Changes in children hair-Hg concentrations during the first 5 years: Maternal, environmental and iatrogenic modifying factors

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Abstract

Children are exposed to Hg from mothers (via placenta and lactation), environment (food), and in many parts of the world by thimerosal-containing vaccines (TCV) during immunization. Neurodevelopment studies based on infant hair-Hg (HHg) have been designed without explicit attention to the factors associated with changes in infant physiology and Hg sources of exposure. A longitudinal study of changes in HHg concentrations from birth to 5 years was done in a sample of children from Porto Velho (Rondonia), Brazilian Amazonia. The study extracted information from the asymmetry associated with maternal and infant HHg changes at specified sampling: birth (fetal exposure), 6 months of exclusive breastfeeding, 36 months (weaning) and 60 months (pre-school). The distribution of HHg in breastfed infants followed a pattern different from their mothers. While mothers had the highest HHg concentrations at childbirth, infants showed the highest HHg values at 6 months after the recommended full schedule (six shots) of immunization with TCV; after that, the downward trend in HHg shown by children coincided with both weaning and less frequent vaccination period (5 years). Extended lactation (up to 36 months) was not significantly associated with HHg of infants or mothers; however, significant association (Spearman’s r) between maternal and infant HHg concentration was seen at birth (r=0.3534; P=0.001), 6 months (r=0.4793; P<0.0001), 3 years (r=0.0122; P=0.012) and 5 years (r=0.0357; P=0.005). Maternal postpartum metabolic changes, infant development and transitional diets and possibly Hg from TCV contribute to the asymmetry of HHg changes between mothers and children.

Keywords: Vaccines; Thimerosal; Hair-Hg; Breastfeeding; Fish consumption; Amazon; Neuro-motor development

1. Introduction

Current studies associating Hg exposure and neurodevelopmental disorders (NDD) in children rely on the metal concentrations in hair. Hair is composed of a protein complex formed from amino acids that avidly binds to methyl-Hg (MeHg). The assumption is that the occurrence of NDD results from consuming fish contaminated with MeHg, which avidly binds to hair (Cernichiari et al., 2007). Indeed, epidemiologic studies dealing with MeHg exposure from fish consumption have used HHg concentrations as a reliable indicator. However, during the first years of life, infants can be exposed to Hg in their changing food sources: from breast milk (or formulas) to weaning foods, and at a latter age, from other dietary sources (that might include fish) when eating adult food. Young children can also be exposed to other forms of organic Hg (ethyl-mercury—EtHg) when immunized with thimerosal (Marques et al., 2007a).

Children of fish-eating populations of the Amazon Basin are exposed to maternal MeHg via placental transfer and breast milk (Barbosa et al., 1998; Barbosa and Dórea, 1998; Marques et al., in press). In this context, the increase in the risk of NDD has been strongly suggested to be associated with MeHg naturally present in fish from the Amazonian rivers (Grandjean et al., 1999). The possibility of
NDD raised by these studies has relied on HHg concentrations (biomarkers of fish consumption). Indeed, variability in HHg of riverine and Amerindian populations has been correctly assumed to be a function of fish consumption. Barbosa et al. (1995) have shown important differences in urine and HHg concentrations due to occupational exposure (gold miners) and fish consumption for native Amazonians. However, we have recently raised the possibility that, at least for the first 6 months of life, HHg concentrations may be influenced by the heavy immunization (with TCV) schedule of urban populations (Marques et al., 2007a). Indeed, during the infant’s first years, susceptibility to modifying factors remains unknown, but physiological and dietary changes (breast milk, weaning and habitual family diets) are likely to influence HHg changes.

