Bull. Environ. Contam. Toxicol. (1999) 62:397-402
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Bulletin of Environmental Contamination and Toxicology

Organochlorine Pesticide Residues in Human Blood Serum of Inhabitants of Veracruz, Mexico

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Received: 1 August 1998/Accepted: 16 February 1999

Since their introduction in agriculture and public health, organochlorine pesticides have provided greatest benefits to humans in the protection of agricultural production and harvests, and in the combat of vectors of born diseases in public health programs. The recognition of the persistence of organochlorine pesticides in the environment has led to their restricted use in favor of less persistent alternative pesticides. Although they have been restricted in industrialized communities, persistent organochlorine pesticides are still used in public health programs in tropical countries to control transmitting organisms of vector-borne diseases prevalent in these regions. Previously restricted, DDT reappeared in the 1980's, being recommended by WHO as the insecticide of choice in the combat of malaria vectors susceptible to DDT (WHO 1984). In Mexico, DDT has been exclusively used in the combat of malaria vectors (DGE SSA, 1996) and Lindane (γ-HCH) in agriculture for soil sanitation and as seed dresser, in livestock to combat ectoparasites in cattle and by the Secretary of Health to combat skin parasites in humans (CICOPLAFEST 1994). The previous monitoring study of human adipose tissue of Mexican residents who lived in areas where DDT has been sprayed by the Secretary of Health, revealed great contamination and exposure to their vapors during spraying and elevated DDE deposits in adipose tissue (Waliszewski et al. 1996).

Estimates of the total exposure to persistent organochlorine pesticides, and the body burden, are useful for epidemiological research and public health monitoring. While the human tissues are difficult to access, blood is one of the biological materials which can be conveniently obtained from human subjects for study of body burden following chronic exposure to organochlorine pesticides. The partitioning of organochlorine pesticides between adipose tissue and serum may be related to variations in the lipid content of the serum (Brown and Lawton 1984), since the organochlorine pesticides are associated with the lipids of blood serum (Bach and Sela 1984, Maliwal and Guthrie 1981). The aim of the present study was to determine the levels of organochlorine pesticides in blood serum of mothers living in Veracruz, admitted to IMSS Hospital for delivery.

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MATERIALS AND METHODS

Blood samples were obtained during the period of October 1997 to June 1998 from sixty-five volunteer mothers admitted to IMSS Hospital for delivery and who lived a minimum of one year in Veracruz or its suburban zone. The blood samples, approximately 10 mL, were collected by venipuncture 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 two days before the caesarean. The samples were analyzed using a method previously described (Waliszewski and Szymczynski 1991). A determined volume of blood serum was passed to a 25 mL tube with stopper and then acetic acid in a ratio of (1:1) was added. The sample with acetic acid was left for 30 minutes to hydrolize and liberate pesticides from complexes with endogenous substances of the blood. Thereafter, organochlorine pesticide residues were extracted three times with 10 mL portions of a mixture of petroleum ether and acetone (9:1). The extracts were collected in a 100 mL separatory funnel and washed twice with 25 mL portions of distilled water to remove traces of acetic acid, acetone and water soluble substances. The organic phase was dried by passing it through a 5 g. sodium sulfate layer. The sodium sulfate was then rinsed with petroleum ether and both extract and rinses were rotary evaporated to about 10 mL. The concentrate was transfered quantitatively with petroleum ether to a glass tube with stopper and 1 mL of concentrated sulfuric acid was added. The contents were vigorously shaken for about one minute and left for about three minutes to ensure good phase separation. The petroleum ether phase was taken out with a pipette and dried by passing it through a sodium sulfate layer to a 50 mL roundbottomed flask. The sodium sulfate was rinsed several times with petroleum ether. Extract with rinses were rotary evaporated to a few drops. The concentrate was transfered quantitatively with petroleum ether to a 1 mL calibrated tube and the final volume was adjusted to 1.0 mL for qualitative and quantitative determinations with ECD gas chromatography.

A Varian 3400 CX gas chromatograph equipped with a ^{63}Ni electron capture detector was used for the chromatographic determinations. A volume of 1 µL was injected in splitless mode into a PTE-5 QTM 15 m x 0.53 mm i.d., 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.

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-Endosulfan and Endosulfan sulfate. The minimum detection limits for the residues analyzed were: 0.1 $ng \cdot mL^{-1}$ for HCH isomers, 0.2 $ng \cdot mL^{-1}$ for Aldrin, Heptachlor, Heptachlor epoxide and pp'DDE, and 0.3 $ng\,mL^{-1}$ for pp'DDT

and op'DDT, pp'DDD and Endosulfans. To determine the quality of the method, a recovery study was performed on ten overspiked replicates of a blank cow blood serum sample, which presented contamination levels below the detection limits. The fortification study, done at 0.5 to 2.0 ng/mL levels, depending on the pesticide, showed mean values from 89% to 95% of recovery (except a and β-Endosulfan, caused by the partial conversion of α-Endosulfan to β-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.

