PCDD, PCDF AND PCB DETERMINATION IN DOLPHINS REVEALS A WORLD HOTSPOT FOR PCBS IN GUANABARA BAY, BRAZIL

Dorneles Paulo[1,2], Lailson-Brito José[1,2], Eppe Gauthier[3], Azevedo Alexandre[2], Azevedo-Silva Claudio[1], Torres João[1], Malm Olaf[1], Lepoint Gilles[4] and Das Krishna[4]

[1] Radioisotope Laboratory, Biophysics Institute, Rio de Janeiro Federal University, Brazil. [2] Aquatic Mammal Laboratory, Oceanography Faculty, Rio de Janeiro State University, Brazil. [3] Centre of Analysis of Residues in Traces, Liege University, Belgium. [4] Laboratory for Oceanology, MARE Centre, Liege University, Belgium.

Introduction

Since the beginning of the industrialization process marine ecosystems have become the final destination for pollutants. Special concern has been raised since the 1960s about the environmental persistence, bioaccumulative capacity, toxicity, as well as past broad utilization of some organochlorine compounds, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). The toxicity of the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to certain laboratory animals and wildlife species has lead the molecule to be called "the most toxic man-made chemical" [1]. Despite the high toxicity of these compounds and the awareness of the scientific community about the environmental problem, there are no previous studies on dioxin levels in marine biota from Brazil.

Guanabara Bay (Rio de Janeiro State, Southeast Brazilian region) is the most anthropogenically disturbed area along the lengthy (around 8500 km) Brazilian coastline. The estuary is bordered by 12000 industries and four cities (including Rio de Janeiro metropolitan area) with a total population of about 11 million people [2]. Despite the anthropogenic pressure, Guanabara Bay supplies food and breeding grounds for marine tucuxi dolphins (*Sotalia guianensis*). This dolphin species occurs exclusively in coastal waters of western Atlantic on South and Central America, from southern Brazil (27°35'S, 48°34'W) to Honduras (15°58'N, 79°54'W) [3]. Regarding Guanabara Bay specifically, population assessment studies, accomplished through photo-identification, have reported a population of about 70 marine tucuxi dolphins [4]. Through these photo-id studies, it was possible to verify that this small population exhibits habitat fidelity, since the same individuals are found year-round in this site [5]. The main reason for the marine tucuxi dolphin residence in Guanabara Bay seems to be the presence of food, since feeding-related activities predominate along the entire year [6].

Determination of organochlorine compounds in cetaceans is of special interest, due to their top position in the food web and their long life-span [2]. Therefore, the main objective of the present study was to determine the current concentrations of PCDDs, PCDFs and PCBs in dolphins from a region of high industrialization and urbanization in the Southern Hemisphere. Since most of these compounds biomagnify in food chains and changes in ratios of stable isotopes of nitrogen ($^{15}N/^{14}N$) have been used to elucidate trophic relationships [7], measurements of these isotopes have been carried out in marine tucuxi dolphins and their prey in Guanabara Bay. Stable isotopes were measured, firstly, to quantitatively assess the trophic level of the dolphin, and, secondly, to provide information for future human risk assessment investigations related to fish consumption.

Material and methods

PCDD, PCDF and PCB determination in blubber of dolphins

Subcutaneous adipose tissue samples were collected from 10 marine tucuxi dolphins that stranded on the beaches of Guanabara Bay from 2000 to 2006 (Table 1). The extraction, purification and measurement methods are described elsewhere [8]. Briefly, blubber samples were extracted by pressurized liquid extraction (PLE) using a Dionex (Sunnyvale, CA, USA) ASE 200 extractor. Conditions were: 33 ml extraction cells filled approximately 5 g of blubber sample and sodium sulphate, 20 ml of hexane per cycle, 5 min cycle time, two cycles per extraction, pressure of 1500 p.s.i. The fat extracts were dried on sodium sulphate prior to complete solvent evaporation. A solution of hexane/dichloromethane was added to aliquots of about 200–300 mg of extracted lipids. The lipid extracts were then spiked with a mixture containing seventeen 13C-labeled 2,3,7,8-substituted dioxins isomers, 4 c-PCBs isomers (EDF-4144, LGC Promochem) and 8 mono-ortho PCB isomers (Campro Scientific WP-LCS). An automated multi-column clean-up was performed on the Power-Prep system (FMS,

