

ASSESSMENT OF PCB CONTAMINATION IN FISH FROM *RIO GRANDE* RESERVOIR OF SÃO PAULO STATE, BRAZIL

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Introduction

Rio Grande Reservoir is one of the most important water bodies in the São Paulo Metropolitan Region. This reservoir was built in the 1920's and initially was one of the branches of the Billings Reservoir. For many years, its waters presented low quality due to contamination with organic sewage from the Metropolitan Region. In 1982, the Rio Grande Reservoir was separated from the Billings Reservoir in an attempt to preserve its water quality. Nowadays, Rio Grande Reservoir is used for drinking water supply, it is also used for leisure and fishing.

It is well known that the region presents Polychlorinated Biphenyl (PCB) contamination¹. In spite of its prohibition all around the world, historically PCBs were used in commercial mixtures such as Aroclors 1242, 1248, 1254 and 1260, these were probably the most used in Brazil, since the majority of them were purchased from "Monsanto"². The PCB commercially used in transformers, called Ascarel, is an oily product that contains 40-60%(w/w) of PCBs. Brazil established the prohibition of use and commercialization of PCBs in 1981, which was regulated by a ministerial ordinance (29/01/81)³.

Once these toxic contaminants reach the water surface, they may concentrate in suspended particulate, sediments, and bioaccumulate in fish⁴. Thus, fish monitoring serves as an important indicator of contaminated sediments and water quality problems. The previous use of PCBs in these areas (*Rio Grande*) and the possibility of unofficial use of these compounds coupled with fish consumption, indicates the necessity of chemical analyses to evaluate such contaminant in fish as part of the comprehensive water quality monitoring program of CETESB (São Paulo State Environmental Protection Agency).

This study is an attempt to evaluate the public health risk related to fish consumption from Rio Grande reservoir, since contaminated food is the main intake route for such compounds like PCBs⁵.

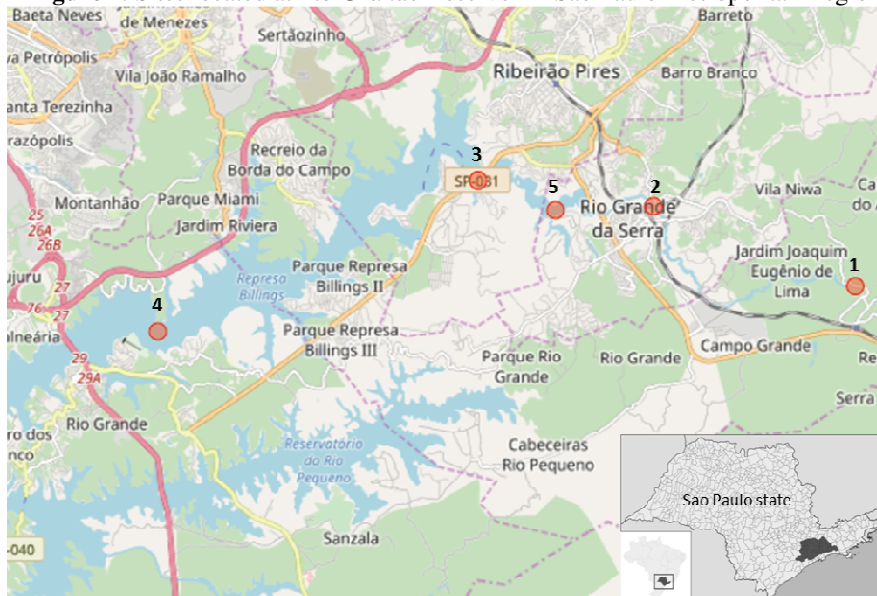
Materials and methods

Fish samples were collected in several sites (figure 1) located in the reservoir *Rio Grande* in three different years (2009, 2015 and 2016). Fish species were caught using different types of nets in order to sample a large variety of species and reflect the community that occupies the studied sites. Fish muscles and visceral material were separated before analysis.

Samples were extracted by ultrasonic extraction (U.S EPA method 3550C)⁶ or using a tissue homogenizer⁷. All the solvents used were organic residue analysis grade. Samples were spiked with surrogate before extraction; TMX and PCB 209 were used with microwave extraction and PCB-198 for tissue homogenizer. The extracts were purified in a mixed column with neutral and acid/basic silica and in some cases were used a second column with florisil, the PCBs were eluted with n-hexane. For each batch a Quality Control Analysis was performed, such as sampling in triplicate, one of the samples being spiked with a standard solution of PCBs, blank, blank spiked with PCBs and reference sample from NIST SRM1946 or NIST SRM1947 were also performed.

The final extracts from 2009 campaign were analyzed in a double electron capture detector gas chromatograph, Agilent model 7890 (GC/ECD). The GC was fitted with a VF-XMS (60m x 0.25mm x 0.25µm). The results were confirmed in the second detector fitted with a CP-Sil 8CB capillary column (60m x 0.25mm x 0.25µm). The final extracts from 2015/2016 campaign were analyzed in a triple quadrupole mass spectrometer, Agilent model 7000 (GC/MS/MS) fitted with a SLB-5MS (60m x 0.25mm x 0.25 µm). The PCB congeners (28, 52, 101, 118, 138, 153 and 180) were determined.

