

# Risk Assessment to the Health of Amazonian Indigenous For the Consumption of Fish, Meat of Hunts and Vegetables Containing Metylmercury

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**Abstract**— This study was to evaluate the exposure to methylmercury (HgMe) and the potential health risk of Tupari Indians through the consumption of their main foods. Were collection of samples of plant foods and muscle tissue from different species of fish and wild animals consumed in three villages of the Rio Branco Indigenous Land in Rondônia, in the Brazilian Amazon. The HgMe was measured in an atomic fluorescence spectrophotometer with gas chromatography. The statistical treatment of the data was performed by software R. Of the six different plant species, only sweet potato (*Ipomoea batatas*) had mean concentrations of HgMe above the limit of detection of the analytical technique for the three villages. There was a significant difference in the levels of HgMe between the species of wild animals and fish belonging to the same alimentary habit. Carnivores presented higher levels of HgMe than those obtained for non-predators, both for fish and for wild animals. The results of the assessment of the potential risk to indigenous health indicated a total HgMe of the weekly ingestion rate (WIR) of between 8.4 and 15.0 µg / kg of body weight for the villages evaluated, extrapolating all reference doses (RfD) regarding for the

Provisional Tolerable Weekly Intake (PTWI). The risk quotients (RQ) varied from 5.3 to 21.4, considerably exceeding the limit ( $RQ \leq 1$ ), which allows to consider the impossibility of toxic effects of HgMe. The fishes accounted for the highest percentage of WIR of HgMe for all villages, with an emphasis on predatory species. Considering the nutritional value of fish meat, it is suggested the continuity of the consumption of this meat in the villages with preference for non-carnivores.

**Keywords**— Indigenous Amazonian; HgMe intake rate; Risk quotient; Fish meat; wild animal meat.

## I. INTRODUCTION

The damages to human health due to the consumption of food and water contaminated by mercury, mainly methylmercury, is the most toxic form of this element are widely reported in the literature, being able to affect the central nervous system and to cause serious health problems to the population exposed to this organometal (AHMED et al., 2018; MILHOMEM FILHO et al., 2016; MOZAFFARIAN; 2009; MARQUES et al., 2007; CHEN et al., 2006; FILLION et al., 2006).

The exposure of traditional populations of the Brazilian Amazon to methylmercury has been reported as a result of the use of mercury in the gold mining (BASTOS et al., 2007), of the soybean advance in this region of Brazil (TUZEN et al., 2009; MALEKI; ZARASVAND, 2008), as well as due to the impoundments of the Amazonian rivers for the construction of hydroelectric power plants (PESTANA et al., 2016; FEARNESIDE, 2014).

According to Dórea et al. (2006) in the Amazonian lifestyle, traditional fish consumption is high, characterizing the largest route of exposure to methylmercury, and may in the Mean and long term pose a risk to the health of these people depending on the concentrations of mercury present in these fish and the amount consumed. In this sense, the health problems caused by contact or ingestion of high concentrations of mercury are potentiated when it comes to the indigenous population of the Amazon that lives in villages around the rivers and therefore has greater exposure to methylmercury, the main source of protein (BASTOS et al., 2007). In addition to fish, Amazonian indigenous peoples still have the habit of feeding meat of hunts, which can also be an important route of exposure to mercury depending on the amount consumed in the villages, as well as how polluted the environment.

Some studies have demonstrated high levels of mercury in fish (BASTOS et al., 2016; LIMA et al., 2015; BASTOS et al., 2008) in the Madeira River region of Rondonia state in the Brazilian Amazon, researchers responsible for the exposure to mercury in the Amazon. When dosing Hg in muscle tissue of different fish species from the Cassipore River basin (state of Amapá), Lima et al. (2015) observed concentrations (0.570 to 0.670  $\mu\text{g}\cdot\text{g}^{-1}$ ) that exceeded the limits set by the World Health Organization (WHO, 1988).

A study conducted by Santos et al. (2003) in the state of Rondonia with Wari Indians pointed out the risk of exposure to Hg contamination, since high levels of this element were identified in hair samples from this population. Barbosa et al. (1998) found average levels of Hg-T in the order of 8.30  $\mu\text{g}/\text{g}$  in the hair of 251 women and indigenous children selected along the Madeira River and Kayapo Reserve, and for 25 of them the concentration exceeded 10.0 mg/g, whereas the legislation regulates a maximum of 5.0  $\mu\text{g}/\text{g}$  (BRASIL, 1998). Santos et al. (2003) verified a high mercury exposure after analyzing mercury in the hair of 910 indigenous people (men, women and children) of TI Pacaas Novos, from different villages, including the Soterio village, with very high values of this heavy metal in children 2 to 5 years old.

Other scientific investigations carried out in the Brazilian Amazon also raised concern after observing weekly intake rates of total mercury and HgMe that

extrapolated the reference doses (RfD) established by the PTWI suggested by different international bodies related to health and human food. For example, Castilhos et al. (2001) found a weekly intake rate (WIR) of 1.33  $\mu\text{g}/\text{kg}$  body weight from the consumption of fish from the Tapajos river in Pará, as well as Mourão (2016) found a mean weekly dose of HgMe intake fish in the Madeira River region in the order of 3.4 and 3.5  $\mu\text{g}/\text{kg}$  bw for infants and juvenile riparian groups and adults in Porto Velho, Rondonia, respectively.

In view of the above, it is relevant to analyze the level of exposure to methylmercury of indigenous Tupari Amazonians living in the Rio Branco Indigenous Land, in the state of Rondonia, in the Brazilian Amazon. This is because, although there are no gold mines in that region, the area is located near a region with soybean expansion and also under the influence of seven hydroelectric plants, which may be contributing to the increase of HgMe concentrations in the soils and vegetables produced and consumed in the villages, as well as the fish and wild animals routinely present in the diet of these peoples.

