DDT and its metabolites in breast milk from the Madeira River basin in the Amazon, Brazil

Antonio Azeredo a, b, João P.M. Torres b, *, Márlon de Freitas Fonseca b, f, José Lailson Britto g, Wanderley Rodrigues Bastos c, Cláudio E. Azevedo e Silva b, Giselle Cavalcanti b, c, Rodrigo Ornellas Meire b, Paula N. Sarcinelli d, Luz Claudio e, Steven Markowitz e, Olaf Malm b

* Colegiado de Ciências Farmacêuticas, Departamento de Saúde, UFC, Brazil
b Laboratório de Radioisótopos Eduardo Penna Franca, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Brazil
c Laboratório de Toxicologia, Centro de Estudos da Saúde do Trabalhador e Ecologia Humana, Escola Nacional de Saúde Pública/FIOCRUZ, Brazil
f Laboratório de Biogeoquímica, UNIR, Campus, BR 364, Km 9.5 CEP: 78900-000, Porto Velho, RO, Brazil
g Laboratório de Radiobiologia, Centro de Estudos da Saúde do Trabalhador e Ecologia Humana, Escola Nacional de Saúde Pública/FIOCRUZ, Brazil
h International Training Program on Environmental and Occupational Health – Mount Sinai School of Medicine/Queens College, New York, USA
i Instituto Fernandes Figueira/FIOCRUZ, Av. Rui Barbosa, 716, Flamengo, Rio de Janeiro, RJ, Brazil
j Aquatic Mammals Laboratory, Oceanography Department, UERJ, Brazil (Rio de Janeiro State University, Av. São Francisco Xavier s/n, Maracana, Rio de Janeiro, RJ)

Abstract

Until the 1990s the 1,1,1-trichloro-bis-2,2'-4(chlorophenyl) ethane (DDT) was sprayed in the walls of the house along the Madeira River basin, Brazilian Amazon, a region well known for its large number of malaria cases. In 1910, Oswaldo Cruz described the presence of malaria in 100% of the population living in some localities from the Madeira River basin. Data available in the literature point to the DDT contamination in fishes captured in Madeira River region. Fish is the major source of dietary protein to these people. DDT tends to accumulate in lipid rich tissues and is being eliminated by different events, including lactation. Considering the importance of feeding breast milk to the children, 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 the samples 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, which was higher than the acceptable daily intake proposed by the WHO.© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Breast milk is the most complete source of nutrients (proteins, carbohydrates, fat and vitamins), immune factors, and other important constituents linked to the immune responses, including those related to the protection against infectious disease (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 (Sauer et al., 1994; Koopman-Esseboom et al., 1995; Patandin et al., 1999; Fitzgerald et al., 2001; Berlin et al., 2002).

The presence of DDT and its metabolites is widely studied in many parts of the world in environmental samples, biota and humans (Olea-Serrano et al., 2002). The interest in the DDT levels in many organisms and in humans is given by its adverse effects such as activity in estrogenic receptors (Guillette et al., 1995; Danston et al., 1997; Gillesby and Zacharenski, 1998; Hekstra et al., 2001), induction of spontaneous abortion (Hart et al., 1972; Palmer et al., 1973; Johnson et al., 1988; Korrick et al., 2001), and apoptosis in mononuclear cells (Teoburi et al., 1998; Perez-Maldonado et al., 2004).

Fish consumption is considered as 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 world (Harris et al., 2001; Torres et al., 2002 and D’Amato et al., 2004). 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 (Cruz and Sampaio, 1910). 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 Fig. 1. The milk samples were collected in wide mouth glass flasks previously decontaminated with acetone and n-hexane, and the milk samples were stored in the freezer until the 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 on the Prapamontol and Stevenson (1991) method with certain modifications. The milk samples were warmed in a hot bath at 37 °C before analysis until complete homogenisation. An aliquot of 1 ml was added to 5 ml of a solution containing ethyl acetate:acetone:methanol (1:2:2), homogenized by vortex mixing (1 min), ultrasonic bath (20 min), of a solution containing ethyl acetate:acetone:methanol (1:2:2), complete homogenisation. An aliquot of 1 ml was added to 5 ml samples were warmed in a hot bath at 37 °C before analysis until complete homogenisation. An aliquot of 1 ml was added to 5 ml samples were warmed in a hot bath at 37 °C before analysis until complete homogenisation. An aliquot of 1 ml was added to 5 ml samples were warmed in a hot bath at 37 °C before analysis until complete homogenisation.

