.746

The results of statistical analyses done in Table 3, as paired T-Test to calculate differences between means, between breast adipose tissue and serum lipid levels of organochlorine pesticides, confirm the previous conclusion of no correlation in organochlorine pesticide concentrations between breast adipose tissue and serum lipids. The mean values and 95% of CI are negative or low indicating incompatibility of both sample groups. Moreover, F-test for equality of variances reveals higher levels for HCB, β-HCH, DDE and op'DDT than 1 and dissimilarity of variances for both sample groups. The significant results of F-test shows that the standard deviations of paired groups are not equal, concluding the existence of differences between means of organochlorine pesticide concentrations in breast lipids and serum lipids.

Table 3. Results of paired T-Test for analyses of differences, T-values mean of differences (X) and 95% CI of differences and F-test results of organochlorine pesticide levels in breast adipose tissue and serum lipids.

Paired T-Test	Differences		F-test
	X	95% CI	
HCB-breast and HCB serum	-0.179	-0.261, -0.097	1.886
β-HCH breast and β-HCH serum	-0.333	-0.632, -0.034	1.784
pp'DDE breast and pp'DDE serum	-2.013	-2.537, -1.489	2.994
op'DDT breast and op'DDT serum	-0.082	-0.163, +0.001	2.111
pp'DDT breast and pp'DDT serum	0.176	-0.039, +0.390	0.060
Σ-DDT breast and Σ-DDT serum	-1.916	-2.530, -1.301	0.001

In light of these results, it can be assumed that there is no correlation between concentrations of persistent organochlorine pesticides in breast adipose tissue and serum lipids. Therefore, the serum sample organochlorine pesticide concentrations make it impossible to draw an unequivocal conclusion related to the correlation between levels of these compounds in human blood serum and breast cancer, making blood sample a poor predictor (Archibeque-Engle et al. 1997, Güttes et al. 1998, Strucinski et al. 2000).

Acknowledgments. We thank CONACYT and SEP/FOMES for economic support of the study.

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