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# DDT and Its Metabolites in Human Milk Collected in Veracruz City and Suburban Areas (Mexico)

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Since 1990, Mexico has used approximately 3000 tons of DDT per year in anti-malaria control programs, mainly in tropic areas (PAHO 1995). This organochlorine pesticide has been used in the malaria-endemic areas of Veracruz, Mexico, thus, for this purpose a spraying regime has been followed in urban and suburban areas of Veracruz City by applying DDT every six months on indoor surfaces and dwellings at a coverage of $2 g/m^2$ (DGE SSA 1996).

Due to their lipophilic properties, DDT and its metabolites are primarily stored in fat-rich tissues and subsequently translocated and excreted through milk fat. Nursed babies are exposed to DDT through the fat in human milk, fact that has caused concern about the health risk to breastfed infants since a significant increase in the number of infants showing hiporeflexia, associated with an increase in the DDE concentration in breast milk, has been reported. These effects became apparent at DDE levels of $\geq 4$ mg/kg in milk fat (Rogan et al. 1986). Nevertheless, Mexican mothers are still positively advised as to breast-feeding by a nationwide program.

The aim of this study was to determine the residue levels of DDT and its metabolites in human milk samples from Veracruz City, suburban and rural areas and to estimate if the residue level findings in human milk exceed the limits recommended by FAO/WHO.

## MATERIALS AND METHODS

A total of 300 (13 to 43 years of age, mean 23.1 ± 5.4 years) participated in the study voluntarily and agreed with its aim. Mothers had lived in the Veracruz area during the preceding one year at least and gave birth at the General Hospital of Veracruz City. The 300 human milk samples were taken from healthy donors and randomly collected over a period of 8 months from August 1996 to March 1997 on the first two months postpartum. The donors were asked to express by a breast pump about

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30-100 mL of breast milk to a chemically clean glass bottle, labeled regarding the day and month of sampling and kept frozen at -20°C until analyzed. Each mother comp leted a questionnaire to provide personal information such as age 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 (Phillipsburg, NJ 08865 USA). 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 (US EPA 1982) a fused silica capillary column 30m x 0.53mm id. and 0.5 mm film was used at the following temperature program: 140°C (for 3 min) to 250°C at 10°C/min, held 10min. Carrier gas was nitrogen at 6.7 mL/min, and direct sample injection of 1µL was employed. The stored human milk samples were left to defrost and then centrifuged at 3000 rpm to separate the fat from milk. The fat layer was removed from the sample and processed according to Waliszewski and Szymczynski method (1982): 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 was performed

on ten replicate overspiked samples of uncontaminated cow milk fat. The control samples were spiked with a solution containing all of the analytes (Bouwman et al. 1989). The fortification levels, recovery mean values, standard deviations and detection limits are presented in Table 1. The mean values ranged from 94% to 97% of recovery and the standard deviations were below 10 indicating excellent repeatability of this method.

Statistical analysis such as frequency, means, standard deviations and ranges were estimated using Minitab 10.5 for Macintosh. Differences among DDT and metabolite levels according to the place of residence were examined using One-way analysis of variance at $P<0.05$. The Tukey-Kramer Test was used to determine the difference among group means due to the place of residence (Minitab 10.5 for Mackintosh).

## RESULTS AND DISCUSSION

Frequency of positive samples, mean and standard deviations and ranges of obtained values are listed in Table 2. The DDT and the metabolites residue levels of breast milk samples are reported on fat basis as it has been considered the most appropriate manner to express contamination with these residues, due to the effects of variations in lipid level during lactation (Noren 1983). Table 2 shows that p,p'-DDE and p,p'-DDT were found in 100% and 99.7% of samples analyzed, respectively, 91.3% contained o,p'-DDT and 38.3% p,p'-DDD. The p,p'-DDE metabolite was the main contributor to Σ-DDTs detected in human milk samples with a mean value of 5.302 mg/kg on fat basis and a maximum level of 78.098 mg/kg on fat basis. This metabolite was followed by p,p'-DDT with a mean value of 2.141 mg/kg on fat basis and a maximum level of 22.468 mg/kg on fat basis, indicating direct exposure to DDT vapors used in sanitary actions.