Differences among the organochlorine pesticide residue values in blood serum were examined using multi-way analysis of variance. The ANOVA test determines the significance of categorical factors on pesticide levels after removing any differences caused by the continuous variables. The Student-Newman-Keuls Test was used to determine the difference among means (Statistica 5).

RESULTS AND DISCUSSION

The frequency, mean levels, standard deviations and ranges (ng/mL) of organochlorine pesticide residues detected in the blood serum samples are presented in Table 1. HCB was found as a ubiquitous contaminant detected in 100% of analyzed samples, although at a low 1.1 ng/mL mean level. It was followed by pp'DDE determined in 98% of samples, a concentration which dominated all other organochlorine pesticides, with a mean level of 14.5 ng/mL. A third pesticide of importance was β–HCH which revealed a frequency of 71% and a mean level of 1.4 ng/mL.

Table 1. Frequency (%), means (x) concentrations (ng/mL), standard deviation (SD) and ranges of organochlorine pesticides detected in 65 human blood serum samples of mothers living in Veracruz, Mexico

PESTICIDE	FRECUENCY (%)	x ± SD	RANGES(ng/mL)
HCB	100	1.1 ± 0.8	0.1 - 3.2
α–HCH	16	0.1 ± 0.2	0 - 1.1
β–HCH	71	1.4 ± 1.8	0 -100.0
γ–HCH	30	0.2 ± 0.6	0 - 4.0
Σ–HCH		1.6 ± 2.0	0 - 10.0
pp'DDE	98	14.5 ± 28.0	0 -190.4
pp'DDD	8	0.3 ± 0.2	0 - 0.9
op'DDT	16	1.1 ± 4.0	0 - 2.3
pp'DDT	41	1.2 ± 3.8	0 - 20.9
Σ–DDT		16.4 ± 30.8	0.7 -212.3

Table 2. Comparison of organochlorine pesticide residue levels (ng/mL) in blood serum samples of different countries.

Pesticide	Honduras 1989 (1)	Kwazulu 1991 (2)	Pakistan 1989 (3)	Kenya 1992 (4)	Nicaragua 1993 (5)	Brazil 1996 (6)	India 1996 (7)	This study Mexico 1998
HCB	0.3	-	-	-	-	-	-	1.0
α–HCH	-	-	0.08	-	-	0.3	6.32	0.1
γ–HCH	-	-	0.29	-	-	0.2	1.75	0.2
β–HCH	0.4	-	1.39	0.67	0.34	3.4	13.44	1.4
Σ–HCH	-	-	-	6.6	-	3.7	21.50	1.6
pp'DDE	42.5	103.4	8.6	-	-	14.3	16.13	14.5
pp'DDD	-	0.2	-	-	-	-	-	0.3
op'DDT	-	-	-	0.8	-	-	-	1.0
pp'DDT	2.7	37.3	0.61	4.7	-	1.5	-	1.8
Σ–DDT	-	140.9	-	12.4	12.46	16.1	20.79	16.4

1. Steinberg KK et al 1989; 2 .Bouwman H et al 1991; 3 Krawinkel MB et al 1989; 4 Kanja LW et al 1992; 5 Rugama R et al 1993; 6 Minelli EV et al 1996; 7 Dua VK et al 1996

The remaining pesticides listed in Table 1 present lower levels and frequencies in the analyzed blood serum samples. Neither Heptachlor, Heptachlor epoxide, Aldrin, α,β-Endosulfans nor Endosulfan sulfate were detected in any of the analyzed samples.

The concentration of organochlorine pesticide residues in blood serum samples make it possible to show the body burden with these substances in the population studied as well as to compare contamination of various populations exposed to these pesticides. Since lipoproteins have been principally implicated in the transport of organochlorine pesticides in human blood, the contamination levels reveal the mobility within adipose tissue where these pesticides were formerly stored.

The comparison of results obtained from monitoring studies of organochlorine pesticides in human blood serum in other countries, where contamination similar to that of Mexico has been expressed, is presented in Table 2. The results reveal DDT as a principal contaminant in monitoring surveys in India, Brazil, Nicaragua and Kenya, at levels comparable to those found in Veracruz, Mexico, where DDT is still used in sanitary actions to combat vector transmitting diseases. Furthermore, the contamination levels of DDT found in Pakistan and Kwazulu, where DDT has been similarly sprayed by the Secretary of Health, were approximately 10 times higher than that determined in Veracruz.

The obtained results confirm the contamination of Veracruz inhabitants as a consequence of DDT use in public health programs. The study showed the need to carry out permanent monitoring surveys for organochlorine pesticide residues in human blood serum in order to treat human exposure after insecticide spraying campaigns and to prevent potential risk to human health because of accumulating properties of organochlorine pesticides in human fat tissue.

Acknowledgments: we thank CONACYT Project 4238PM for economical support of this study.

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