Waltham, MA, USA). All analyses were performed by GC–HRMS using a MAT95XL high-resolution mass spectrometer (Finnigan, Bremen, Germany) and a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph. The following dioxins and furans were targeted for analysis: 2, 3, 7, 8 – Tetra CDD; 1, 2, 3, 7, 8 – Penta CDD; 1, 2, 3, 4, 7, 8 – Hexa CDD; 1, 2, 3, 6, 7, 8 – Hexa CDD; 1, 2, 3, 7, 8, 9 – Hexa CDD; 1, 2, 3, 4, 6, 7, 8 – Hepta CDD; Octa CDD (OCDD); 2, 3, 7, 8 – Tetra CDF; 1, 2, 3, 7, 8 – Penta CDF; 2, 3, 4, 7, 8 – Penta CDF; 1, 2, 3, 4, 7, 8 – Hexa CDF; 1, 2, 3, 6, 7, 8 – Hexa CDF; 1, 2, 3, 7, 8, 9 – Hexa CDF; 2, 3, 4, 6, 7, 8 – Hexa CDF; 1, 2, 3, 4, 6, 7, 8 – Hepta CDF; 1, 2, 3, 4, 7, 8, 9 – Hepta CDF; Octa CDF (OCDF). Concerning dioxin-like PCBs, the following congeners (IUPAC numbers) were targeted for analysis: 77; 81; 126; 169; 105; 114; 118; 123; 156; 157; 167 and 189. Toxic equivalents (TEQ) for the sum of all the abovementioned organochlorine compounds (Σ PCDD/Fs/DL-PCBs) were calculated. Concentrations below detection limits were considered as zero (lower bound TEQ). Levels of indicator-PCBs (IUPAC numbers: 28, 52, 101, 138, 153, 180) were also measured.

Stable isotope measurements

Measurements of stable isotopes of nitrogen were carried out in muscle samples from marine tucuxi dolphins and fish that according to the literature constitute prey species of this mammal in Guanabara Bay [9], as well as in Rio de Janeiro state as a whole [10]. The dolphins sampled for stable isotope measurements, comprising males (n=11) and females (n=9) stranded on the beaches of Guanabara Bay from 1995 to 2007. Measurements were also carried out in samples from fish species collected from Guanabara Bay in summer 2006. The fish species were: Atlantic anchoveta (*Cetengraulis edentulus*, n=8), mullet (*Mugil* sp., n=7), whitemouth croaker (*Micropogonias furnieri*, n=7), largehead hairtail (*Trichiurus lepturus*, n=8) and smooth weakfish (*Cynoscion leiarchus*, n=6). In addition, seston samples were collected in Guanabara Bay (also in summer 2006) using a 75-nm-mesh plankton net. After being freeze-dried, the samples were ground into a homogeneous powder. Nitrogen gas originated from the sample was analysed on a V.G. Optima (Micromass) IR-MS coupled to an N-C-S elemental analyser (Carlo Erba). Stable isotope ratios were expressed in δ notation according to the following: $\delta^{15}N = [(^{15}N/^{14}N_{sample} / {}^{15}N/^{14}N_{atmospheric\ N2}) - 1] \times 1000$. *Shapiro-Wilk's W* test was used in order to test data normality. Since only normal distributions were observed, inter-species comparisons were carried out by the *Student's t-test*.

Results and discussion

PCDD, PCDF and PCB concentrations in blubber of dolphins

Among all the compounds targeted for analysis, only 2, 3, 7, 8 – TetraCDD; 1, 2, 3, 7, 8 – PentaCDF; and 1, 2, 3, 7, 8, 9 – HexaCDF (LOQs of 0.064, 0.086 and 0.096 pg/g, respectively) were not detected in any of the samples. Table 1 shows different summations of polychlorinated contaminant concentrations. The highest ΣPCDD/F concentration observed among females was lower than the lowest level verified among males (Table 1). However, the same does not hold for the sum of the dioxin-like PCBs (ΣDL-PCBs), for the sum of organochlorine compounds other than indicator PCBs (Σ PCDD/Fs/DL-PCBs), for WHO-TEQ values, for the sum of indicator PCBs (ΣindPCBs), as well as for the sum of all PCBs (ΣDL-PCBs/indPCBs).