## II. METHODOLOGY

### Sample collection

The collections were carried out in the villages of Serrinha, Trindade and Nazare, with a total of 116 indigenous, located in the Rio Branco Indigenous Land, in the state of Rondonia, Brazilian Amazon, after authorization of the Chico Mendes Institute for Biodiversity Conservation (47500-1, 47500-2, 47500-3 and 47500-4), indigenous representatives (indian chief) from the villages where the study was to be conducted by means of a signature in a letter of authorization from the National Indian Foundation (Nº 58/AAEP/PRES/2016), as well as from the Committee (approximately 150 g of the muscle tissue of 86 fish from 19 different species with carnivorous, detritivorous and preferably herbivorous dietary habits and approximately 300 g of tissue muscular of 57 wild animals from 16 different species belonging to the carnivorous, omnivorous and exclusively herbivorous trophic levels. Were collected 150 g of bark-free mass of 6 units of 6 different species of vegetal foods produced and consumed in the three villages studied, thus adding 108 vegetables samples.

### Preparation and chemical digestion of samples

For the extraction of HgMe we weighed 0.05 g for fish samples, 0.5 g for meat of hunts and 1.0 g for vegetables. For the samples of fish and of the hunts was considered the wet weight whereas for the vegetables the dry weight. Samples were digested with 3.0 mL of 25 % potassium hydroxide in methanol Mean and taken to the oven at 68 °C for 6 hours for fish, hunts and vegetables with shaking every hour. At the end of the chemical

extraction, the samples were stored sheltered from light to avoid any degradation of HgMe and analyzed two days later, sufficient time for the chemical stabilization of the samples (PICHET et al., 1999).

### Quantification of HgMe

The quantification of HgMe was carried out with the addition of buffer solution with 4.5 hydrogen ionic potential consisting of 300 µL of acetic acid and sodium acetate followed by the addition of 30 µL of the extract from each sample and 50 µL of sodium tetraborate 1 %. The samples were measured with Milli-Q water in inverted meniscus in the 40 mL vials. The HgMe determination was performed on the gas chromatograph coupled to the atomic fluorescence spectrophotometer (CG-AFS, Brooks Rand). The operating conditions of the equipment were: gas flow in the MERX purge trap (Ar) of

45 mL.min<sup>-1</sup>, flow of the Traps (Ar) drying gas of 30 mL.min<sup>-1</sup> and flow of the GC (Ar) of 32 mL.min<sup>-1</sup> (TAYLOR et al., 2011; ALMEIDA, 2012).

### Quality control of the analytical technique

The Analytical Technique Detection Limit (TDL) was calculated by averaging the standard deviation of control whites, multiplied by three, with values of 0.00000005 mg.kg<sup>-1</sup> for vegetables, 0.000001 mg.kg<sup>-1</sup> for the fish and 0.00000013 mg.kg<sup>-1</sup> for the hunts. The experimental results for the certified reference samples showed good agreement with the certified values, as well as satisfactory recovery percentages, indicating the high reproducibility of the analytical method. The experimental results for the certified reference samples are show in Frame 1.

Frame 1 - Reference values and values found for certified reference material, and percentages of recovery of HgMe for the different matrix.

Matrix	Certified sample	Reference value (mg.kg <sup>-1</sup> )	Value found (mg.kg <sup>-1</sup> )	Recovery (%)
Vegetables	IAEA 356	0.0055	0.0050	106
Fish	Tuna fish	2.85	2.58	90
Hunts				

### Characterization of the health risk due to the ingestion of HgMe

The potential health risk of the populations of the three villages was estimated from the values calculated for the HgMe Weekly Intake Rate (WIR) consumption of fish, game and sweet potatoes (*Ipomoea batatas*) collected and analyze, and their comparisons with reference values (RfD) established by the Provisional Tolerable Weekly Intake (PTWI) Rate suggested by three international regulatory institutions - 1.6 µg. HgMe/kg bw/week (JECFA, 2014), 1.3 µg. HgMe/kg (EFSA, 2014) and 0.7 µg. HgMe/kg bw/week (US EPA, 2001), as well as by the calculation of the Risk Quotient (RQ). For the WIR calculation it was necessary to know the average consumption in the villages for each food analyzed, as well as the average body weight of the population of each village.

### Weekly average food consumption analyzed

The estimate of the average weekly consumption of the different species of fish and wild animals analyzed, as well as for the vegetables was obtained through a questionnaire answered by a representative from each family and from each of the three villages.

### Weekly Intake Rate (WIR) of HgMe

The HgMe WIR for each of the villages was calculated through Equation 1, fish and hunts, and also

for sweet potatoes (*I. batatas*), since this was the only plant that presented HgMe>TDL concentration for the three villages.

$$WIR = \text{HgMe} (\mu\text{g.kg}^{-1}) \times QW (\text{kg}) / \text{bw} \text{ Eq.1[1]}$$

Where:

WIR = Weekly Intake Rate of HgMe in µg.kg<sup>-1</sup>

HgMe = Concentration of HgMe in food in µg.kg<sup>-1</sup>

QS = quantity of food consumed per week in kg

bw = Average body weight of the population in kg

The mean body weight of the populations was calculated based on data (weight in kilograms) recently collected (March 2018) and provided by the Special Secretariat of Indigenous Health, Base Base of the High Forest of the West and was therefore considered a body weight average of 66.4 kg for the Serrinha Indians, 68.3 kg for the Trindade population and 69.7 kg for the residents of the village of Nazaré. The WIR results are expressed in microgram of HgMe ingested per kilogram of body weight per week (µg. HgMe/kg bw/week) and per person in each of the villages.

### Risk Quotient (RQ)

The RQ is the ratio between the weekly exposure to HgMe (WIR) and the reference dose (PTWI) for weekly intake of HgMe per kilogram of body weight.

Therefore, Equation 2 (US EPA, 2004) was used to calculate it.

$$RQ = WIR / PTWI \text{ Eq. [2]}$$

Where:

RQ = Health risk quotient by HgMe intake

WIR = HgMe weekly intake rate per kilogram of body weight

PTWI = Reference dose for tolerable weekly intake of Hg Meper kilogram of body weight

**Statistical analysis**

The statistical tests were performed by software R (R CORE TEAM, 2013), considering 5 % of

significance. Statistical analyzes were performed using the non-parametric Bootstrap method, with simulation of 100,000 resamples from the master sample, using the Accelerated Addition Correction (CBa) method. These methods are indicated in the case of reduced sample numbers and guarantee reliable results, mainly because they do not require any probabilistic assumptions.

