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Mercury and Methylmercury in the children hair and fish mostly consumed in Cubatão, São Paulo State, Brazil

Mercúrio e Metilmercúrio em cabelos de crianças e em peixes mais consumidos em Cubatão, estado de São Paulo, Brasil

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ABSTRACT

This study evaluated Hg and MeHg concentrations in fish muscle and children hair from Cubatão, using as strategies: i) Hg and MeHg determinations in the mostly consumed fish species, ii) Hg and MeHg determinations in 93 children hair samples (from 04 to 14 years old), and iii) analysis on the children diet consumption questionnaire answered by the respective parents. The results obtained in a similar study in Cananeia were used as references. These two regions show different environmental anthropogenic impacts and distinct eating habits. The quantity and the frequency of fish consumption were the most important factors of Hg bioaccumulation in children hair. In both cities, 95 % of mercury contents in hair were >1 $mg.kg^{-1}$ (US EPA reference) in analyzed children, and MeHg were between 67 and 83 % of total Hg. The median for total Hg concentration in fish muscle (wet basis) ranged from 10 to 179 $\mu g.kg^{-1}$ for Cananéia and 10 to 181 $\mu g.kg^{-1}$ for Cubatão, values lower than the Brazilian limits for Hg in fish. Considering the Hg contents detected in the mostly consumed fish and hair, they indicate that the children are not at risk for Hg and MeHg contamination.

Keywords. mercury, methylmercury, children, hair, fish, food habit.

RESUMO

Este estudo avaliou a concentração de Hg e MeHg em amostras de peixes e de cabelos de crianças de Cubatão, usando-se como estratégia: i) Hg total e MeHg em peixes mais consumidos; ii) Hg total e MeHg em cabelos de 93 crianças (04 a 14 anos); iii) análise do inquérito alimentar respondido pelos pais. Os dados obtidos em estudo similar realizado em Cananéia foram usados como referência. Essas duas regiões estuarinas, sob diferentes graus de impacto ambiental, apresentam diferentes hábitos alimentares. A quantidade e a frequência de consumo de peixes foram os fatores mais importantes na bioacumulação de Hg nos cabelos. Em ambas as cidades, 95 % das concentrações de Hg total nos cabelos foram abaixo de 1 $mg.kg^{-1}$ (limite US EPA) e o percentual de MeHg ocorreu entre 67 e 83 % em relação ao teor de Hg total. As medianas para Hg total nos músculos (base úmida) variaram de 10 a 179 $\mu g.kg^{-1}$ para peixes de Cananéia e 10 a 181 $\mu g.kg^{-1}$ de Cubatão, inferiores ao limite da legislação brasileira para Hg. A partir dos teores para Hg total e MeHg em peixes mais consumidos e cabelos, as crianças parecem não estar em risco de contaminação.

Palavras-chave. mercúrio, metilmercúrio, cabelo, crianças, peixe, hábito alimentar.

INTRODUCTION

Recent studies of European and Asian fish consumers revealed significant associations between high fish consumption and high mercury (Hg) and methylmercury (MeHg) levels[1,2]. Fish consumption is considered the primary pathway for human accumulation of Hg and MeHg. This, in turn, results in statistically significant differences between high and low fish consumer groups[3]. In spite of the possible negative effects upon human health, fish consumption remains a very important protein source in the human diet. Fish is also a primary source of nutrients for child development. Many articles[4,5] and a review[6] discussing the risk-benefit considerations of fish consumption have been published in recent years. In the Mozaffarian and Rimm[4] study, the conclusion presented was that for major health outcomes among adults, based on both the strength of the evidence and the potential magnitudes of the effects, the benefits of fish intake outweigh the potential risks. According to Mahaffey et al study[5], essential polyunsaturated fatty acids, such as docosahaxaenoic acid (DHA), confer benefits to brain and visual system development in infants and reduce risks of certain forms of heart disease in adults which mitigate against CH_3Hg effects. For women of childbearing age, benefits of modest fish intake, with the exception of a few selected species also outweigh risks. Myers et al [7] presented a review about nutrient and MeHg exposure from consuming fish giving emphasis to the findings from a large prospective cohort study of a population that consumes fish daily, the Seychelles Child Development Study. Preliminary findings from this study suggest that the beneficial influence of nutrients from fish may counter any adverse effects of MeHg on the development nervous system. Facing this dilemma of risk and benefit of fish consumption FAO and WHO held an Expert Consultation Workshop in 2011[8]. Some conclusions from this workshop were: consumption of fish provides energy, protein and a range of essential nutrients; eating fish is part of the cultural traditions of many peoples and in some populations, fish is a major source of food and essential nutrients; and among infants, young children and adolescents, evidence is insufficient to derive a quantitative framework of health risks and benefits. However, healthy dietary patterns that include fish consumption and are established early in life influence dietary habits and health during adult life. Some recommendations were also described such as develop, maintain and improve existing databases on specific nutrients and contaminants, particularly methylmercury and dioxins, in fish consumed in their region and develop and evaluate risk management and communication strategies that both minimize risks and maximize benefits from eating fish[8].

