Bull. Environ. Contam. Toxicol. (1996) 57:22-28

Bulletin of Environmental Contamination and Toxicology

Organochlorine Pesticide Residues in Human Breast Milk from Tropical Areas in Mexico

S. M. Waliszewski,[1] V. T. Pardio Sedas[2] J. N. Chantiri P.,[1] R. M. Infanzon R.,[1] J. Rivera[1]

[1]Instituto de Medicina Forense, Universidad Veracruzana Framboyanes, Esq. Reyes Heroles, 91950 Veracruz, Ver., Mexico
[2]Laboratorio de Control de Agua y Alimentos, Secretaria de Salud, Edificio F Hospital General de Veracruz, Avenida 20 de Noviembre 1074, 91700 Veracruz, Ver., Mexico

Received: 15 August 1995/Accepted: 10 February 1996

The use of persistent organochlorine pesticides in tropical areas has particular implication regarding environmental quality and human health. This is caused by the specific conditions of high temperature, high humidity and the socioeconomic status of inhabitants. Because humans are at the top of the food chain, obviously human tissues may contain relatively high amounts of such bioaccumulating chemicals.

The concentration of the organochlorine pesticide residues in human milk has been the subject of many studies (Bouwman et al. 1990: Skaare et al. 1990; Alawi et al. 1992; Hernandez et al. 1993; Somogyi and Beck 1993) due to its importance as the first food for the newborn child. Although food intake is the main route of exposure, inhalator-y and dermal routes might have importance for mothers living in countries where sanitary actions are the main source of contamination. The concentration of DDT, which is the principal pollutant of breast milk in these areas, has caused concern about the health risk to breastfeed infants (Wolf 1983; Sant'Ana et al. 1989). Attention must be drawn to the fact that in countries lacking strict bans on the sprayed applications of the organochlorine insecticides, residue levels of these chemicals may be present in human milk at considerably high levels.

The aim of this study was to determine the residue levels of 14 organochlorine pesticides in human milk samples from the state of Veracruz as an indicator of human body burden in tropical areas of Mexico and to assess if the residue level findings in human milk exceed the limits recommended by FAO/WHO.

MATERIALS AND METHODS

A total of 43 mothers (17 to 41 years of age, mean 28.7 years) participated in the study voluntarily and agreed with its aim. Mothers had lived in the Veracruz area during the preceding 3 years. The 43 human milk samples were taken from healthy donors and randomly collected over a period of 6 months from November 1994 to March 1995 on the 15th day postpartum. The donors were asked to manually express about 30 mL of breast milk to a chemically clean glass bottle, labeled regarding

Correspondence to: S. M. Waliszewski

the day and month of sampling and kept frozen at -20°C until analyzed. Each mother completed a questionnaire to provide personal information such as age, number of previous births and place of residence.

The following reagents -petroleum ether (b.p.40-50°C), acetone, sulfuric acid of analytical grade, anhydrous sodium sulfate (heated overnight at 650°C)- were purchased from J.T.Baker. Before analysis reagents were tested for impurities by gas chromatography. Analytical standards were purchased from Supelco, Inc. The glassware was washed with chromic mixture, rinsed with distilled water and then with distilled acetone and petroleum ether to prevent contamination of analyzed samples and to make it suitable for pesticide residues analysis.

The analyses were carried out on a gas liquid chromatograph Varian model 3300 equipped with a ^{63}Ni electron capture detector and a Varian model 4400 integrator. For pesticide separation according to the US EPA Method 608 a glass column 200cm x 2mm id. packed with 1.5% SP-2250 + 1.95% SP2401 on 100/200 mesh Supelcoport was employed. Operating conditions were as follows: nitrogen carrier gas at 20 mL/min: temperatures: column 200°C, injector 250°C, detector 300°C and 1µL direct injection volume. A fused silica column PTE-5 QTM 15m x 0.53mm id., 0.5µm fillm was used for confirmation of the presence of pesticides at the following temperature program: 140°C (for 3 min) to 250°C at 10°C/min, held 10 min. Carrier gas was helium at 6.7 mL/min, and direct sample injection of 1 µL was employed.

