Biol Trace Elem Res (2013) 151:30–37
DOI 10.1007/s12011-012-9542-2

Mercury Concentration in Breast Milk and Infant Exposure Assessment During the First 90 Days of Lactation in a Midwestern Region of Brazil

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Received: 30 August 2012 / Accepted: 31 October 2012 / Published online: 15 November 2012

Abstract Breast milk samples collected from 18 nursing mothers between the 15th and 90th day of lactation were digested in nitric acid in a microwave, and total mercury (THg) levels were quantified by atomic fluorescence spectrometry. Participants responded to a 24-h dietary recall questionnaire on the 74th and 76th day of lactation and to a Food Frequency Questionnaire querying the frequency of fish intake over the last 90 days. Usual intake was estimated using the PC-SIDE software package. A meal of fish was offered on the 75th day of lactation. Mothers' individual mean THg levels ranged from <0.76 to 22.7 ng/mL during the period, and the mean level for all samples (n=142) was 6.47±6.04 ng/mL. The multilevel mixed linear model used showed high heterogeneity of the mercury levels among the mothers, and THg levels did not change significantly over the period under study. However, a significant increase in THg levels was observed after the intervention with the fish meal. Exposure increased for most infants on the 90th day of lactation, with intakes exceeding the THg provisional tolerable weekly intake (PTWI) at least once during the period for 77.8 % of samples. Mothers consumed mostly food from the fat and grain groups, and a significant correlation was detected between consumption of food of these groups and breast milk THg levels (p=0.006 and 0.007). A significant correlation was also found between vegetable consumption and carbohydrate intake and THg levels in the samples (p= 0.015 and 0.045, respectively). No correlation was found between mothers' daily fish consumption frequency and THg levels. Although this study showed that mercury intake by infants during lactation may exceed the toxicologically safe exposure level (PTWI), we nevertheless believe that the benefits of lactation for both the mother and the infant outweigh the eventual risks that this exposure may represent.

Keywords Mercury · Breast milk · Food consumption · Infant exposure · Brazil

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Introduction

Breast milk is the most complete food in the human diet, providing almost all the necessary nutrients for the baby and protection against a variety of diseases, including diarrhea, acute respiratory infections, and otitis [1]. Exclusive breastfeeding during the first 6 months of life can reduce child mortality rates [2] and the chance of developing diabetes, hypertension, and cardiovascular diseases in adulthood [3–5]. However, milk is an excretion pathway in mammals and may contain toxic compounds to which the mother has been exposed, mainly from dietary sources [6].

Mercury is a neurotoxic element widely present in the environment from natural and anthropogenic sources [7]. Mercury concentrations in the water, sediment, and biota of the Brazilian Amazon are above global levels, largely from natural sources and natural biogeochemical processes [8]. Fish are considered the main vehicle of dietary exposure to mercury, with mean total mercury (THg) levels in predator fish in the Amazon region ranging from 0.1 to 0.8 μg/kg in most studies [9]. Similar levels have been found in predator cutlass fish (*Trichiurus lepturus*) captured on the Brazilian coast [10], but lower levels were found in farmed tilapia (up to 0.03 μg/kg [11]) and in sea catfish (*Cathorops spixii*) raised in estuaries in Southern Brazil (up to 0.39 μg/kg [12]). At least 90 % of THg found in fish was present as methyl mercury (MeHg) [13, 14].

Some studies conducted worldwide have shown that chronic exposure to MeHg, mainly during gestation and lactation, may impair fetus and infant neurodevelopment [15–17]. A provisional tolerable weekly intake (PTWI) for MeHg of 1.6 μg/kg BW for women of child-bearing age and a PTWI for THg of 5 μg/kg BW were established by the JECFA [18].

The consumption of fish has many beneficial effects on human health, being a source of high-quality protein, B vitamins, minerals, and long-chain polyunsaturated fatty acids, including pre-formed omega-3 fatty acids [19]. Fish intake is linked to lower risk of coronary heart disease and to better offspring neurodevelopment during pregnancy and lactation [19, 20]. However, exposure of the mother to MeHg could offset the benefits of fish-borne nutrients for nursing babies [21].

Mean levels of mercury in breast milk worldwide are mostly within the range of 0.5 to 7 ng/mL [22–26]. Factors that affect these levels include the mother's area of residence, active/passive smoking, anemia, and diet [22, 25–27]. In most studies, milk samples were collected from each mother on a single occasion during lactation.