Generally in Brazil, the communities such as indigenous groups, riverside populations and coastal dwellers (low-income groups) mainly obtain protein from fish consumption due to the fact of cheap and higher supply and cultural fishing habits. On the other hand, high-income communities have more protein options and for this reason do not frequently consume large amounts of fish. A similar trend was observed in the study conducted by Farias et al[9] that studied children from 3 public schools (PS_1, PS_2 and PS_3) in Cananéia city observing a direct relation between total Hg content in hair and the low economic level of the family. In general, most families of the 3 schools studied presented lower income (1 to 5 minimum salaries, US$ 300 to 1,500/ month). These low-income families showed a greater dependency of protein intake from aquatic origin, in relation to bovine protein due to a greater availability and lower cost in the coastal region[9]. The majority of the studied families stated that fish consumption is derived from the families´ fishing activities. Garcia[10] in his study also observed the same relationship.

Continuing the study started in Cananéia, similar study was developed in Cubatão, considering the importance of assessing mercury levels in hair and commercial fish in coastal populations and their association with eating habits[11].

The purpose of this study was to evaluate total Hg and MeHg concentration in fish muscle and children hair, using as strategy: *i)* total Hg and MeHg determinations in the most consumed fish species; *ii)* total Hg and MeHg determination in 93 children hair samples (aged 4 to 14) in Cubatão and *iii)* analyses of answers to a diet consumption questionnaire by the parents of the children participating in the study. The results obtained for Hg in children hair in a previous study in Cananéia[9] were used for comparison and discussion purposes. These two estuarine regions are undergoing different environmental anthropogenic impacts[12-15]. Cananéia is considered a naturally preserved area and recognized as a World Biosphere Reserve by UNESCO. Cubatão is located in an area under strong environmental impact caused by complex industrial activities. Eating habits

of these local populations are also undergoing different socio-economic changes. From the results of this study it is hoped that an estimation of the children population exposure to Hg and MeHg by means of hair analysis and fish intake can be obtained. Since, there are no references values for total Hg in children´s hair, this study can also be used as a contribution for a pool of referential values in hair for Brazilian coastal children populations. In Brazil, total Hg levels in children hair have mostly been focused on different Amazonian communities[16,17].

MATERIAL AND METHODS

Studied areas

The Baixada Santista is a coastal area located in São Paulo State that includes four important coastal cities: São Vicente, Santos, Guarujá and Cubatão. Santos city has the busiest South American port. Cubatão city is one of the country´s most important industrial poles with a population of 118,720 inhabitants[18]. Santos Bay, Santos and São Vicente estuarine channels and rivers are part of an important hydrological polluted system, which includes Cubatão city´s industrial center and complex with over a hundred industries including the largest Brazilian steel plant[19]. Nowadays, there has been a reduction of hydrological pollution but not enough to reestablish the base-line values. In regards to Hg, some studies offer some environmental data for this region[20-22] revealing Hg contamination in sediments.

Cananéia city is located in the south coast of São Paulo state, far from the Santos-Cubatão system and is a part of the estuarine-lagoon complex of Cananéia-Iguape. In the southern part, Cananéia is surrounded by a preserved estuarine system[15,23] that includes the Comprida, Cananéia and Cardoso Islands. This integral system is recognized by UNESCO (United Nations Educational, Scientific and Cultural Organization) as a World Biosphere Reserve. Cananéia city is also one of the oldest cities in Brazil and has one of the largest preserved natural Brazilian mangrove areas. The city has two main economic activities, which are tourism, and fishing and its 12,226 inhabitants make their living primarily of these two activities[18]. The extensive mangrove provides a rich breeding and feeding ground for an extremely wide variety of aquatic fauna and flora[10,23]. This scenario contributes to the presence of a large fishing population, which consumes large quantities of fish and seafood. Figure 1 presents the regions studied.

