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Influence of Breastfeeding Time on Levels of Organochlorine Pesticides in Human Milk of a Mexican Population

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Abstract This study was conducted with the objective of determining whether there is a depuration of organochlorine pesticides in breast milk according to breastfeeding time. In total, 171 samples from mothers that lived in the State of Guerrero, Mexico were analyzed. There was a weak negative relationship between *pp*'DDE ($r = -0.216$) and Σ-DDT ($r = -0.222$) concentrations with the days of lactation. In a comparison analysis, a statistically significant decrease of *pp*'DDT and *pp*'DDE levels was observed, as well as the Σ-DDT from the first to the fifth week of lactation. A reduction of 0.188 mg/kg lipid of *pp*'DDE and 0.181 mg/kg lipid of Σ-DDT per week was obtained. HCB, β-HCH and *op*'DDT concentrations were low and had no major fluctuations between subgroups. The low levels found and the observed reduction in time involve less exposure to the infant to these pollutants. Through this methodology changes in levels of certain organochlorine pesticides in various stages of human milk production may be shown.

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Breast milk is the most important food for human in the first 6 months of life because it has a rich content in nutrients and substances that provide protection against infections (WHO 2003; Newton 2004). The study of this biological matrix is useful to determine exposure to different pollutants such as organochlorine pesticides, which are highly persistent in the environment and accumulated in the food chain (Massart et al. 2008). Also, these compounds are important for their potential negative effects on human health (IPCS 2011; Mnif et al. 2011).

Due to its lipophilic character theses pesticides are accumulated in body fat deposits where they remain for several years and can be excreted through breast milk (Ritter et al. 1995). The chemical composition of human milk varies from the initial discharge, called colostrum, lasting a few days or weeks, and then becoming mature milk (Moltó-Puigmartí et al. 2011; Antonakou et al. 2013). During this process differences in concentrations of organochlorine pesticides are produced as a result of the changing profile of fat and protein contents in milk (Yu et al. 2007; Waliszewski et al. 2009).

The present study is derived from research on persistent organic pollutants (POPs) in breast milk carried out in the State of Guerrero, located in southern Mexico (Chávez-Almazán et al. 2014), an area with a history of intensive use of organochlorine pesticides in agriculture and vector control of malaria (Gallardo 2000), which led to an exposure of the population through the environment (Alegría et al. 2008; Wong et al. 2008) and food (Gutiérrez et al. 2012). This research focused on the spatial distribution of organochlorine pesticides and the association between various factors such as maternal (age, parity, lactation, eating habits) and environmental (region and locality of origin), with the degree of contamination by these compounds in samples.

The aim of this study was to determine variations in organochlorine pesticide levels in breast milk through the grouping of donor according to the stage of lactation in which they were in, and to determine whether this methodological approach can be used as a viable alternative to that of previous studies on this topic.

Materials and Methods

A group of mothers who have been resident in the State of Guerrero (located in southern Mexico) during last 10 years were invited to participate in this monitoring study. Those who agreed to participate donated a sample of breast milk only one time, and they were instructed in the collection of the sample, either manually or by suction device.

Samples (around 30–50 mL) were collected in clean glass bottles and transported to the laboratory; they were first centrifuged at 3000 rpm for 15 min to separate the fat and then were stored at −20°C until analysis. The pesticides extraction was made in accordance with Waliszewski et al. (2004); the milk fat samples (2–3 g) were placed on a ceramic container and mixed with sodium sulfate anhydrous (Sigma-Aldrich, St. Louis, MO, USA) until a dry powder was obtained; afterwards this material was placed in a glass column (1 cm inner diameter, 50 cm long) and the fat was extracted with 100 mL of hexane (JT-Baker, Center Valley, PA, USA). The eluate was concentrated to 30 mL in a rotatory evaporator at 40°C.

The determination of lipid content of the sample was conducted by the gravimetric method (Waliszewski et al. 2004), in which 10 mL of concentrated extract was placed in a pre-weighed round bottomed flask of 50 mL and solvent was evaporated. The lipid content correspond to the difference between both weights.