**III. RESULTS AND DISCUSSION**

Figure 1 shows the mean concentrations of HgMe in sweet potatoes (*Ipomoea potatas*), per village studied.

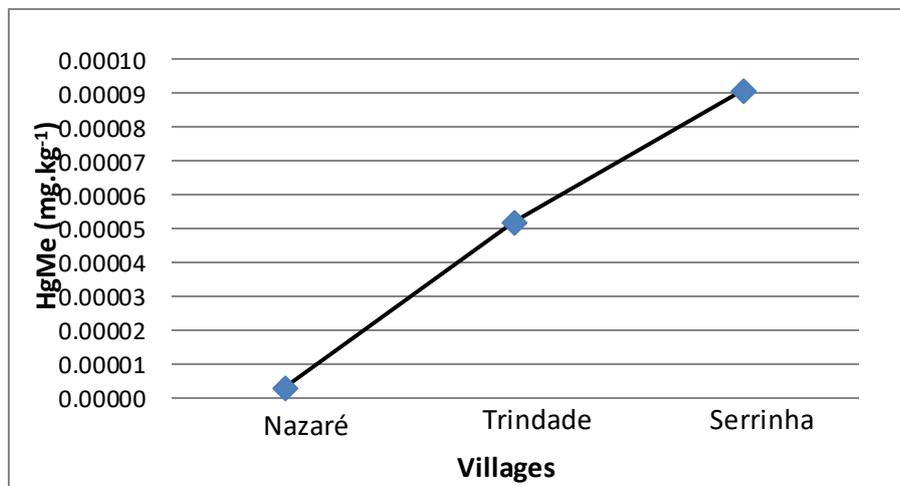


Fig. 1 - Mean concentrations of HgMe in *Ipomoea batatas* produced and consumed in the villages.

Table 1 shows the differences between the HgMe averages obtained for each species of fish per feeding habit, as well as the minimum and maximum values for each species analyzed.

Table.1 - Descriptive and discriminative statistics for HgMe concentrations in fish of different species belonging to the same food habit

CARNIVOROUS ESPECIES						
Scientific name	Popular name	N	Min.	Max.	*Mean±SD	Mean Test
<i>P. nattereri</i>	Red piranha	6	0.557	1.725	1.150±0.002	A
<i>P. corruscans</i>	Pintado	6	0.562	1.116	0.921±0.004	B
<i>P. hemiliopterus</i>	Pirarara	3	0.685	0.976	0.815±0.002	B
<i>S. marginatus</i>	White piranha	6	0.416	0.711	0.577±0.004	B
<i>P. pirinampu</i>	Barba-Chata	4	0.397	0.979	0.575±0.001	B
<i>A. brevifilis</i>	Mandubé/Palmito	8	0.106	0.620	0.389±0.023	C
<i>P. fasciatum</i>	Surubim/Cachara	4	0.312	0.404	0.364±0.002	C
<i>Z. jahu</i>	Jaú	2	0.320	0.330	0.325±0.003	C
<i>C. kelberi</i>	Tucunaré-Amarelo	4	0.179	0.357	0.265±0.003	C
<i>A. falcistrotris</i>	Dog-fish	1	-	-	0.074	-
DETRITIVOROUS SPECIES						
Scientific name	Popular name	N	Min.	Max.	*Mean±SD	Mean Test
<i>P. altamazonica</i>	Branquinha	6	0.299	1.007	0.577±0.080	A
<i>P. nigricans</i>	Curimatã	9	0.010	0.440	0.185±0.015	B
<i>L. pardalis</i>	Acari-Bodó	6	0.020	0.083	0.063±0.005	C
<i>C. callichthys</i>	Cascudo	2	0.016	0.055	0.035±0.012	C

HERBIVOROUS ESPECIES						
Scientific name	Popular name	N	Min.	Max.	*Mean±SD	Mean Test
<i>M. aureum</i>	Silver pacu	3	0.176	0.311	0.262±0.010	A
<i>P. brachypomus</i>	Pink pirapitinga	4	0.022	0.862	0.245±0.098	A
<i>A. ocellatus</i>	Cará-Açú	4	0.134	0.260	0.214±0.005	A
<i>L. freiderici</i>	Piau	4	0.031	0.058	0.048±0.003	B
<i>C. macropomum</i>	Tambaqui	4	0.025	0.039	0.031±0.002	B

\*Mean concentration of HgMe expressed in  $\text{mg.kg}^{-1}$ . N: Number of samples. SD: Standard deviation of the sample. Min. And Max.: Minimum and maximum values expressed in  $\text{mg.kg}^{-1}$ . Means followed by equal letters in the same column for species of the same food habit do not differ ( $p > 0.05$ ) from each other.

As shown in Table 1, there was a significant difference between the fish species of the different feeding habits, and no influence of the size of the species on the MeHg levels was verified. These results are not compatible with those found by Ahmad et al. (2015), who observed that total mercury (T-Hg) levels increased significantly ( $p < 0.002$ ) in larger fish ( $> 20$  cm in length). On the other hand, as in this research, Bastos et al. (2016) did not detect an influence of the size of omnivorous fish in their concentrations of Hg-T, when they measured this element in 3182 specimens of different dietary habits collected in the Madeira river basin in Rondônia, in the Brazilian Amazon.

The mean levels of HgMe found in this work for *C. Kelberi* (carnivorous) and *A. ocellatus* (preferably herbivorous) were higher than those found by Kehrig et al. (2008) in samples from the same species (0.245 and  $0.062 \text{ mg.kg}^{-1}$ , respectively) of the Balbina reservoir, also in the Brazilian Amazon, reinforcing the possibility of influence of the hydroelectric plants installed upstream of the studied villages in the methylation of Hg present in fish of the river Branco, consumed by the Tupari Indians. Some species of fish analyzed in this research presented MeHg levels higher than those found in samples collected in the Tapajos and Madeira river basins (BARBOSA et al., 2003), rivers known for their gold mining history, unlike the River White basin that has no history of mining and where the fish were collected for this study. This fact can be explained by the fact that the mercury associated with the Amazonian soil is released into the atmosphere and/or the river system, often favored by deforestation, or even due to natural processes and / or those from anthropic activities (ROULET et al., 1998).

