Legacy Organochlorine Pesticides and Methylmercury in Amazon Catfish (*ageneiosus brevifilis*): Risk Assessment for Riverside People

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1. Introduction

Contamination by anthropological sources has been taken as a worldwide concern issue, since last decades, even in remote or pristine areas. Although, there still is a lack of information about the real threats regarding high polluted areas or places that had a massive historical use of harmful substances. Mainly if considered the possible reactions in the environment and its metabolites [1].

Since 2001, most of the OCPs have been regulated under the Stockholm convention as persistent organic pollutants (POPs). The Stockholm convention restrictive measures started from 2004 including 12 substances, and keep working to listing new POPs [2]. The production of pesticides in industrial scales made Brazil one of the main exporters of pesticides in South America [3].The most used OCP in Brazil, perhaps in the world, is the Dichlorodiphenyltrichloroethane (DDT) [4]. In 1998, the Brazilian Ministry of Health stated that there was no longer the use of this compound in the country, but its total ban only took place in 2009. However, recent studies suggest a recent input of DDT in Brazilian Amazon [5] and point out the presence of unstudied DDT related compounds in the southeast coast of Brazil [1, 6].

Mercury occurs naturally in the environment under several forms, but its massive use for different purposes has become an environmental concern issue due its concentration increase at specific places [7]. Regarding Hg speciation, the most common organic form of mercury is the methylmercury (MeHg), which is also one of the six most toxic substances in the world and, like the POPs, increases in concentrations up ward trophic levels, reaching high concentrations in top-chain organisms [8].

Despite the restrictive measures adopted along past years, organochlorine pesticides (OCPs) and mercury (Hg) continue to act as important threats in the environment because of its high potential for long-range transport, bioaccumulation, environmental persistence and high toxicity [9]. In the Amazon biome, most of these contaminants came from mining, agro-chemical and forest burning [10].

Fishes are considered good indicators to chronic contamination for both organochlorine and methylmercury pollutants and the main via to human exposure, especially those at high trophic level due to its contaminants biomagnifications potential. Hence, carnivorous catfishes seem to be good indicators of environmental contamination and a good tool to access the exposure of riverside people, once it is one of the cheapest and commonly consumed fish by this population.

This study aimed to expand the knowledge about environmental contamination by massive used contaminants in Brazil, being able to indicate potential threats to Amazon riverside people regarding their main dietary.

2. Materials and Methods

Barcelos is the biggest city of Amazonas state and the second biggest of Brazil, in terms of area (122,476 km²). Located upside the Rio Negro (0° 58′ 30″ S, 62° 55′ 26″ W), its weather is typically tropical. Its population is estimated in 27.589 habitants, which means a population density of 0.23 hab/km², extremely dependent on fishing to their economy and feeding [11]. This part of Brazilian Amazon has a massive historical use of both contaminants: OCPs due to the fight against vectors of tropical diseases and Hg due to the intensive mining activity.

The *Ageneiosus brevifilis*, commonly known as Mandubé, is a leather fish commonly found in the Amazon basin freshwater. This species can reach up to 50 cm (total length) and 2.5 kg when mature. They have living habits at river bottom in muddy water, considered a carnivorous and nocturnal species that feed themselves mainly through fishes and invertebrates [12]. Caught in abundance and sold by small prices at most of Amazon fish markets, this species is a very important source of protein to riverside population, mainly to the poorest people.

Fish samples (n=12) were bought at the local fish market in Barcelos city, Amazonas, Brazil (September, 2015). They were freeze-dried by liofilization and only muscle tissue was used to measure the contaminants. OCPs determination was based on a miniaturized method optimized for exhaustive extraction and simultaneous purification of halogenated pollutants in fat containing matrices [13, 14], with modifications. 100µL of the internal standard PCB 103 and 198 was added to ~0.5 g of catfish samples. Extracts were dried and then reconstituted using 100 µL of injection standard (TCMX 100 $ng.g^{-1}$) for chromatographic analysis.

Organochlorine pollutants were determined using a 7890N gas chromatograph equipped with a BD5 fused silica capillary column (60m x 0.25 mm x 0.25 µm film thickness) and coupled with a 5975C quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA). The equipment operated in SIM mode for target compounds, 25 substances related to legacy OCPs from the standard solution Pesticide Mix 1, AccuStandard, New Haven, USA; and in full scan mode to evaluate the presence of non-target compounds, both with electron capture negative ionization (ECNI).