Later, 10 mL of concentrated extract was transferred to a tube with stopper, and 1 mL of sulfuric acid was added (JT-Baker, Center Valley, PA, USA). The tube was stopped up and vigorously shaken for 1 min to fat precipitation. Supernatant was dried by passing it through a 5 g layer of sodium sulfate anhydrous and washed with hexane. The extract was concentrated to a few drops and quantitatively transferred to a volumetric tube in which the final volume was adjusted to 1.0 mL with hexane.

Chromatographic analysis was done on a 3400 CX Varian Gas Chromatograph equipped with an Electron Capture Detector (Palo Alto, CA, USA); a manual sample injection of 1 μL was applied in splitless mode. The organochlorine pesticides separation was performed on a capillary column DB-608 (0.32 mm inner diameter, 30 m in length; J&W Scientific, USA) with nitrogen flow rate at 6.3 mL/min. Oven temperature was 193°C for 7 min followed by an increase of 6°C/min until 250°C and held for 20 min.

Organochlorine pesticides in samples were identified and quantified, comparing data to a standard solution of HCB, isomers α-, β- and γ-HCH, *pp*'DDE, *op*'DDT and *pp*'DDT (Supelco Inc. Bellefont, PA, USA). The quantities of organochlorine pesticides in breast milk were expressed as milligrams per kilogram of lipid base (mg/kg lipid).

To determine the quality of the analytical method, samples of bovine milk, free of organochlorine pesticides, were spiked in 10 replicates and analyzed to calculate the recovery percentage. The fortification study, done at 0.01–0.04 mg/kg lipid, showed mean values from 87 % to 101 %. Also, the precision was acceptable due to the coefficient of variation being <11 % for all compounds. The minimum detection limits for the analyzed residues on lipid basis were: 0.001 mg/kg lipid for HCB, α-HCH, and γ-HCH, and 0.002 mg/kg lipid for β-HCH, *pp*'DDE, *op*'DDT and *pp*'DDT.

To determine the influence of lactation on pesticide concentrations, a Pearson correlation analysis (r value) and a scatterplot showing the pesticide levels with respect to days of lactation were performed. In addition, participants were classified into five subgroups according to the weeks of lactation when the sampling was conducted (number of samples in each subgroup: first week = 13, second week = 48, third week = 43, fourth week = 33 and fifth week = 34). Concentrations of pesticides in each subgroup were reported as median because the data were not normally distributed according to the Kolmogorov–Smirnov test. Differences in pesticide concentrations were calculated by means of a Kruskal–Wallis test with a confidence level of 95 %. In addition, a linear regression model was applied. The statistical tests were performed using SPSS, version 20 (IBM Corporation, Armonk, NY, USA).

This protocol was approved by the Committee on Research Ethics of State Laboratory of Public Health, Health Department of Guerrero. Informed consent was obtained from all individual participants included in the study.

Results and Discussion

A total of 171 samples of breast milk were analyzed. HCB and β-HCH showed a low frequency, with 37 % and 36 %, respectively. *op*'DDT and *pp*'DDT were present in 76 % and 98 % of all analyzed samples. *pp*'DDE was found in 100 % of participants. Meanwhile the isomers α- and γ-HCH, were not detected.

The pesticide concentrations showed a tendency to decrease with increasing days of lactation. This relationship was weak in most of the compounds, with the exception of *pp*'DDE and Σ-DDT (Total DDT), which had values of correlation coefficient of −0.216 and −0.222, respectively ($p < 0.01$) (Figs. 1, 2).

The compounds that had the lowest concentrations were HCB and β-HCH (Overall median = 0.009 and 0.006 mg/kg lipid, respectively). DDT isomers revealed higher concentrations and a trend of decreasing concentration with the lapse of weeks, mainly the *pp*'DDE (Table 1); however, this was not statistically significant ($p > 0.05$). In a linear regression analysis it is shown that the decreased concentration of this metabolite was 0.188 mg/kg lipid per week (Fig. 3).

Likewise Σ-DDT showed a depuration time: Week 1 = 1.222, Week 2 = 1.057, Week 3 = 0.805, Week 4 = 0.693 and Week 5 = 0.498 mg/kg lipid. A weekly progressive decrease (in the regression analysis: 0.181 mg/kg lipid, Fig. 4) is indicated. These results demonstrated that the time of lactation has an influence on concentrations present in breast milk.