According to Tuzen et al. (2009) deforestation of forest areas followed by burning is another important factor contributing to the increase of mercury levels in the environment. This is because, the burning of biomass releases the mercury into the atmosphere and facilitates the evaporation of part of the mercury contained in the soil.

Herrmann (2004) corroborated with the aforementioned authors adding that when removing the vegetation the erosion process is increased and, consequently, leaching of the mercury contained in the soil, facilitating the entry of this element in the rivers. In this sense, it is worth compensating to add that the mercurial compounds that may constitute some agricultural pesticides used in large soybean plantations in the area around Rio Branco Indigenous Land that is with a notable agricultural expansion may also have cooperated for the presence of mercury in the Branco river, where it is possible which has come through leaching during heavy rains in the region and therefore contaminated in fish and other aquatic animals such as alligators and some chelonians, such as turtles and tracajás, commonly found in Amazonian rivers.

This hypothesis reinforces the importance and the need for further investigations on the presence of Hg in areas that, without prior extraction of gold, are close to agricultural areas or downstream from hydroelectric plants.

Figure 2 shows a comparison of mean HgMe levels in fish muscle tissues by eating habits, showing a higher mean ( $0.604 \text{ mg.kg}^{-1}$ ) for carnivorous fish compared to detritivorous ( $0.250 \text{ mg.kg}^{-1}$ ) and herbivorous ( $0.155 \text{ mg.kg}^{-1}$ ), which did not differ significantly between them. This result is due to Oliveira et al. (2010) to the process of biomagnification in the organism of predatory fish. According to Poste et al., (2015) this is due to predatory fish feeding on other fish or aquatic animals that were already contaminated with this metal that has a cumulative effect on the organism of all living beings. Due to the high proportion of organic mercury in the total inorganic mercury present in fish meat, Dórea (2003) advised the consumption of non predatory species, since they normally present lower levels of Hg-T and also of HgMe, favoring a reduction in the exposure of the population.

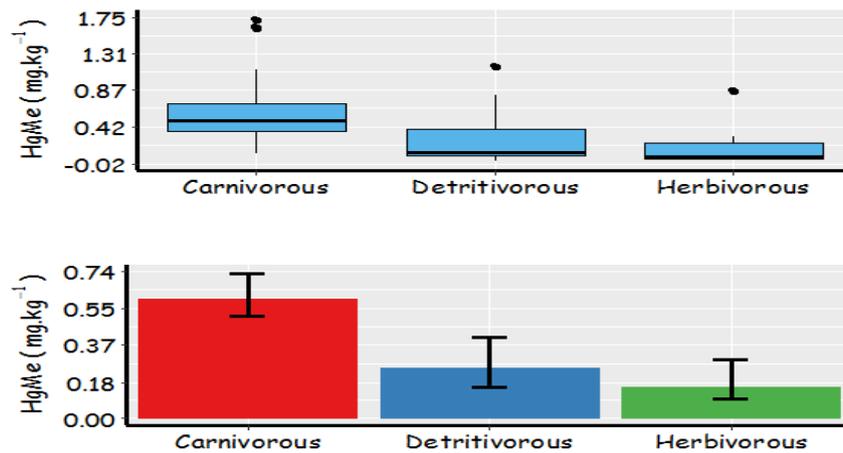


Fig. 2 - Mean concentrations of HgMe in fish muscle tissue, by dietary habit

Table 2 shows the differences between the HgMe averages obtained for each species of hunting by food habit, as well as the minimum and maximum values for each species analyzed.

Table.2 - Descriptive and discriminative statistics for the concentrations of HgMe in the hunts of different species belonging to the same food habit

CARNIVOROUS ESPECIES						
Scientific name	Popular name	N	Min.	Max.	*Mean±SD	Mean Test
<i>M. niger</i>	Alligator-açu	3	0.0009	0.3320	0.156±0.011	A
<i>C. crocodilus</i>	Jacaretinga	2	0.0019	0.1850	0.094±0.006	B
ONIVOROUS ESPECIES						
Scientific name	Popular name	N	Min.	Max.	*Mean±SD	Mean Test
<i>T. pecari</i>	Queixada	7	0.0004	0.0017	0.0008±0.012	A
<i>T. tajacu</i>	Cateto	6	0.0001	0.0016	0.0005±0.014	B
DETRITIVOROUS ESPECIES						
Scientific name	Popular name	N	Min.	Max.	*Mean±SD	Mean Test
<i>C. apela</i>	Nail monkey	2	0.0083	0.0096	0.0089±0.001	A
<i>C. parvirostris</i>	Nambu	1	-	-	0.0064	-
<i>P. unifilis</i>	Tracajá	3	0.0006	0.0085	0.0055±0.012	B
<i>P. expansa</i>	Tartaruga	8	0.0025	0.0113	0.0054±0.024	B
<i>H. hidrochaerus</i>	Capybara	3	0.0033	0.0062	0.0054±0.001	B
<i>A. paca</i>	Paca	7	0.0002	0.0065	0.0018±0.005	C
<i>D. agouti</i>	Cotia	3	0.0008	0.0018	0.0012±0.003	C
<i>A. macao</i>	Macaw	2	0.0001	0.0014	0.0008±0.005	CD
<i>M. americana</i>	Veado mateiro	2	0.0003	0.0005	0.0004±0.016	D
<i>D. novemcinctus</i>	Armadillo	2	0.0002	0.0004	0.0003±0.004	D
<i>T. terrestres</i>	Tapir	5	0.0002	0.0003	0.0002±0.012	D
<i>O. benzoarticus</i>	Pampas deer	1	-	-	0.0002	-

\*Average concentration of HgMe expressed in mg.kg<sup>-1</sup>. N: Number of samples. SD: Standard deviation of the sample. Min. and Max. : Minimum and maximum values expressed in mg.kg<sup>-1</sup>. Means followed by equal letters in the same column for species of the same food habit do not differ (p > 0.05) from each other.