Total Mercury (HgT) determination was carried out following the method described by Bastos *et al.*, 1998 [15]. Which consists in acid digestion (H_2SO_4 + HNO_3 - 3:1) followed by potassium permanganate oxidation ($KMnO_4$) of ~0.4 g (freeze-dried sample). Atomic absorption spectrophotometry coupled with cold steam generator, was used to measure the HgT concentrations (FIMS 400 Perkin-Elmer). Methylmercury concentrations were measured from ~0.03 g (freeze-dried sample) by KOH methanol (25%) digestion followed by ethylation with Sodium tetraethylborate as a Derivatizing Agent (NaBEt4), according to Bloom, 1992 [16] and EPA, 2001 [17]. Gas chromatography coupled with atomic fluorescence spectrometrywas used to measure the MeHg concentrations (MERX™ Automated Methylmercury Analytical System – Brooks Rand).

Regarding the chronic exposure to some harmful substances, the World Health Organization (WHO) proposes an Acceptable Daily Intake (ADI) as the maximum quantity of a toxic substance that offers no risk even if daily ingested. The ADI value is not definitive and is expressed in $\mu g.kg^{-1}$ (body weight). Brazilian data for fish consumption is estimated in 10.6 kg/habitant/year which reflect a medium value of 29 g/habitant/day [18]. Although, previous studies reports that riverside people eat much more fish than the national average and suggests a medium value of 200 g/habitant/day [19]. Thus, the Estimated Daily Intake (EDI) was measured according to the local fish consumption for riverside people and the adult medium body weight of 70 kg then compared to each ADI value [20].

$$Conc.\ \left(\frac{\mu g}{gp.u.}\right) * \frac{g/habitant/day}{70\ \mathrm{kg}} = \mathrm{IDE}\ \left(\frac{\mu g.\mathrm{kg}^{-1}}{\mathrm{day}}\right)$$

For Quality assurance and Quality control (QA/QC), 3 blanks were included to the analytical batch to perform blank correction from their average. Method recovery for OCPs, based on PCB 103 and 198 concentrations, was satisfactory with medium recovery of 93% and standard deviation of 12%. To evaluate the accuracy of the mercury and methylmercury-based methods, a certified reference material from the National Research Council (Dorm-3 - fish protein) was used, with satisfactory results, obtaining a 98% recovery for HgT and 112 % for MeHg. The method limit of quantification (LOQ) was calculated by the medium value of the blanks plus 3 times its standard deviation value.

3. Results and Discussion

All samples showed contamination levels of Hg and some OCPs. The values were expressed in $\mu g.kg^{-1}$ (wet weight) in order to access the fishes' contamination levels as they are consumed. Results were grouped by the sum of related substances for pesticides and only MeHg concentrations were considered in this study because of its predominance in muscle tissues of analyzed samples. The average percentage of methylmercury regarding the total mercury for all samples was of 98 ± 6%. Each concentration value is shown in Table 1. The sums of pesticides should be read as ∑-HCH $=\alpha + \beta + \delta + \gamma$ –HCH; ∑-chlordane= *Cis* and *Trans*-chlordane + oxychlordane; ∑-DDX = *o,p'* and *p,p'*-DDD + DDE + DDT. There was no detectable concentration of any non-target compound related to legacy pesticides besides pentachloroanisole (PCA). PCA is a common metabolite of pentachlorophenol (PCP) in fish, although it is not well documented, in Brazil, PCP is used only in wood protection, since 1998, before that it was used as fungicide, algaecide and insecticide [21].

Data dispersion among each contaminant group is represented in Figure 1. It is important to highlight that the greatest medium values were measured for MeHg (1127.37µg.kg^{-1}) and ∑-DDX (600.64 µg.kg^{-1}), followed by methoxychlor (235.81 µg.kg^{-1}), ∑-chlordane (71.97 µg.kg^{-1}), heptachlor (70.31 µg.kg^{-1}), ∑-HCH (54.05µg.kg^{-1}) and HCB (47.88 µg.kg^{-1}). The contamination profile of OCPs was quite similar to most of the samples. These results can be explained by the massive input of DDT and mercury in the Amazon basin, mainly related to the fighting against vectors of tropical diseases and mining, respectively [10]. While the others OCPs could be more related to agro-chemicals input, lower usage in sanitary campaigns or even atmospheric deposition [22].