Blanks were done in parallel and did not show the presence of peaks in their chromatograms. The recovery was performed using spiked samples of breast milk. The used extraction method presented the recovery of 88.4%, 102.6%, and 82.2% with the 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 blanks, and the obtained values for p,p′-DDE, p,p′-DDD, and p,p′-DDT were 0.0040 ng/ml, 0.0340 ng/ml, and 0.0040 ng/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 the individual samples. The calculation of the infant daily intake (IDI) was done according to Marien and Lafllame (1995) that proposed the following equation: IDI = (BMLC × MC × 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 in the terms MC, PMF, and BW were estimated as 1 kg/day, 4%, and 5 kg respectively, according to the adopted by Marien and Lafllame (1995). The estimated values were compared with the proposed limit of 0.020 mg 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 the meals by 98% of the interviewees. 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. An additional point that should be considered is the absence of pesticide use in the Amazonian agriculture and no mother had a 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 from the mothers of communities from Madeira River basin. Some characteristics of the studied mothers are shown in Table 1.

3.2. DDT status

The chromatograms showed the residues of DDT and its metabolites in all the analysed samples. The concentration ranged from

<table>
<thead>
<tr>
<th>Range of age</th>
<th>Parity</th>
<th>Mean ± SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–20</td>
<td>1–3</td>
<td>1.9 ± 0.70</td>
<td>11</td>
</tr>
<tr>
<td>21–25</td>
<td>2–8</td>
<td>4.2 ± 1.89</td>
<td>15</td>
</tr>
<tr>
<td>26–30</td>
<td>1–11</td>
<td>5.01 ± 2.67</td>
<td>16</td>
</tr>
<tr>
<td>31–35</td>
<td>1–12</td>
<td>7.43 ± 3.03</td>
<td>14</td>
</tr>
<tr>
<td>36–40</td>
<td>2–12</td>
<td>6.40 ± 4.16</td>
<td>5</td>
</tr>
</tbody>
</table>

* SD: standard deviation.
10.7 to 7271.5 ng/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. Fig. 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 from 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 the decrease in these compounds during its course was observed (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 in the concentrations of DDT in breast milk with age was observed in the previous studies (Stacey et al., 1985; Mussalo-Rahamaa et al., 1988; Dewally et al., 1996).

The \( p,p' \)-DDT and its metabolites \( p,p' \)-DDE and \( p,p' \)-DDD did not occurred independently in the samples. The average of \( p,p' \)-DDE/\( p,p' \)-DDT ratio was 6.3, and the individual values are shown in the Fig. 3. This fact suggests a non-recent use of DDT, the same observed by Gladden 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 the diet rich in fish, once considering the statement of Harris et al. (2001) 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 DDT contamination in fish captured in the Madeira River region previously described by Torres et al. (2002) associated to the high consumption of fish in the diet of the donors can explain the presence of DDT in the analyzed samples.

In 19 of the 20 localities presented, total DDT contamination ranges 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. Table 2 presents the geometric mean of the contamination expressed 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 values of DDT in breast milk doned by mothers from Guatemala in the decade of 1970 (Olszyna-Marzys et al., 1973) presented mean value 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 localities were 2150 (ranging from 411 to 11500), 4070 (from 1570 to 12210), and 1840 ng of total DDT/ml (from 342 to 4970 ng of total DDT/ml). The values of the individual samples of the Olszyna-Marzys et al. (1973) work were higher than those 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 and the donor mothers were from cotton culture areas, where DDT contamination presented higher values than those obtained in areas without cotton culture.
Table 2
Geometric means of the p,p'-DDT, p,p'-DDE and p,p'-DID and total DDT concentrations (values in ng/g of lipid), and the number of samples collected in different localities