The stored human milk samples were left to defrost and then centrifugated at 3000 rpm to separate the fat from milk. The fat layer was removed from the sample, transferred to a mortar and ground with a sufficient amount of anhydrous sodium sulfate to obtain a coarse powder. The sample was then transferred to a chromatographic column of 1 cm id. and 50 cm length and the organochlorine pesticide residues were extracted with 150 mL of petroleum ether. The eluate was concentrated by a rotary evaporator to approximately 30 mL. Ten milliliters of the concentrated extract were transferred into a previously weighed round-bottomed flask of 50 mL and the solvent was rotary evaporated to determine the fat content gravimetrically. Concentrated extract, containing a maximum of 500 mg of fat, was transferred into a 10 mL tube (with a glass stopper) and 1 mL of concentrated sulfuric acid was added. The tube was tightly stopped and vigorously shaken for 30 seconds. The content was left to reach a good phase separation and then the supernatant was dried by passing it through a 3 to 5 g layer of sodium sulfate and washed with petroleum ether. The ether exctract, with rinses, was rotary evaporated to a few drops and quantitatively transferred to a 1 mL volumetric tube. The volume was adjusted with petroleum ether to 1 mL and 1µL of aliquot was injected for gas chromatographic analysis. All samples were analyzed in duplicate and results represent the aritmetic mean.

To determine the quality of the method, the recovery study as performed on ten replicate overspiked samples of uncontaminated cow milk fat. The

fortification levels, recovery mean values, standard deviations and detection limits are presented in Table 1. The mean values ranged from 91% to 99% of recovery and the standard deviations were below 10 indicating excellent repeatability of this method. Significant differences among the recovery values of α– and β– endosulfane resulted from the partial conversion of β– endosulfane to a- endosulfane under the influence of concentrated sulfuric acid during the clean-up step. Fortification study was not performed for dieldrin, endrin and methoxichlor because these pesticides are destroyed during sample treatment by the action of concentrated sulfuric acid.

Table 1. Fortification levels, mean and standard deviation and detection limits (mg/kg fat weight) from fortification study.

PESTICIDE	FORTIFICATION LEVELS	X ± S D	DETECTION LIMITS
HCB	0.010	95.8 ± 5.4	0.001
α-HCH	0.010	96.9 ± 7.0	0.001
ß-HCH	0.020	91.0 ± 8.9	0.002
γ-HCH	0.020	99.1 ± 9.9	0.002
aldrin	0.020	90.1 ± 7.2	0.002
heptachlor	0.020	90.8 ± 7.8	0.002
heptachlor-epoxide	0.020	91.4 ± 6.9	0.002
p,p'-DDE	0.020	96.9 ± 5.1	0.002
o,p'-DDT	0.040	97.1 ± 5.0	0.003
p,p'-DDD	0.030	94.3 ± 5.9	0.003
p,p'-DDT	0.030	95.7 ± 4.9	0.003
α–endosulfane	0.030	132.8 ± 9.1	0.003
ß-endosulfane	0.030	71.3 ± 9.6	0.003
endosulfane-sulfate	0.030	94.3 ± 5.9	0.003

RESULTS AND DISCUSSION

Frequency of positive samples, mean of obtained values and ranges are listed in Table 2. The pesticide residue levels of breast milk samples are reported on fat basis as it has been considered the most appropiate manner to express contamination with these residues, due to the effects of variations in lipid levels during lactation (Noren 1983). Table 2 shows that HCB, β-HCH, p,p'-DDE and p,p'-DDT were found in 100% of samples analyzed and 96% contained o,p'-DDT. The p,p'-DDD was present in 9% of the samples. Both isomers α– and γ–HCH were determined in lower frequency of 40% and 52% respectively. Within the detection limits no sample was found to contain aldrin, heptachlor and its epoxide and endosulfanes. p,p'-DDE was the main contributor to ΣDDTs detected in human milk samples with a mean value of 5.017 mg/kg fat weight and a maximum level of 12.833 mg/kg fat weight. The p,p'-DDE

isomer was followed by p,p'-DDT with a mean value of 1.271 mg/kg fat weight and a maximum level of 13.926 mg/kg fat weight, indicating direct exposure to DDT vapor used in sanitary actions. Among isomers of HCH, the highest residue correspond to the most persistent and lipophilic β–HCH that reached a mean value of 0.561 mg/kg fat weight and a maximum level of 1.661 mg/kg fat weight. Differences in persistence and bioaccumulation potential between isomers change the ratio of different HCH isomers from the start of food chain until the excretion in human milk; with a five times slower rate of elimination in comparison to the γ–HCH, β-HCH has been considered as the predominant and persistent isomer in human milk (Johansen et al. 1994). Other HCH isomers presented considerably lower levels approximated to hundredth parts of mg/kg. Similar levels of HCB were found in 100% milk samples despite the fact that it has been restricted for agricultural uses. This pesticide is recognized as a by-product of some industrial chlorination process, and probably the main source of human and environmental contamination.