PCB levels observed in the present study were in the range reported by Kuehl et al [11] and Berggrena et al [12], in blubber samples from bottlenose, common and white-sided dolphins from the U.S. Atlantic Coast [11], as well as from male harbour porpoises from the Baltic Sea, the Kattegat-Skagerrak Seas and the West Coast of Norway [12]. In the former study the total PCB concentrations varied from 17.4 to 195 μg/g lipid, and in the latter, the levels varied between 2.2 and 78 μg/g lipid (sum of CB52, CB101, CB118, CB138, CB153 and CB180). The concentration range reported by Kuehl et al [11] comprises the sum of one hundred PCB congeners, much more than the 18 PCB congeners determined in the present study. In the cited investigations, cetaceans were collected from 1978 to 1990. During the 1970s and 1980s, the manufacturing of PCBs was terminated in most industrialized nations. This implies further that not only had the animals analyzed by Kuehl et al [11] and Berggrena et al [12] lived in a highly polluted area but also in a period of elevated environmental contamination by polychlorinated biphenyls.

The sample set analysed by Kuehl et al [11] included bottlenose dolphins obtained after an unusual mortality event. Although the impact of these strong immunosuppressive agents [13] is not fully known, their role as causative

agents in that mass mortality was considered by the authors [11]. The toxicological significance of the total PCB concentrations in marine tucuxi dolphins from Guanabara Bay can be evaluated through the fact that the levels exceed established thresholds for effects of PCBs on reproduction in harbour seals (25 μg/g) and mustelids (7.5 μg/g), immune function in harbour seals (17 μg/g), and endocrine effects (thyroid hormone and vitamin A) in river otters (4 μg/g) and harbour seals (17 μg/g) [14].

The possible public health problem

The possibility of occurrence of a human health problem due to consumption of fish from Guanabara Bay is raised when data on stable nitrogen isotope ratios are considered. With the only exception of the comparison between Atlantic anchoveta and mullet, all the remaining pairwise inter-species comparisons yielded significant differences in $\delta^{15}N$ values (Fig. 1). Interestingly, significant higher $\delta^{15}N$ values were verified in whitemouth croakers than in marine tucuxi dolphins ($p=0.007$), implying further that this fish species feeds higher than marine tucuxi dolphins in Guanabara Bay food web. A recent study on digestive content analyses of marine tucuxi dolphins from Guanabara Bay revealed that the whitemouth croaker constituted the prey species of highest frequency of occurrence in the stomachs [9]. An explanation for these seemingly contradictory data may be found if the size of the whitemouth croaker specimens is considered. The body length on which marine tucuxi dolphins exert predation fits between 6.3 and 32.2 (14.4 ± 4.4) [9]; however, all the whitemouth croakers obtained for stable isotope measurements were lengthier than 50 cm. The higher $\delta^{15}N$ values observed in whitemouth croaker tissues strengthen the possibility of occurrence of high PCB concentrations in this fish species, which is marketed for human consumption, including the body length size obtained for stable isotope measurements.

The longevity of the species may play a key role in the observation of higher concentrations in predator marine mammals [15]. It is expected that contaminants accumulate with increasing age [15], and cetaceans are frequently older than their prey [16]. However, the maximum estimated age for marine tucuxi dolphins was 30 years [17], and the longevity of the whitemouth croaker was estimated to be 35 years [18]. Consequently, the possibility of the human health problem due to consumption of highly contaminated fish is reinforced if the longevity of this fish species is taken into account. Therefore, risk assessment studies on human exposure to PCBs through consumption of fish from Guanabara Bay are of fundamental importance.

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Table 1. Concentrations of PCDDs, PCDFs and PCBs in dolphins from Guanabara Bay, Brazil.

