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

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Abstract

Until the 1990's the 1,1,1-trichloro-bis-2,2′-(4chlorophenyl) ethane (DDT) was sprayed in the walls of the house in the along the Madeira River basin, Brazilian Amazon, a region well known by its large number of malaria cases. In the 1910, the relate of Oswaldo Cruz about health conditions in Madeira River region describes the presence of malaria in rates ranging until 100% of infected people in some localities. Data available in the literature points to the DDT contamination in fishes captured in Madeira River region. Fish is the major source of dietary protein to this people. DDT tends to accumulate in lipid rich tissues being eliminated by different events, including lactation. Considering the importance of the breast milk to the children feeding, the associated risks of DDT exposure via breast milk intake to children must be assessed. This is the main objective of this work: to analyse the presence of the p,p′-DDT and its metabolites p,p′-DDE and p,p′-DDD in 69 human milk samples and to estimate the intake of DDT and its metabolite in terms of total DDT (total DDT = p,p′-DDE + p,p′-DDD+ p,p′-DDT). All sample showed contamination with DDT and its metabolites ranging from 25.4 to 9361.9 ng of total DDT / g of lipid (median=369.6 ng of total DDT / g of lipid) and 8.7 % of the Estimated Daily Intake (EDI), in terms of total DDT, was higher than the Acceptable Daily Intake proposed by the WHO. Key words: DDT, breast milk, children, organochlorine pesticide, fish.

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1. Introduction

Breast milk is the most complete source of nutrients (proteins, carbohydrates, fat and vitamins), immune factors, and other important constituents linked to immune responses, including in the protection against infectious dieseases (Institute of Medicine, 1991; Oddy, 2001). Unfortunately, breast milk is not free of contaminants and its ingestion represents an important exposure pathway to organochlorine pesticides and other environmental and pharmaceutical chemicals to children (Berlin et al., 2002; Fitzgerald, et al., 2001; Koopman-Essembom et al., 1995; Patandin et al., 1999; Saver et al., 1994).

The presence of DDT and its metabolites is widely studied in many parts of the world in environmental samples, biota and humans. The interest in the DDT levels in many organisms and in humans is given by its adverse effects such as activity in estrogenic receptors (Daston et al., 1993; Gillesby and Sacarewsk, 1998; Guillette et al., 1995; Hoekstra et al., 2001), induction of spontaneous abortion (Hart et al., 1972; Palmer et al., 1972; Johnson et al., 1988; Korrick et al., 2001), and apoptosis in mononuclear cells (Pérez-Maldonado et al., 2004; Teoburi *et al.,* 1998).

Fish consumption is considered an important source of DDT and other organochlorine pesticides to humans, since these compounds are frequently detected in a wide range of fish types from many parts of the word (Harris et al., 2001). Torres et al., (2002) and D'Amato et al., (2004) reported DDT contamination in various fish species from Madeira River. The presence of the DDT in these fishes can be explained by its widespread use in the Brazilian Amazon in the control of vector-borne diseases such as malaria. The Ministry of Health of Brazil reported that it ended the use of DDT in 1992 (Oliveira Filho, 1997), but still permitted its use in the control of leishmaniasis, that is also endemic in the Amazon region.

2. Material and methods

2.1. Samples

Samples of human milk (n=69) were collected between Porto Velho City and the locality of Axinim in two trips to the Madeira River in 2001 and 2002 totalling 20 localities. The sampling area is shown in the Figure 1. The milk samples were colleted in wide mouth glass flasks previously decontaminated with acetone and n-hexane and the milk samples were stored in the freezer until analysis. This study was previously approved in the Committee of Ethics in Research and conformed to meet high standards regarding human samples and other ethic guidelines (process n° 026/02 CEP-NESC/UFRJ).

