Concentrations of Organochlorine Pesticides in Milk of Nicaraguan Mothers

MARTHA L. LACAYO ROMERO
Universidad Autonoma de Nicaragua
Managua, Nicaragua
JOSE G. DOREA
Department of Nutrition
and
Department of Chemistry
Universidade de Brasilia
Brasilia, Brazil

ADELA C. CRUZ GRANJA
Universidad Autonoma de Nicaragua
Managua, Nicaragua

ABSTRACT. Breast-milk samples from 101 mothers from the basin of Rio Atoya, Nicaragua, were collected on two occasions within the first trimester of lactation. Milk samples were analyzed for 13 organochlorine pesticides: (1) *p,p′*-dichlorophenyldichloroethylene; (2) *p,p′*-dichlorophenyltrichloroethane; (3) *p,p′*-dichlorophenyldichlorodiene; (4) α-hexachlorocyclohexane; (5) β-hexachlorocyclohexane; (6) γ-hexachlorocyclohexane; (7) δ-hexachlorocyclohexane; (8) toxaphene; (9) dieldrin; (10) endrin; (11) aldrin; (12) heptachlor; and (13) heptachlor-epoxide. Organochlorines of the dichlorodiphenylethane class (i.e., *p,p′*-dichlorodiphenylethane and *p,p′*-dichlorodiphenylethane) were found in all samples and at the highest mean concentrations observed in the study. Chemicals in the hexachlorocyclohexane family (i.e., α- and δ-hexachlorocyclohexane) were not found at all (0%), and the other hexachlorocyclohexane compounds (i.e., β > γ) were found in less than 6% of the samples. Twenty percent or less of the sample contained chlorinated cyclodienes (i.e., dieldrin > endrin > heptachlor-epoxide > heptachlor). No measurable concentrations of α-hexachlorocyclohexane, aldrin, *p,p′*-dichlorophenyldichlorodiene, and toxaphene were found in the breast milk samples. Analysis of variance demonstrated that only the concentration of *p,p′*-dichlorophenyldichloroethylene *p,p′*-dichlorophenyltrichloroethane, and endrin were affected significantly by maternal age. Overall, with the exception of *p,p′*-chlorophenyldichloroethylene, and *p,p′*-dichlorophenyltrichloroethane, the mean concentrations of the analyzed pesticides were low. Total *p,p′*-dichlorophenyltrichloroethane concentrations that exceeded the allowed daily intake set by the World Health Organisation were found in 5.9% of the samples.

HUMAN MILK is an ideal food source for infants. In addition to its nutritional and immunological protection, it also helps the psychological well-being of mothers and infants.[1] Investigators, however, have expressed growing concerns about the presence of pesticides and environmental pollutants in our food chain, and this has prompted the worldwide study of levels of such pollutants in human milk—especially pesticides that enhance agricultural production and/or combat disease vectors.[2] Whereas the beneficial effects of human milk on the growth and well-being of babies are recognized, the adverse/toxicological effects of organochlorine pesticides at levels typically found in breast milk have also been considered.[3]

Pesticides in the food chain can reach everyone; however, women who live and work in agricultural areas characterized by intensive use of pesticides likely have a greater chance of exposure to higher concentrations of pesticides. Thus, during the childbearing life of a woman, the load of lipophilic pesticides accumulated through years of exposure could be of particular concern with respect to fetal development and well-being of breast-fed babies.

Human milk offers an important tool for monitoring chemical exposure. It provides a measure of maternal contamination and, in addition, it offers a unique opportunity for estimation of total chemical intake by infants during breast-feeding. Milk can be collected without invasiveness; therefore, it is suitable for large-population studies. To monitor the level of contamination and to generate data for possible toxicological evaluation, we studied the concentrations of organochlorine pesticides in breast milk of Nicaraguan mothers who lived and/or worked in areas where agricultural pesticides were used intensively.

Method

We selected 101 mothers who lived in the urban and rural areas of the Rio Atoya basin at least 3 y to participate in the study. We explained the objective of our study to the mothers and obtained their consent for participation. Each mother then answered a questionnaire about duration of residence in the area, pesticide exposure, and biocharacteristics (i.e., age, height, weight [before and after pregnancy], number of children, and number of previous lactations).