Table 2. Organochlorine pesticide residues (mg/kg fat weight) in human milk samples.

PESTICIDE	FREQUENCY	MEAN	RANGES
HCB	100%	0.047	0.005 - 0.123
α-HCH	40%	0.018	0.002 - 0.045
ß-HCH	100%	0.561	0.140 - 1.661
γ-HCH	52%	0.022	0.001 - 0.082
Σ-HCH		0.579	0.141 - 1.661
aldrin	not detected		
heptachlor	not detected		
heptachlor-epoxide	not detected		
p,p'-DDE	100%	5.017	0.884 - 12.833
o,p'-DDT	96%	0.265	0.025 - 1.472
p,p'-DDD	9%	0.107	0.011 - 0.364
p,p'-DDT	100%	1.271	0.133 - 13.926
Σ-DDT		6.440	0.990 - 26.872
α–endosulfane	not detected		
ß-endosulfane	not detected		
endosulfane-sulfate	not detected		

Table 3 presents the mean values of pesticide contamination according to parities. In the primagravidas group the Σ-DDT level reached 9.473 mg/kg fat weight and diminished with a subsequent child to 4.531 mg/kg fat weight and up to 3.927 mg/kg fat weight in mothers with a third breastfed child. A significant difference was found at $p<0.05$ between Σ-DDT mean levels of the number of children.

Regression analysis showed a significant influence of parity on DDE and

Σ-DDT ($p<0.0001$). This supports the theory that the total body load of organochlorines via the breast milk are shed with each successive pregnancy and lactation. During lactation, fat mobilization could take place from adipose tissues within which the organochlorine pesticides are stored, followed by excretion with the milk (Rogan et al. 1986; Skaare et al.1 990; Spicer and Kereu 1993; Weisenberg et al. 1985). This pattern was not observed among HCH isomers and HCB.

Table 3. Mean levels of organochlorine pesticides (mg/kg fat weight) in human milk samples according to number of parities.

	NUMBER OF PARITIES		
PESTICIDE	ONE	TWO	THREE
HCB	0.030	0.083	0.055
α-HCH	0.013	n.d.	0.019
ß-HCH	0.640	0.551	0.445
γ-HCH	0.014	0.027	0.029
Σ-HCH	0.649	0.566	0.480
p,p'-DDE	7.166	3.639	2.821
o,p'-DDT	0.297	0.164	0.289
p,p'-DDD	0.122	0.045	0.118
p,p'-DDT	1.909	0.731	0.732
Σ-DDT	9.473	4.531	3.927

n.d. = not detected

Table 4. Levels of organochloine pesticides (mg/kg fat weight) in human milk according to place of residence.

PESTICIDE	VERACRUZ-URBAN	VERACRUZ-SUBURBAN
HCB	0.065	0.021
α-HCH	0.018	n.d.
ß-HCH	0.387	0.805
γ-HCH	0.025	0.006
Σ-HCH	0.387	0.806
p,p'-DDE	2.709	8.253
o,p'-DDT	0.109	0.470
p,p'-DDD	0.061	0.159
p,p'-DDT	0.422	2.460
Σ-DDT	2.963	11.301

n.d. = not detected

Table 4 shows the pesticide residue levels in breast milk samples obtained from mothers living in urban and suburban areas of Veracruz.

The group denominated “Veracruz-suburban” refers to those mothers living in the surrounding areas of the city, which are characterized by swamps. In these areas DDT is extensively used as an insecticide of choice against rural endemic vector disease. The DDT vapors of sprayed preparations are inhaled by the mothers causing extensive exposure, uptake and eventual elimination via breast milk. Significant differences were found in Σ-HCH ($p<0.05$) and Σ-DDT ($p<0.001$) mean levels in human milk originating from urban and suburban areas of residence of Veracruz. DDT compounds were more abundant in milk samples collected from suburban areas compared with those from urban areas.

Mean values of the pesticides detected in analyzed human milk samples showed differences in contamination levels between both groups. In tropical areas, the area of residence is one of the most important factors for exposure assessment. Other factors such as maternal age, diet, and smoking habits have a major influence in those countries where the source of organochlorine pesticide residues exists only at residual levels, due to the lack of a direct exposure source such as vapors (Duarte-Davidson et al., 1994). Due to insignificant contributions in explaining the levels of contamination, the factors mentioned above were not evaluated in our study, since the only parameter influencing and outweighing these pesticide levels of milk from mothers living in areas where vector disease control programs were applied, is the source of DDT, which is still used by public health authorities on a large scale.

