

DDT AND ITS METABOLITES IN BREAST MILK FROM THE MADEIRA RIVER BASIN IN THE AMAZON, BRAZIL

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Introduction

Breast milk is the best source of nutrition for infants, it contains the optimal balance of fats, carbohydrates and proteins for the developing babies, also providing other benefits related to growth, development and immunity. The finding of toxic chemicals in breast milk raises important issues for pediatric practices, for the practice of public health, and for the environmental health research community¹.

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 studies². The presence of organochlorines, including PCBs in human breast milk is diminishing in developed countries. Results from Brazil include surveys in São Paulo, Rio de Janeiro and Paraná States. DDT was used in vector control in Brazil, especially malaria control in the Amazon region from 1945 until 1997, and fishes from the Madeira River are known to present relatively high levels of this pesticide and its main metabolites³. The presence of DDT in edible fishes from this region was evaluated in samples collected from 1991 to 2002 and revealed a reduction trend along time⁴.

In order to describe for the first time the human contamination with DDT in the Amazon environment were the contamination is clearly related to vector control activities, in this work we analyzed 63 breast milk samples collected in different riverine communities along 1.500 km of the Madeira River from Porto Velho (RO) to Itacoatiara (AM) in May of 2002 (Fig. 1).

Other studies reported that DDT in breast milk is closely related to the DDT content in the women adipose tissue and during lactation approximately 30% of the DDT body burden is passed to the milk^{5,6}.

Material and Methods

All of the samples were taken using acetone rinsed large mouth flasks, after the nursing mothers were informed for the research purposes and signed an informed consent term. They were interviewed for their food habits regarding fish consumption.

The samples were extracted by means of solid phase extraction. In brief, 1 ml of breast milk is mixed to 5 ml of ethyl acetate/methanol/acetone (2:4:4 v/v/v), this mixture is submitted to 1 minute in a vortex and after that stays 20 minutes in a ultrasonic bath and is centrifuged at 200 rpm for 15 minutes. The organic phase is put on a pre-conditioned (hexane, ethyl acetate, methanol and distilled water) C-18 SPE cartridge that is cleaned two times with a solution of acetone and water (3:1). After drying for 40 minutes the C-18 cartridge is eluted with 1 ml of n-hexane (pesticide grade). The eluate is put on the top of a pre-conditioned (dichloromethane - DCM, ethyl acetate, petroleum ether and hexane) FLORISIL cartridge that is later eluted with 10 ml of n-hexane and 5 ml petroleum ether-acetone (15%). The internal standard DCN is added to the cleaned extract and the volume is reduced to 1 ml.

Chromatographic conditions: We used a GC-ECD, from Hewlett Packard model 5980, with capillary columns HP-5 (5% phenyl groups). The i.d. is 0,320 µm and film thickness of 0,25 µm. The injection port, set at 240 °C, is operated in the split-less mode. The detector temperature is 300 °C. The carrier gas was helium (1ml/min) and the make up gas (ultra-pure N₂, 62,4 ml/min). The temperature program rise from 110 °C to 280 °C (1st step 3°C/min to 168 °C, 2nd step 1,5 °C/min to 230°C, 3rd step 60°C/min ATÉ 280°C – hold for 10 minutes). Total run time is 70 minutes.

All of the results were adjusted for an estimate fat content of 3%.

Results and Discussion

The results are presented in Table 1. Total DDT sample concentrations ranged from 53 ppb to 12 ppm. This higher level is more than

the double accepted level stipulated by WHO. Since 1997, the Health Ministry is not using DDT, thus, the contamination with the metabolite DDE and the parent compound DDT is clearly occurring via fish intake³.

Table 1. DDT in Breast milk from the Madeira R. Valley (N=68)

	DDE	DDD	DDT	DDT-T	3% fat DDT-T
Average	24,7	2,1	7,2	34,0	1133,5
Median	8,6	0,9	2,1	14,4	481,0
Max	290,9	16,0	101,4	374,5	12482,6
Min	0,4	0,2	0,1	1,0	33,8
Std. Dev.	44,1	2,8	16,1	59,7	1991,3
RSD%	178,8	137,0	222,9	175,7	175,7

The prevalent congener in the vast majority of the samples was DDE (DDE/DDT = 3).

As is noted from other studies^{7,8}, in our study there is a relation between total DDT levels in milk with age of the mother and the number of children they had. For example, the mother who had the first baby with more than 29 five year, showed the maximum residue level (12 ppm in a fat basis).

Fortunately, there is no link between critical maternal body residue levels linking DDE to fetal or infant toxicity. However a subtle biological effect may occur due to exposure to high amounts of this pesticide metabolite can not be discarded, especially during embryogenesis.

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References

1. Landrigan PJ, Sonawane B, Mattison D, McCally M and Garg A. (2002). *Environmental Health Perspectives*. 110(6) A313-A315.
2. Romero MLL, Dorea JG and Granja AC. (2000). *Archives of Environmental Health*, 55(4)274-278.
3. Torres, JPM, Pfeiffer WC, Markowitz S, Pause R, Malm O, Japenga J. (2002). *Environmental Research A*. 88, 134-139.
4. D'Amato C, Torres JPM, Malm O, Bastos WR, Cláudio L, Markowitz S. (2004). *Organohalogen Compounds* Vol. 66.
5. Dorea JG, Granja AC and Romero MLL. 1997. *Annals of Nutrition and Metabolism*, 41:250-254.
6. Dorea JG, Granja AC, Romero MLL and Cuadra-Leal. 2001. *Environmental Research A*. 86, 229-237.
7. Sarcinelli, PN, Pereira, ACS, Mesquita SA, Oliveira-Silva, JJ, Meyer A, Menezes, Alves SR, Mattos, RCOC, Moreira J and Wolff M. 2003. *Environmental Research* 143-150.
8. Delgado IF, Barreto HHC, Kussumi TA, Alleluia IB, Baggio CA and Paumgarten FJR. 2002. *Cad. Saúde Pública*, 18 (2):519-524.