EVALUATION OF FISH EXPOSURE TO POP-LIKE (ORGANOTIN) COMPOUNDS IN SEPETIBA BAY (RIO DE JANEIRO STATE, BRAZIL) THROUGH HEPATIC TOTAL TIN CONCENTRATIONS

Paiva TC[1], Schilithz PF[2], Bisi TL[2], Lepoint G[3], Azevedo-Silva CE [1], Azevedo AF[2], Flach L[4], Lailson-Brito J[2], Das K[3]*, Dorneles PR[1,3]

[1]Laboratório de Radioisótopos, Instituto de Biofísica, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brasil; [2]Laboratório de Mamíferos Aquáticos e Bioindicadores (MAQUA), Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, RJ, Brasil; [3] Laboratoire d'Océanologie, Université de Liège - MARE, Liège, Belgique; [4]Instituto Boto-cinza, Mangaratiba, RJ, Brasil.

Introduction

The contamination of marine environments by organotin compounds (OTs) has raised substantial concern due to the toxicity of these compounds, as well as due to the fact that they are easily bioaccumulated. In fact, some investigations have drawn attention to a POP-like environmental behaviour of OTs, particularly triphenyltin (TPhT)[1]. OTs have been produced for a number of industrial and agricultural processes; however, the use that has threaten the highest number of non-targeted species is related to the presence of TBT (tributyltin) and TPhT as active ingredient of inks applied to ship hulls and other submerged metallic surfaces in order to prevent the incrustation of molluscs and crustaceans[2].

TBT and TPhT have been used as antifouling agents since the 1970s. Due to the abovementioned environmental concern, many developed nations adopted restrictions during the early 1980s; however, the international ban of such use of these molecules only occurred more than two decades later. Developed nations use only tin-free antifouling paints nowadays; conversely, the same does not occur in all developing countries. The Brazilian Navy prohibited such use of OTs in the country; nevertheless, it is legal to buy TBT in Brazil. Theoretically, TBT is sold in Brazil for other purposes; nonetheless, the compound is still being used as antifouling agent in the country[3].

Industrial development and population growth have been significant along the Brazilian coast. Consequently, many estuaries receive discharges of pollutants from domestic, industrial and agricultural wastewaters. Regarding these activities, Sepetiba Bay (SB), in Rio de Janeiro state (RJ), should be highlighted as one of the most impacted estuaries along the Brazilian coast. This bay seems to be on the same degradation path once followed by another estuary that also bathes Rio de Janeiro city, Guanabara Bay, which constitutes the most dramatic example of man-made degradation along Brazilian coast[4-10]. Despite the anthropic pressure, fishing is an important economic activity for the region, which emphasizes the importance of this kind of research, since critical parameters for human exposure to toxic substances can be identified.

Top marine predators, such as fish, seabirds and cetaceans, are regarded as valuable indicators of persistent bioaccumulative toxicant (PBT) levels accumulated in marine environment, due to their top position in the food web and their long life span[4-10]. Stable isotope measurements have been used in a number of studies for investigating the behavior of contaminants along the food chain[11-13]. Furthermore, the approach has already being adopted for evaluating mercury trophic flow in Guanabara, Sepetiba and Ilha Grande bays, using carbon and nitrogen stable isotopes ratios ($\delta^{13}C$, $\delta^{15}N$)[14].

The present study focuses on evaluating the exposure of marine vertebrates to OTs through the determination of ΣSn concentrations. This approach is based on the fact that, unlike OTs, which are readily bioconcentrated and found in high concentrations in biota, inorganic tin species are practically not absorbed through mucous membranes. Therefore, it can be concluded being possible to evaluate which species / population of vertebrates would be more exposed to OTs through hepatic total tin concentrations. Some studies from our research team have already demonstrated the efficiency of this approach[2, 7,15]. For that reason this investigation deals with analyses of samples from fish, using also crude literature[15] data on hepatic tin concentrations of SB dolphins and nitrogen stable isotope values obtained from these samples[14], for comparison purposes. Therefore, vertebrates from an impacted estuary (fish and dolphins), were used herewith as sentinel species of environmental health. Additionally, the hepatic ΣSn concentrations of nektonic organisms from SB specifically were also used for (1) evaluating possible variations along the food chain, i.e., for investigating the possible occurrence of trophic dilution or biomagnification, as well as for (2) evaluating the potential contribution of different Guiana dolphin prey species to the OT burden of this predator.

Materials and methods

Fish sampling was performed in SB. It included items from the diet of Guiana dolphins (*Sotalia guianensis*) in RJ waters. