FISH CONTAMINATION WITH DDT DUE TO MALARIA CONTROL IN THE BRAZILIAN AMAZON

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Introduction

The DDT, a term used to refer to Dichlorodiphenyltrichloroethane, is an organochlorine pesticide first synthesized in 1874, but its properties as insecticide were discovered only in the late 1930's by the chemist Paul Muller, who won the Nobel Prize in 1948. Since its discovery, DDT use revolutionized the control concepts against malaria and other tropical insect-borne diseases. A large-scale industrial production started in 1943 and it was used in great quantities mainly for the agricultural and forest pest control. A smaller quantity of the world production (20-30%) was used in tropical disease control[1]. In 1946 it was established a regular system of DDT applications in Amazon houses[2]. Its use became common in malaria vector control and other tropical diseases, like leishmaniasis. DDT began to be restricted after the discovery of its toxicity against wild animals, especially top predators and due to potential toxic effects against humans[3]. The DDT restrictive measures in Brazil started in 1971[4]. In 1985 DDT was prohibited for agricultural purposes, but continued to be use for Public Health Campaigns, under the responsibility of FUNASA, the Brazilian National Health Foundation[5]. An investigation conducted by Vieira *et al.*[6] detected $\sum$DDT in soil, sediments and chicken eggs from an area seven and nine years after its last application for leishmaniasis vector control near the sprayed sites. Today DDT is recognized as one of the twelve Persistent Organic Pollutants - POPs[7].

Concerning DDT in food, based on clinical observations as well as experimental animals, the annual Joint FAO/WHO Meetings on Pesticide Residues held in 2000[8] estimated a Provisional Tolerable Daily Intake (PTDI) for DDT in 0.01 mg/kg/day. Marien and Laflamme[9] have proposed a Tolerable Daily Intake (TDI) for breast feedings infants of 5 x 10^{-3} mg/kg/day, and conducted an assessment to evaluate the public health significance of eating ∑DDT contaminated fish, accomplished by establishing a daily intake level of DDT for the population of greatest concern, like breastfeeding infants. Their results indicated that mothers who frequently consume contaminated fish could have breast milk DDT concentrations highly enough to expose their infants to levels above the TDI. The aim of this study was to evaluate the ∑DDT (*o,p'*-DDT + *p,p'*-DDT + *o,p'*-DDE + *p,p'*-DDE + *o,p'*-DDD) levels in commercial fish samples from distinct Brazilian Amazon sites, which are consumed by the riverine populations, and to assess the potential health impacts from eating these fishes, especially for breastfeeding infants.

Materials and methods

Study Area: The sampling points are situated in Brazilian Amazon: The city of Boa Vista, capital of Roraima State, and along Madeira River and Tapajós River Basin and at the Balbina Lake. A lot of Amazonian edible species was bought on fish markets or from fishermen, without a specific division in food habits into the total group. There were carnivorous, omnivorous, detritivorous and herbivorous fishes, in a total of 31 edible species from Amazon Ecosystem.

Sampling and analysis: Fish samples were collected between October 1990 and May 2000 (n=105). The edible parts (muscle tissue) were frozen and transported to the Radioisotopes Laboratory of the Biophysics Institute of the Federal University of Rio de Janeiro, where they were freeze-dried. All samples were weighed before and after it in order to calculate the dry matter, based on the weight lost with the evaporated water. An extraction method for the analysis of organochlorine compounds in sediments[10] was adapted for fish samples. A Shimadzu Gas Chromatographer-14B with a ^{63}Ni electron capture detector (ECD) was used for the analysis.

Results and discussion

Tables 1 to 6 describes the results obtained in the fish samples analyzed according to place and the DDT concentrations (dry-weight, wet weight and lipid weight levels). On the Table 1 is the levels determined in a single sample, a carnivorous fish bought in a market from Boa Vista. Its level was sufficiently high to expose breastfeeding infants from fish consuming mothers. Table 2 gives the levels detected in Tapajós Rio Basin. This river presented largest levels than Madeira River, although in lipid weight basis the DDT levels were low. The city of Itaituba, whose samples were obtained in March 1991, presented the largest fish sample concentration of all work, showing 396.0 ng.$^{-1}$ (dry-weight) and 150.3 ng.g^{-1} (wet-weight).

Table 1. Sum of DDT in *Hoplias malabaricus* from Boa Vista, Roraima State, October 1991 (ng.g^{-1}).

Dry-weight level	Wet weight level	Lipid weight level (µg.g^{-1})
67.3	16.8	4.206

Table 2. Sum of DDT in fishes from Tapajós River Basin. (ng.g^{-1}).