Figure 1. Sites located at *Rio Grande* Reservoir – São Paulo Metropolitan Region (Brazil)



Results and discussion

Among 187 fish samples, three species were selected to be evaluated in relation to risk consumption: *Astyanax* sp, *Hoplias malabaricus*, *Rhamdia quelen*. The choice was based on the different eating habits and habitats of these species, and to the fact that the local population may consume them. PCB results measured in the muscles and visceral material are shown in Table 1.

Table 1. Concentration of PCB Σ (PCB 28, 52, 101, 118, 138, 153 and 180) in fish species ($\mu\text{g/kg}$ wet weight)

Fish species		Site 1*	Site 2*	Site 3*	Site 4*	Site 5 (2015)	Site 5 (2016)
<i>Astyanax</i> sp	Muscle	25.21	57.82	72.92	NC	NC	NC
	Viscera	89.76	343.96	471.79	NC	NC	NC
<i>R. quelen</i>	Muscle	NC	342.00	275.45	8.16	12.99	25.09
	Viscera	NC	350.20	545.70	NA	68.39	91.41
<i>H. malabaricus</i>	Muscle	NC	76.43	65.00	1.68	3.39	9.94
	Viscera	NC	2587.00	1065.70	85.92	65.08	54.58

Note: NC = Specie not caught in this site; NA = Not analyzed; *2009

The collected organs were: liver (hepatopancreas in some fishes), kidney and spleen, they reflect not only the latest body metabolism, but also the beginning of the processes of bioaccumulation and biomagnification of contaminants. Concentration found in visceral material was much higher than that found in muscle, indicating a recent contamination, except for *R. quelen* in site 2, in which it was detected at almost the same level in both tissues. *H. malabaricus*, a carnivorous species, tends to present higher bioaccumulation in the visceral material than in the muscles, up to, approximately, 15 times higher in the visceral material than in the muscle in Site 3. Although, due to its small size, *Astyanax* sp may be consumed as whole, including head, bones and viscera, usually, only the muscle of *Rhamdia quelen* (catfish) and *Hoplias malabaricus* (trahira) is prepared for cooking.

Risk evaluation of fish consumption

To evaluate the health risk through consumption of fish, the maximum allowable fish consumption rate (CRLim) were estimated for three groups of population - general population (adults), children from 1 to 4 years old, and children 5 up to 11 years old - for both carcinogenic and non carcinogenic effects, by means of equations 1 and 2,

respectively. For the calculation, the concentration sum of PCB congeners in the muscles of the sample fish analyzed (C_{PCB}) was used, Table 1.

$$CR_{lim}(Kg / d) = \frac{ARL \times BW}{CSF \times C_{PCB}} \quad (\text{Equation 1})$$

$$CR_{lim}(Kg / d) = \frac{RfD \times BW}{C_{PCB}} \times RSC \quad (\text{Equation 2})$$

Where:

The average body weights are: adult = 70 kg (USEPA, 2000)⁸, children between 1 to 4 years old = 14.4 kg and 5 to 11 years old = 26.4 kg (Health Canada, 2007)⁹.

Cancer slope factor (CSF): 2 per mg/kg-d (USEPA, 1997)¹⁰

Maximum acceptable cancer risk level (ARL): 1 in 100000 (10^{-5})

Reference dose for PCBs (RfD): 2×10^{-5} mg/kg-d (USEPA, 1996)¹¹

Relative source contribution (RSC): 50% (Voorspoels et al., 2008)¹²

Table 2 presents the daily consumption limits for the three considered groups.

Table 2. Daily fish consumption limits for three groups of population (kg/d)