Sex	ΣPCDD/Fs Mean pg/g ±S.D. Median Min - Max	ΣDL-PCBs Mean µg/g ±S.D. Median Min - Max	Σ PCDD/Fs/DL-PCBs Mean µg/g ±S.D. Median Min - Max	WHO-TEQ Mean pg/g ±S.D. Median Min - Max	Σind PCBs Mean µg/g ±S.D. Median Min - Max	ΣDLPCBs/ind PCBs Mean µg/g ±S.D. Median Min - Max	N
M	52 ±36 32 27 - 123	13 ±3 13 8 - 16	13 ±3 13 8 - 16	2390 ±626 2259 1358 - 3096	87 ±44 65 49 - 146	100 ±45 78 56 - 160	7
UI	69	31	31	6375	249	279	1
F	13 ±11 9 5 - 26	9 ±5 8 5 - 14	9 ±5 8 5 - 14	1355 ±751 1180 706 - 2178	42 ±17 33 30 - 62	51 ±22 41 35 - 76	3

UI, unidentified sex; "ind PCBs", indicator polychlorinated biphenyls.

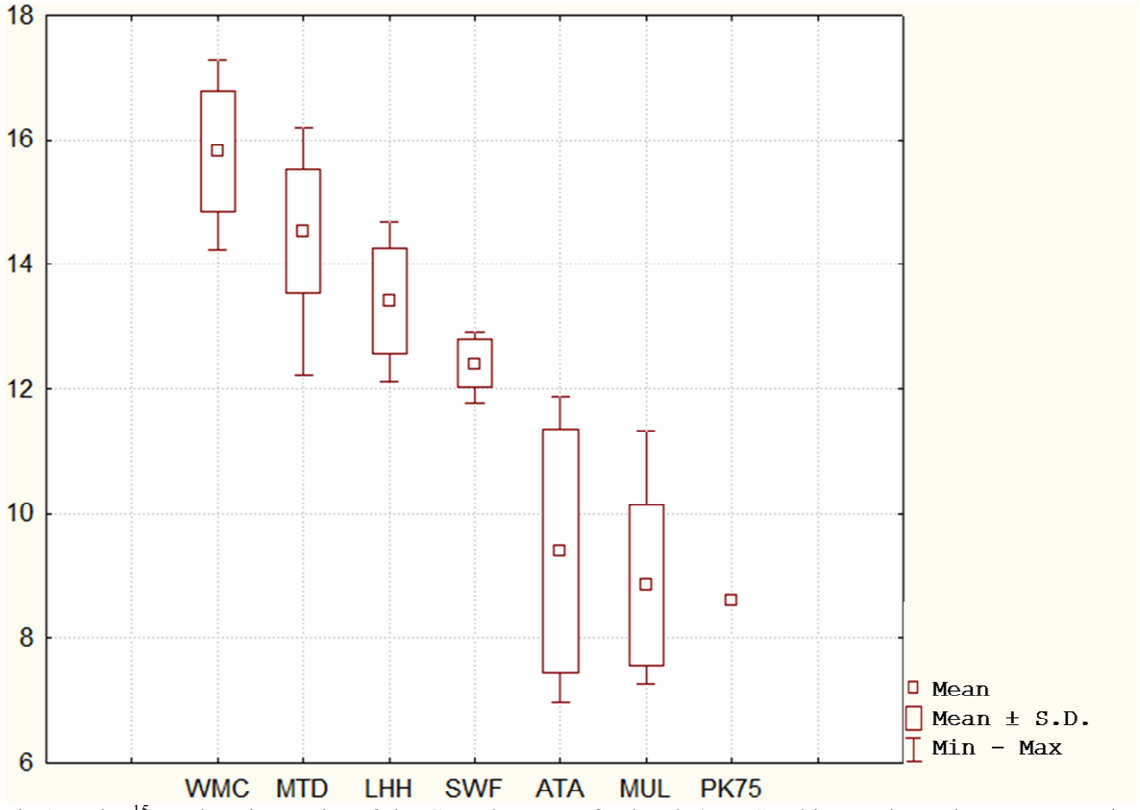

Fig.1. Delta ^{15}N values in species of the Guanabara Bay food web (WMC, whitemouth croaker; MTD, marine tucuxi dolphin; LHH, largehead hairtail; SWF, smooth weakfish; ATA, Atlantic anchoveta; MUL, mullet; PK75, seston samples collected using a 75-nm-mesh plankton net).