From the group of carnivorous habitats the highest average concentration of HgMe was for the larger size species (*Melanosuchus niger*) compared to *Caiman crocodilus*. The individuals representative of the species collected for this research measured between 1.84 and

3.41 meters in length and the largest specimen responded by the highest level of HgMe obtained for this species. including above the maximum value (0.5 mg.kg<sup>-1</sup>) delimited by the WHO (1988) for fish with the same alimentary habit, much, although it is another type of

animal. A similar situation occurred for the species of omnivorous fighters when the average concentration of HgMe for *Tayassu tajacu* was lower than the levels found for *Tayassu pecari*, the latter with individuals around two times larger than *T. tajacu*, therefore, require a higher consumption of both plant and animal (remnants of other animals). which could have contributed to the higher levels of HgMe bioaccumulated over time in the muscle tissue of this larger species.

The results obtained for the herbivorous fighters surprised, therefore, it was expected higher concentrations of HgMe for the species *P. unifilis* and *P. expansa*, because they are chelonians that feed on algae and other vegetal proteins deposited in the sediment of the river, in the same way that a greater concentration of capybara was suspected, since this animal usually lives in regions bordering rivers and uses them as a hiding place and protection against natural predators, as well as for reproduction, thus, it has a greater contact with the sediment of rivers. This result was expected since other

studies (Almeida et al., 2014; Vergotti et al., 2009; Gomes et al. 2006; Lechler et al., 2000) showed that the bottom sediments are the abiotic accumulates mercury in the Amazonian rivers. It is suggested, therefore, the importance of conducting further research aimed at understanding the behavior of mercury in the wild animal organism.

Unlike the predatory and omnivorous species. the species *Tapirus terrestris* that reaches adulthood (as captured) between 0.9 and 1.4 meters in length and weighs up to 250 kilos was significantly different among the species that fed exclusively on plants with the lowest concentrations of HgMe obtained for this huntsing group, assuming that the highest levels found for the herbivorous species may have to do with the age of the animals (bioaccumulation) in a greater proportion than with the size.

Figure 3 shows a comparison of the mean levels of HgMe in the muscle tissues of the hunts, by alimentary habit.

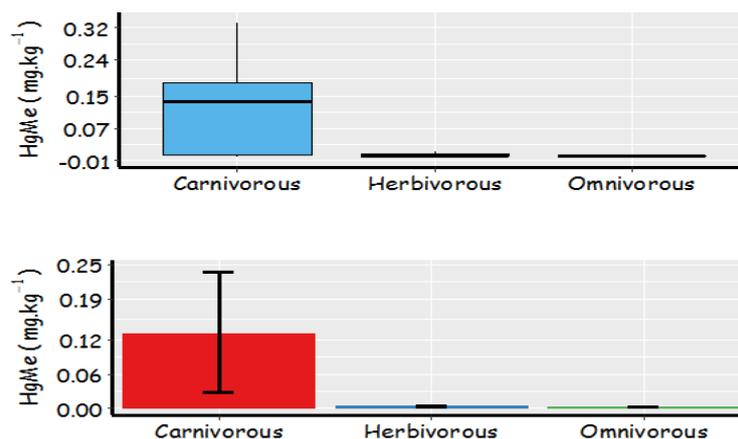


Fig. 3 - Mean concentrations of HgMe in the muscle tissue of the hunts, by food habit.

As for the fish, the carnivorous species had the highest average concentration of HgMe (0.131 mg.kg<sup>-1</sup>) compared to the means found for the herbivorous and omnivorous species, which did not differ among themselves with mean levels of 0.003 and 0.001 mg.HgMe.kg<sup>-1</sup>, respectively. This result can also be explained by the processes of biomagnification and/or bioaccumulation over time, as reported by Lavoie et al. (2013).

Wren et al. (1980) found percentages of HgMe in total inorganic mercury between 78.6 and 87.8 % in muscle tissues of wild beavers (herbivorous) collected in a bay in the Muskoka District, in the Canadian province of Ontario. These percentages are therefore higher than those obtained in this study for non-predatory huntsing species, even for carnivorous species.

In general, the fish presented Hg-T and HgMe concentrations more significant than the fowl and vegetal foods, being therefore the main source of food exposure to these heavy metals for the Tupari Indigenous, who have meat of fish as their main source of animal protein, if not the most consumed food in the villages, while game meat can be the second main source of mercury food exposure, followed by vegetables.

However, this hypothesis can only be confirmed by taking the quantity of these different foods consumed for each of the villages, and from there calculate the HgMe intake rate due to consumption of fish, meat of hunts and vegetables and their ratio between the reference doses for weekly ingestion per kilogram of body weight (PTWD), thus obtaining the health risk quotient (RQ) (US EPA. 2004).

Figure 4 shows the estimated average amount for weekly consumption of the analyzed fish and meat of

huntss, as well as sweet potatoes (*I. batatas*), per village.

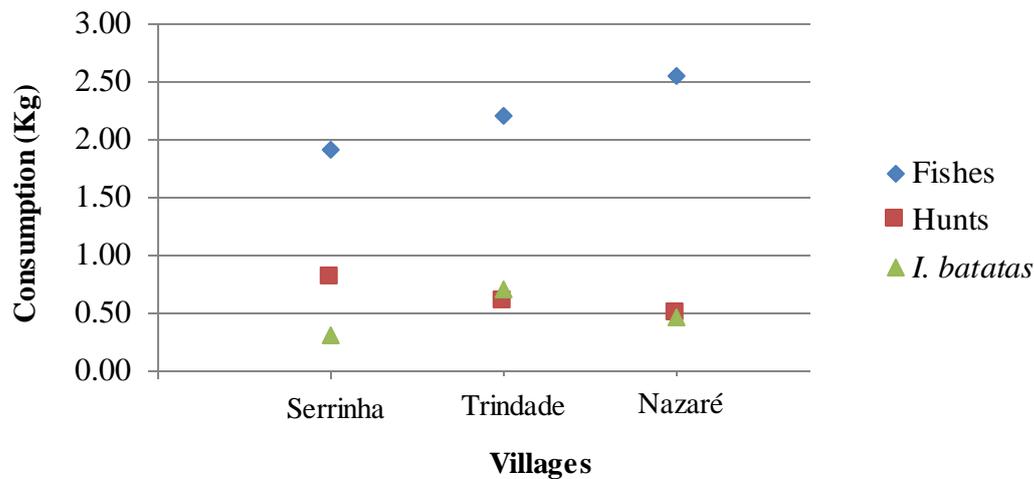


Fig. 4 - Estimated weekly average consumption of fish, game meat and sweet potatoes (*I. batatas*), per village

Several methodologies were used to collect information in studies that evaluated the consumption of fish in communities in the Brazilian Amazon region, including quantification of home consumption (OLIVEIRA et al., 2010), individual measures (PASSOS et al., 2008) and measures of consumption from the concentration of mercury in the hair (DOREA. 2003; DOREA et al., 2005). Among the studies that used the methodology of family consumption of fish with average per capita measures for fish consumption. Cerdeira et al. (1997) verified an average of 369 g/day in Monte Alegre (PA) and Boischio and Henshel (2000) of 243 g/day for the riverine population of the Madeira river (RO), values close to those verified in this research.