In order to evaluate the human exposure to the studies contaminants throughout catfish consumption, the EDI was compared to the ADI for each contaminants group. The ADI provisory values for OCPs were estimated in 0.6 µg.kg^{-1}/day for HCB, 5 µg.kg^{-1}/day for ∑-HCH, 0.5 µg.kg^{-1}/day for ∑-chlordane, 10 µg.kg^{-1}/day for ∑-DDX, 0.1 µg.kg^{-1}/day for heptachlor, 10 µg.kg^{-1}/day for methoxychlor and 0.1 µg.kg^{-1}/day for Hg. For most OCPs the EDI do not reach the respective ADI proposed value. Only heptachlor EDI average (0.15µg.kg^{-1}/day) exceeded the ADI, with half of the samples (n=6) ranging from 0.2 to 0.36 µg.kg^{-1}/day. Nevertheless, 100% of catfish samples exceeded the reference acceptable value for mercury, ranging from 1.79 to 5.74 µg.kg^{-1}/day, with an average value (3.22 µg.kg^{-1}/day) 30 times higher than the ADI.

As depicted by our results, the higher concentrations of ∑-DDX and MeHg, up to one order of importance than the others contaminants, reflect and endorse the massive use of DDT and Hg in the region. Even though the recent input of legacy pesticides could be dismissed, these chemicals can still be a threat for those who live in contaminated areas and have local fishes as their main protein source.

Table1: Contaminants concentrations (µg.kg^{-1}w. w.) in fish muscle tissue from Barcelos, Amazonas, Brazil.*Ab* refers to the species, *Ageneiosus brevifilis.*

Sample	Lenght	HCB	∑-HCH	∑-Chlordane	∑-DDX	Heptachlor	Methoxychlor	MeHg
***Ab* 1**	35 cm	< LOQ	< LOQ	< LOQ	100.72	< LOQ	80.57	986.61
***Ab* 2**	37 cm	44.33	83.74	88.67	512.29	73.89	344.81	1027.77
***Ab* 3**	39 cm	58.29	47.69	127.18	392.15	100.69	296.76	2009.46
***Ab* 4**	38 cm	56.67	77.28	164.87	2060.83	108.19	278.21	1112.37
***Ab* 5**	35 cm	43.02	52.58	47.80	200.78	71.71	229.46	1216.04
***Ab* 6**	41 cm	26.62	37.27	63.89	532.41	5.32	31.94	1624.68
***Ab* 7**	38 cm	55.75	55.75	70.96	537.27	15.21	293.98	628.93
***Ab* 8**	35 cm	< LOQ	45.73	10.16	233.71	< LOQ	< LOQ	982.42
***Ab* 9**	37 cm	26.45	15.87	42.33	476.19	15.87	< LOQ	755,05
***Ab* 10**	41 cm	83.07	27.69	44.30	420.88	116.30	276.90	949.14
***Ab* 11**	33 cm	33.42	81.16	47.74	381.91	< LOD	171.86	1281.72
***Ab* 12**	30 cm	51.18	69.79	83.75	1358.58	125.62	353.60	954.26

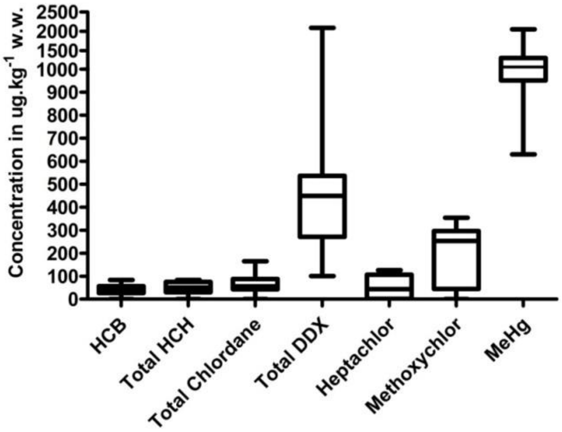

Figure 1: Boxplot graphic illustrating the data dispersion among each contaminant group for catfish samples.

4. References

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