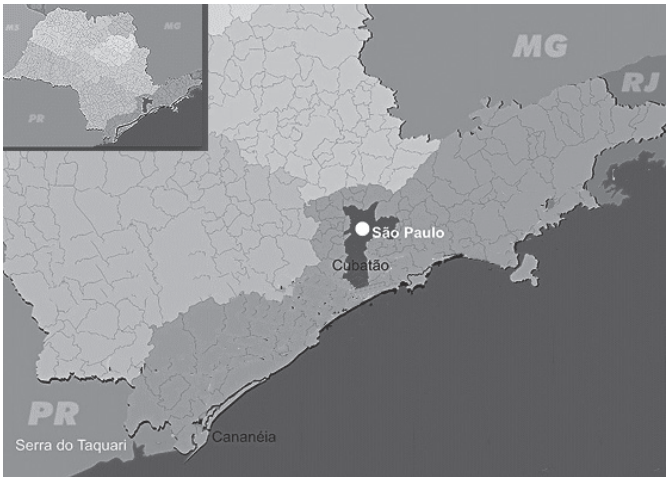

Figure 1. Map from Cananéia and Cubatão cities, Brazil

Sample Collection

Hair sampling and preparation

The hair samples from the children (4-14 years old) from the two cities were collected at the schools indicated by the Cities Educational Departments observing that sampling was to be in communities close to the estuarine system and/or where the students were members of a fishing family. In Cubatão, hair (93) and fish (58 individuals) samples were collected in March 2008 and in Cananeia[9], 105 hair samples and 53 fish samples were obtained in August 2007. The most adequate communities to participate in this study were located at: i) Jardim Casqueiro and Ilha Caraguatá in Cubatão and ii) Vila Cabana, Acarau and Carijo in Cananéia. These communities were also chosen based on the number of fishing families located in each region. Children from public schools (PS) in both cities were invited to participate in the study. All parents and/or guardians signed a consent agreement for their children´s participation in the study. Excluding factors were those children whose parents did not sign the consent agreement and children whose hair was too short to cut, mainly boys. In order to ascertain the daily dietary intake a questionnaire was filled out by the participating families about fish weekly intake, fish species most consumed, age, gender, social and economic status. This kind of questionnaire was used in a previous study[9]. The children selected had to present the signed consent agreement, have enough hair for collection and also a filled out questionnaire. Children hair samples were collected with a single cut from the

occipital region very close to the scalp with steel scissors, in the approximate amount of 1g. A small recipient containing 1 g of hair sample was used for comparison during *in situ* sampling. The study was approved by the Ethics Committee of the Nuclear and Energy Research Institute (IPEN/CNEN-SP) (Project n^0 109/CEP-IPEN/CNEN-SP)[9].

Fish selection and preparation for analysis

Tabulated data from this study identified the 5 most consumed fish species in Cubatão which were: three carnivorous species: *Macrodon ancylodon (*King weakfish – Pescada**), Menticirrhus americanus (*Southern king croaker - Perna de Moça**) and Micropogonias furnieri (*Whitemouth croaker – Corvina**)* and two planktivorous species, *Mugil liza (*Mullet – Tainha**) and Sardella braziliensis* species *(*Sardine – Sardinha**).* In Cananéia only four fish species were cited on the questionnaires which were: three carnivorous species, *Macrodon ancylodon (*King weakfish – Pescada**), Centropomus parallelus* (Fat snook – Robalo peba*) *and Micropogonias furnieri (*Whitemouth croaker – Corvina**)* and one planktivorous species, *Mugil liza (*Mullet – Tainha**)*. The families that took part in this study informed that they usually bought fish from the local markets, except the fishing families. Due to this, all the fish samples analyzed in this study were bought in local markets as well. (*Popular fish name in Portuguese).

The fish samples were bought directly from local street markets in Cubatão during March of 2008 and in Cananéia city in August 2007. All samples were assessed for quality indicators and external organoleptic characteristics (eyes, gills and scales). Fish were conditioned in isothermic boxes in crushed ice (-4 °C) and then identified before registering the biometric information (total length, total weight and body weight). In the laboratory, the samples of fish muscle were obtained from the 58 fishes from Cubatão and the 53 from Cananéia. The samples were dried at 40 °C in a ventilated oven until constant weight. Dried samples were ground, homogenized and prepared for total Hg determination. These dried samples were then analyzed and consequently, the results for total Hg and MeHg were obtained on a dry weight basis. After that, dry weight results obtained were transformed to wet weight basis by using humidity %.