A milk sample was collected from each mother at 1 mo of lactation and 1 mo later, between May 1994 and February 1995. Nurses assisted in manual collection of breast milk following a brief stimulation by the infant's sucking. The milk samples were collected in *n*-hexane–washed flasks, refrigerated, protected from light and taken to the laboratory, sealed, and frozen at –20 °C until analysis.

We determined levels of organochlorine pesticides (OCPs) by gas chromatography—after we isolated the fat from the milk, extracted the OCPs from the fat, and cleaned the OCPs extract. Briefly, after thawing, we homogenized the samples by vigorous shaking, and 2 g of breast milk was weighed in a test tube in which 5 ml of acetone:hexane (1:1 volume by volume) was added; the mixture was then shaken vigorously for 1 min. Separation of phases was achieved by centrifugation at 4,000 rpm for 5 min. The organic phase was transferred with a disposable pipette, and the aqueous phase was extracted twice more with the same solvents. The organic phases were combined, evaporated to approximately 0.5 ml, and diluted to 2 ml with *n*-hexane. We then evaporated an aliquot of organic milk extract to dryness for the gravimetric determination of milk fat content. We then transferred 2 ml of the *n*-hexane extract to a glass column that contained 16 g of deactivated florisil. The column was then eluted with 30 ml of hexane-diethylether (93:7 volume by volume). A second elution was made with 50 ml hexane diethylether (85:15 volume by volume), and it was collected in a second flask. Both fractions were collected individually in a 250-ml volumetric flask, concentrated, and the organic solvents evaporated to approximately 2 ml. Each fraction was then transferred individually to a test tube. The volumetric flasks were again washed with 10 ml of *n*-hexane. Both fractions were also evaporated individually.

A 1-µl aliquot of each fraction was injected into a Varian 3400 (Varian [Palo Alto, California]) gas chromatographer equipped with an Autosampler 8100 (Varian) and an electron capture detector and integrator (Varian). We used a DB-5 30-m × 0.32-mm capillary column, supplied by J & W Scientific (Tolsom, California), to identify and quantify the OCPs. The operating conditions were as follows. Hydrogen was the carrier gas and nitrogen the cleaning gas. The respective temperatures of the injector and detector were 80 °C (1 m), 4 °C/min until 200 °C reached, 1 °C/min until 230 °C reached; and 15 °C/min until 250 °C reached (5 min). The final temperatures of the detector and injector were 350 °C and 250 °C, respectively.

Chromatography samples were run with hexachlorobenzene (HCB), which served as internal standards, and recoveries were between 75% and 120%. We identified peaks by comparing the retention times with those of analytical standards (Supelco Inc. [Bellefonte, Pennsylvania]). We used a calibration curve and internal standards to calculate the concentrations of the OCPs.

We used a SAS computer program for personal computers (SAS Institute [Cary, North Carolina]) to perform data handling and statistical analysis. To calculate analysis of variance, we used a multifactorial model that included first-order interactions following log transformation of the pesticide concentrations. Probability levels below .05 were considered statistically significant.

Results

To standardize our study, in which different populations were compared, we adhered to the following recommendations for each subject who provided breast-milk samples[4]: (a) during the study, only 1 child was breast-fed per mother, (b) milk was provided between 4 and 8 wk following delivery, (c) each mother's pregnancy was free of complications, (d) both mother and infant appeared apparently healthy, and (e) the mother lived for at least 3 y in the area of study. We also incorporated other relevant maternal factors recommended by Sim and McNeil[5] (e.g., maternal age, parity, fat content of breast milk).

Characteristics of the mothers in our study are shown in Table 1. Although 28.5% of the mothers lost net body weight during pregnancy, overall there was a mean weight gain of 1.9 kg. The average number of children per mother was 2. In our study group, breast-feeding was practiced often inasmuch as the average number of lactations approximated the number of children.

The frequency of occurrence of pesticides in human milk is shown in Figure 1. Whereas *p,p'*-dichlorophenyldichloroethylene (*p,p'*-DDE) and *p,p'*-dichlorophenyltrichloroethane (*p,p'*-DDT) were found in almost all samples (i.e., 100% and 74% of samples, respectively), some pesticides showed up in 20% of the samples (dieldrin), but others were not detected (i.e., α-hexachlorocyclohexane [HCH], δ-HCH, aldrin, *p,p'*-dichlorophenyldichlorodiene [*p,p'*-DDD], and toxaphene).

