High organochlorine concentrations in a threathened Guiana dolphin population

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Introduction

Persistent Organic Pollutants (POPs) are recognized for its wide utilization around the world in the past¹. Organochlorine compounds were used mostly in electrical equipment and as vector control^{2,3}. Nowadays, its use is prohibited in many countries mainly due to the toxic effects observed in many organisms, which includes immune and reproduction disorders¹. Organochlorine compounds are stable, persistent and can be found in high concentrations in aquatic organisms, especially in odontocete species. Organochlorine compounds includes polychlorinated biphenyl (PCBs) and pesticides, such as dichlorodiphenyltrichloroethane (DDT) and Mirex^{2,4}.

Guiana dolphin (*Sotalia guianensis*) is a coastal delphinid found in the Western Atlantic Ocean, with north limit in La Mosquita, Honduras (14°00'N 83°20'O) and south limit in Baía Norte, Santa Catarina, Brazil (27°35'S 48°34'O)⁵. In Brazilian waters, Guiana dolphins can be founded mainly in estuarine environments, such as in Guanabara Bay, located in Rio de Janeiro, South-eastern Brazil, where it is found resident population⁶. Guiana dolphin abundance in Guanabara Bay has been decreasing steadily in recent years, with less than 40 individuals currently⁷. Due to its presence near the coast, their high trophic level and long-lived Guiana dolphin is exposed to chemical pollution^{4,8}. It has been showed that Guiana dolphins from Guanabara Bay presented high organochlorine compounds concentrations and are comparable with other species from Northern Hemisphere^{8,9,10}.

The objective of the present study was determinate organochlorine compounds concentrations in the subcutaneous adipose tissue (blubber) of Guiana dolphin from Guanabara Bay.

Materials and methods

Blubber samples were obtained from 15 Guiana dolphins, *Sotalia guianensis*, that were incidentally captured in gillnets or find stranded in Guanabara Bay between 2008 and 2015. Guanabara Bay is in Rio de Janeiro state coast, Brazil (22°50S; 43°10'W). It is a coastal bay heavily polluted due the high population density and the massive presence of many industries^{8,12}. Guanabara Bay is one of the areas with strongest anthropogenic pressure in Southern Henisphere^{8,12}.

Samples were stored in aluminum and kept frozen (-20°C). Briefly, aliquots of approximately 1.0 g of blubber were homogenized with anhydrous sodium sulfate (1:5), an internal standard (PCB 103 and PCB 198) was added and extracted by continuous soxhlet apparatus with 100 mL of a mixture of hexane:dicloromethane (1:1) for 8 hours. The extract was reduced to approximately 5 ml and then were mixed with sulphuric acid for the clean-up. Lipid content was measured gravimetrically. After centrifugation and hexanic phase separation, an internal standard (2,3,5,6-tetrachloro-m-xylene/TCMX) was added for the quantification^{4,11}. Analysis was performed in a gas chromatographer with an 63 Ni electron capture detector (*Agilent Technologies*, 7890). Integration and acquisition of the data were performed in Ezchrom 3.2.1 Software System and organochlorine concentrations were expressed in µg. g⁻¹ lipid weight (lw). The organochlorine compounds analysed were: PCBs and pesticides. Pesticides were expressed as the sum of DDT and metabolites and HCH isomers (o.p'-DDE, p.p'-DDE, o.p'-DDD, p.p'-DDD, o.p'-DDT, α , β , $\gamma \in \delta$, repectively) besides HCB and Mirex. The sum of PCBs was represented by 25 congeners (IUPAC numbers 8, 28, 44, 97, 101, 105, 118, 132, 138, 141. 151, 153, 158, 174, 177, 180, 183, 187, 194, 195, 199, 203, 206, 209). Blanks were used in each batch and the recovery of the samples were satisfactory, ranging between 84% and 120%. Standards solutions were PCB Congener Mix for West Coast Fish Studies and WHO/NIST/NOAA Congener List for PCBs and Pesticide Mix for pesticides, both from AccuStandard® Laboratory. The analytical method was validated using two certificated materials (Cod Liver Oil-SRM:1588 and Whale Adipose Tissue -SRM-1945) from the National Institute of Standards and Technology-NIST). The detection limits of the method varied from 0.08 to 2.2 μ g/L for PCBs and from 0.29 to 0.52 μ g/L for pesticides.

Statistical analyses were performed by STATISTC 7.0 Software System for Windows. Data was tested for data normality by Shapiro Wilk's test (p < 0.05), and non-parametric test was used. Mann-Whitney's test was applied to verify possible sex differences and sexual maturity in relation to organochlorine concentrations.

Results and discussion:

The organochlorine concentrations and biological data are presented in table 1. \sum PCB were the predominant compounds. This pattern was also found in delphinids in other studies in Brazilian coast^{2,4,8,13}. PCBs have a wide use and greater persistence in the environment due its physical-chemical properties^{2,4,14}. Especially in this study, it was expected higher concentrations of \sum PCB than \sum DDT since Guanabara Bay is an estuary highly impacted mostly by industrial sources and it is surrounded by large human population densities^{8,12}. Among PCBs, PCB 153 was the predominant congener, followed by PCB 138 and PCB 180, agreeing with other studies^{2,4,8}. Due their high degree of chlorine substitution and high molecular weights, these congeners are persistent in the environment¹⁴. Besides the large use of this compounds in commercial mixtures in Brazil^{2,4}. For DDT, p.p'-DDE were the predominant metabolite, reflecting a not recent use of DDT in Rio de Janeiro's coast².