The Nazaré village had the highest average daily consumption (364 g/day) of fish, however, it was lower than the per capita consumption of 406 g/day found for an isolated community in Lake Puruzinho, Amazonas state (OLIVEIRA et al. 2010). On the other hand, due to the fact that 50 % of consumption in the Trindade village is of carnivorous species, this population may present a potential risk to high health, since the results showed higher concentrations of HgMe for this group of species compared to the species not predatory. This information corroborates with Ebinghaus et al. (2007), who stated that because of the biomagnification effect the carnivorous fish offer greater exposure to mercury,

The differences observed between the villages for the consumption of fish and meat of huntss can be related to the location of the villages in river Branco. In

the case of fish, for example, the lowest consumption was obtained for the Serrinha village, which has terrestrial access and is considered the point of reference and contact with the other IT villages. This is why there is a daily flow of vehicles from Funai and Sesai in the village. With a constant flow of vehicles, the indigenous residents of this village have greater access to the municipality of Alta Floresta do Oeste, where they usually hitchhike to buy other foods, which may have contributed to the reduction of fish consumption compared to the consumption in Trindade and Nazaré, the latter, is the most distant river along the studied estuaries, and perhaps because of this reason it had the highest average fish consumption.

Also due to land access, some natives of the Serrinha village have a motorcycle, which makes it easier to move to more distant forest areas within the TI where they usually go huntssing. This observation may have influenced the higher huntssing consumption observed for this village compared to Trindade and Nazaré, which were significantly the same as the average weekly meat consumption of the wild animals analyzed in this study. The greater consumption of sweet potatoes (*I. batatas*) for the village Trindade may be due to this vegetable being consumed by some families of this village with sugar after meals, therefore habitually consumed in the village as dessert. Proportional percentages and estimated amounts of fish and game consumption, by food habit and by village, are shown in Table 3.

Table.3 - Quantity (kg) and percentage proportion (%) of estimated consumption by food habit of fish and hunts analyzed, by village.

Food	Food habit	Village		
		Serrinha kg (%)	Trindade kg (%)	Nazaré kg (%)
Fishes	Carnivorous	0.475 (25)	0.660 (30)	1.275 (50)
	Detritivorous	0.475 (25)	0.660 (30)	0.765 (30)
	Herbivorous	0.950 (50)	0.880 (40)	0.510 (20)
Hunts	Carnivorous	0.040 ( 5 )	0.030 ( 5 )	0.025 ( 5 )
	Detritivorous	0.320 (40)	0.210 (35)	0.150 (30)
	Herbivorous	0.440 (55)	0.360 (60)	0.325 (65)

According to the literature, mercury in the muscle of fish and other predatory animals is predominantly in the organometallic form that is the result of the bioaccumulation and biomagnification of HgMe over time and as the trophic level increases (POSTE et al., 2015; LAVOIE et al. al., 2013). Based on this assumption and the toxicity of this chemical, it is inferred that the greater the proportion of meat consumption of carnivorous animals in the diet, the greater the human exposure to HgMe. According to Dórea et al. (2006) in the Amazonian lifestyle, traditional fish consumption is

high. characterizing the greatest route of exposure to HgMe, and in the medium and long term can pose a risk to the health of these people depending on the concentrations of mercury present in these fish and the amount consumed. Table 4 shows the calculated values for the HgMe WIR through the consumption of sweet potatoes (*I. batatas*) and of the fish and wild animal analyzed by food habit, as well as their percentages in relation to the JECFA reference (PTWI) (2014), EFSA (2015) and US EPA (2001), by village.

Table 4 - Comparison between the HgMe WIR by the consumption of sweet potatoes (*I. batatas*), fish and meat of hunts and the different reference doses (PTWI), by village.

Food	Food habit and vegetable specie	WIR ( $\mu\text{g}/\text{kg bw}/\text{week}$ )		
		Serrinha *(66.4 kg)	Trindade *(68.3 kg)	Nazaré *(69.7 kg)
Fish	Carnivorous	4.32078	5.83660	11.04878
	Detritivorous	1.78840	2.41581	2.74390
	Herbivorous	2.21762	1.99707	1.13415
Hunts	Carnivorous	0.07892	0.05919	0.04699
	Omnivorous	0.01445	0.00949	0.00646
	Herbivorous	0.00663	0.00008	0.00466
Vegetable	<i>I. batatas</i> (Sweet potatoes)	0.00041	0.00003	0.00033
<b>Total WIR (<math>\mu\text{g}/\text{kg pc}</math>)</b>		<b>8.4<sup>c</sup></b>	<b>10.3<sup>b</sup></b>	<b>15.0<sup>a</sup></b>
% PTWI JECFA		525	644	937
% PTWI EFSA		646	792	1154
% PTWI US EPA		1200	1471	2143

WIR: HgMe weekly intake rate through the estimated consumption of fish, meat of hunts and sweet potatoes (*Ipomoea batatas*) analyzed. WIR Total: Sum of WIR obtained for different foods. \* Average body weight of the population per village. % PTWI JECFA: Percentage of total WIR over reference dose of 1.6  $\mu\text{g}/\text{kg bw}$ . % PTWI EFSA: Percentage of total WIR in relation to the reference dose of 1.3  $\mu\text{g}/\text{kg bw}$ . % PTWI US EPA: Percentage of WIR over reference dose of 0.7  $\mu\text{g}/\text{kg bw}$ . Means followed by equal letters in the same line do not differ from each other ( $p > 0.05$ ) in relation to the total WIR obtained for the populations of the three villages studied.