2.2. Extraction method

The extraction procedure used was based in the Prapamontol and Stevenson (2001) method with certain modifications. The milk samples were warmed in a hot bath at 37°C before analysis until complete homogenisation. An aliquot of 1mL was added to 5mL of a solution containing ethyl acetate:acetone:methanol (1:2:2), homogenized by vortex mixing (1 minute), ultrasonic bath (20 minutes), and centrifuged for 15 minutes at 2000 RPM. To the supernatant was added 10mL of ultra pure water and the mix was passed through a C_{18} Solid Phase Extraction cartridge (C_{18} SPE) previously conditioned with n-hexane (2 x 1mL), ethyl acetate (2 x 1mL), methanol (2 x 1mL) and ultra pure water. The C_{18} SPE was washed twice with 1 mL of solution containing 25% of in water and kept in vacuum to dry completely. The DDT and its metabolites were eluted with 1mL of n-hexane, volume that was submitted to the cleanup step using Florisil SPE conditioned with of chlorine methylene (25mL), ethyl acetate (10mL), solution of 15% of petroleum ether in acetone (10ml), and n-hexane (10ml). After elution of the extracts the Florisil SPE was washed with 10mL of n-hexane and 5 mL of a solution containing 15% of petroleum

ether in acetone. Both C_{18} and Florisil SPE were purchased by SUPELCO™ (USA). The combined was added 10μL of the standard dichloronaphtalene (DCN) and gently reduced to 1mL in flow of nitrogen. An aliquot of 3μL of the final extract was injected in a GC-ECD and the quantification was performed by internal standard method using DCN.

2.3. Quality control

Blanks were done in parallel of the samples and did not shown the presence of peaks in their chromatograms. The recovery was performed using spiked samples of breast milk. The used extraction method presented recovery of 88.4%, 102.6%, and 82.2% with coefficient of variation of 3.1%, 2.2%, and 2.7% for p,p′-DDE, p,p′-DDD, and p,p′-DDT respectively. The limit of detection was calculated as three times the standard deviation of the, and the obtained values for p,p′-DDE, p,p′-DDD, and p,p′-DDT were 0.0040ng/mL, 0.0340ng/mL, and 0.0040ng/mL respectively.

2.4. Estimative of the DDT daily intake

The estimative of the DDT daily intake by infant was performed using the calculated concentrations of total DDT in individual samples. The calculation of the Infant Daily Intake (*IDI*) was done according to Marien and Laflame (1995) that proposed the following equation: $IDI = (BMLC \times MC \times PMF) / BW$, where: *BMLC* = Breast Milk Lipid Concentration; *MC* = Milk Consumption, *PMF* = Percent Milk Fat, and *BW* = Body Weight of the nursing infants. The values used to the terms *MC*, *PMF*, and *BW* were estimated in 1kg/day, 4%, and 5 kg respectively, conform adopted in the work of Marien and Laflame (1995). The estimated values were compared with the proposed limit of 0.020mg of DDT/kg of body weight (WHO, 1984).

3. Results and discussion

3.1. Population data and diet assessment

The diet of the mothers was based in cassava flour, some fruits and fish according to the data of the applied questionnaire. Fishes represented the main source of proteins of the donor's mothers, being consumed in all meals by 98% of the interviewed. The consumption of milk and meat (bovine, swine or poultry meat) was not declared in the same 98% of the applied questionnaires, a fact considered common for traditional Amazonian population. A additional point of view that should be considered is the absent of pesticide use in the Amazonian agriculture and no one mother had previous contact with DDT for agricultural use, a fact that was also identified in the questionnaires. The breast milk samples collected in the present work were obtained of mothers of communities from Madeira River basin. Some characteristic of the studied mothers are showed in the Table 1.

3.2 DDT status

The chromatograms showed residues of DDT and its metabolites in all analysed samples. The concentration ranged from 10.7 a 7271.5ng/g of lipid for p,p′-DDE, from just a value lower than the limit of quantification to 400.7 ng/g of lipid for p,p′-DDD, from 3.0 to 2534.1 for p,p′-DDT, and 25.4 to 9361,9 to total DDT. The Figure 2 shows the distribution of average and geometric mean of the total DDT values in the different studied localities. The obtained geometric mean of the total DDT in the different studied localities ranged from 118.3 in Santa Rosa to 1005 ng of total DDT/g of lipid in Cachoeirinha. Geometric mean of total DDT/g of lipid of the localities showed a good correlation ($r = 0.993$) with the average values. The geometric mean/average ratio was 0.76.

The highest value of total DDT (9361.9 ng of total DDT/g of lipid) was obtained of a primipara mother aging 27 years old. Parity is pointed as a factor in the organochlorine amounts in breast milk Harris et al. (2001). Lactation is potentially the most significant activity in the reduction of the stored organochlorine in the human body, once it was observed the decrease of these compounds during its course (Haggyard et al., 1973; Bakken and Siep, 1976; Rogan et al., 1986; Skare and Polder, 1990; Quinsey et al., 1996). Age has been noted as one of the most significant contributors of organochlorine pesticides in breast milk. The increase of the concentrations of DDT in breast milk with age was observed by previous studies (Stacey, et al., 1985; Mussalo-Rauhamaa et al., 1988; Dewailly, et al., 1996).