Total Hg and MeHg determination

Total Mercury and MeHg determinations were performed by Cold Vapor Atomic Absorption Spectrometry (CV AAS), using a FIMS (Flow Injection Mercury System) from Perkin Elmer. The experimental procedure applied in hair and fish samples have already been described in previous papers[9,24]. The methodology validation for total Hg and MeHg determinations was carried out using human hair reference material (IAEA-086), Dogfish muscle (DORM-1, NRCC) and Dogfish liver (DOLT-3, NRCC), for fish samples. The detection limit (DL) for total Hg determination is 0.001 mg.kg^{-1} and quantification limit (QL), 0.010 mg.kg^{-1} [24].

All analyses were performed at the Neutron Activation Analysis Laboratory in Nuclear and Energy Research Institute – LAN/IPEN, SP-Brazil.

Statistical treatment

The statistical analysis was processed by the IBM SPSS Statistics Version 17.0 software (Tulsa, OK, USA).

For fish and total Hg in children hair from the different public schools investigated, the Shapiro Wilkis data treatment was used. However, since no normal Hg level distribution was found, the non-parametric Kruskal Wallis was applied to verify the significance of the differences in the Hg levels in the fish samples. The Sperman correlation test was used for the Hg and other variables.

RESULTS AND DISCUSSION

The precision and accuracy for total Hg and MeHg determinations were checked by means of reference material analyses in fish muscle (Dorm-1 – Dogfish muscle and Dolt-3 – Dogfish liver) and hair (IAEA-086). The results for total Hg and MeHg determinations in the IAEA-086 reference material showed relative standard deviation (RSD) of 3.6 and 6.3 % (n=10) and relative error (RE) of 1.0 and 1.5 %, respectively. For fish samples, the results showed RSD from 4.2 to 11.7 and RE from 0.9 to 12.0% (n=10). These results showed good precision and accuracy for both analytical methodologies in both matrices.

The fish samples were submitted to a biometric procedure that was applied to each individual before the muscle sampling and mercury analyses. Table 1 presents the total body length, feeding habits, median and interval for total Hg and MeHg (%) concentrations in fish muscle of the different species analyzed from Cananéia and Cubatão cities. In general, fish from a non-polluted costal region (without effective Hg contamination) present total Hg concentrations around

0.15 mg.kg^{-1} (wet weight) whereas fish in contaminated regions can reach total Hg levels up to 2 mg. kg^{-1}(wet weight). Benthic species can reach concentrations from 10 to 20 mg.kg^{-1}, values considered lethal for fish[25]. In the present study , the higher total Hg levels found in Cananéia and Cubatão fishes, were 0.442 mg.kg^{-1} and 0.348 mg.kg^{-1} (wet weight), respectively, both in *Micropogonias furnieri* (Whitemouth croaker), a detritivorous species. These values are lower than the maximum levels established by the current Brazilian legislation (2013)[26] considering predatory and non-predatory fishes, 1.0 and 0.5 mg.kg^{-1}, respectively. In the present study, total Hg values for the individuals analyzed ranged from < 0.010 to 0.442 mg.kg^{-1} (median of 0.010 mg.kg^{-1} for *Mugil liza (*Mullet) to 0.179 mg.kg^{-1} for *Micropogonias furnieri (*Whitemouth croaker), for fish available for consumption in Cananeia city. For Cubatão, the values ranged from < 0.010 to 0.348 mg.kg^{-1} (median of 0.010 mg.kg^{-1} for *Mugil liza* to 0.181 mg.kg^{-1} for *Micropogonias furnieri*). All data are presented in wet weight. According to the limits proposed for fish and fish products, considering the predatory and non-predatory species sampled, none surpassed the limits established by Brazilian legislation[26].

The percentage of MeHg determined in fish muscle showed values ranging from 14 % to 99 % taking into account the total Hg level. In general, the predominant form of Hg in fish muscle is methylmercury[27]. In other tissues such as liver and kidney Hg presents itself as it inorganic form exceeding the values of Hg found in the muscle[25,28]. An exception is the Pacific blue Marlin that presents a 20 % level of MeHg in its muscle tissue. In general, the % of MeHg follows the pattern: muscle>

Table 1. Total length, weight, feeding habits, median and range for total Hg and MeHg (%) content in different fish species analyzed from Cananéia and Cubatão