Body retention of mercury and its hair uptake depend on dietary and physiological factors. In children, there are drastic physiological changes during the first years that affect the toxicokinetics of Hg. Mercury is taken up from the bloodstream during hair formation. Newborn hair essentially represents Hg exposure during fetal development. During the first 6 months, at least in breastfed infants, the origin of Hg is still maternal, but it is not through the fetal circulation but enteral through breast milk. Enteral exposure to Hg from weaning food has not been explored for fish-eating populations, but it is expected to be different from an adult’s diet. However, for children with access to health services, another source of Hg exposure has been unaccounted for in fish-eating populations, that is, immunization with TCV (Marques et al., in press).

Easy collection and preservation make the minimally invasive HHg sampling a method of choice in evaluating MeHg exposure from fish consumption. Indeed, HHg has made it possible to estimate fish consumption more reliably than with conventional dietary recall methods (Richardson and Currie, 1993; Gosselin et al., 2006); it has been conveniently applied in differentiating occupational and environmental (fish intake) exposures (Barbosa et al., 1995) and to overcome difficulties of communication involving cross-cultural studies of Amazonian Amer-Indians (Dorea et al., 2005) and isolated communities of riverine populations (Alves et al., 2006).

Ponce et al. (1998) compared Hg exposure estimates based on self-reported fish intake and measured fish-Hg concentrations and HHg concentrations; they showed bias and random error as components of uncertainty regarding HHg exposure estimates. Indeed, Canuel et al. (2006) demonstrated significant regional differences in HHg kinetics; therefore, generalization of HHg models needs to take into account not only the organic form of Hg and source of exposure but also physiological differences and genetic diversities of individuals within populations.

Both immunization (primary prevention) and breastfeeding (secondary prevention) share desirable health outcomes: prevention of infectious diseases (vaccines) and long-term health CNS and general health indicators warranted by breastfeeding. However, such health-related procedures are also the initial way infants and young children are exposed to Hg (Marques et al., in press). Infant routes of Hg exposure entail a variety of sources and a corresponding chemical species: placenta (MeHg), breast milk (MeHg and inorganic Hg), TCV (EtHg) and weaning foods (MeHg and inorganic Hg); each source of exposure is part of a unique set of events at specific time which influences substantially the toxicokinetics of Hg.

The underlying metabolic pathways attendant on the changing anatomical and physiological functions of early infancy, as well as the interactions among sources (Hg forms, dose and duration) make young children a heterogeneous population in respect to the toxicokinetics of Hg. Additionally, during this time there are special circumstances of intense iatrogenic exposure during the immunization with TCV that still prevails in some countries; these interactions are likely determinants of Hg retention in children’ hair. The data collected for this study were conceived for evaluating the neurodevelopment of children exposed to pre-natal exposure Hg from maternal fish consumption (Marques et al., 2007a). When the research project started we were not aware of the TCV issue in pediatric vaccines; this was brought up during the revision process of the parent publication (Marques et al., 2007a). Because we had all records of immunization we could conceptualize the assessment of thimerosal-Hg in the young children. Therefore, we took advantage of our cohort to study longitudinal changes in HHg of mothers and respective children from birth to key ages of 6, 36 and 60 months.

2. Materials and methods

2.1. Sample description

The city of Porto Velho, capital of the state of Rondonia (West Amazonia), has experienced significant demographic changes with people coming from many other Brazilian regions. During the last 30 years, it has changed from a traditional Amazonian city to one under the impact of heavy migration brought by agricultural projects in the south-west region and especially the influx of prospectors seeking alluvial gold along the banks of the Rio Madeira basin. The present population has both traditional families that base their diets on fish and starchy foods and city dwellers with more cosmopolitan food habits. In this changing environment, we investigated the health status of breastfed infants with special reference to food habits and possible Hg exposure due to fish consumption.

The research protocol was approved by the Ethics Committee for Human Studies of the Universidade Federal de Rondonia and details have already been published (Marques et al., 2007a,b).