Place	Dry-weight mean ± SD (Range)	Wet w. Mean ± SD	Lipid w. (µg/g)
Alta Floresta,Teles Pires R. Jan91, Sep93, Jan96 (n = 7)	105.7 ± 97.6 (18.8 – 286.5)	26.0 ± 25.5	1.435 ± 1.587
Itaituba, March 1991 (n = 4).	230.3 ± 127.9 (85.8 – 396.0[1])	73.4 ± 56.4	2.940 ± 1.405
Santarém, Aug.91[1]and Sep93[2] (n=7)	31.7 ± 13.6 (17.9 – 56.9)	7.9 ± 3.5	0.549 ± 0.249
Brasília Legal, Aug92 (n = 7)	28.8 ± 29.9 (4.0 – 79.9)	8.6 ± 11.9	0.291 ± 0.295
Rato River, Tapajós tributary Nov92 (n = 2)	16.2 (13-3 – 19.2)	3.5	0.250
Jacareacanga, March and Sep93 (n = 6)	26.4 ± 18.9 (11.2 – 57.0)	6.4 ± 4.6	0.580 ± 0.505
Tapajos river average	68.9 ± 91.1	19.3 ± 30.5	0.959 ± 1.208

Alta Floresta, located in the Teles Pires River tributary, presented the second largest contamination with mean DDT levels of 105.7 ± 97.7 ng.g^{-1} (dry-weight) and 26 ± 25.5 ng.g^{-1} (wet-weight). These two places presented DDT concentrations considered to dangerous.

Table 3 gives the DDT levels from the three Madeira River Basin places were fish samples have been collected in 1990, 1991 and 1993. Porto Velho, the capital of Rondonia State, presented the largest levels and the largest range. Observing the three places, the largest DDT levels were in fish samples collected upstream.

Table 3. Sum of DDT levels observed in fishes from Madeira River Basin, Rondonia and Amazonas States ($ng.g^{-1}$).

Place	Dry weight mean ± SD (Range)	Wet w. ($\mu g.g^{-1}$)	Lipid w. ($\mu g.g^{-1}$)
Manicoré, Oct90 (n = 1) [2]	32.5	9.9	0.471
Humaitá, Oct91 and Aug93 (n = 10) [3]	36.8 ± 21.1 (13.7 – 71.1)	8.4 ± 4.9	0.974 ± 0.857
Porto Velho, Oct91 and Aug93 (n = 12)	83.4 ± 92.0 (10.1 – 310.6)	21.2 ± 24.9	1.341 ± 1.244
Madeira river average	60.9 ± 70.7	15.2 ± 19.0	1.220 ± 1.082

[2] Sample composed from two fishes of the same specimens.
[3] One of the samples was composed by a pool of five fishes from the same specimen (*Serrasalmus sp*).

In Table 4, there are the DDT levels from the fish collected in Balbina Lake. In this place only two species were studied. None of the samples presented levels considered dangerous.

Table 4. Sum of DDT levels observed in fishes from Balbina Lake, March 1996 ($ng.g^{-1}$) (n=5)

Dry weight mean ± SD (Range)	Wet weight level	Lipid weight levels ($\mu g.g^{-1}$)
6.9 ± 6.4 (1.4 - 14.3)	1.6 ± 1.5	0.118± 0.089

Tables 5 and 6 shows the fish samples collected or bought in Municipal markets from cities along the Madeira River and fish samples bought in two markets of Itacoatiara, a city situated at the right Amazon River border, near Manaus.

Table 5. Sum of DDT observed in fishes from Madeira River, May 2002 ($ng.g^{-1}$)

Place	Dry weight mean ± SD (Range)	Wet w. (µg/g)	Lipid w. (µg/g)
Humaitá (n=2)	6.1 (ND - 12.1)	2.45	0.378
Novo Aripuanã (n=1)	14.9	3.02	0.466
Vila do Caiçara (n=4)	9.5 ± 18.2 (ND - 36.9)	2.0 ± 3.8	0.190 ± 0.312
Sto Antonio da B do Madeira (n=6)	2.1 ± 2.3 (ND - 5.4)	0.5 ± 0.7	0.028 ± 0.034
Nova Olinda do Norte (n=10)	1.4 ± 2.7 (ND - 8.4)	0.4 ± 0.8	0.037 ± 0.070
Madeira river average	6.1 ± 9.5	1.4 ± 2.0	0.117 ± 0.189

ND: Under the limit of detection

Table 6. Sum of DDT levels observed in fishes from Itacoatiara, Amazon River, May 2002 ($ng.g^{-1}$)

Itacoatiara (n=20)	4.9 ± 4.3 (ND - 17.8)	1.4 ± 1.3	0.077 ± 0.066

ND: Under the limit of detection

The DDT levels were significantly different from the levels detected in the other samples. Like in Balbina Lake samples, none of then presented concentrations considered dangerous.