Fish species (n)*	Feeding Habits	Weight (g)	Total lenght (mm)	Total Hg (mg.kg^{-1}) (wet weight)	Total Hg (mg.kg^{-1}) (dry weight)	% MeHg (interval)
					Muscle	Muscle
CANANÉIA						
Whitemouth croaker (11)	Detritivorous	1134 832 - 1711	454 414 - 507	0.179 0.114-0.442	0.812 0.516 – 2.008	63 (34 – 85)
King weakfish (16)	Carnivorous	298 220 - 613	320.5 200 - 385	0.039 0.012– 0.100	0.180 0.056 – 0.456	66 (37 – 99)
Fat snook (12)	Carnivorous	481 312 - 584	399.5 318 - 424	0.032 0.015-0.178	0.129 0.061 – 0.712	45 (18 – 67)
Mullet (14)	Planctivorous	1514 807 - 1936	567.5 432 - 600	0.010 <0.010-0.025	0.032 <0.010 - 0.098	---
CUBATÃO						
Whitemouth croaker (11)	Detritivorous	720 607 - 909	426 360 - 453	0.181 0.041–0.348	0.789 0.178 – 1.512	37 (18 – 61)
Southern king croaker (12)	Carnivorous	1736 1446 - 2170	324 301 - 385	0.105 0.043-0.184	0.499 0.206 – 0.878	53 (22 – 68)
King weakfish (16)	Carnívorous	304 234 - 374	355 200 - 368	0.046 0.012–0.062	0.193 0.051–0.257	42 (18 – 61)
Sardine (16)	Planctívorous	104 91 - 120	223 210 - 235	0.027 <0.010–0.040	0.091 0.026 – 0.135	31 (14 – 61)
Mullet (5)	Planctívorous	1416 1302 - 1710	547 520 – 555	0.010 <0.010–0.023	0.029 0.013 – 0.090	56 (56 – 68)

n: number of samples analyzed; --- : Values for MeHg < 0.010 mg.kg^{-1}

gills> kidneys > liver[29]. Some studies verified that the % of MeHg in muscle tissues is higher in upper trophic levels. In marine ecosystems the % of MeHg was higher in tertiary carnivorous species (C_3) than secondary ones (C_2) while their total Hg levels were of the same order. These observations demonstrated the biomagnification capacity of Hg throughout the trophic food chain and reflect the fact that the upper trophic fish present higher levels of MeHg[30].

Observing the data assembly according to Kruskal-Wallis statistical treatment, the differences between Total Hg observed in function of the fish species analyzed (*carnivorous and non-carnivorous*) presented a high significant difference ($p < 0.05$), as expected, once carnivorous species are exposed to a more intense Hg bioaccumulation rate than the non-carnivorous species[30]

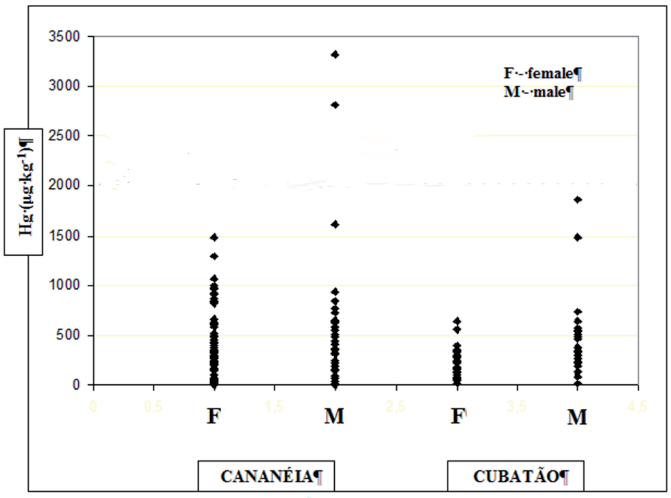

Figure 2. Total Hg concentrations distribution in hair samples in female and male groups from Cananéia and Cubatão cities, Brazil

Table 2. Total Hg and % MeHg results in children´s hair from Cubatão public schools and weekly fish intake