<table>
<thead>
<tr>
<th>Localities</th>
<th>n</th>
<th>p,p'-DDT</th>
<th>p,p'-DDE</th>
<th>p,p'-DID</th>
<th>Total DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auxiliadora</td>
<td>3</td>
<td>456.7</td>
<td>52.8</td>
<td>108.0</td>
<td>643.1</td>
</tr>
<tr>
<td>Boa Ventura</td>
<td>2</td>
<td>526.3</td>
<td>48.4</td>
<td>188.9</td>
<td>771.4</td>
</tr>
<tr>
<td>Bom Jesus</td>
<td>3</td>
<td>422.2</td>
<td>46.1</td>
<td>99.8</td>
<td>605.2</td>
</tr>
<tr>
<td>Cachoeirinha</td>
<td>6</td>
<td>1005.0</td>
<td>54.0</td>
<td>163.9</td>
<td>1050.5</td>
</tr>
<tr>
<td>Caicara</td>
<td>2</td>
<td>421.9</td>
<td>37.5</td>
<td>110.8</td>
<td>615.1</td>
</tr>
<tr>
<td>Caranapanutuba</td>
<td>2</td>
<td>513.4</td>
<td>18.6</td>
<td>42.7</td>
<td>588.4</td>
</tr>
<tr>
<td>Cararé</td>
<td>4</td>
<td>265.5</td>
<td>37.0</td>
<td>43.4</td>
<td>376.3</td>
</tr>
<tr>
<td>Espirito Santo</td>
<td>4</td>
<td>206.7</td>
<td>26.7</td>
<td>38.8</td>
<td>284.8</td>
</tr>
<tr>
<td>Livramento</td>
<td>2</td>
<td>277.9</td>
<td>67.1</td>
<td>84.8</td>
<td>447.9</td>
</tr>
<tr>
<td>Moanense</td>
<td>5</td>
<td>216.9</td>
<td>26.8</td>
<td>34.5</td>
<td>433.8</td>
</tr>
<tr>
<td>Paquiu</td>
<td>3</td>
<td>181.2</td>
<td>26.9</td>
<td>19.5</td>
<td>134.4</td>
</tr>
<tr>
<td>Porto Velho</td>
<td>4</td>
<td>460.6</td>
<td>27.3</td>
<td>88.7</td>
<td>619.4</td>
</tr>
<tr>
<td>Remanso</td>
<td>2</td>
<td>383.1</td>
<td>34.1</td>
<td>67.3</td>
<td>283.8</td>
</tr>
<tr>
<td>Rosarina</td>
<td>2</td>
<td>315.1</td>
<td>39.7</td>
<td>156.4</td>
<td>545.4</td>
</tr>
<tr>
<td>São Pedro</td>
<td>3</td>
<td>336.5</td>
<td>36.1</td>
<td>96.5</td>
<td>512.7</td>
</tr>
<tr>
<td>São Sebastião</td>
<td>4</td>
<td>222.1</td>
<td>14.7</td>
<td>28.3</td>
<td>272.7</td>
</tr>
<tr>
<td>Santa Rosa</td>
<td>2</td>
<td>91.1</td>
<td>14.5</td>
<td>12.4</td>
<td>118.3</td>
</tr>
<tr>
<td>Santo Antônio do Pau Queimado</td>
<td>4</td>
<td>103.1</td>
<td>28.8</td>
<td>30.4</td>
<td>178.6</td>
</tr>
<tr>
<td>Uiracurituba</td>
<td>4</td>
<td>132.5</td>
<td>16.7</td>
<td>27.6</td>
<td>183.4</td>
</tr>
<tr>
<td>Val Paraíso</td>
<td>2</td>
<td>264.2</td>
<td>44.5</td>
<td>42.2</td>
<td>356.1</td>
</tr>
</tbody>
</table>

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 2610 ng of DDT/g in whole milk basis with an average value of 278 ng of DDT/g in milk. The 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. The values reported by Lara et al. (1982) were obtained before the prohibition of the DDT use in agriculture by the Brazilian Government (that occurred only in 1985), and were higher than the observed in the present study.

Paumgarten 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 1700 ng 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 higher level (534.3 ng of DDD/g of lipid) than that observed by Paumgarten et al. (2000). Other DDT levels around the world are shown in Table 3.

The geometric mean of the total DDT (p,p'-DDT + p,p'-DDE + p,p'-DID = 2100 ng/g of lipid) in milk samples donated by mothers living in urban area of São Paulo State, Brazil since 1979 to 1981 was 180 ng of DDT/g of lipid, which was lower than the observed in the present study. The mean value (2100 ng 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 the developed countries such as Sweden and Germany present a perceptible decrease in the DDT levels (Schade and Heinzow, 1998; Norén and Meironytė, 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 is not observed in Latin America by the 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 primipara mother were 698.8 ng of p,p'-DDE/g of lipid, 32.5 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 values for multipara mothers who presented the contamination of p,p'-DDE = 238.2; p,p'-DDT = 31.5; p,p'-DDT + 52.1, and total DDT = 346.7 ng/g of lipid.