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

| PESTICIDE | FORTIFICATION LEVELS | $\overline{x} \pm SD$ | DETECTION LIMITS |
|---|---|---|---|
| p,p'-DDE | 0.020 | $96.9 \pm 5.1$ | 0.002 |
| o,p'-DDT | 0.040 | $97.1 \pm 5.0$ | 0.003 |
| p,p'-DDD | 0.030 | $94.3 \pm 5.9$ | 0.003 |
| p,p'-DDT | 0.030 | $95.7 \pm 4.9$ | 0.003 |

**Table 2.** DDT and its metabolites residue levels (mg/kg on fat basis) in human milk samples

| PESTICIDE | FREQUENCY (%) | $\bar{X} \pm SD$ | RANGES |
|---|---|---|---|
| p,p'-DDE | 100.0 | 5.302 ± 6.070 | 0.054 - 78.098 |
| o,p'-DDT | 91.3 | 0.310 ± 0.472 | 0.000 - 4.166 |
| p,p'-DDD | 38.3 | 0.088 ± 0.194 | 0.000 - 1.932 |
| p,p'-DDT | 99.7 | 2.141 ± 3.110 | 0.000 - 22.468 |
| Σ-DDT | | 7.815 ± 8.655 | 0.515 - 99.338 |

Table 3 shows the DDT and metabolite residue levels, the ratio DDE/DDT and the Estimated Daily Intake (EDI) in breast milk samples obtained from mothers living in urban and suburban areas, and rural communities around Veracruz City. The group denominated "Veracruz-suburban" refers to those mothers living in the surrounding areas of the city, which are characterized by swamplands. In these areas DDT is extensively used as an insecticide of choice against malaria vector mosquito, causing the exposure of lactating women, uptake and further excretion via breast milk, the major route of DDT elimination. Results of the ANOVA at $P<0.05$ confirmed significant differences among Σ-DDT mean levels and DDE/DDT ratio in human milk originating from urban and suburban areas and rural communities around Veracruz City. DDT and its metabolite levels were higher in milk samples collected from nursing mothers living in suburban areas followed by those detected in lactating women from the surrounding rural communities. DDE/DDT ratio was higher in milk samples from the urban area, indicating previous exposures and the consumption of contaminated food with DDE.

**Table 3.** Mean levels of DDT and metabolites (mg/kg on fat basis), DDE/DDT ratio in human milk samples and Estimated Daily Intakes (EDI) (µg/kg body weight/day) of breastfed infants according to place of residence

| | PLACE OF RESIDENCE | | |
|---|---|---|---|
| PESTICIDE | VERACRUZ-URBAN | VERACRUZ-SUBURBAN | RURAL COMMUNITIES |
| p,p'-DDE | 1.979 ± 1.341 | 6.941 ± 7.030* | 5.597 ± 4.433 |
| o,p'-DDT | 0.127 ± 0.181 | 0.401 ± 0.554* | 0.296 ± 0.397 |
| p,p'-DDD | 0.018 ± 0.059 | 0.127 ± 0.233* | 0.068 ± 0.138 |
| p,p'-DDT | 0.500 ± 0.783 | 3.006 ± 3.511* | 1.957 ± 3.010 |
| Σ-DDT | 2.578 ± 1.945 | 10.461 ± 9.764* | 7.914 ± 6.798 |
| DDE/DDT | 6.909 ± 11.989* | 4.221 ± 4.308 | 5.601 ± 5.565 |
| EDI | 14.437 | 58.582 | 43.318 |

* Values statistically different at ($p<0.05$) between columns

On the contrary, human milk samples from mothers living in suburban areas and rural communities showed lower DDE/DDT ratios, denoting recent exposure to DDT vapors.

FAO and WHO have set the acceptable daily intake (ADI) at 20 µg/kg body weight/day for Σ-DDT (FAO/WHO 1993). Estimates of infant dietary intake of pesticides due to contamination of human millk might be obtained from human milk data, and this estimates, in turn, may be compared with international guidelines (Dogheim et al. 1996). Considering a Mexican infant with a mean body weight of 5 kg (Lara et al. 1995) and a mean milk daily intake of 0.8 L (Cisneros and Flores 1995), a breast milk from Mexican mothers with a mean fat content of 3.5% (Stafford et al. 1994), and considering the Σ-DDT mean level of each group of mothers according to place of residence, the Estimated Daily Intake (EDI) of Σ-DDT by the infants from Veracruz suburban area is 58.582 µg/kg body weight/day, 43.318 and 14.437 µg/kg body weight/day for the infants from rural communities and Veracruz City, respectively. It should be noted that the EDI of the first two groups of children is estimated to exceed the ADI of Σ-DDT by 2.93 and 2.22 times.

From a toxicological point of view, the mean levels of DDT compounds found in this study indicate the higher exposure of mothers in suburban and rural areas than in Veracruz city and consequently must be considered as a possible health risk to their infants. Because of the resulting levels exceed the ADI limits, the known benefits of breast-feeding might be analyzed, outweighing possible adverse health effects. This study reveals that implementing a yearly monitoring program for DDT and other organochlorine pesticides in human milk is urgent in these areas.

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