(Total Hg – mg.kg^{-1})	CUBATÃO		CANANÉIA[a]		
	PS_{Ct1}	PS_{Ct2}	PS_{Ca1}	PS_{Ca2}	PS_{Ca3}
Female (n)	31	36	7	26	35
Mean ± std	0.236 ± 0.145	0.338 ± 0.345	0.041 ± 0.035	0.462 ± 0.258	0.461 ± 0.361
Median	0.235	0.324	0.033	0.394	0.326
range	0.016 – 0.637	< 0.010 – 1.330	< 0.010 - 0.112	< 0.010 – 0.959	0.038 - 1.479
Age (median) (range)	11 (9-14)	10 (7-13)	5 (4-9)	8 (5-12)	6 (4-12)
% MeHg (median)	74	71	74	69	79
Male (n)	13	13	8	12	17
Mean ± std	0.458 ± 0.169	0.483 ± 0.554	0.249 ± 0.262	0.636 ± 0.881	0.745 ± 0.787
Median	0.467	0.296	0.203	0.379	0.506
range	0.220 – 0.741	<0.010 – 1.859	< 0.010 - 0.770	0.076 – 3.321	< 0.010 – 2.814
Age (median) (range)	10 (9-12)	10 (8-12)	6 (4-8)	9 (3-10)	7 (5-10)
% MeHg (median)	83	72	68	83	83
Total (n)	44	49	15	38	52
Mean ± std	0.301 ± 0.182	0.376 ± 0.362	0.152 ± 0.216	0.641 ± 0.537	0.576 ± 0.547
Median	0.270	0.319	0.043	0.384	0.405
Range	0.016 – 0.741	< 0.010 -1.859	<0.010 - 0.770	<0.010 – 3.321	< 0.010 – 2.814
age (median) (range)	11 (9-14)	10 (7-13)	5 (4-9)	8 (4-12)	7 (4-12)
% MeHg (median)	74	71	72	70	81
Fish weekly intake (%)					
Rarely	34	44	13	8	7
1 - 2 times/week	57	49	48	45	43
> 2 times/week	9	7	39	47	50

a: Farias et al [9]; n: number of individuals; $PS_{Ct1, Ct2}$: are public schools from Cubatão; $PS_{Ca1, Ca2, Ca3}$: are public schools from Cananéia

in function of its position in the food chain.

Table 2 shows the mean, median and interval for the total Hg results in children´s hair, as well as, median age, MeHg (%) and fish weekly intake for female and male groups, from Cananéia and Cubatão communities. Some data for total Hg in children hair from Cananéia city obtained in a previous study of our group were already published[9]. Hair mercury content was lower than the US EPA reference limit[31] of 1 $mg.kg^{-1}$ in 95 % of the studied children; 4 % presented levels between 1 and 2 $mg.kg^{-1}$ and 1 %, higher than 2 $mg.kg^{-1}$. The normal level of Hg in hair is 1-2 $mg.kg^{-1}$, however those who consume fish one or more times per day may have Hg levels in hair exceeding 10 $mg.kg^{-1}$ [32].

Figure 2 shows the total Hg concentrations distribution in hair samples in female and male groups from Cananéia and Cubatão cities. The mean values for male groups were higher in both counties (Table 2). Nakagawa (1995), *apud* Yasutake et al[33] observed that gender can be an important variable for total Hg concentration in hair and verified that the content in males is much higher than in females. On the other hand, there are studies where a significant correlation between gender and total Hg content in hair was not observed[34]. Presently, there is still no agreement about the influence of gender on the total Hg content in hair.

In the present study, a negative correlation between age and total Hg among the entire sampled group was also found. The Spearman correlation coefficient was found to be r_s= -0.336, indicating a weak negative correlation between age and total Hg content in children´s hair.

The PS_{Ct1} School individuals sampled from the Cubatão region presented a total Hg mean of 0.236 $mg.kg^{-1}$ for females and 0.458 $mg.kg^{-1}$ for the male group. At the PS_{Ct2} School, the mean was 0.338 $mg.kg^{-1}$ for females and 0.483$mg.kg^{-1}$for the male group. Cananéia region, at the PS_{Ca1} School, individuals presented total Hg mean of 0.041 $mg.kg^{-1}$ for the female group, 0.249 $mg.kg^{-1}$ for the male group; PS_{Ca2} School, 0.462 $mg.kg^{-1}$ for the female group, 0.636 $mg.kg^{-1}$ for the male group and PS_{Ca3} School, 0.461 $mg.kg^{-1}$ for the female group and 0.745 $mg.kg^{-1}$ for the male group. Table 2 also shows the median of MeHg (%) in the hair samples analyzed. The MeHg % ranged from 71 to 83 % for both groups in Cubatão and from 68 to 83 %, in Cananéia. In the literature, the average value of 80 % of MeHg in relation to total Hg content in hair is commonly found[35].

According to the questionnaire answers regarding eating habits in Cananéia city (Table 2), a frequency of 1-2 times/week of fish and seafood intake occurred in 48 %, 45 % and 43 % for children from PS_{Ca1}, PS_{Ca2} and PS_{Ca3}, respectively. Furthermore, according to the questionnaire data 39, 47 and 50 % reported consuming fish > 2 times/ week, respectively. Rare fish consumption was reported by 13 %, 8 % and 7 %, respectively, showing the importance of fish in the diet of the local population. In general, the sampling revealed that most families (67.6 %) belonged to a low socio-economic level, receiving 1 to 5 minimum salaries in Brazil (US$ 300 to 1500), contributing to a higher fish and seafood consumption due to the fact that most families make their living by fishing[9].