The objectives of this study were to determine the levels of mercury present in breast milk samples collected over a 90-day period from nursing mothers in a low fish-eating population in Midwestern Brazil and to correlate these levels with the mothers' food consumption.

Material and Methods

Study Population

The study population was a sub-sample from a previous study which aimed to evaluate eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in breast milk samples of mothers who received a fish portion for consumption and who were enrolled in an exercise protocol. The women were contacted through private maternity clinics in Brasilia, Brazil, in 2002 and 2005 during their prenatal follow-up. Sixty-three mothers agreed to take part in the study, but 31 decided early on not to proceed. Of the 32 mothers who agreed to donate milk samples according to the project's protocol (over a 90-day lactation period, eight sampling days), only 18 completed the proposed tasks (at least four sampling days). Inclusion criteria were: between 20 and 40 years of age, body mass index before pregnancy between 20 and 30 kg/m^2, in good health, non-smokers, primiparous, exclusively breastfeeding, with a healthy infant born at term (38–41 weeks of gestation), and at least a high school level of education. Maternal body weights and heights were measured at the residence according to the standardized protocol [28]. Infant weights at birth were obtained from the clinics' records. Infant weights (naked) were measured at 30, 60, and 90 days by a trained person using a pediatric Sohlene scale (nearest 100 g). Whenever the weight of any infant at any time was not available, the value was estimated by adding the weight at birth to the mean weight gain for the period obtained for the other infants of the same sex.

The study was approved by the Ethics Committee on Human Studies from the University of Brasilia (Proj. 120/09), and all participants signed the consent form.

Breast Milk Samples

Breast milk samples (approx. 2 mL) were collected on the 15th, 30th, 45th, 60th, 74th, 75th, 76th, and 90th day of lactation by hand expression into verified metal-free glass vials on the morning prior (foremilk) and after (hind milk) breastfeeding. The participants were instructed to wash both hands and to clean the nipple with filtered water before collecting the samples. Samples were kept frozen in the mother's home freezer and transported in thermal boxes to the laboratory within a maximum of 15 days after collection, where they were maintained at −26 °C until analysis.

Each mother was asked to annotate the time spent breastfeeding for each sample collection point. The volume consumed by the infant was estimated assuming a milk flow of 13.5 mL/min [29]. The data were adjusted to not exceed 1,100 mL/day for children up to 30 days of age and 1,300 mL afterwards, according to the expected milk volume increase through lactation [29, 30]. When breastfeeding time information was not available, the volume was estimated based on the mean volume estimated for the other infants of the same sex, at each collection time.

Mercury Analysis

Prior to analysis, the samples were defrosted at ambient temperature, and the two samples collected from each mother for each day were combined and thoroughly homogenized. A 1-mL aliquot of the homogenized milk sample was transferred to a Teflon vessel, 2 mL of Suprapur nitric acid 65 % (Merck, USA) added, the system closed, and introduced into a Microwave DGT-100 (Provecto Systems, Brazil) for digestion. The digest was transferred to a 25-mL volumetric flask and diluted with Nanopure water (Ultrapure Water System, Barnstead). THg in the breast milk was quantified by atomic fluorescence spectrometry in a PSA 10.023 Merlin system (PS Analytical, Kemsig, Sevenoaks, UK) using a 2 % stannous chloride solution as a reduction agent. Duplicate aliquots of each homogenized sample were digested and analyzed. A 1,000 mg/L mercury standard solution (Merck, Germany) was used to prepare daily standard curves in water in the range of 0.01 to 0.2 μg/L (five points). The correlation coefficient for each curve was always greater than 0.99. The performance of the method was verified with certified skim milk powder reference material containing 9.4±1.7 ng/g THg (BCR®-150; Institute for Reference Material and Measurements, Belgium). The estimated uncertainty (0.94 ng/g, n=7) was within the acceptable limit provided by the manufacturer, with a coefficient of variation of 9.9 %. The limits of detection (LOD) and quantification (LOQ) were estimated based on the instrument response of a blank solution (B, n=10). LOD ($10\times B+3$ sd) was 0.26 ng/mL and LOQ ($10\times B+8$ sd) was 0.76 ng/mL.