Conform expected the p,p′-DDT, p,p′-DDE, and p,p′-DDD did not occurred independently. The average of p,p′-DDE/p,p′-DDT ratio was 6.3, and the individual values are shown in the Figure 3. It means a non-recent use of the insecticide DDT, conform observed by Gladen et al. (1999) that obtained the DDE/DDT ratio of 7.1.

There is sufficient evidence that the presence of the DDT and its metabolites in the analysed milk samples is due to diet rich in fish, once considered the Harris et al. (2001) statement that the consumption of contaminated food represents an important source of organochlorine pesticides to humans. The population living in the Madeira River region is characterised by high consumption of fish meat in their diet. The presence of DDT observed in previous works (Torres et al., 2002) and its high consumption can be the source of DDT in the studied human milk samples.

In 19 of the 20 localities presented total DDT contamination ranging from 118 to 771.4 ng of total DDT/g of lipid. Just one locality (Cachoeirinha) presented geometric mean for total DDT higher than 1000 ng of total DDT/g of lipid. The Table 2 presents the contamination expressed in geometric mean for all sampled localities.

3.3 Comparison of the DDT contamination with literature data

A large number of studies involving the DDT contamination in breast milk are available in the literature. The observed contamination in breast milk donned for mothers from Guatemala in the 1970's (Olszyna-Marzys *et al.*, 1973) presents values higher than the observed in the present study. The authors analyzed breast milk samples from La Bomba, Cerro Colorado, and El Rosario. The mean values in these locality were 2150 (ranging from 411 to 11500), 4070 (from 1570 to 12210), and 1840 ng of total DDT / mL (from 342 to 4970ng of total DDT / mL). The values of the individual samples of the Olszyna-Marzys *et al* (1973) work were higher than the calculated in the present study. The magnitude of the DDT contamination in the Olszyna-Marzys *et al* (1973) can be explained by its allowed agricultural use in the 1970 decade and the donor mothers were from cotton culture areas, where DDT contamination presented higher values than the obtained in areas without cotton culture.

Lara et al. (1982) studied DDT levels in 25 human milk samples from São Paulo State, Brazil since 1979 to 1981. The observed amounts of DDT ranged from 16 to 2610μg of DDT/l in whole milk basis with average value of 278 μg of DDT/l in this work. Values of 400, 65250, and 7175 ng of total DDT/g of lipid were obtained (to minimum, maximum, and average, respectively) when adjusted to lipid basis assuming 4% of lipid in whole milk. These values were higher than the observed in the present work, but is important to consider that DDT was banned in Brazilian Government in 1985, fact that could explain the amounts found in the occasion of this 1982 work.

Paumgartten et al. (2000) investigated the levels of DDT and its metabolites in mothers living in the urban area of Rio de Janeiro, Brazil. The mean values found were 180 ng of DDT/g of lipid, 1520 ng of DDE/g of lipid, 6 ng of DDD/g of lipid, and 1700ng of total DDT/g of lipid.

These values of DDT, DDE, and total DDT were higher than the observed geometric means in the present study. In this work, only DDD presented level higher (534.3ng of DDD/g of lipid) than the observed by Paumgartten et al., (2000). Other DDT levels around the world are shown in the Table 3.

The mean concentration of total DDT (p,p′ DDT+ p,p′ DDE+ p,p′ DDD) in samples of breast milk donned by Chinese women (Kunisue et al., 2004). The mean value (2100ng of total DDT/ g of lipid) was higher than the contamination expressed in terms of geometric mean in milk samples from Madeira River. The authors hypothesized that population might be mainly exposed to DDT via seafood intake.

Some values of DDT contamination in developed countries such as Sweden and Germany presents a perceptible decrease in the DDT levels (Schade and Heinzow, 1998; Norén and Meironyté, 2000; Solomon and Weiss, 2002.). The principal explication to the phenomena is the prohibition of the DDT use in agriculture by these countries, mainly in the decade of 1970. Solomon and Weiss (2002) reported a decrease of 81% of the detectable residue levels of DDT in Germany. The same tendency of decrease in human milk contamination levels are not observed in Latin America by lack of contamination data that support this kind of comparison and by a latter prohibition of DDT in the developing countries.

