Organochlorine Pesticides (OCPs) in commercial fish from Guanabara Bay of Rio de Janeiro State, Brazil

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Introduction

Fishing is an activity of great importance for Brazil because of its vast coastal extension and economic relevance. Estimates of per capita consumption by the First Directory of Fisheries and Aquaculture in Brazil show an increase in intake from 9,03 kg/person/year in 2009 to 11,17 kg/person/year in 2011 [1]. The consumption of fish has been promoted in Brazil based on its nutritional and functional properties, because is a source of proteins with high biological value, the high amount of fat soluble vitamins such as vitamin D and water soluble vitamins such as B complex, as well an omega-3 polynsaturated fatty acids, recognized for anti-inflammatory potencial and cardioprotective effect [2]. With population growth and industrial activity increase, the demand for chemicals to improve food production has increased [3]. High levels of contaminants present in fishing bays in the state of Rio de Janeiro can affect fauna, water quality and fish. Among the contaminants can be highlighted the presence of Persistent Organic Pollutants (POPs), which are lipophilic substances and therefore tend to accumulate in the food chain, like Organochlorine Pesticides (OCPs) [4]. Most of these contaminants already have their banned use, but due to their characteristic of environmental persistence they represent a potential source of contamination of hydrographic bays and coastal areas [5]. The present study aims at investigating the occurence of OCPs in sardine *(Sardinella brasiliensis)*, whitemouth croaker *(Micropogonias furnieri)* and mullet *(Mugil liza)* from Guanabara Bay, located in the state of Rio de Janeiro, Brazil.

Materials and methods

The samples of sardine (n=20), whitemouth croaker (n=19) and mullet (n=16) were collected from January to December of 2015 directly with fishermen from Guanabara Bay at three different áreas: Gradim, located in the municipality of São Gonçalo, Barreto, located in the municipality of Niterói and Suruí, located in the municipality of Magé. All samples were conditioned in thermal bags and transported to the Eduardo Penna Franca Radioisotope Laboratory of the Carlos Chagas Filho Institute of Biophysics, where this analysis is routinely performed.

The analytical procedure for extraction, purification and chromatographic analysis of the samples followed the methodology described by Botaro et al. [6]. Initially all the samples were lyophilized and ground for the analysis. Briefly, approximately 3g of sardine muscle, 6g of whitemouth croaker and 6g of mullet were extracted in Soxhlet's system for 8 hours with a mixture of n-hexane and dichloromethane (1: 1) (v / v). After extraction the samples were concentrated under heating and nitrogen flow, purified by acid attack with the addition of concentrated sulfuric acid and centrifuged for 20 minutes. The clean-up was carried out through an open chromatographic column filled with anidrous sodium sulphate, activated alumina, acidic silica gel (44%) were eluted with 120 mL of hexane / dichloromethane (1:2) (v/v). Finally, the sample was concentrated under nitrogen flow and transferred to vial, where it is evaporated to dryness and resuspended with 50 µL of isoctane and 50 µL of TCMX (tetrachloromethoxyene) (200 ppb) and injected into a system in negative chemical ionization mode (GC-ENCI-MS) for identification and quantification of the OCPS. The system used was the Agilent 7890 gas

chromatograph coupled to an Agilent 5975C MSD mass spectrometer, with automatic injection in the *splitless* mode using a chromatographic column of 60m x 0.25mm x 0.25µm. Helium was used as carrier gas with a pressure of 16 psi. The chromatographic conditions of temperature were: 90 ° C for 1 minute, 10 ° C / min to 150 ° C, 3 ° C / min to 240 ° C for 5 minutes, 10 ° C / min to 300 ° C for 8 minutes.

Results and discussion

All results are expressed in ng OCP per gram of wet weight (ng/g w.w.) (Table 1). The fish samples evaluated had at least one OCP and the sum of concentration of these compounds (Σ OCP) ranged from 6.6 ng/g w.w., 7.4 ng/g w.w. and 2.8 ng/g w.w. for sardine, whitemouth croaker and mullet respectivelly. However, no significant difference was found between the concentration of the sum of these compounds among the three fish species studied. Among the 26 investigated substances only 7 were not found in any samples, they are: $^{\delta}$-HCH, Isodrin, Endrin, Oxyclordane, trans-heptachlor epoxide, β-endosulfan and Mirex.