Food Consumption

Participants of the study responded to a 24-h dietary recall questionnaire on the 74th, 75th, and 76th day of lactation. All data were carefully revised with each participant to ensure the accuracy of the dietary information. On the 75th day, a 200-g portion of ready-to-eat salmon (359 kcal, 17.5 g fat, 47.2 g protein, 0 g carbohydrate, 0.58 mg EPA, and 1.9 mg DHA) was given to each mother to be consumed during lunch. The dietary data were transferred to the Nutrition Data Systems for Research software (Nutrition Coordinating Center, University of Minnesota, USA). The usual intake was estimated after a within-person variability correction was applied to the data of the 74th and 76th days. The PC-SIDE (Iowa State University, version 1.0) software package was used to transform and back-transform the dietary data based on Nusser et al. [31]. A food frequency questionnaire (FFQ) was applied on the 90th day postpartum to obtain fish consumption information over the past 3 months, and the data obtained were used to estimate daily fish consumption frequency. Discrete values for the frequency of fish consumption were 0.011 (1 day out of 90 days), 0.033 (1 day out of 30 days), and 0.143 (1 day out of 7 days). We did not have information on the type of fish consumed by the population.

Mercury Intake and the Risks from Exposure

Mercury intakes for each infant were calculated for the 30th, 60th, and 90th day of lactation by multiplying the THg level found in the mother's breast milk on each day by the breast milk consumption (volume) per body weight estimated for that day. The risk from exposure to mercury at each point was estimated by comparing the intake with the THg PTWI (5 μg/kg BW or 7.1 μg/kg BW/day) and expressed as percent PTWI.

Statistical Analysis

SPSS version 20 was used for the non-parametric Spearman correlation tests between variables, and the significance level was set at $p<0.05$. Multilevel mixed linear model analysis using SAS 9.2 was used to evaluate the behavior of mercury levels in breast milk over time. Multilevel mixed linear model was applied to the entire period of investigation and specifically to the period after the fish intervention (74 to 90 days).

Results

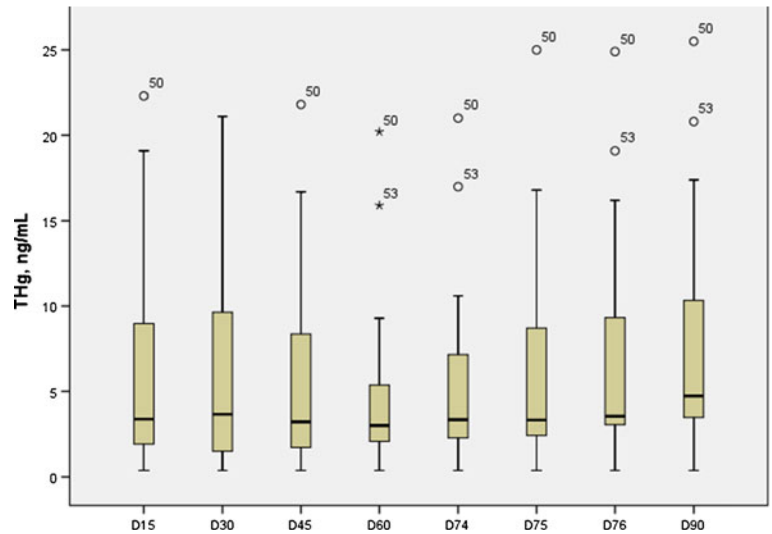
Mercury Levels in Human Milk and Infant Exposure

The participants of the study were 20–39 years of age and, on average, gained 15.3 kg during pregnancy. The infants weighed, on average, 3.3 kg at birth, gaining 2.5 kg during the first 3 months of life (Table 1). Half the infants were male.

Total mean mercury (THg) levels over the entire period (day 15 to 90 postpartum) for each mother ranged from <LOQ to 22.7 ng/mL, and the mean level for all samples (n=142) was 6.47±6.04 ng/mL (highest level of 25.5 ng/mL). Figure 1 shows the box plot of the data and the mean THg levels for the period considered. Multilevel mixed linear model analysis showed that although there was a slight tendency for THg levels to increase during the period, this was not significant, mainly due to the high heterogeneity of mercury levels among the mothers (initial mean log mercury level of 1.2, with an increase of 0.004 points at each time interval; p=0.09). When the analysis was applied to the period after the fish intervention, there was a significant smooth increase (initial mean log mercury level of