The levels of OCPs found in sampled breast milk and descriptive statistics are shown in Table 2. The highest mean concentrations of pesticides found in our samples were for *p,p'*-DDE and *p,p'*-DDT; one-third of the samples (33/91) had *p,p'*-DDE/*p,p'*-DDT ratios between 5 and 10, whereas for another one-third (32/91), the pesticide ratios were between 31 and 40. The remaining samples (36/91) had ratios that exceeded 60. The results for the majority the samples suggest that there was a persistent use of DDT in the area of study. Details of the DDE/DDT ratio relative to place of residence and occupational exposures are presented in Table 3. The mean ratio of DDE/DDT between rural and urban residents, the variable showing a significant effect, showed comparable values.

The summary of statistical results (Table 4) provided no evidence of a significant effect of the measured variables on pesticide concentrations. We found, however, a statistically significant ($p < .005$) effect for maternal age on concentrations of *p,p'*-DDT, *p,p'*-DDE, and endrin. Place of residence (i.e., mothers who lived in rural vs. urban locations) was associated significantly with DDE ($p = .059$) and dieldrin ($p = .026$) levels.

On the basis of the average concentration of milk fat

Table 1.—Summary of Background Information on Mothers Studied

Variables	*n*	$\bar{x}$	*SD*	Range
Duration of residence (y)	101	8.3	3.5	3–11
Place of residence				
Urban	51			
Rural	50			
Exposure				
Agricultural work	15			
Housewife	86			
Age (y)	101	23.9	6.05	14–42
No. children	101	3	2	1–13
No. lactations	101	2	1.7	1–13
Prepregnancy weight (kg)	101	55.7	9.4	38.5–87.0
Postpregnancy weight (kg)	101	57.6	8.8	39.5–81.2
Milk fat (g/100 g)	101	2.5	2.5	0.4–14.6

Notes: $\bar{x}$ = mean, and *SD* = standard deviation.

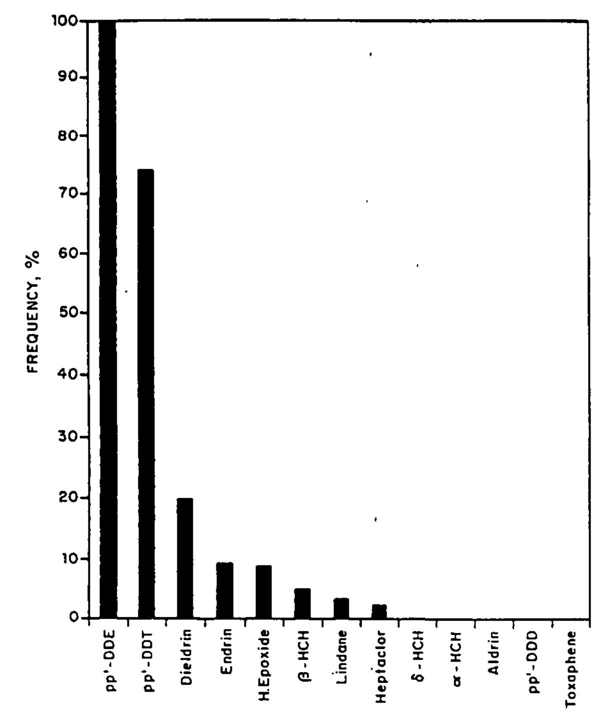

Fig. 1. Distribution of organochlorine pesticides in milk of Nicaraguan mothers. (DDE = *p,p'*-dichlorophenyldichloroethylene; DDT = *p,p'*-dichlorophenyltrichloroethane; DDD = *p,p'*-dichlorophenyldichlorodiene; and HCH = hexachlorocyclohexane.)

Table 2.—Distribution of Concentrations (μg/g of Milk Fat) of Organochlorine Pesticides (OCPs) in 101 Samples of Human Milk of Nicaraguan Mothers

OCP	$\bar{x}$	*SD*	Range
p,p'-DDE	2.805	3.069	0.030–23.2
p,p'-DDT	0.129	0.227	0–1.595
p,p'-DDD	0		0–0
Dieldrin	0.018	0.054	0–0.355
Endrin	0.003	0.033	0–0.280
Aldrin	0		0–0
Heptachlor-epoxide	0.006	0.004	0–0.340
Heptachlor	0.001	0.009	0–0.100
α-HCH	0		0–0
β-HCH	0.006	0.034	0–0.310
Lindane	0.001	0.013	0–0.130
δ-HCH	0		0–0
Toxaphene	0		0–0

Notes: DDT = dichlorophenyltrichloroethane, DDD = dichlorophenyldichlorodiene, DDE = dichlorophenyldichloroethylene, HCH = hexachlorocyclohexane, $\bar{x}$ = mean, and *SD* = standard deviation.