Table 1: Mean, standard deviation, median and minimum and maximum (min-max) of biological data and organochlorine concentrations ($\sum PCB$, $\sum DDT$, $\sum HCH$, HCB and Mirex) in Guiana dolphins from Guanabara

	Bay, R	io de Janei	ro, Brazil, e	expressed in	µg.g⁻¹ lipid w	eight.	
Т	'L (cm)	Lipids	∑ PCB	$\sum DDT$	$\sum HCH$	HCB	Mirex

		(%)					
Females							
Mean \pm SD	177 ± 19	19 ± 11	65 ± 77	6 ± 5	0.004 ± 0.3	0.03 ± 0.02	0.5 ± 0.2
Median	183	18	25	4	0.003	0.002	0.5
Min-Max	136-195	6-39	18-229	2-17	0.0004-1	< 0.3- 0.09	0.008-1
Males							
Mean \pm SD	157 ± 36	30 ± 24	76 ± 104	4 ± 2	0.1 ± 0.09	0.002 ± 0.008	1 ± 3
Median	161	20	46	4	0.1	0.002	0.1
Min-Max	82.5-191	6-70	6 - 303	0.9-10	0.02-0.3	< 0.3- 0.01	0.1-8

PCBs concentrations in Guiana dolphins from Guanabara Bay were very high and can be compared to delphinids from industrialized areas of the Northern Hemisphere^{8,9,10}. For instance, in bottlenose dolphin (*Tursiops truncatus*) from South Carolina, USA, mean \sum PCB concentrations were in the same other of magnitude (94 µg.g⁻¹ lw)¹⁰. In another delphinid, the rough-toothed dolphin (*Steno bredanensis*) collected in Mediterranean Sea, it was in about 25 times higher (mean \sum PCB concentrations: 2.5 µg.g⁻¹ lw)⁹.

Comparing with previous study⁸, PCBs concentrations stands out, since, it has been seen a continuous raise of these concentrations in Guiana dolphins from Guanabara Bay. In relation to a previous period (sampling from 2000 to 2005)⁸, median \sum PCB concentrations in Guiana dolphin from Guanabara Bay raised in about 1.3 times in females (from 19 to 25 µg.g⁻¹ lw) and twice in males (from 29 to 47 µg.g⁻¹ lw)⁸. A 3-year-old immature male showed \sum PCB concentrations (303 µg.g⁻¹ lw) similar to a killer whale (*Orcinus orca*) stranded in Rio de Janeiro's coast (257 µg.g⁻¹ lw)². Besides, a mature female monitored during her lifetime in the study area for 20 years, which had five calves in the period, still showed high \sum PCBs concentrations (67 µg.g⁻¹ lw). These concentrations were in the same order of magnitude for \sum PCB that was found in a male with 14 years (90 µg.g⁻¹ lw) in the present study.

In relation to other compounds, a different pattern was seen. Median \sum DDT concentrations were very similar in Guiana dolphins from Guanabara Bay (from 5 to 4 µg.g⁻¹ lw in females and from 9 to 4 µg.g⁻¹ lw in males)⁸. HCB concentrations were similar between a previous study also (from 45 and 29 µg.g⁻¹ lw in females and 51 to 26 µg.g⁻¹ lw in males)⁸. And yet, \sum HCH and Mirex were not determined in the previous study⁸, and therefore, this comparation cannot be done.

It was expected to have significative differences between males and females organochlorine concentrations and sexual maturity. This could be related to the transfer of organic pollutants during gestation and mostly during lactation^{15,16}. However, in the present study, it was not found significative differences between sex for all compounds (Mann-Whitney's Test, p > 0.05). This result is probably due the reduced number of adults in relation to young individuals in the sampling.

The high organochlorine concentrations (especially PCBs) in Guiana dolphins from Guanabara Bay may contribute for the decline in Guiana dolphin population in Guanabara Bay. This decline was reported to be of 37% in 16 years, which occurs especially due the low survival of young individuals with less than 5 years old⁷. It is well known that organic pollutants can affect negatively immune and reproductive systems of organisms¹ by

impairing the development of young individuals. In this sense, organochlorine compounds can contribute to premature deaths⁷ or can impair reproduction. In fact, in this population has been seen a mature female that have only 1 calve in 12 years of monitoring (MAQUA-UERJ, unpublished data).

Since this Guiana dolphin population lives in a degraded area, with great pollution discharges^{8,12}, organochlorine compounds can be an important threath for Guiana dolphin conservation. Therefore, in this critical and reduced population which has the first confirmed decline of a delphinid population in Brazilian waters⁷, a continuous monitoring of pollutants contamination is extremely important.

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