2.2. Study protocol

Mothers were introduced to the study and invited to participate by a nurse during their routine visits to the Pre-natal Clinics of three hospitals in Porto Velho: Hospital de Base, Hospital Panamericano and Hospital Regina Pacis. One-hundred and sixty potential participants received plain-language information about the study and a written consent form was presented and signed by the 155 that agreed to participate—115 were eligible; the written consent stated that participation was voluntary, their
2.3. Hair-Hg analysis

Sample preparation and analytical procedures were done according to our standardized protocol for Hg determination in hair as described elsewhere (Marques et al., 2007a). Hair analysis was done on the first centimeter closest to root end. Sample preparation and Hg determination were done according to routine procedures previously established at the Universidade Federal do Rio de Janeiro (Bastos et al., 1998). We followed routine procedures after adaptation of analytical protocol used for Hg determination in previous studies. Briefly, the hair samples were comminuted with stainless steel scissors, weighed, and digested before analysis. Human hair samples were washed with EDTA 0.01%, dried in an oven at 50 °C, weighed and digested with 5 mL of HNO₃/H₂SO₄ (1:1) and 4 mL of 5% KMnO₄ using a digestion block at 80 °C for 40 min. The determination of total Hg in the digested samples was done by cold vapor atomic absorption spectrometry with a flow injection system-FIMS (CV-AAS, Perkin-Elmer—FIMS 400, Ueberlingen, Germany).

All glassware used in the analytical protocol was washed clean, rinsed with 5% EDTA and double distilled, and left to rest in 5% HNO₃ overnight. Then it was rinsed again in double distilled water, and dried at 100 °C for 12 h. Precision and accuracy of Hg determinations were assured by the use of internal standards, use of triplicate analyses of samples and certified reference materials (IAEA-085 and 086, Vienna, Austria) with recoveries of 92%. The limit of detection for the procedure was determined at <0.01 μg/g and there was hair-Hg determination below the limits.

2.4. Immunization vaccines and schedule

Infants up to 6 months of age received the full immunization scheme recommended by the Brazilian immunization program. After that time mothers were oriented about the importance of immunization and followed recommendations of available pediatric services. Among the vaccines taken by infants during the 5 years of the study some were preserved with 0.01% thimerosal (which metabolizes into EtHg). The Hg concentration of the doses delivered through vaccines was 25 μg Hg/0.5 mL. The immunization schedule with TCV and respective intake of Hg as stated by manufacturers is summarized in Table 1. These were: hepatitis-B (Korea Green Cross Corporation, Kijeung-Eup Yougoin-Goon Kiyunggi-Do, Korea; Euvax B injectable, LG Life Sciences, Jeonbuk-Do, Korea), diphtheria, tetanus and pertussis-DTP (Triple Antigen, Serum Institute of India Ltd, India; Vacina Triplice, Instituto Butanta, São Paulo, Brazil), Hib: Haemophilus influenzae type b (HibTITER®, Lederle-Praxis), Flu: influenza (VAXIGRIP, Pasteur Mérix Connaught, Sáo Paulo, Brazil). Children that were immunized against Hib within the study were comminuted against Hib within the first year (two doses) received the booster dose 6–12 months after the last dose, otherwise received only one dose in the second year of life. The recommendation for the Flu vaccine is to be taken between the ages of 6 and 35 months in two doses of 0.25 mL (1 month apart) and one single dose (0.5 mL) the following year; although the vaccines were taken the ages given represent approximations of the recommended date.

The exposure to EtHg derived from vaccines was based on the current national immunization program of the Ministry of Health of Brazil (Table 1). After 6 months only 20 of the 82 infants were given a fourth dose of DTP between 9 and 12 months. We estimated breast-milk Hg exposure from weight gain up to 6 months, when the infants were exclusively breast-fed, and discussed it elsewhere (Marques et al., 2007a).