Table 3.—Details of Breast Milk DDE/DDT Ratios Relative to Place of Residence and Occupational Exposure of Nicaraguan Mothers

	DDE/DDT ratios			
	Place of residence		Occupational exposure	
Statistic	Rural	Urban	Yes	No
$\bar{x}$	29.8	28.1	23.5	29.5
SD	29.9	25.7	12.1	28.7
Median	32.7	23.8	11.6	2.0
Minimum	5.9	2.0	19.8	25.0
Maximum	176.5	142.0	44.8	176.5

Notes: Statistical analysis of data presented appears in Table 4. DDE = dichlorophenyldichloroethylene, DDT = dichlorophenyltrichloroethane, $\bar{x}$ = mean, and *SD* = standard deviation.

(Table 1), as well as on infant weight and milk-consumption data provided by the World Health Organisation[6] (WHO [i.e., 600 ml milk consumed by a 4.91-kg infant 2 mo of age]), we estimated the daily intake of pesticides (Table 5) by a breast-fed infant. We found that in 5.94% of the samples, the estimated consumption of total DDT exceeded the allowed daily intake (ADI) proffered by WHO.

Discussion

Despite great interest in the assessment of contamination levels of infants and maternal body load of pesticides via analysis of breast milk, the lack of uniformity in data collection makes comparison of results difficult.[5] The lipophilic nature of OCPs enables them to approximate the lipid metabolism of mothers. The pregnant woman accumulates fat during pregnancy to guarantee fetal development and lactation. The mobilization of body fat during lactation varies greatly among women,[8] depending on the prevailing socioeconomic and/or nutritional status. Mobilization of fat differs at various sites, and the rate of loss of weight gained during pregnancy will depend on maternal nutrition. In other studies of pesticide concentrations in milk fat, investigators have seldom considered such dynamic changes in lipid metabolism that occur during lactation.

Given the importance of breast-feeding for underprivileged populations, the fact nevertheless exists that comparatively fewer studies of pesticide contamination of human milk have been done in less-developed countries[2] than in more-developed countries. In addition, the most important pesticide that has been found in these studies is DDT (and its metabolites). In our study, DDT was present in all samples and at concentrations that were higher than the other pesticides studied (Table 2). Our results are within the range of reports for both industrialized and nonindustrialized countries.[2] It is noteworthy that the concentrations of these compounds found in less technically developed countries are higher than in industrialized counties. Although banned for agricultural use, DDT in the environment is still finding its way to human milk. On the basis of biotransformations of DDT, Jensen[2] has proposed that the ratios of DDE/DDT usually found in human milk fat are an indicator of past and present exposure to these dichlorodiphenylethane compounds. Although the range of variation was wide, it appears that DDT is nonetheless used intensively.

The use of DDT has been banned in many countries, including Nicaragua; however, enforcement is different from country to country, especially when the sanitary necessities of tropical countries come into play. In fact, many surveys have shown a decrease in DDT contamination of human milk fat over the years in many countries.[2] The levels of DDT found in our study were high, compared with levels in countries in which the ban is enforced. However, in comparison with other Latin American and African countries, our results are low.[2]

As noted by Jensen,[2] although dieldrin and heptachlor are the most abundantly used cyclodienes, the levels found in this study are much lower than for DDE. Although lipophilic in nature, the OCPs show the greatest differences in metabolism and bioconcentration.[9] We found chlorinated cyclodiene compounds (i.e., dieldrin, endrin, heptachlor-epoxide, heptachlor) in less than 20% of our samples—a result that comports with published data[2] and is in keeping with the world trend; in addition, we did not find aldrin, but, instead, its more-persistent metabolite, dieldrin (i.e, < 20% of samples), at levels that exceeded the detection limit (i.e., 0.01–0.355 μg/g fat). Endrin, which is rarely reported in the literature,[2] was present in only 9.4% of the samples. Heptachlor was found in only 2.3% of the samples and, according to Jensen,[2] it theoretically is transformed to epoxide derivatives by living organisms and is subsequently eliminated. Heptachlor-epoxide was the prevalent chemical form (i.e., 8.9% of samples).