Table 1: Average OCP concentrations ± SD (ng/g w.w.) in muscle of sardine, whitemouth croaker and mullet and P value using *Kruskal Wallis* test and post *Dunn* test*.

	Sardine (*n=20)*	Whitemouth Croaker (*n=19)*	Mullet *(n*=16)	P
HCB	0.016 ± 0.027[a]	0.005 ± 0.009[a]	0.006 ± 0.008[a]	0.1362
α-HCH	0.005 ± 0.007[a]	0.003 ± 0.006[a]	0.004 ± 0.007[a]	0.4847
β-HCH	<LOD	0.003 ± 0.010	<LOD	
$^{\gamma}$-HCH	0.041 ± 0.122[a]	0.018 ± 0.035[a]	0.025 ± 0.051[a]	0.8098
Σ HCH	0.046 ± 0.128[a]	0.024 ± 0.051[a]	0.029 ± 0.056[a]	0.6456
Heptachlor	0.011 ± 0.039[a]	0.036 ± 0.076[a]	<LOD	0.0570
Aldrin	0.001 ± 0.003	<LOD	<LOD	
Dieldrin	<LOD	<LOD	0.005 ± 0.019	
cis-Heptachlor epoxide	<LOD	0.003 ± 0.011	<LOD	
cis-Chlordane	0.004 ± 0.015[a]	0.015 ± 0.040[a]	0.007 ± 0.020[a]	0.3680
trans-Chlordane	0.014 ± 0.027[a]	0.011 ± 0.021[a]	0.019 ± 0.043[a]	0.7575
Σ Chlordane	0.018 ± 0.042[a]	0.026 ± 0.060[a]	0.025 ± 0.057[a]	0.9464
α-Endosulfan	0.000 ± 0.001[a]	0.001 ± 0.003[a]	0.002 ± 0.005[a]	0.2599
p,p'-DDE	0.766 ± 2.342[a]	0.695 ± 1.146[a]	0.286 ± 0.522[a]	0.3565
o,p'-DDE	0.429 ± 0.946[a]	0.018 ± 0.044[a]	0.343 ± 0.722[a]	0.1443
o,p'-DDD	0.068 ± 0.122[a]	0.014 ± 0.049[b]	<LOD	0,0224
p,p'-DDD	0.007 ± 0.031	<LOD	<LOD	
o,p'-DDT	0.459 ± 0.588[a]	0.005 ± 0.022[b]	0.008 ± 0.033[b]	<0.0001
p,p'-DDT	0.180 ± 0.400[a]	0.119 ± 0.199[a]	0.027 ± 0.090[a]	0.0665
t,t'-DDT	0.114 ± 0.096[a]	0.344 ± 0.492[a]	0.160 ± 0.217[a]	0.8049

Σ DDT	2.023 ±	4.526[a]	1.196 ±	1.953[a]	0.824 ±	1.244[a]	0.2284
Metoxichlor	4.462 ±	13.261[a]	6.162 ±	9.225[a]	1.955 ±	3.810[a]	0.2365
Σ OCP	6.576 ±	18.027[a]	7.454 ±	11.389[a]	2.846 ±	4.894[a]	0.1762

*Different letters in the same row differ statistically at the significance level of 5% ($p<0.05$), SD (Standard Deviation), <LOD (bellow detection limit), Σ HCH (α-HCH + β-HCH + ɣ-HCH), Σ Clordane (*cis*-Clordane + *trans*-Clordane), Σ DDT (p,p'-DDE + o,p'-DDE + o,p'-DDD + p,p'-DDD + o,p'-DDT + p,p'-DDT + t,t'-DDT), Σ OCP (Sum of Organochlorine Pesticides).

A second work carried out in Guanabara Bay investigated the presence of 25 OCPs this time in whitemouth croaker (n = 11) and mullet samples (n = 12), in the whitemouth croaker samples were found 23 OCPs and in mullet samples were found 20 pesticides in question [7]. The other results present in the study are expressed in ng/g of fat, making it difficult to compare the datas. Pesticides present in higher concentration of whitemouth croaker samples were Heptachlor, followed by β-endosulfan and Endrin, different from found in the current study where the pesticides found in higher concentrations in the whitmouth croaker samples were Metoxichlor, p, p'-DDE and p, p'-DDT. The same difference was found in the results in relation to mullet, in the results of Silva et al. where found in higher concentrations the p, p'-DDT, Heptachlor and Aldrin, already in the current study were found Metoxichlor, o, p'-DDE and p, p'-DDE, respectively.