Significant reductions were observed in the concentrations of the isomers of DDT from the first to the fifth week, with variations >60 %, as in the case of the metabolite *pp*'DDE. In addition, decreased levels of HCB and β-HCH indicate a low exposure level by breastfeeding to these POPs in the State of Guerrero (Chávez-Almazán et al. 2014). The results obtained in this study were lower than those found in other Mexican States such as Yucatán (β-HCH, 0.612 mg/kg lipid, *pp*'DDE, 3.042 mg/kg lipid and *pp*'DDT, 0.210 mg/kg lipid, Rodas et al. 2008), Veracruz (*pp*'DDE, 3.230 mg/kg lipid, *pp*'DDT, 0.510 mg/kg lipid, Waliszewski et al. 2002) and Chiapas (*pp*'DDT, 0.277 mg/kg lipid, López et al. 2006).

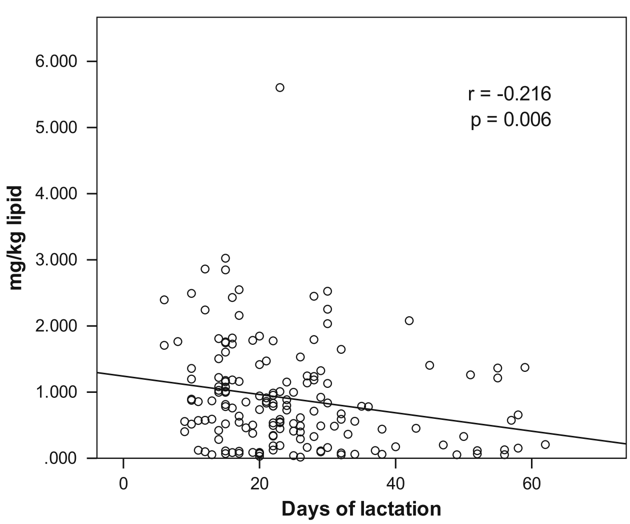

Fig. 1 *pp*'DDE levels versus days of lactation

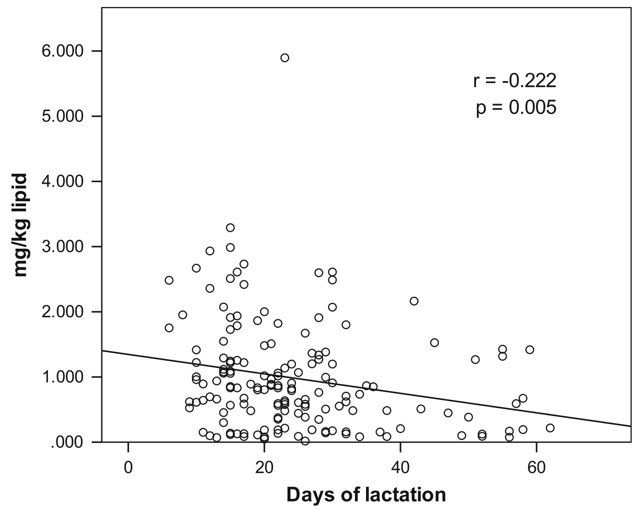

Fig. 2 Σ-DDT levels versus days of lactation

Table 1 Median of organochlorine pesticide levels in breast milk (mg/kg lipid) for different weeks of lactation

	Weeks of lactation				
	1	2	3	4	5
HCB	0.008	0.008	0.010	0.011	0.007
	n = 7	n = 15	n = 18	n = 13	n = 10
β-HCH	0.003	0.004	0.003	0.007	0.007
	n = 6	n = 18	n = 16	n = 10	n = 12
pp'DDE*	1.197	0.997	0.729	0.613	0.447
	n = 13	n = 48	n = 43	n = 33	n = 34
op'DDT	0.019	0.017	0.016	0.017	0.012
	n = 12	n = 38	n = 32	n = 25	n = 23
pp'DDT*	0.060	0.048	0.035	0.050	0.036
	n = 13	n = 47	n = 42	n = 32	n = 34
Σ-DDT	1.222	1.057	0.805	0.693	0.498
	n = 13	n = 48	n = 43	n = 33	n = 34

n, number of positive samples

* $p < 0.05$ of Kruskal–Wallis test

The variation in organochlorine pesticide levels by breastfeeding time has also been demonstrated by Ennaceur and Driss (2013), who analyzed *pp*'DDE, *pp*'DDT, HCB, HCH and dieldrin for 10 months in breast milk samples from women in Tunisia; a gradual decrease in concentrations of *pp*'DDE and *pp*'DDT was observed. Meanwhile Waliszewski et al. (2009) studied such variations over a shorter period of 4–30 days of initial breastfeeding and also found a decrease in values of β-HCH, *pp*'DDE and *pp*'DDT. By contrast, Yu et al. (2007) found