2.5. Statistical analysis

Of the 100 original enrolled mothers, only complete data of 82 mother–infant pairs could be obtained at the end of the study (Marques et al., in press). The statistical packages contained in Excel and Prism were used for data summarization (means, standard deviation, changes in mean Hg concentrations) and correlation analysis. Repeated measurements analysis was run to test sampling effect on HHg on infant and maternal Hhg concentrations; this statistical analysis was performed using SAS release 9.1.3 (SAS Institute Inc., Cary, North Carolina). We accepted a value of <0.05 as statistically significant.

3. Results

A summary of the estimation of Hg forms in each of the exposure media (TCV and breast milk) that can contribute to HHg is shown in Table 1. During the first 6 months, infants received up to six shots of TCV (and were exposed to 150 μg Hg from thimerosal); the TCV corresponded to Hepatitis B and DTP; subsequently the immunization with TCV were for influenza and Hib with a coverage ranging from 2.5 to 25% of the children. Age of children corresponds to recommended vaccination schedules, but only 6-month-olds had the full schedule of immunization and the corresponding EtHg exposure. It is important to note that the exposure to TCV-Hg from the first vaccine taken at birth (0 day) is the highest and the most challenging dose; at this time the small body mass of neonates takes an impact equivalent to the double doses (of DTP and hepatitis B) at 6 and 12 months. The estimation of total Hg exposure from breast milk was possible only up to 6 months when mothers were closely monitored and psychologically supported to keep on with exclusive breastfeeding; indeed, most mothers (66%) breastfed for 12 months and some reported to breastfeed up to 60 months (Fig. 1). However, the effect of lactation on HHg concentrations of mothers (excreting Hg for 60 months) and children (absorbing Hg) over the 36 months is illustrated in Fig. 1: there was no statistically significant association between HHg and length of lactation, either for mothers or for children.

Table 2 summarizes HHg concentrations of children and mothers during the study period. The higher mean HHg at 6 months coincided with both Hg exposure from breast milk and the heavy vaccine schedule. Indeed, the distribution of HHg in infants followed a different pattern of their mothers (Fig. 2). After 6 months, exclusively breastfed infants receiving a full load of immunization (five types of vaccine, 150 μg Hg) showed an expansion of HHg concentrations greater than HHg at birth and at 3 and 5 years of age. HHg in their respective mothers showed the highest concentrations at childbirth, but a similar pattern at 6 months and 3 years later. Frequency of fish consumption (0–1 servings/week vs. compared to 2–7 servings/week) was presented and discussed previously (Marques et al.,...
in these urban mothers fish consumption depended on the species available at market (season and price are strong determinants).

Fig. 3 can be interpreted as difference in Hg transfer rates between placenta and mammary gland. The profile of ratios of mother:infant HHg clearly indicate that mothers’ decreases while infants’ increases. However, the association between maternal and infant HHg (Spearman’s $r$) illustrated in Fig. 4 was significant at all times: statistical significant association between maternal and infant HHg concentration was seen at birth ($r = 0.3534; P = 0.001$), 6 months ($r = 0.4793; P < 0.0001$); Spearman’s $r$ values at 3 years ($r = 0.0122; P = 0.012$) and 5 years ($r = 0.0357; P = 0.005$) although significant were low and indicative of less perfect correlations. Indeed, sampling time was sta-

Table 1
Children immunization schedule, type of vaccines, and corresponding EtHg intake (as Hg) during the first 60 months

<table>
<thead>
<tr>
<th>Age (months)$^a$</th>
<th>Vaccine</th>
<th>% coverage</th>
<th>Type$^b$</th>
<th>μg Hg/dose</th>
<th>Breast milk$^c$</th>
<th>μg Hg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>Hp-B</td>
<td>25.0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>Hp-B</td>
<td>25.0</td>
<td>30.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>DTP</td>
<td>25.0</td>
<td>44.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>DTP</td>
<td>25.0</td>
<td>91.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>DTP + Hp-B</td>
<td>50.0</td>
<td>111.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Hg injected

| 8                | 2.4     | Hib        | 25.0    | NE         |
| 10               | 8.5     | Hib        | 25.0    | NE         |
| 12               | 8.5     | Hib + Flu  | 50.0    | NE         |
| 15               | 25.5    | DTP        | 25      | NE         |
| 24               | 10      | Flu        | 12.5    | NE         |
| 36               | 5.0     | Flu        | 12.5    | NE         |
| 48               | 2.4     | Flu        | 25      | NE         |

NE, not estimated.