In relation to the difference between the OCP concentration among the species, this difference was significant only for o, p'-DDE, which presented higher concentration in the sardines when compared to the whitemouth croaker and o, p'-DDT that presented higher concentration also in the sardines in relation to the whitemouth croaker and mullets, not having significant difference between the two last ones. This difference may be due to the fact that the DDT molecule has a high liposolubility, with octanol/water partition coefficient (Kow) equal to $9.6x10^5$, which also reflects its bioaccumulation potential [8]. And sardines are the species with the highest lipid percentage between the three species studied, the possibility of these compounds accumulating in their lipid tissue is higher than in the other species.

Due to the reduction of the use of DDT by its prohibition, in addition to the physicochemical characteristics of the molecule, the ratio between the concentrations of the metabolite DDE/ΣDDT is used as an indication of the recent use of this pesticide; reasons lower than 0.6 are indicative of the recent use of the same [9]. In the present research this ratio was higher than 0.6 for the three species studied, indicating that the contamination by this pesticide is probably not by recent use.

In addition, when we evaluated the proportion of DDT metabolites in the analyzed samples, we found DDE in a higher proportion than the other metabolites (DDT and DDD), with values of 59%, 60% and 76% for sardines, whitemouth croaker and mullet respectively. These results can be explained not only by the reduction of the recent use of this pesticide but also by the rapid biotransformation of DDT in DDE described in fish [10]. Another determining factor for the difference in concentration between the DDT metabolites is the half-life of these molecules, when the p,p' DDT molecule is incorporated into the fish tissue it has a half-life of approximately 8 months, lower than the half-life of the p,p' DDE molecule, which has a half-life of about 7 years in fish [11].

In relation to the metabolite t, t'-DDT, that although it was found in low concentrations in the studied samples, it was found more frequently in all of them in relation to the other compounds. The t, t'-DDT is a very poorly studied and discovery for the first and only time in raya samples (*Gymnura altavela*, Linnaeus, 1758) from Guanabara Bay, with a concentration of 324.4 ng/g of fat in the first sample and 1,146.1 ng/g of fat in the second sample. In this paper the authors suggest that this metabolite is possibly formed by the transformation of the o, p'-DDE molecule with the loss of HCl by a biotransformation involving UV irradiation [12]. Therefore, the present study is one of the pioneers in the identification and quantification of this metabolite.

The concentrations of OCPs found in the samples of the present study are low compared to results referring to other species of fish and other Bays, which may be associated to the fact that the species studied migrate from Guanabara Bay by a long stretch of the Brazilian coast, the sardine is a species whose population is distributed in the Southeast Brazilian coast that goes from Cabo de São Tomé/RJ to the cape of Santa Marta Grande/SC [13]. In the case of whitemouth croaker, Guanabara Bay represents an important estuary for the first stages of life of the species, being captured in this Bay a percentage of approximately 83% juveniles and a smaller percentage of adult fishes [14]. As all the compounds investigated in the present study are Persistent Organic Pollutants, therefore, characterized by their potential for bioaccumulation and biomagnification, the younger organism have lower concentrations of these compounds accumulated [4].

Regarding the collection areas of the present study (Gradim, Barreto and Suruí), no significant difference was found between the points in relation to the ΣOCP in the three species. This result can be explained not only due to the small number of samples of each species per collection area, but mainly due to the environmental characteristics of the species that cross the entire Bay during its life cycle, in this way, the area where each sample was collected is not able to interfere in the total content of OCP [13].

Other studies carried out in Guanabara Bay investigating not only the presence of OCPs but also Polychlorinated Biphenyls (PCBs), another class of POP originating basically from the industrial residue of capacitors and energy transformers, found higher concentrations of ΣPCB in relation to ΣDDT, Suggesting a greater industrial impact in this area in relation to the impact caused by chemical residues from agriculture [5].

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