Japanese data on the contamination of DDT and its metabolites (Kunisue et al., 2006) were lower than those found in the samples collected from Madeira River region for both primipara 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 (330 ng/g of lipid) and for multipara mothers the contamination of p,p'-DDT, p,p'-DDD, and p,p'-DDE were 10.67, and 220 ng/g of lipid, respectively. These values were lower than the obtained values in the present study and a possible explication is that DDT was used in the Brazilian Amazon until the decade of 1990 in the vector control of malaria. Another interesting study from Russia reports breast milk contamination total DDT. The Russian mean concentrations of with total DDT (p,p'-DDT + p,p'-DDE + p,p'-DID) in breast milk collected in Kargopol were 991 and 1065, in Severodvinsk were 1131 and 804, in Arkhangelsk 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 the milk samples collected in all the localities from Madeira River region. The data of DDT contamination of primipara milk samples from Poland (Szyrwińska and Lulek, 2007) showed comparable mean values.

Table 3
Data of ng DDT/g lipid in some countries

<table>
<thead>
<tr>
<th>Locality</th>
<th>DDT</th>
<th>DDE</th>
<th>DDD</th>
<th>Total DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>72</td>
<td>343.4</td>
<td>42.1</td>
<td>492.8</td>
</tr>
<tr>
<td>Brazil (prec.)</td>
<td>2009.3</td>
<td>7262.7</td>
<td>0.03</td>
<td>9266.6</td>
</tr>
<tr>
<td>Brazil</td>
<td>0.12</td>
<td>2.53</td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Brazil</td>
<td>0.07</td>
<td>0.32</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>China</td>
<td>390</td>
<td>2480</td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>3330</td>
<td>19166</td>
<td>194.6</td>
<td>5965.7</td>
</tr>
<tr>
<td>Kazakhstan</td>
<td>19.5</td>
<td>421</td>
<td>37.5</td>
<td>8124</td>
</tr>
<tr>
<td>Thailand</td>
<td>2313</td>
<td>4840</td>
<td>194</td>
<td>5965.7</td>
</tr>
<tr>
<td>Ukraine</td>
<td>822</td>
<td>2457</td>
<td></td>
<td>11225.7</td>
</tr>
<tr>
<td>USA</td>
<td>217</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results originally in ng/ml of whole milk corrected to ng/g of lipid, adopting 3.5% of fat in milk.

** Mothers from urban area.

*** Mothers from rural area.

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

It is reported that the 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 the function of age and parity in the present study. The correlation showed very low $r^2$ (0.0078 and 0.0614, respectively), the same was observed by other authors (Kunisue et al., 2004; Minh et al., 2004; Sudaryanto et al., 2005). Tanabe and Kunisue (2007) 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 women in these countries may have many children during their 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. Mes et al. (1993) analyzed 412 milk samples from Canada mothers and observed low correlation between age of the mothers and organochlorine levels. Vannuchi et al. (1992) and Spicer and Kereu (1993) reported low correlation when considered number of sons and organochlorine contamination in human milk.

3.6. Estimated infant daily intake

With the results for total DDT, an estimative of the infant daily intake was calculated. 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 a daily intake of the sum of DDT exceeding the tolerable daily intake (TDI) of 0.020 mg 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 results in the present study, and ranged from 0.004 to 0.007 mg of DDT/g of body weight/day, and no child exceeded the TDI proposed by WHO.

4. Conclusions

According to the obtained results it is important to establish a systematic monitoring program of DDT levels in breast milk from mothers of the Madeira River basin, as well as from the other basins in the Amazon region characterized by high fish consumption, since the DDT is pointed as an endocrine disruptor, and may also have a negative impact upon the nervous system development of the exposed lactent. 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.

Acknowledgments

The authors are indebted to the reviewers for their valuable comments that helped us very much to improve the manuscript. We need also to acknowledge the technicians of CESTH/FIOCRUZ Laboratory located at Rio de Janeiro, Brazil. Antonio Azeredo was supported by Brazilian Research Council (CNPq process n° 142118/2001–0), João P. M. Torres is Level II Research Fellow of CNPq and is Advance Selikoff Fellow at the Mount Sinai School of Medicine and Queens College in New York and is partially supported by Grant 1 D43 TW00640 from the Fogarty International Center of National Institute of Health of the United States of America (NIH-USA).

References