$^a$ All 82 infants had a full immunization schedule during the first 6 months; the fourth dose of DTP was taken after the first year only by 25.5%. The Hib and influenza vaccines taken after 6 months were at the recommended age by the specified percentage of the children; the age of immunization after 6 months are approximations based on current recommendations.

$^b$ Hp-B: Hepatitis B (0.01% thimerosal/dose. Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, 0.01% thimerosal [LG Life Sciences, Jeonbuk-do, Korea]; DTP (Serum Institute of India Ltd; Vacina Triplice, 0.01% thimerosal/dose [Instituto Butanta, Sao Paulo, Brazil]; Hib: Haemophilus influenzae type b (HibTITER®, 0.01% thimerosal/dose, Lederle-Praxis); Flu: influenza (VAXIGRIP 0.01% thimerosal/dose, Pasteur Mérieux Connaught, Sao Paulo, Brazil). Children that were immunized against Haemophilus influenzae type b (Hib) within the first year (two doses) received the booster dose 6–12 months after the last dose, otherwise received only one dose in the second year of life. The recommendation for the anti-flu vaccine is to be taken between the ages of 6 and 35 months in two doses (1 month apart) of 0.25 mL and one single dose (0.5 mL) the following year; although the vaccines were taken the ages given represent approximations of the recommended date.

$^c$ Integrated total Hg intake adapted from Marques et al. (2007a); we used the data of breast milk-Hg concentrations (adapted from Dorea (2004)) to estimate Hg exposure during breastfeeding: infant mean weight × mean daily breast milk consumption (140 mL/kg) × number of days × mean total Hg concentration in breast milk (1.9 μg/L).
tistically significant for mothers (<0.0001) but not for children (0.0987).

The distribution of HHg in infants followed a pattern different from their mothers. While mothers had the highest concentrations of HHg at birth, breastfed children experienced the highest HHg concentrations after the heaviest schedule (six shots) of immunization with TCV. Extended lactation (>36 months) was not significantly associated with changes in HHg of infants or mothers; both maternal and children HHg followed a downward trend.

4. Discussion

The profiles of distribution of maternal and infant HHg concentrations during the first 5 years revealed differences in type of Hg exposure. The chronology of infant hair sampling matched events associated with diet (that included breastfeeding) and immunization schedules. We found a clear trend in infant HHg coinciding with vaccine schedule but cannot prove a cause–effect relationship. The intercurrence of TCV-EtHg and the transitional weaning diets may have contributed to the pattern of infant HHg changes. Different cumulative distribution of HHg (mother:infant) ratios extracted information in response to short-term changes in source and dose of Hg exposure: the effects of the short-term but heavy schedule of immunization with TCV were likely modifying factors.

Based on HHg concentrations, the urban mothers in this study had a much lower consumption of fish than Amazonian “ribeirinho” women; we reported before that 57/82 of these mothers ate at most one fish meal a week showed a lower mean hair-Hg concentration than mothers that consumed more than 2 times a week (Marques et al., in press). HHg concentrations were lower than those observed for
“ribeirinho” women of the Rio Madeira in 1991 by Barbosa et al. (1998). Mean HHg concentrations of studies in fish-eating Amazonians have been reported to vary between 6.5 and 34.2 ppm (Barbosa et al., 2001); however, adjusting for inherent HHg variability or long-term studies of HHg changes (within individuals) are rare. Because of HHg variability, the effects of pregnancy and lactation may not impact the mean HHg deposition in Amazonian high fish eaters (Barbosa et al., 2001) but the relative changes (percent adjusted) within mothers show that it decreases during pregnancy (Barbosa et al., 1998); thus, in agreement with the present findings. The percent decline in maternal HHg concentrations observed in this and previous study (Barbosa et al., 1998) may reflect the metabolic changes associated with lactation which includes transfer of Hg to milk.