3.4. Differences in total DDT concentration between primipara and multipara mothers

The observed geometric mean for DDT and its metabolites for premipara mother were 698.8 ng of p,p′ DDE /g of lipid, 32.5 ng of p,p′, DDD/g of lipid, 85.1 ng of p,p′, DDT /g of lipid, and 838.7 ng of total DDT /g of lipid . These values were higher than the calculated for multipara mothers that presented contamination of p,p′-DDE = 238.2; p,p′-DDD = 31.5, p,p′-DDT = 52.1, and total DDT = 346.7ng/g of lipid.

Japanese data contamination of DDT and its metabolites (Kunisue et al., 2006) were lower than the found in the samples collected from Madeira River region for both premipara and multipara mothers. The values of contamination to primipara p,p′-DDT(13 ng/g of lipid), p,p ′-DDD (1.2 ng/g of lipid), and p,p′-DDE (330ng/g of lipid) and for multipara mothers the contamination of p,p′-DDT, p,p′-DDD, and p,p′-DDE were 10, 0.67, and 220 ng/g of lipid respectively. These values were lower than the obtained in the present study and a possible explication is that DDT was used in the Brazilian Amazon until the decade of 1990 in vector control of malaria. Another interesting study from Russia reports breast milk contamination with total DDT. The Russian mean concentrations of with total DDT (p,p′-DDE + p,p′-DDD + p,p′-DDT) in breast milk collected in Kargopol was 991 and 1065, in Severodvinsk were 1131 and 804, in Arkahangelsk were 1392 and 1086, and in Naryan-Mar were 1103 and 757 ng of total DDT/g of lipid for primipara and for multipara mothers respectively (Polder et al., 2003). These values, except the mean value for multipara mother from Naryan-Mar, were higher than the geometric means observed in milk samples collected in all localities from Madeira River region. Data of DDT contamination of primipara milk samples from Poland (Szyrwińska and Lulek, 2007) showed comparable mean values.

3.5. Correlations between total DDT in breast milk vs. age and vs. parity

It is reported that concentrations of organochlorine pesticides in human breast milk vary with factors such as age of the mother and parity (Harris et al., 2001; LaKind et al., 2001). No significant correlations were found to total DDT in function of age and parity in the present study. The correlation showed very low r^2 (0.0078 and 0.0614 respectively), the same observed by other authors (Kunisue et al. 2004; Minh et al., 2004). Tanabe and Kunisue (2006) considered in their work that the absence of correlations between organochlorine compounds levels and age/parity of the donor mothers living in Asian developing countries cannot be

clearly explained. The authors point as a possible reason that most woman in these countries may have many children during her life and the first infant is born at a young age of the mother, the same fact is observed in the mothers participating in the present study. In a study of organochlorine contaminants in 412 milk samples from Canada the authors observed low correlation between organochlorine levels and age of the mothers (Mes et al., 1993), the same observed in other works for organochlorine compounds and sons number (Vannuchi *et al.*, 1992; Spicer e Kereu, 1993).

3.6. Estimated infant daily intake

With the results for total DDT, it was calculated an estimative of the Infant Daily Intake. The individual values of *IDI* ranged from 0.00023 to 0.8322 and the geometric mean was 0.00329 mg of total DDT/g of body weight/day. According to the calculated values of the children 8.7% presented daily intake of the sum of DDT exciding the Tolerable Daily Intake (TDI) of 0.020mg of total DDT/kg of body weight proposed by WHO (1984). Polder et al., (2003) estimated the *IDI* of the DDT for Russian children. Their results were lower than the observed in the present study, and ranged from 0.004 to 0.007 mg of DDT/g of body weight/day, and no one child exceeded the TDI proposed by WHO.

4. Conclusions

According to the obtained results that shown considerable DDT contamination and some individual estimated *IDI* higher than the TDI proposed by WHO, it is important to establish of a systematic DDT monitoring program in breast milk from Madeira River Basin as well as to all of the other Amazon rivers, since this contaminant is pointed out as a endocrine disruptor and may have also a negative impact upon the development of the child nervous system. It is also important to do more research to investigate and develop other parameters which may be indicative of any disturbance caused by DDT in the exposed children. These data should be discussed by the overall Amazonian society, by the scientific community and Official Public Health organisms in order to help to construct decisions in the future use of the DDT in the Madeira River Basin, that is still presenting large numbers of cases of malaria each year together with other diseases transmitted by insect vectors. Nevertheless, despite of the obtained data showing the prevalence of DDT contamination, breast feeding should not be discouraged since it warrants the most complete nutrient supply to children.