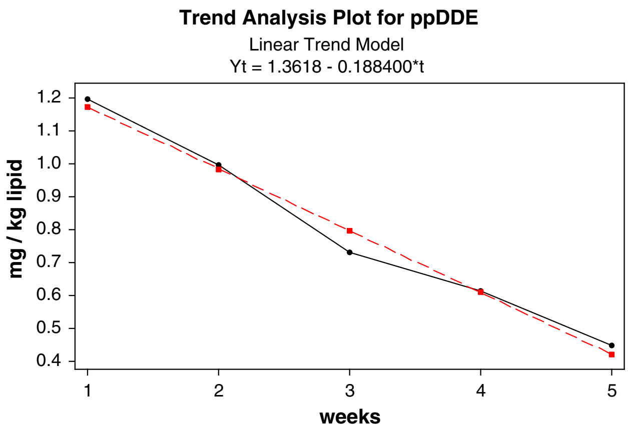

Fig. 3 Trend over time of concentrations of *pp*'DDE

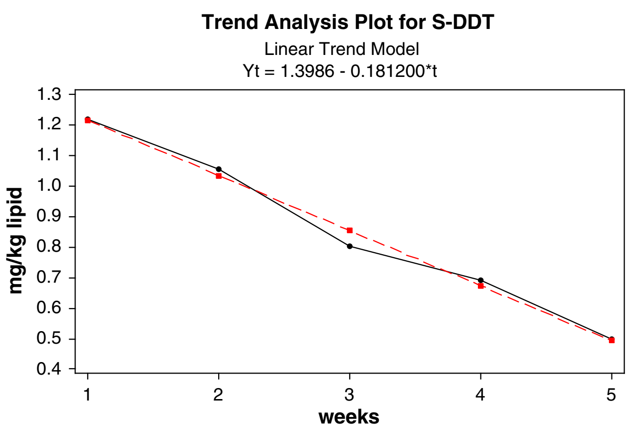

Fig. 4 Trend over time of concentrations of Σ-DDT

no statistically significant differences in concentrations of HCB, β-HCH, *pp*'DDE and *pp*'DDT in samples taken at 4–5 days postpartum with respect to those obtained at day 14, which indicated changes in lipid and protein contents between colostrums and mature milk. In this work the tendency of lipid levels was not studied during the time, but according to Jensen (1996) and Pérez and Pérez (1984), these levels decrease with time and remain stable in mature milk after 14 days of lactation.

The methodology proposed in this monitoring study was put forward in order to determine the role of breastfeeding time on organochlorine pesticide levels in the human body by means the grouping of donors according to the weeks of breastfeeding. This approach differs with respect to the studies developed by Ennaceur and Driss (2013), Waliszewski et al. (2009) and Yu et al. (2007), in which pesticide levels were determined longitudinally. The main advantage of this work was it sampling design which possibility collection of a large number of samples from participants during a single occasion. In conclusion, the results obtained in the work should be considered as an additional contribution in evaluating breastfeeding period as a factor which can influence the presence of these POPs in human milk.

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References

Alegría H, Wong F, Jantunen LM, Bidleman TF, Salvador-Figueroa M, Gold-Bouchot G, Ceja-Moreno V, Waliszewski SM, Infanzon R (2008) Organochlorine pesticides and PCBs in air of southern Mexico (2002–2004). Atmos Environ 42(38): 8810–8818

Antonakou A, Skenderi KP, Chiou A, Anastasiou CA, Bakoula C, Matalas AL (2013) Breast milk fat concentration and fatty acid pattern during the first six months in exclusively breastfeeding Greek women. Eur J Nutr 52:963–973

Chávez-Almazán LA, Diaz-Ortiz J, Alarcón-Romero M, Dávila-Vazquez G, Saldarriaga-Noreña H, Waliszewski SM (2014) Organochlorine pesticide levels in breast milk in Guerrero, Mexico. Bull Environ Contam Toxicol 93:294–298