Table 1 Maternal and infant characteristics

	Mean±sd	Range
Maternal (n=17)		
Age (years)	27.3±5.0	20–39
Height (m)	1.63±0.06	1.54–1.75
Weight, post-gestation (kg)	80.4±8.8	69.6–100.1
Weight gain during gestation, kg	15.3±4.8	7.0–24.1
Infant, 50 % male		
Weight at birth, kg (n=18)	3.3±0.4	2.8–3.9
Weight gain (0–90 days), kg (n=14)	2.5±0.7	1.6–3.5
Breastfeed volume, mL		
30 days (n=17)	935±203	500–1,100
60 days (n=15)	1,103±171	660–1,300
90 days (n=13)	1,261±73	1,053–1,300



Mean: 6.66	6.03	6.02	5.31	6.01	6.52	7.29	7.89
Sd: 6.51	5.60	6.22	5.26	5.48	6.36	6.66	6.92

Fig. 1 Breast milk total mercury concentration (THg) in the first 90 days of lactation in Brasilia, Brazil. *Numbers* of the outliers correspond to the mother's identification: *severe outlier; °moderate outlier

0.44, with an increased 0.01 point at each time interval; p=0.004).

There was a significant correlation between maternal weight postpartum and THg at 15 days (p=0.018) and a marginal correlation (p=0.052) between maternal weight gain and THg at 15 days, but not between weight after 15–90 days and the respective THg levels. No significant correlation was found between infant weight at any time and respective THg levels, nor with infant weight gain during the first 90 days of life.

Figure 2 shows the daily levels of exposure to THg of the infants of the 18 participating mothers during the first 3 months of lactation, expressed as percent PTWI. Most infants (77.8 %) had intakes exceeding the PTWI (>100 %) at least once during the period. Seven infants had intakes exceeding the PTWI at the 30th, 60th, and 90th day of lactation (between 140 and 813 % PTWI).

Maternal Food Consumption and Nutrient Intake

Dietary information is shown in Table 2. The mothers mostly consumed food from the fat and grain groups (over seven portions/day), with a significant correlation between consumption and breast milk THg (p=0.006 and 0.007, respectively). A significant correlation was also found between vegetable consumption and THg (p=0.015). Carbohydrate, protein, and fat intakes during lactation were appropriate for all mothers [19]. Among macro and micronutrients, significant correlation was found only between mercury levels and carbohydrate intake (p=0.045).

The intake of DHA and EPA was low, reflecting low fish consumption by the studied population. FFQ data showed that one mother consumed no fish during the entire period of the study, eight mothers (47 %) consumed fish at least once during the 3-month period, and two consumed one to two

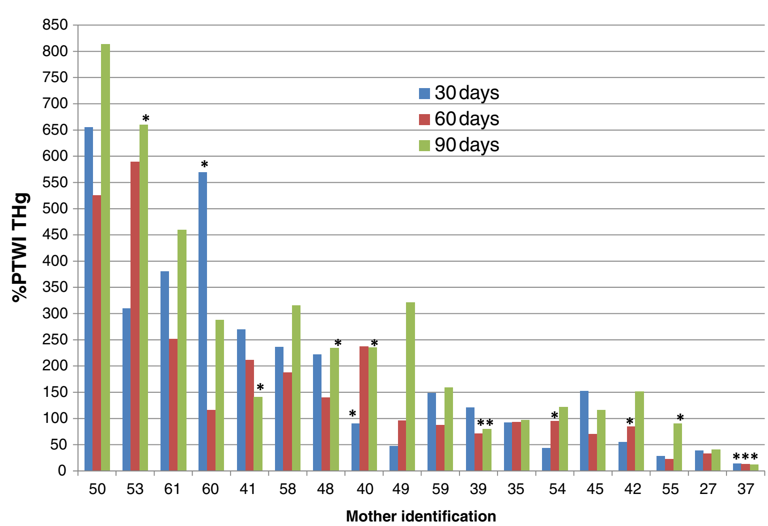


Fig. 2 Infant daily exposure level to THg during breastfeeding, expressed in percent of PTWI. The percent PTWI with an asterisk indicates that, due to the lack of information, the intake was calculated using body weight and/or milk volume estimated using data for the other infants of the same sex

Table 2 Usual food group consumption and macro and micro nutrient intake (per day) of 17 participants and their correlation with total mercury milk levels