Events related to pregnancy and lactation accelerate maternal-Hg metabolism (Greenwood et al., 1978), but Hg transfer rates in utero (pregnancy) and ex utero (breast-feeding) differ depending on the organic (MeHg) or inorganic Hg form (Dorea, 2004). As discussed elsewhere (Dorea, 2004), both Hg forms are equally transferred through milk but MeHg is more readily transferred across the placenta than inorganic Hg. Indeed, inorganic Hg absorption through milk is not a significant source of Hg exposure to breastfed infants (Sandborgh-Englund et al., 2001). But, compared to inorganic Hg, Bjornberg et al. (2005) reported that MeHg seems to contribute more to infant exposure via breast milk; additionally, total Hg in breast milk decreased significantly from day 4 to 6 weeks, remaining unchanged thereafter (Bjornberg et al., 2005). Studies of lactating mothers (after the accidental poisoning in Iraq) showed that the milk-Hg was 5% of blood-Hg but the organic fraction of milk-Hg was only 3% of blood-MeHg (Bakir et al., 1973); this indicates that the placenta plays a greater role in Hg transfer than the mammary gland (Bjornberg et al., 2005). The HHg ratios clearly showed differences in Hg transfer rates between pregnancy and lactation (Fig. 3).

In the present study, the length of breastfeeding does not seem to be a significant determinant of HHg concentrations of mothers or infants, thus showing that the mammary gland is a more effective barrier for Hg transfer than the placenta. Indeed, we had showed that during lactation mothers increased HHg to pre-pregnancy levels (Barbosa et al., 1998). Studies relating length of breastfeeding with HHg concentrations of mothers and infants are rare and results of correlation analysis are not always concurrent. There are differences between populations: infants’ HHg and duration of breastfeeding were significant correlated in the Amer-Indians (of eastern Amazonia) but not in the “ribeirinho” of Rio Madeira studied by Barbosa et al. (1998).

The Hg found in hair of breastfed infants comes from both exogenous (breast milk) and endogenous (metabolic turnover of fetal tissues) sources; because of postnatal hair change and growth, the contribution of residual fetal tissue may become negligible at a certain age: nonetheless, HHg in breastfed infants (if immunized with thimerosal-free vaccines) originates from maternal sources. This close association between maternal and children HHg remains longer after breastfeeding: at weaning and at 5 years (Fig. 2). Irrespective of the level of HHg the mother–infant dyad remains in very close association; this indicates that dietary characteristics (type of fish or frequency of consumption) are strong within families (Fig. 2). These observations are in agreement with results obtained with high fish-eating population living at the banks of the Rio Madeira. Barbosa et al. (1998) reported a statistically significant correlation between “ribeirinho” mothers and breastfed infants’ HHg of older age. This significant correlation was reported by some (in Madrid—Gonzalez et al., 1985) but not by others (Fujita and Takabatake, 1977). Nevertheless, our finding of significant association between maternal and fetal hair is in agreement with studies in other parts of Amazonia (Mohan et al., 2005) and in low fish-eating mothers of other countries (Sikorski et al., 1986; Lindow et al., 2003).