In Cananéia, the children hair samples from public school PS_{Ca1} presented median values for total Hg, for both male and female groups, much lower than the PS_{Ca2} and PS_{Ca3} values. Furthermore, schools PS_{Ca2} and PS_{Ca3} presented the higher number of fishermen as parents (45 %). This fact may explain why these children consume more fish/seafood and as a result presented higher total Hg levels in their hair than the children from PS_{Ca1}.

In Cubatão, the children hair samples from public schools PS_{Ct1} and PS_{Ct2} presented very similar median values for total Hg (0.270 and 0.319 $mg.kg^{-1}$) and higher mean values for the male group in both schools as well (Table 2).

According to eating habits in Cubatão city, the present study verified, observing the parents answers to the questionnaire (Table 2), that a 1-2 times/week of fish and seafood intake frequency occurred in 57 % and 49 % for PS_{Ct1} and PS_{Ct2}, respectively. Furthermore, 9 and 7 % reported consuming fish more than 2 times/ week. Rare fish consumption was observed in 34 % and 44 %, respectively. These results indicate a different frequency in fish and seafood consumption in Cubatão when compared to the fish consumption data from Cananéia. This difference may also explain the higher mean Hg levels in children´s hair (female and male groups) in Cananéia (except for PS_{Ca1}) than Cubatão as Hg concentration in fish species most consumed were similar in both communities. This seems to confirm the influence of higher fish intake frequency in Cananéia.

In Cubatão, the sampling also revealed that most families (59 %) belonged to a low socio-economic level, receiving 1 to 5 minimum salaries. The majority of the parents interviewed answered that they consumed fish acquired in the weekly open street market.

Table 3. Total Hg concentrations in children´s hair from different localities in Brazil and other countries

Brazil	Total Hg (mg kg^{-1})	
	Mean ± s.d.	Interval
São Luiz do Tapajós – age group: 0 - 5 years (Pará State – Northern Brazil) [25]	21.06 ± 14.38	0.10 – 94.5
Barão do Melgaço – age group: 3 – 7 years (Mato Grosso State – Central Brazil) [38]	5.37 ± 3.35	0.58 – 17.14
River Madeira basin: children and adults (Amazon region – Northern Brazil) [17]	15.2 ± 9.6	0.36 – 150
Manaus city – age group: 2 – 7 years (Amazon region- Northern Brazil) [16]	1.93 ± 3.90	0.02 – 34.4
Cubatão – age group: 1- 10 years (São Paulo State - Southeastern Brazil) [19]	0.88 ± 0.61	–
Cananéia – age group: 4 - 12 years (São Paulo State- Southern Brazil) [9]	0.46 ± 0.35	< 0.01 – 3.33
Cubatão – age group: 4 - 14 anos (São Paulo State - Southern Brazil) (present study)	0.34 ± 0.29	< 0.01 – 1.86
Other countries	Total Hg (mg kg^{-1})	
	Mean ± s.d.	Interval
Spain – age: 4 years [2]	0.99 ± 0.20	< 0.01 – 5.63
Japan – age: 7 years [33]	1.4 ± 0.89	0.45 – 6.32

The available information in regards to the Hg content in children hair from different Brazilian regions is focused primarily on data from the Amazon region[16,17]. Hacon et al[36] and Passos and Mergler[37] published two reviews showing the mercury concentration in the Amazon.

Table 3 presents total Hg levels in children hair from Cananéia[9] and Cubatão (present study) and children living in the Amazon region and two other countries. Total Hg levels in children hair found in the present study were much lower when compared to values obtained from the Amazon region and lower than values published by ACPO[19]. The values observed in this study were also lower than values from other countries, despite the difficulty for comparison based on age differences between participants. However, considering these different studies, fish consumption is often cited as the most significant factor for human mercury bioaccumulation, particularly in its organic form. The results presented in this study suggest that the concentration of total Hg and MeHg found in children hair samples were mainly dependent on eating habits of the population, the same observed by Tavares et al[38] and Marques et al[39] in studies developed with children in Brazil as well as low economic level families.