The most predominant HCH compound in human milk is the β-isomer, which often makes up 90% of total HCH compounds.[2] In our study, although HCHs were found in less than 6% of sample, the predominant form found was β-HCH (i.e., 5.4%), which ranged in concentration between 0.01 and 0.31 μg/g of milk fat. The occurrence of HCH compounds above detection limits in this study was not only lower—but it occurred less frequently—in samples than has been reported in other Latin American countries.[2] There is a large difference in bioconcentration fractions of OCPs.[9] The pattern of

Table 4.—Summary Results of Analysis of Variance of Organochlorine Pesticides in Human Milk Relative to Constitutional and Environmental Variables

	Probability (*p*)					
Variables	*p,p′*-DDT	*p,p′*-DDE	β-HCH	HPTCL-EP	Dieldrin	Endrin
Duration of residence	.875	.223	.306	.428	.821	.401
Maternal age	.0002	.001	.514	.638	.384	.005
Parity	.835	.609	.071	.405	.964	.484
Occupational exposure	.374	.490	.557	.061	.395	.848
Place of residence	.189	.059	.627	.285	.026	.193
Parity × Maternal Age	.546	.490	.557	.061	.860	.215

Notes: DDE = dichlorophenyldichloroethylene, DDT = dichlorophenyltrichloroethane, HCH = hexachlorocyclohexane, and HPTCL-EP = heptachlor-epoxide.

Table 5.—Samples for which Calculated Daily Intake Exceeded Acceptable Daily Intake (ADI) Recommended by WHO*

Pesticides	ADI (mg/kg BW)	NOAEL	Safety factor	Infants (%)
Aldrin	0.0001	0.025	250	0
Dieldrin	0.0001	0.025	250	0
Endrin	0.002	0.025	125	0
Toxaphene	NE			
Lindane	0.008	0.75	100	0
DDT	0.02	0.25	10	5.94
HPTCL-EP	0.0001	0.025	250	0

**Notes:* NOAEL = no observable adverse effect level, NE = nonexistent, DDT = dichlorophenyltrichloroethane, HPTCL-EP = heptachlor-epoxide, BW = body weight, and WHO = World Health Organisation.
*Adapted from the study by Lu[7] (1995).

occurrence in our study group agreed with the expected differences between DDT and HCH—and within the HCH family.[9] The γ-isomer, lindane, is the most toxic in the HCH family, but in the current study, it was found in 3.5% of our samples. Although it is largely spread as a global pollutant, the presence of toxaphene is seldom reported in human milk[2] or human fat[10]—perhaps resulting from the complexity of toxaphene compounds and from the low sensitivity of electron capture detectors for such compounds. Boer and Wester[11] noted that 177 known compounds under the general name of "toxaphene" (i.e., polychlorinated monoterpenes) require special analytical treatment for isolation and quantification. Toxaphene, which has been identified with gas chromatography/mass spectrometry[11] and photodegradation of interfering substances,[12] has been reported in a few samples of human milk in Nicaragua, but it was not detected on our study. In Jensen's comprehensive review on pollutants of human milk, he reported that in only one publication was toxaphene referenced.

Inasmuch as the population we studied lived in an area characterized by intense agricultural activity and pesticide use—and Nicaragua is a site of pesticide occupational/accidental poisonings[13]—we found levels of pesticides in milk compatible with levels reported in other parts of the world. Calculation of the ADI in our study revealed safe limits were exceeded in several samples[14,15]; in fact, 6 samples had levels of total DDT (i.e., DDE + DDT) that exceeded safe limits set by WHO.[7]

If one considers the diversity of constitutional variables (e.g., age, reproductive history [i.e., number of pregnancies; number of lactations and duration of each], nutritional status), as well as environmental variables (e.g., pesticide exposure, including type of diet), then the analysis of variance shows that only maternal age was a significant determinant in organochlorine pesticide accumulations in breast milk. The pattern of the organochlorine pesticide distribution and the complex interaction among the variables did not draw our attention to any other measured variable as a possible determinant factor in pesticide contamination of human milk. The mobilization and redistribution of lipophilic pesticides caused by lactation is a potential health hazard to the suckling infant. Therefore, the monitoring of lipophilic pesticides in breast milk is a great environmental and public health concern.

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Requests for reprints should be sent to Jose Dorea, C.P. 04322, Universidade de Brasilia, 70910-979, Brasilia, Brazil.

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