FA0 and WHO (1985) have set the acceptable daily intake (ADI) at 20 µg/kg body weight/day for Σ-DDT, 10µg/kg body weight/day for γ-HCH and 0.1 µg/kg body weight/day for HCB. Assuming an infant with a mean body weight of 5 kg, a mean milk fat content approximately of 3.5%, a mean milk daily intake of 0.8 L, a Σ-DDT mean level of the whole group of 6.440 mg/kg milk fat weight, the estimated daily Σ-DDT intake for the infants (whole group) via breast milk is 36.06 µg/kg body weight/day, the dalily γ-HCH intake is 0.12 µg/kg body weight/day and the daily HCB intake for the same group is 0.26 µg/kg body weight/day. The exposure to the children is estimated to exceed the ADI of Σ-DDT by 2 times and the estimated daily intake of HCB also exceeds the ADI by 2.6 times whereas the daily intake of γ-HCH is below the ADI.

The higher mean levels of DDE found in this study, which depend on both external uptake and internal metabolism from DDT in the maternal body, must be considered as a possible health risk to the infant. The risk must be balanced against the benefits of the breastfeeding, especially in developing countries. For this reason it would be appropriate to continue monitoring breast milk samples and the extent of human exposure to organochlorine pesticide residues to see if a downward trend is achieved. If higher contamination tendency is detected these pesticides should be temporarily banned and substituted for less toxic insecticides by the Health Bureau.

REFERENCES

Alawi MA, Ammari N, Al-Shuraiki Y (1992) Organochlorine pesticide contaminations in human milk samples from women living in Amman, Jordan. Arch Envrion Contam Toxicol 23:235-239.

Bouwman H, Reinecke AJ, Cooppan RM (1990) Factors affecting levels of DDT and metabolites in human breast milk from Kwazulu. J Toxicol Environ Hlth 31:93-115.

Duarte-Davidson R, Wilson SC, Jones KC (1994) PCBs and other organochlorines in human tissue samples from the Weilsh population: II Milk. Environ Poll 84:79-87.

FAO/WHO (1985) Joint FAO/WHO meeting on pesticide residues. Pesticide residues in food 1984: Teport Paper 62. FA0 Plant Production and Protection. 24 September-3 October.

Hernandez LM, Fernandez MA, Hoyas E. Gonzalez MJ, Garcia JF (1993) Organochlorine insecticide and polychlorinated biphenyl residues in human breast milk in Madrid (Spain). Bull Environ Contam Toxicol 50:308-315.

Johansen HR, Becher G, Polder A, Skaare JU (1994). Congener-specific determination of polychlorinated biphenyls and organochlorine pesticides in human milk from Norwegian mother living in Oslo. J Toxicol Environ Hlth 42: 157-171.

Norén K (1983) Some aspects of the determination of organochlorine contaminants in human milk. Arch Environ Contam Toxicol 12:277-283.

Rogan EJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tingelstan K, Tully M (1986) Polychlorinated biphenyls (PCBs) and dichloridiphenyl dichloroethane (DDE) in human milk: Effects of maternal factors and previous lactation. Am J Publ Hlth 76:172-177.

Sant'Ana LS, Vassilieff I, Jokl L (1989) Levels of organochlorine insecticides in milk of mothers from urban and rural areas of Botucatu, SP, Brazil. Arch Environ Contam Toxicol 42:911-918.

Skaare JU, Polder A (1990) Polychlorinated biphenyls and organochlorine pesticides in milk of norwegian women during lactation. Arch Environ Contam Toxicol 19:640-645.

Somogyi A, Beck H (1993) Nurturing and breast-feeding; exposure to chemicals in breast milk. Environ Hlth Perspect Suppl 101(suppl 2):45-52.

Spicer PE, Kereu RK (1993) Organochlorine insecticide residues in human breast milk: A survey of lactating mothers from a remote area in Papua New Guinea. Bull Environ Contam Toxicol 50:540-546.

Weisenber E, Arad I, Sahm Z (1985) Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch Environ Contam Toxicol 14:517-521.

Wolf MS (1983) Occupationally derived chemicals in breast milk. Australian J lndust Med 4:259-281.