			Daily fish consumption limit (kg/d)					
Site	Fish species	PCB (µg/kg)	Limit per Day (kg/d)			Limit per Day (kg/d)		
			Carcinogenic effects			Noncarcinogenic effects		
			Gen	Children (1 - 4 yrs)	Children (5 -11 yrs)	Gen	Children (1 - 4 yrs)	Children (5 -11 yrs)
1	<i>Astyanax</i> sp	25.21	1.39x10 ⁻²	2.86x10 ⁻³	5.24x10 ⁻³	2.78x10 ⁻²	5.71x10 ⁻³	1.05x10 ⁻²
2	<i>Astyanax</i> sp	57.82	6.05x10 ⁻³	1.25x10 ⁻³	2.28x10 ⁻³	1.21x10 ⁻²	2.49x10 ⁻³	4.57x10 ⁻³
	<i>R. quelen</i>	342.00	1.02x10 ⁻³	2.11x10 ⁻⁴	3.86x10 ⁻⁴	2.05x10 ⁻³	4.21x10 ⁻⁴	7.72x10 ⁻⁴
	<i>H. malabaricus</i>	76.43	4.58x10 ⁻³	9.42x10 ⁻⁴	1.73x10 ⁻³	9.16x10 ⁻³	1.88x10 ⁻³	3.45x10 ⁻³
3	<i>Astyanax</i> sp	72.92	4.80x10 ⁻³	9.87x10 ⁻⁴	1.81x10 ⁻³	9.60x10 ⁻³	1.97x10 ⁻³	3.62x10 ⁻³
	<i>R. quelen</i>	275.45	1.27x10 ⁻³	2.61x10 ⁻⁴	4.79x10 ⁻⁴	2.54x10 ⁻³	5.23x10 ⁻⁴	9.58x10 ⁻⁴
	<i>H. malabaricus</i>	65.00	5.38x10 ⁻³	1.11x10 ⁻³	2.03x10 ⁻³	1.08x10 ⁻²	2.22x10 ⁻³	4.06x10 ⁻³
4	<i>R. quelen</i>	8.16	4.29x10 ⁻²	8.82x10 ⁻³	1.62x10 ⁻²	8.58x10 ⁻²	1.76x10 ⁻²	3.24x10 ⁻²
	<i>H. malabaricus</i>	1.68	2.08x10 ⁻¹	4.29x10 ⁻²	7.86x10 ⁻²	4.17x10 ⁻¹	8.57x10 ⁻²	1.57x10 ⁻¹
5 (2015)	<i>R. quelen</i>	12.99	2.69x10 ⁻²	5.54x10 ⁻³	1.02x10 ⁻²	5.39x10 ⁻²	1.11x10 ⁻²	2.03x10 ⁻²
	<i>H. malabaricus</i>	3.39	1.03x10 ⁻¹	2.12x10 ⁻²	3.89x10 ⁻²	2.06x10 ⁻¹	4.25x10 ⁻²	7.79x10 ⁻²
5 (2016)	<i>R. quelen</i>	25.09	1.39x10 ⁻²	2.87x10 ⁻³	5.26x10 ⁻³	2.79x10 ⁻²	5.74x10 ⁻³	1.05x10 ⁻²
	<i>H. malabaricus</i>	9.94	3.52x10 ⁻²	7.24x10 ⁻³	1.33x10 ⁻²	7.04x10 ⁻²	1.45x10 ⁻²	2.66x10 ⁻²

Note: "Gen" refers to general adult population

The daily consumption limit of fish can be also expressed as number of meals a month ($T_{ap} = 30.44$ days). Therefore, the limits presented in the table 2 were converted into number of meal (CR_{mm}) for the three population groups (Table 3). Assuming that a meal based on fish is equivalent MS (Meal Size) to 0.227 kg, portion adopted by USEPA. The number of meals was obtained by equation 3:

$$CR_{mm}(\text{meals} / \text{month}) = \frac{CR_{lim} \times T_{ap}}{MS} \quad (\text{Equation 3})$$

Table 3. Monthly Fish Consumption Limits for the three population groups

Site	Fish species	PCB (µg/kg)	Number of fish meals (meal/month)					
			Carcinogenic effects			Noncarcinogenic effects		
			Gen	Children (1 - 4 yrs)	Children (5 -11 yrs)	Gen	Children (1 - 4 yrs)	Children (5 -11 yrs)
1	<i>Astyanax</i> sp	25.21	1.86	0.38	0.70	3.73	0.76	1.40
2	<i>Astyanax</i> sp	57.82	0.81	0.17	0.31	1.62	0.33	0.61
	<i>R. quelen</i>	342.00	0.14	0.03	0.05	0.27	0.06	0.10
	<i>H. malabaricus</i>	76.43	0.61	0.13	0.23	1.23	0.25	0.46
3	<i>Astyanax</i> sp	72.92	0.64	0.13	0.24	1.29	0.27	0.49
	<i>R. quelen</i>	275.45	0.17	0.03	0.06	0.34	0.07	0.13
	<i>H. malabaricus</i>	65.00	0.72	0.15	0.27	1.44	0.30	0.54
4	<i>R. quelen</i>	8.16	5.75	1.18	2.17	11.50	2.36	4.34
	<i>H. malabaricus</i>	1.68	27.89	5.75	10.54	55.91	11.49	21.05
5 (2015)	<i>R. quelen</i>	12.99	3.61	0.74	1.36	7.23	1.49	2.73
	<i>H. malabaricus</i>	3.39	13.84	2.85	5.22	27.69	5.70	10.44
5 (2016)	<i>R. quelen</i>	25.09	1.87	0.38	0.71	3.74	0.77	1.41
	<i>H. malabaricus</i>	9.94	4.72	0.97	1.78	9.44	1.94	3.56

Note: "Gen" refers to general adult population

Table 3 shows the monthly number of meals that can be consumed by the three population groups without risk to the health. Since most of the numbers of meals that may be safely eaten is so low, we concluded that there is a potential risk in consuming all species analyzed. The risk assessment results gave support to the decision makers of Health and Environment Secretariat for an integrated management of this area. Further studies are necessary for a better understanding of the contamination level and evolution in this region.

Acknowledgements

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