The mean HHg values of our breastfed children are lower than the means reported for 1-year-olds of several Eastern Amazonian communities of the Rio Tapajós (Pinheiro et al., 2007). Although there are no studies of infant HHg (due to breastfeeding and TCV) our mean values (Table 2) are within HHg concentrations predicted from Redwood et al. (2001) TCVI-exposure model. All infants in our study received a substantial load of parenteral EtHg concentrated in the first 6 months; only a small percentage (2.5–25%) of children received an equivalent load (175 µg Hg) distributed over 42 months at a much older age (12–48 months) and larger body weight. The profile of the mother:infant HHg ratios indicates that the breastfed infants’ hair retains more Hg than the fetus (Fig. 4); however, since we do not have speciation of Hg chemical forms (EtHg or MeHg), it can not be solely attributed to TCV. Our findings suggest that the significant association between mothers’ and infants’ HHg at all sampling times reflect the family environmental exposure through fish-Hg.

Neonate metabolic pathways and attendant excretion rates of Hg vary according to stage of development mainly because of differences in gut development and bile secretion as well as source of Hg exposure. The wider distribution (greater variability) of infant HHg at 6 months can be attributed to the fact that in addition to Hg from breast milk, HHg values can be influenced by the EtHg breakdown from TCV, thus increasing organic Hg exposure. Predictive models of hair-Hg concentrations in infants have assumed low or no Hg excretion up to 6 months (Redwood et al., 2001). Therefore, positive Hg balance in infants contrasting with negative balance of mothers can be interpreted from Fig. 4. Nevertheless, additional research is necessary to understand the relationship between infant early development (weight gain and tissue differentiation), and differences in the metabolism of Hg from such distinct sources—Hg intrinsic to breast milk and parenteral EtHg from TCV. The physiological barriers that breast-milk Hg has to cross are bypassed by parenteral-administered
thimerosal-EtHg. This particular aspect of HHg change was discussed in the parent publication (Marques et al., 2007a).

After exclusive (up 6 months) and extended breastfeeding (36 months), infant mean HHg values declined toward the maternal values; this reflects both, decreased Hg exposure from weaning foods and a greatly reduced frequency of TCV-EtHg exposure (Table 1). Overall, the decrease in infant HHg during weaning was relatively less compared to maternal HHg; but the mean HHg of infants at 5 years is equivalent to maternal HHg, thus possibly reflecting exposure to the same dietary Hg sources. Since infants were exposed to higher doses of EtHg during the first 6 months, infant’s increase in HHg may result from higher transfer rates of EtHg-to-hair compared to subsequent sampling periods. HHg speciation that could differentiate MeHg from EtHg would be necessary to ascertain the origin of total HHg.

Fluctuation in maternal HHg concentrations has been an unverified event that might occur as a result of altered metabolism during pregnancy. Because the epidemiological evidence suggests a causal link between exposure to fish-Hg and NDD, avoidance of fish consumption during pregnancy and lactation has been warranted for NDD prevention (Ronchetti et al., 2006). The Harvard Center for Risk Analysis panel used maternal HHg to quantify the impact of pre-natal MeHg chronic exposure on infant cognitive development; the panel concluded that MeHg exposure sufficient to increase maternal HHg by 1 μg/g decreases intelligence quotients by 0.7 points (Cohen et al., 2005). Therefore, fluctuation in maternal HHg is an important issue with implications for public health policies.

The strengths of this study include its prospective design and the high rate of retention of mother–infant pairs at key chronological sampling: birth (fetal exposure), 6 months of exclusive breastfeeding, 36 months (weaning) and 60 months (pre-school); additionally we identified input of other organic Hg sources. However, our limitation in analytical capability to determine the TCV-EtHg in hair is an important constraint in interpreting these aspects of the results. A common problem faced by Hg toxic effects or susceptibility at sub-clinical levels is the difficulty of accurately reconstructing responses to sources after the exposure event. This is especially the case with young infants and accompanying changes in diets intertwined with attendant sources of Hg (which might include extrinsic EtHg) such as in the present study; additionally, there are ethical considerations related to breastfeeding and immunization that limit group (without such life-saving features) comparisons, thus making exposure studies uniquely difficult.

5. Conclusion

Unverified changes in maternal and infant HHg are important issues relating this marker to studies of exposure, effects and susceptibility. Maternal postpartum metab-