	Mean	sd	Correlation	*p* value[a]
Fat, servings	7.9	2.7	0.595	0.006
Grains, servings	7.5	0.85	0.586	0.007
Fruits, servings	4.02	1.7	−0.185	0.222
Vegetable, servings	2.5	0.7	0.554	0.015
Energy, kcal	2,643	123	−0.194	0.228
Carbohydrate, g	334.4	40.0	0.424	0.045
Fat, g	105.8	8.5	0.093	0.361
Protein, g	93.5	4.3	0.068	0.398
Phosphorus, mg	1,396	221	0.110	0.337
Zinc, mg	11.5	2.8	0.037	0.444
Copper, mg	2.1	1.1	−0.012	0.481
Vitamin C, mg	156.7	19.9	−0.199	0.222
DHA, mg	0.06	0.04	−0.03	0.455
EPA, mg	0.03	0.03	−0.106	0.343
Fish frequency, times	0.100	0.14	−0.027	0.914

sd standard deviation, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

[a]One tail

meals with fish per month. Two mothers consumed fish three times per week, and four mothers normally consumed one to two meals per week containing fish. The mean daily fish consumption frequency was 0.1, with no correlation with THg levels in breast milk (Table 2).

Discussion

THg levels in breast milk from mothers living in Brasilia (Midwestern Brazil) were similar ($p>0.05$) to those found previously for the city (5.73±5.43 ng/g [24]) and in studies conducted in the Brazilian Amazon region (5.8±5.9 ng/g [32, 33]). These levels were higher than those found in Austria (1.59±1.21 μg/L [22]), Spain (0.45–0.62 μg/L [26]), Slovenia (mean of 0.3 ng/g, median of 0.2 ng/g [34]), Turkey (3.42±1.66 μg/L [25]), and Saudi Arabia (3.1±4.0 μg/L [23]). The higher THg levels found in Brazil are probably due to the naturally rich presence of mercury in Brazilian ecosystems in comparison with other countries [8, 9]. About 50 % of the THg levels in breast milk were found as MeHg by Orkarsson et al. [35], while Miklavcic et al. [34] found these to be, on average, 38 % ($P10=3$ %, $P90=70$ %), a much lower rate than found in mothers' hair (89 %) and in cord blood (94 %).

In this study, no significant changes were detected in mercury levels in the breast milk of most mothers during the 15–90-day postpartum period. According to Dorea [36], breast milk Hg concentrations are likely to be correlated with protein concentration and may be higher in colostrums, which was not available in the present study. Indeed, Drexler and Schaller [37] in Germany found higher levels of mercury in colostrums (mean of 1.37±2.14 μg/L) than in 60-day postpartum milk (0.64±1.46 μg/L). In Sweden, Björnberg et al. [38] found that THg in breast milk decreased significantly between 4 days (0.06–2.1 μg/L) and 6 weeks postpartum (0.07–0.37 μg/L), but remained unchanged thereafter (up to 13 weeks).

Various nutritional factors have been shown to modulate the toxicokinetics and dynamics of MeHg. In an extensive review of this topic, Chapman and Chan [39] reported that wheat bran fiber alters MeHg demethylation by intestinal flora in mice, affecting mercury reabsorption and excretion. Passos et al. [40] found that, for the same number of fish meals, Amazon riparians consuming fruit more frequently had significantly lower blood and hair Hg concentrations, suggesting a possible protective effect of the fruit. Our study found a significant positive correlation between breast milk THg and the consumption of grain, fat, and vegetable groups (but not with the fruit group). There was also a significant positive correlation with carbohydrate intake. Gundacker et al. [22] also found an association with cereal consumption. Mean background levels of mercury in cereals, grains, and vegetables are mainly between 1 and 6 μg/kg [41–43]. About half of the mercury found in rice grain, a staple food in Brazil, was shown to be present as MeHg in China [42]. Gundacker et al. [22] found that mothers with lower body weights (<60 kg) had significantly higher breast milk mercury levels than the others, while our study showed a positive correlation between maternal body weights and mercury levels.