It was expected that the highest Hg and MeHg levels were to be found in children hair living in Cubatão in relation to those observed in children living in the Cananéia city due to the association of the fish source from the polluted estuarine system in Cubatão (industrial activities and the largest Brazilian steel plant) as mentioned before. However, this was not the case. The fish consumed in Cubatão did not necessarily come from local waters. Furthermore, there are fewer fishing families in this industrial region. On the other hand, the number of fishing families in Cananéia city was much higher than in Cubatão, probably resulting in the higher fish consumption in Cananéia. In both locations, the families were classified as low-income varying from 1 to 5 minimum salaries (67.6 % in Cananéia and 59 % in Cubatão) according to POF (Family Budget Survey -

POF 2008-2009 (the Brazilian census[18]). This fact also seems to favor fish/seafood consumption for protein in coastal cities. It should be noted that Cubatão city offers more options for protein sources than Cananéia. This behavior could be associated to the high price of fish and to the pollution estuarine history of Cubatão which may contribute to lower fish consumption. On the other hand, Cananéia estuary has an extensive mangrove area that provides a rich breeding and feeding ground for an extremely wide variety of aquatic fauna and flora, producing a large amount of fish and seafood.

In relation to total Hg fish concentrations, both Cananéia and Cubatão fish presented levels below the fish Hg limits established by Brazilian legislation for human consumption (predatory and non-predatory fishes, 1.0 and 0.5 $mg.kg^{-1}$, respectively). In Cubatão city, the origin of the fish in the local markets was verified by questioning the sales person during the purchase. They stated that most commercialized fish originated from the coastal areas of Santa Catarina State, in the southern Brazilian region, far from this polluted region.

Researchers from Duke University, USA, recently published a study about photolytic degradation of MeHg in brackish waters where the Hg concentrations in freshwater are much greater than those found in salt water. However, when fish are ingested, it is the saltwater fish that offer greater risk to human health[40]. The MeHg is potentially more dangerous than inorganic Hg forms and in the water system is associated with dissolved organic matter and in the sea, is also associated with chloride, ie, salt. One way nature transforms the MeHg in a less toxic form is through photochemical reactions promoted by sunlight. When MeHg is associated to dissolved organic matter such as plants or animals detritus, the sunlight breaks the molecule. However, in ocean water, the MeHg remains strongly linked to chloride and is not easily degraded by sunlight. Since MeHg is not broken down by sunlight in sea water, its residence time is much greater in saltwater than in freshwater, which increases the rate of intake by marine animals[40]. Fish and seafood have a natural tendency to store MeHg in their bodies characterized therefore as the main source of metal intake in humans. In the marine environment, bacteria can methylate the Hg and transform it into an easily assimilated form especially in surface marine sediments. According to the same study it is also important to direct research and policy control of Hg for saltwater, mainly in the coastal areas those are submitted to anthropogenic inputs. To date, most efforts have been directed to the presence of the metal in freshwater. This fact is particularly true in Brazil, given that most studies with Hg are located in the Amazon region.

Finally, the results obtained in the present study indicated the necessity of obtaining more data concerning Hg values for different coastal regions of Brazil in order to contribute to establishing values considered characteristic for different parts of the country and in relation to human populations. Children, in particular, need intensive and continuous studies because they are most sensitive to the deleterious effects of this element.

CONCLUSION

The fish species analyzed were the most consumed by the coastal populations studied and presented Hg levels in muscles lower than the limit established by Brazilian legislation (0.5 – non-carnivorous species and 1.0 $mg.kg^{-1}$ – carnivorous species, respectively) in both studied regions. The median values for total Hg levels in the fish species analyzed were very similar in both coastal cities.

The Hg concentrations in children hair were lower than the US EPA reference limit of 1 $mg.kg^{-1}$ in 95 % of the studied children. This value was used as reference since at present there is no value set for children. The mean values for total Hg in children's hair (female and male groups) in Cananéia were higher than in Cubatão, except for PS_{Ca1} School. From these results, it seems that the quantity and frequency of fish ingested are the main factors that contribute to the exposure and bioaccumulation of Hg, as the total Hg levels in fish were similar in both communities.

To complement information about Hg in communities outside the Amazon region it is important to determine the profile of Hg and MeHg distribution in Brazil, considering the wide diversity of ecosystems, cultural patterns and socio-economic conditions particularly considering the frequency and type of fish people consume.

The results from this study can also be used as a contribution for referential values for total Hg levels in hair for Brazilian coastal children populations.

Considering the Hg levels in those fish species most consumed and hair, children from both communities studied appear not be at risk of Hg and MeHg contamination.

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