The DDT wet-weight level of the samples, which are the real levels ingested by human consumers, is obtained multiplying the DDT dry-weight level dry matter percentage of the sample. It is necessary calculate this level to estimate the breast-feeding infants intake.

Menone and co-workers[11], analyzed organochlorines in tissues of silverside (*Odontesthes bonariensis*), a freshwater fish specie, from Mar Chiquita coastal lagoon, Argentina. The DDT mean levels in muscle was 1.7 ± 0.6 $ng.g^{-1}$ wet-weight (average 1.0-3.0 $ng.g^{-1}$), showing levels near to the observed by us in Balbina Lake and in the places analyzed in 2002. According to Batista and co-workers[12] fish is the most important source of animal protein in the Amazon region, with an average consumption rate up to 550 g day^{-1} for traditional populations like the riverines that have fish as a single source of protein. With the obtained results, it is possible to assess the mothers and breastfeeding infants exposure risk, based in the calculation of the three parameters proposed by Marien and Laflamme (1995)[8]:

1. Daily Intake Estimate for Mothers (MDI): MDI = (FC x FI)/BW: Where MDI = Mothers Daily Intake (mg/kg-day), FC = DDT Fish Concentration (mg/ kg), FI = Fish Daily Intake (kg). Accordingly to Batista[12], it is up to 0.550 kg, BW = Bodyweight for adult female (estimate of 60 kg). Example: Porto Velho fishes (n = 12), with an average sum of DDT concentration of 0.021 mg/kg.MDI = (0.0021 x 0.550)/ 60 = 1.95×10^{-4} mg/ kg/day. None of the samples had DDT contents that could surpass the FAO/WHO Tolerable Daily Intake of 0.01 mg/kg (2000). Consequently, the adults were free from any measurable risk.

2. Breast Milk Lipid DDT Concentrations (BMLC): The infant daily intake of ∑DDT depends on the concentration of these compounds in the breast milk of mother. Based on previous studies (Durhan *et al.* 1965)[13], Marien and Laflamme[9] estimated that breast milk lipid DDT concentration follows a linear algorithm: **ln BMLC = 0.7 ln (MDI x BW) + 3.** Example: ln $BMLC_{Porto\ Velho}$ = 0,7ln (1.92 x 10^{-4} x 60) + 3 = -0.11 Exp. (-0.11) = BMLC = 0.891 mg/ kg.

3. Infant Daily Intake (IDI): IDI =BMLC x MC x PMF)/BW: Where BMLC = Breast Milk Lipid Concentration of ∑DDT (mg/kg) as a function of the Mothers Daily Intake (MDI), MC = Consumption of breast milk (estimate of 1 kg/day), PMF= Percent milk fat in breast milk (we estimated in 3 %) and BW = Bodyweight for nursing infants (it was estimated in 4.5 kg). Ex: IDI Porto Velho = (0.891 x 1 x 0.03)/4,5 = 5.94 x 10^{-3} mg/kg/day, exceeding the proposed TDI of 5.0 x 1 0^{-3}. 1.06 x 10^{-3}, 0.80 x 10^{-3}, and 1.41 x 10^{-3} mg/kg (wet-weight levels). These fish levels are not sufficiently high to expose adults or children to ∑DDT levels considered dangerous.

On table 7 there are the localities where fishes had DDT levels sufficiently high to expose breast feed infants to dangerous levels of ∑DDT higher than 5.0 x 10^{-3} mg/kg/day Accordingly to Marien and Laflame[8]. The others samples collected between 1990 and 1996 did not present an average sum of DDT levels sufficiently high to expose the breastfeeding infants, having concentrations respectively of 9.94 x 10^{-3}, 8.45 x 10^{-3}, 7.91 x 10^{-3}, 8.56 x 10^{-3}, 3.54 x 10^{-3}, 6.40 x 10^{-3} and 1.65 x 10^{-3} mg/kg (wet-weight levels). And the samples of 2002 from Humaitá, Novo Aripuanã, Vila do Caiçara, Nova Olinda do Norte and Santo Antonio da Boca do Madeira, at Madeira River, and Itacoatiara, at Amazon River, presented an average sum of DDT levels respectively of 2.61 x 10^{-3}, 3.02 x 10^{-3}, 2.78 x 10^{-3}.`