THg levels in breast milk were not correlated with fish consumption in our low fish-eating population over the 3-month period. However, when the correlation analysis was restricted to the period 1 day before and after the fish (salmon) intervention (74–90th day), a significant increase in THg levels was observed, indicating that salmon consumption had an immediate impact on mercury levels in the excreted milk. In a study conducted in Austria with a population that consumes fish only occasionally, no correlation was found between fish intake and milk mercury levels [22]. Significant correlations, however, were found in high fish-eating populations, such as on the Faroe Islands [27], in Germany [37], and in Iran [44]. A study with a high fish-eating population in the Brazilian Amazon region showed that infant hair Hg was not significantly correlated with breast milk Hg, but was with maternal hair Hg, indicating that the placenta plays a greater role in Hg transfer than breast milk, even in cases of prolonged breastfeeding (1.5 years) [32]. Sakamoto et al. [45] reported that blood Hg levels in infants at 3 months of age were significantly lower than those at birth. Thus, exposure to mercury, especially high during gestation [15], indeed decreases during breastfeeding.

In the Faroe Islands cohort study, breastfeeding was not associated with deficits in neuropsychological performance measured for 7-year-old children who had a relatively high prenatal exposure to mercury [16]. Myers et al. [46] found several associations between postnatal MeHg biomarkers and Seychelles children's developmental endpoints. However, no consistent pattern of association emerged to support a causal relationship.

The nutritional benefits of fish consumption in high fish-eating populations need to be weighed against the possibility of adverse effects due to mercury exposure [18]. Grotto et al. [47] found an association between mercury levels in blood and hair and oxidative stress in Amazon communities, but they also found a beneficial effect from fish consumption regarding the same endpoint. In the USA, government authorities recommend that women of childbearing age, nursing mothers, and young children do not consume fish with high mercury content and not consume more than 340 g of other fish per week [48]. Lando et al. [49] found that the targeted groups of women were aware of the FDA/EPA guidelines; however, the mothers and their children may not be receiving the health benefits of eating a sufficient amount of fish, as nearly all of them consumed much less low-mercury fish per week than what is recommended. Our studied population also did not consume the recommended amount of fish, although the reasons are probably not related to the awareness of possible mercury contamination, as there are no national guidelines regarding this issue in Brazil.

This study had some strengths and limitations that should be addressed. The main strength regarded the number of samples taken from each mother during the period of the study, which allowed us to confirm that mercury excretion in milk 15 to 90 days postpartum does not change significantly. We were able to estimate individual volumes of milk consumed by the infants, leading to a more realistic estimation of the mercury intake during the period. In most published studies, this intake was calculated using a constant milk volume taken for all children.

A limitation of this study was the relatively low number of women that agreed to fully participate in the study. This was probably due to the many tasks required, including registering the time spent breastfeeding, collecting milk samples at specific points in time, and answering dietary diaries. We also highlight that the women who participated in this study belonged to a specific strata of the population (primiparous, aged 20 to 40 years, body mass index before pregnancy between 20 and 30 kg/m^2, and at least high school level of education) and cannot be taken as representative of the entire population of the Federal District. Another limitation is that the results of this study reflect the levels of THg in breast milk collected over 7 years ago (2002–2005). Although we have no evidence that mercury emission levels in the Federal District, either from anthropogenic or natural sources, have changed during this period, current levels in breast milk may have changed due to changes in the diet. Household budget survey data have shown that there has been a 30 % increase in the amount of fish acquired for consumption between 2002–2003 and 2008–2009 (5.5 to 7.1 g per capita/day) [50].

Conclusion

Mercury is a widespread element in nature, and human exposure is inevitable. Mercury levels in breast milk in the low fish-eating population investigated in this study were correlated with the consumption of fats, grains, and vegetables, frequent ingredients in their diet. Mercury levels were not correlated with fish consumption frequency during the 3-month study period, but a fish meal did have an impact on mercury levels for at least the following 15 days. Although some studies have shown that in the uterus exposure to MeHg may impair fetus neurodevelopment, this adverse effect has not been proven in infants as a consequence of exposure during lactation. This study has shown that mercury intake by infants during lactation may exceed the toxicological safe exposure level (PTWI). However, we also believe that the benefits of lactation outweigh the eventual risks that this exposure may represent.

Acknowledgments We would like to thank the CNPq (National Technological and Scientific Development Council) for providing a master's scholarship to L.R. Cunha. We would also like to thank Dr. Alisia L. Curriquiry for providing access to PC-Side used in the dietary analysis and Prof. Jurandir R. de Souza for his support during mercury analysis at the Laboratory of Analytical and Environmental Chemistry. We greatly appreciate the suggestions and comments provided by Prof.

Jose G. Dorea and the help of Prof. Eduardo Freitas da Silva with the statistical analysis.

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