As can be observed in Table 4, the highest ( $p < 0.05$ ) WIR value of HgMe was obtained for the population of the Nazaré village, which is directly related to the amount of consumption of carnivorous fish species, which 73.3 % of the total WIR (11.04  $\mu\text{g.HgMe}/\text{kg bw}$ ) for this village. From the analyzed foods, the fish

accounted for the highest percentages of the total WIR calculated for the three villages studied, in the order of 98.8, 99.0 and 99.3 % for Serrinha, Trindade and Nazaré, respectively, followed by the answered for the second place in percentage relative to the total WIR of HgMe, for all the villages.

With a total WIR of 15.0  $\mu\text{g.HgMe/kg bw}$  the population of Nazaré village reached 937, 1154 and 2143 % reference doses (PTWI) of JECFA (2014), US EPA (2001) and EFSA (2014), respectively. After the village of Nazaré, the highest total WIR was obtained for the Trindade population (10.3  $\mu\text{g.HgMe/kg bw}$ ) with total WIR of 644, 792 and 1471 % in relation to the PTWI established by the different comparative same order as that for Nazaré for the reference doses used for the comparison.

Despite the fact that it presented the lowest total WIR in this study (8.4  $\mu\text{g/kg bw}$ ), the Serrinha population exceeded 327, 496 and 1713 times the maximum doses (PTWI) suggested by JECFA (2014), EFSA (2015) and US EPA (2001), respectively. At the same time that these results are mainly reflections of the consumption of fish they surprise and concern, therefore, they can directly imply in the increase the risk to the health of these natives due to the possibility of bioaccumulation of HgMe in the organism with the course of the years. However, it is worth adding that the bioaccumulation of mercury in the human organism is dependent on its rates of ingestion and elimination (POSTE et al., 2015).

The WIR results obtained for the fish analyzed in this study are considerably higher than those found by Castilhos et al. (2001), when they obtained a daily intake of Hg-T of 190 ng/kg bw/day. equivalent to 1.33  $\mu\text{g/kg bw/week}$  from the consumption of fish collected in areas contaminated by the gold mining of the Tapajós river, state of Pará, Amazon. In this same study the authors verified a WIR 0.56  $\mu\text{g/kg bw}$  in regions where such activity is not practiced. The difference between the studies occurred due to the quantity consumed, since the

consumption verified for the villages analyzed in this research was superior to those verified by Castilhos et al. (2001).

Mourão (2016) found a mean exposure dose of HgMe by the consumption of fish in the Madeira river region of 3.4 and 3.5  $\mu\text{g/kg bw/week}$  for the infantile-juvenile and adult groups of Porto Velho, state of Rondônia also in Amazonia, respectively, therefore, less than half of the exposure values (WIR) calculated by the intake of fish verified in this study for Serrinha (8.3  $\mu\text{g/kg bw/week}$ ) and lower than the exposure in Nazaré village (14.9  $\mu\text{g/kg bw week}$ ) at least three times. These authors concluded that even with concentrations of mercury in fish below national limits, the quantity and frequency of fish consumption in the riverine communities of the Madeira river are sufficient to maintain a high exposure dose for all age groups.

Considering that the comparison between WIR and PTWI allows only an estimation of the level of exposure to HgMe or other toxic compound due to the ingestion of contaminated foods (PASSOS et al., 2008), and not directly and directly the health risk of these, aiming to characterize the potential health risk of the populations of the three Tupari villages studied. Through the WIR of HgMe obtained for the fish, hunts and vegetal analyzed, was estimated RQ for the health of the populations of the three villages based on PTWI of 1.6, 1.3 and 0.7  $\mu\text{g.HgMe/kg bw}$ , which indicate doses of chronic oral exposure of methylmercury. The results compared so that  $RQ > 1$  showed a potential risk of contamination (US EPA, 2004) by HgMe in medium and long term. The values of RQ calculated according to US EPA (2004) based on the PTWI are shown in Figure 5.

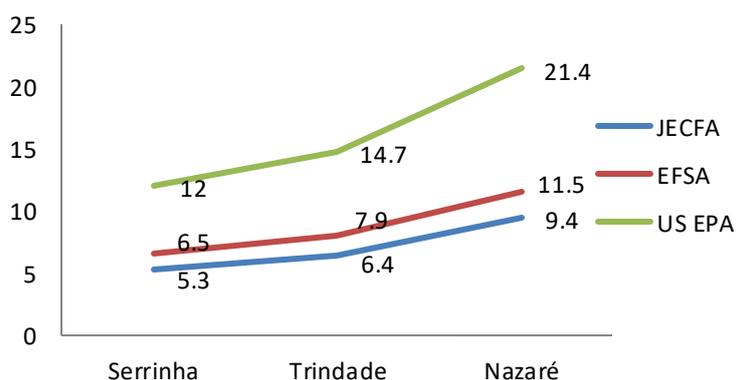


Fig. 5 - Risk quotient (RQ) from the intake of HgMe through the consumption of all foods analyzed, based on the reference doses (RfD) suggested by PTWI of JECFA (2014), EFSA (2015) and US EPA (2001).

Ranges varying between 5.3 and 21.4, the RQ values differed between the three villages studied based on the PTWI of the different regulatory institutions used as parameters for the comparison, so that the greater potential risk of health problems due to the ingestion of

HgMe was always checked for the population of Nazaré, followed by Trindade and Serrinha. Considering the proportion (50 %) of fish consumption at the top of the food chain in the village of Nazaré (Table 3), a higher weekly intake rate (WIR) of HgMe was already expected

for this population and, consequently, risk (RQ) of suffering adverse health effects compared to other villages.

Even using the reference dose of JECFA (2014), less rigorous (PTWI 1.6  $\mu\text{g}/\text{kg}$  bw) among the organizations that establish maximum tolerable values for weekly intake of HgMe, the RQ obtained for the populations of Serrinha, Trinity and Nazaré behaved higher than 4.3, 5.4 and 8.4 times, respectively, the value of RQ understood as safe limit ( $\text{RQ} \leq 1$ ) so that health problems due to oral exposure to this organometallic compound do not occur.