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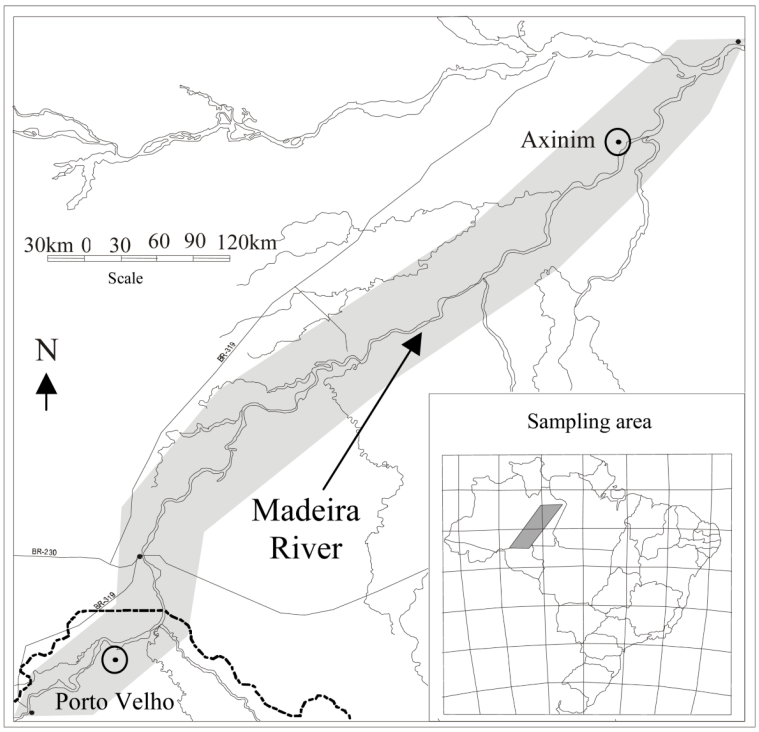

Figure 1.
Sampling area.

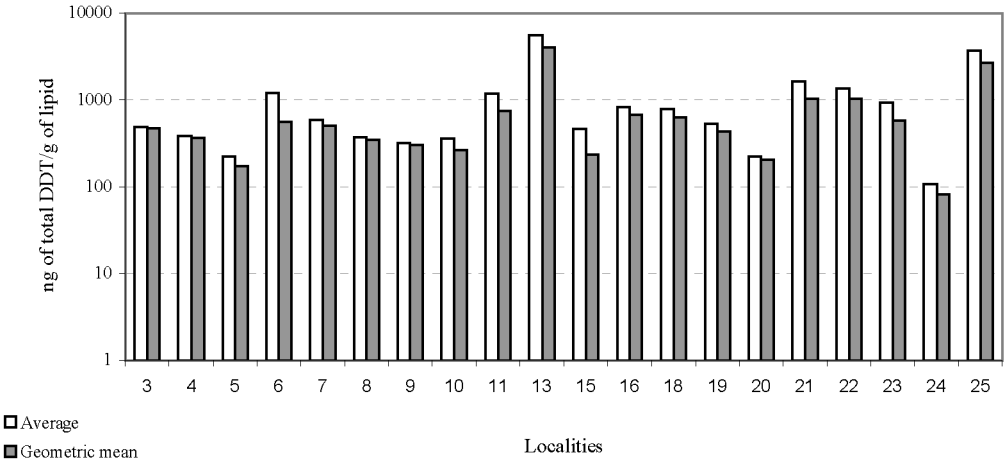

Figure 2.
Total DDT in different localities of the Madeira River.