Ennaceur S, Driss MR (2013) Time course of organochlorine pesticides and polychlorinated biphenyls in breast-feeding mothers throughout the first 10 months of lactation in Tunisia. Environ Monit Assess 185:1977–1984

Gallardo EG (2000) Situación actual de la malaria y el uso de DDT en Mexico. Pan American Health Organization-Health Department of Mexico, Mexico

Gutiérrez R, Ruiz JL, Ortiz R, Vega S, Schettino B, Yamazaki A, Ramírez ML (2012) Organochlorine pesticide residues in bovine milk from organic farms in Chiapas, Mexico. Bull Environ Contam Toxicol 89:882–887

International Programme on Chemical Safety (IPCS) (2011) DDT in indoor residual spraying: human health aspects. WHO Press, Geneva

Jensen RG (1996) The lipids in human milk. Prog Lipid Res 35:53–92

López D, Yáñez L, Athanasiadou M, Bergman A, Herrera C, Diaz F (2006) Determinación de niveles de DDT, DDE y DDE-$MeSO_2$ en leche materna y sangre de poblaciones contaminadas por DDT en Mexico. Acta Toxicol Argent 14:34–46

Massart F, Gherarducci G, Marchi B, Saggese G (2008) Chemical biomarkers of human breast milk pollution. Biomark Insights 3:159–169

Mnif W, Hadj-Hassine AI, Bouaziz A, Bartegi A, Thomas O, Roig B (2011) Effect of endocrine disruptor pesticides: a review. Int J Environ Res Public Health 8:2265–2303

Moltó-Puigmartí C, Castellote AI, Carbonell-Estrany X, López-Sabater MC (2011) Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. Clin Nutr 30:116–123

Newton ER (2004) Breast milk: the gold standard. Clin Obstet Gynecol 47(3):632–642

Pérez GJ, Pérez GP (1984) Biosíntesis de lípidos de la leche. Glóbulos grasos. In: Bioquímica y microbiología de la leche. Limusa, Mexico, pp 24–29

Ritter L, Solomon KR, Forget J, Stemeroff M, O'Leary C (1995) Persistent organic pollutants. Report prepared for The

International Programme on Chemical Safety. www.chem.unep.ch/pops/ritter/en/ritteren.pdf. Accessed 29 Oct 2014

Rodas JP, Ceja V, González RL, Alvarado J, Rodríguez ME, Gold-Bouchot G (2008) Organochlorine pesticides and polychlorinated biphenyls levels in human milk from Chelem, Yucatán, Mexico. Bull Environ Contam Toxicol 80:255–259

Waliszewski SM, Bermúdez MT, Infanzón RM (2002) Niveles de DDT en tejido adiposo materno, suero sanguíneo y leche de madres residentes en Veracruz, Mexico. Estudio 1997–1999. Rev Int Contam Ambient 18:17–25

Waliszewski SM, Gómez-Arroyo S, Carbajal O, Villalobos-Pietrini R, Infanzón RM (2004) Uso del ácido sulfúrico en las determinaciones de plaguicidas organoclorados. Rev Int Contam Ambient 20(4):185–192

Waliszewski SM, Melo-Santiesteban G, Villalobos-Pietrini R, Gómez-Arroyo S, Amador-Muñoz O, Herrero-Mercado M, Carvajal O (2009) Breast milk excretion kinetic of β-HCH, pp'DDE and pp'DDT. Bull Environ Contam Toxicol 83(6):869–873

Wong F, Alegría H, Jantunen LM, Bidleman TF, Salvador-Figueroa M, Gold-Bouchot G, Ceja-Moreno V, Waliszewski SM, Infanzon R (2008) Organochlorine pesticides in soil and air of southern Mexico: chemical profiles and potential for soil emissions. Atmos Environ 42(37):7737–7745

World Health Organization (2003) Global strategy for infant and young child feeding. WHO Press, Geneva

Yu Z, Palkovikova L, Drobna B, Petrik J, Kocan A, Trnovec T, Hertz-Picciotto I (2007) Comparison of organochlorine compound concentrations in colostrums and mature milk. Chemosphere 66:1012–1018