When used in this study, the reference dose of US EPA (2001) to calculate the ratio between WIR and PTWI suggested, the RQ obtained in this study extrapolated between 11 and 20.4 times, which occurred as a function of the dividend (PTWI) is lower (0.7  $\mu\text{g}/\text{kg}$  bw) than PTWI (1.6  $\mu\text{g}/\text{kg}$  bw) recommended by JECFA (2014). Another important observation regarding the evaluation of the risk of exposure of these indigenous populations to HgMe is that when considering only the sum of the WIR obtained for the game and the analyzed fish, the RQ would be on the order of 0.14, 0.10 and 0.08 ( $<1$ ) for the villages Serrinha, Trindade and Nazaré, respectively, even considering PTWI of 0.7  $\mu\text{g}/\text{kg}$  bw, the most rigid of them (US EPA, 2001). Thus, it can be understood that the consumption of sweet potatoes (*I. batatas*) and meat of hunts in the villages studied is not a potential source of risk to the health of the indigenous Tupari due to exposure to HgMe, with fish being the greater risk factor found for this people.

Compared with the international literature, few studies with populations of the Brazilian Amazon evaluated the health risk of diet through the calculation of RQ. These studies include Mourão (2016), who verified a RQ above 1 between 51 % and 97 %, 55 % and 87 % and 54 % and 96 %, respectively, in the groups of children and adolescents, women and adults. These results are lower than those obtained in this research, which is directly related to the higher consumption of fish in the Rio Branco Indigenous Land in comparison to the consumption of the populations studied by the author.

With weekly T-Hg ingestion rates of 31.5 to 44.8 ( $\mu\text{g}/\text{kg}$  bw), Boischio and Hanshel (1996) found values of RQ between 21 and 64 for riverine infants, women of childbearing age, and children under 5 years old, living along the Madeira river, state Rondônia. The authors suggested that children in this riverbank population were taking doses of Hg that could cause neurological damage. Hacon et al. (1997) also evaluated the potential health risk of Amazonian populations using the RQ based on the ratio between the estimated intake rate and the reference dose (PTWI) selected for that study (2.1  $\mu\text{g}/\text{kg}$  bw/week),

with an estimated risk index (RQ) of 9.3 and contribution of 92 % of fish intake.

Although Santos et al. (2000) observed no signs or symptoms of mercury intoxication, the authors observed higher levels of mercury in fish from the Tapajós river region in the state of Pará, and also in the Brazilian Amazon. The authors warned that the high rates of fish consumption in that region raise concerns about the possibility of effects arising from chronic exposure, especially among children and women of childbearing age.

Still in the Brazilian Amazon, Farias (2006) observed high levels of risk for pre-school children in the Jaú National Park region in the state of Amazonas, when for many of them the T-Hg was higher than 5  $\mu\text{g}/\text{kg}$  bw/week, which was PTWI then regulated by the WHO at the time of this study.

The recent international literature is vast of studies by which the risks were estimated by the ingestion of fish containing Hg, however, the majority is marine fish. For example, the studies carried out by Anual et al. (2018) in crustaceans, cephalopods and fish from Malaysia and by Ahmed et al. (2018) on starfish species used for food in regions of the Arabian Sea coast. Although these authors evaluated the risk of eating fish and/or seafood, they found that weekly intake values were considerably lower than those obtained in this study, therefore, with a lower risk of mercury exposure compared to the consumption of fish collected in tropical rivers of the Brazilian Amazon.

Although the effects of mercury on Amazon populations are not as clear, studies in the region have evaluated the effects of mercury exposure on the neurological development of children (MARQUES et al., 2007), prenatal exposure (MARQUES et al., 2013) and adults (YOKOO et al., 2003). These studies have reported that fish consumption, maternal schooling, and nutritional status are all possible factors that may mask the relationship of exposure to mercury with effects on the central nervous system.

The scientific restlessness regarding the human exposure to different organomercurial compounds such as HgMe is mainly due to its slow elimination by the organism, and according to Ertas et al. (2014) extend for years in the brain and according to Kim et al. (2015) also in the kidneys. Several studies have demonstrated the neurotoxic effects of HgMe in populations exposed to this contaminant. The results obtained in the riverside population (HACON et al., 2008) of the Amazon basin exposed to HgMe due to high fish consumption. Through sensitive neurofunctional tests these researchers observed a decrease in visual and motor functions as the levels of mercury in the individuals' hair increased.

In addition to the health problems resulting from the high HgMe ingestion rate mentioned above this metal has also been linked to heart disease. Studies published in the New England Journal of Medicine (2002) reported a direct association between HgMe and myocardial infarction (GUALLAR et al., 2002; YOSHIZAWA et al., 2002).

Contrary to what was observed by Hacon et al. (2008), in a clinical evaluation performed by Dórea et al. (2005) in different indigenous villages of the Brazilian Amazon did not detect neurological complaints such as paraparesis, numbness, tremor and failure of balance, compatible with the exposure to mercury. These authors and their collaborators warned, however, that exposure to HgMe by ingestion fish of sweet-water is a minor problem compared to endemic infectious diseases occurring in the Amazon, such as malaria. The authors added that although fish are abundant in the indigenous diet, they have been consumed without apparent problems to the health of these Amazonian peoples.

The diet based on foods containing considerable amounts of selenium (chestnuts) and fibers (cassava meal) may be helping to avoid the occurrence of perceptible and/or other cardiotoxic effects in the riverine and indigenous populations (ROCHA et al., 2014; LEMIRE et al., 2010). As discussed in this paper and reported by Passos (2003), these compounds have antioxidant and cardioprotective effects, which aid in the production of substances with (KHAN; WANG. 2009) and thus reducing its deleterious effects on the health of these traditional populations of the Brazilian Amazon susceptible to the high RQ by fish ingestion.

#### IV. CONCLUSION

The results of the risk assessment using the MeHg intake rate through consumption of fish, game meat and sweet potatoes indicated that the populations of the indigenous villages studied are exposed to the risk of suffering medium and long term health problems due to the ingestion of these foods, mainly due to the consumption of carnivorous fish. However, although fish have been considered the main sources of exposure to HgMe for the indigenous studied in this research, they have also been a source of selenium in the diet of these populations, such as chestnuts and cassava, which may be improving the chronic risk to the health of the Tupari by the ingestion of foods containing HgMe.

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