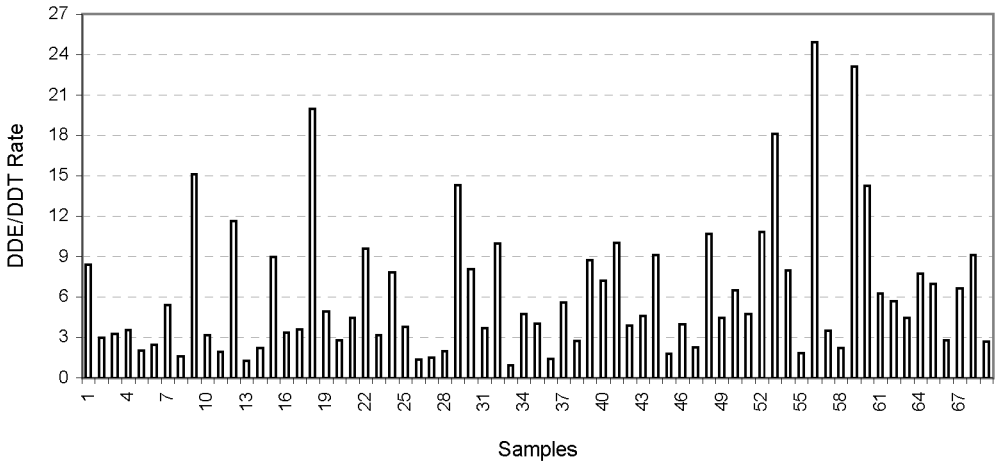

Figure 3.
p,p′-DDE/p,p′-DDT rates in individual milk samples from Madeira River.

Table 1

Age, parity, and number of the studied mothers.

Range of age	Parity		n
	Range	Mean ±SD*	
15 – 20	1 – 3	1.9 ± 0.70	11
21 – 25	2 – 8	4 ± 1.89	15
26 – 30	1 – 11	5.01 ± 2.67	16
31 – 35	1 – 12	7.43 ± 3.03	14
36 – 40	2 – 12	6.40 ± 4.16	5

* – SD: standard deviation

Table 2

Geometric means of the p,p′ DDT, p,p′ DDE. and p,p′ DDD and total DDT concentrations (values in ng / g of lipid), and number of samples collected in different localities.

Localities	n	p,p′ DDT	p,p′ DDE	p,p′ DDD	Total DDT
Auxiliadora	3	456.7	52.8	108.0	643.1
Boa Ventura	2	526.3	48.4	188.9	771.4
Bom Jesus	3	422.2	46.1	99.8	605.2
Cachoeirinha	6	1005.0	54.0	163.9	1005.0
Caiçara	2	421.9	37.5	110.8	615.1
Carapanatuba	2	513.4	18.6	42.7	588.4
Carará	4	266.5	37.0	43.4	376.3
Espírito Santo	4	206.7	26.7	38.8	284.8
Livramento	2	277.9	67.1	84.8	447.9
Moanense	5	216.9	26.8	34.5	433.8
Paquiquí	3	181.2	26.9	19.5	134.4
Porto Velho	4	460.6	27.3	88.7	619.4
Remanso	2	383.1	34.1	67.7	283.6
Rosarina	2	315.1	39.7	156.4	554.6
São Pedro	3	336.5	36.1	96.5	512.7
São Sebastião	4	222.1	14.7	28.3	272.7
Santa Rosa	2	91.1	14.5	12.4	118.3
Santo Antônio do Pau Queimado	4	103.1	28.8	30.4	178.6
Uricurituba	4	132.5	16.7	27.6	183.4
Val Paraíso	2	264.2	44.5	42.2	356.1

Table 3

Data of ng of DDT/g of lipid in some Countries.

Locality	DDT	DDE	DDD	Total DDT	Estimative	Reference
Brazil	72	343.4	42.1	492.8	Geometric mean	Present study
Brazil	2009.3	7262.7		9266.6	Mean	Lara et al., 1982
Brazil	0.12	2.53	0.03		Mean	Beretta and Dick, 1994
Brazil	0.07	0.32		0.39	Mean	Oliveira and Dores, 1998
China	390	2480			Mean	Wong et al., 2002
China		3330[a] 1916[b]			Geometric mean	Sun et al., 2005
Kazakhstan		1955 1500			Mean Median	Lutter et al., 1998
Thailand	169.4* 287.5*	69.4* 90.9*		208.8* 329.9*	Median Mean	Stuetz et al., 2001
Ucraine	822	2457			Median	Gladen et al., 1999
USA		217			Mean	Kostiniak et al., 1999

* - results originally in ng/mL of whole milk corrected to ng/g of lipid, adopting 3% of fat in milk.

[a] – mothers from urban area.

[b] – mothers from rural area.