Table 7. Estimated infant daily intake (mg/kg/day)

	Average fish flesh levels (mg/kg wet weight)	Infant Daily Intake
Itaituba (n=4)	7.34×10^{-2}	1.42×10^{-2} mg/kg/day
Alta Floresta, MT (n=7)	2.60×10^{-2}	6.85×10^{-3} mg/kg/day
Porto Velho (n=12)	2.12×10^{-2}	5.94×10^{-3} mg/kg/day
Boa Vista, RR (n=1)	1.68×10^{-2}	5.05×10^{-3} mg/kg/day

The IDIs for breastfeeding infants were respectively of 3.49×10^{-3}, 3.12×10^{-3}, 2.98×10^{-3}, 3.15×10^{-3}, 1.69×10^{-3}, 2.57×10^{-3}, 0.99×10^{-3}, 1.37×10^{-3}, 1.52×10^{-3}, 1.43×10^{-3}, 0.52×10^{-3}, 0.77×10^{-3}, and 0.89×10^{-3} mg/kg/day, indicating that they are under the proposed IDI of 5.00×10^{-3} mg/kg/day by Marien and Laflamme[9].

As can be observed, there was a reduction on ΣDDT levels in the environment along the years, like was observed by Bignert and co-workers in 1998[14] in Sweden. We can see in Madeira River a strong reduction on the ∑DDT levels, from a mean wet-weight value of 15.2 ± 19.0 $ng.g^{-1}$ in samples collected in 1990, 1991 and 1993, to just 1.4 ± 2.0 $ng.g^{-1}$ in 2002, indicating a reduction around ten. In the city of Humaitá, however, whose samples were obtained in the two periods, the samples presented a mean level (wet-weight) of 8.4 ± 4.9 $ng.g^{-1}$ in 1991 and 1993 (n=12), that was reduced to 2.6 $ng.g^{-1}$ (n=2) in 2002. Mean levels (wet-weight) in Tapajós Basin was 19.3 ± 30.5 $ng.g^{-1}$, 16.8 $ng.g^{-1}$ in the single sample from Boa Vista but only 1.6 ± 1.5 $ng.g^{-1}$ mean values in Balbina Lake, a number that approach to the detected levels in the year 2002, with the Amazon River place of Itacoatiara presenting mean levels of 1.4 ± 1.3 $ng.g^{-1}$. One possibility is because besides all the Balbina samples have been collected in 1996, little time after the other samples be collected, except one from Teles Pires River, this artificial lake is isolated from the other places.

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References

1. Turusov, V.; Rakitski, V.; Tomatis, L., (2002). *Env. Health Perspec.*. **110**, 125-128.
2. Wagley, C. 1953. *Amazon town*. Ed. The MacMillan Co. 305 p.
3. Oliveira Filho, A., M. 1997. Proc. Int. Wsp on Org Micropol in the Environment, Rio de Janeiro, Brasil. P. 1-3.
4. Brasil. Ministério da Agricultura. Portarias nº 356 e 357, de 13 de outubro de 1971.
5. Brasil. Ministério da Agricultura. Portaria nº 329, 2 de setembro de 1985.
6. Vieira, E. D. R.; Torres, J. P. M.; Malm, O. (2001). *Environ. Res*. **86**, 174-182.
7. UNEP: Obtained on Internet: http://www.chem.unep.ch/pops/
8. WHO: Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Geneva, 20-29 September 2000.Obtained on Internet: www.who.int/pcs/jmpr/2000%20recommendations.pdf
9. Mariën, K; Laflamme D, M. (1995). *Risk. Anal.* **15**, 709-717.
10. Japenga, J; Wagenaar, W. J.; Smedes, F.; Salomons, W. (1987). *Environ Technol. Lett.* **8**, 9-20.
11. Menone, M. L.; De Moreno, J. E. A.; Moreno, V. J.; Lanfranchi, A. L.;Metcalfe T. L. and Metcalfe, C. D. (2000). *Arch. Env. Cont. Tox.* **18**, 202-208.
12. Batista, V. S., Inhamuns, A.J., Freitas, C.E.C., Freire-Brasil, D. (1998). *Fish.Man.Ecol.* **5**, 419-435.
13. Durham, W. F.; Armstrong, J.F.; Quinby, G.E., (1969). *Arch. Environ. Healt.*.**18**, 641-647.
14. Bignert, A.; Olsson, M.; Persson, W; Jensen, S.; Zakrisson, S.; Litzen K.; Eriksson, U.; Haggberg, L.; Alsberg. T. (1998). *Environ. Poll*. **99**, 177-198.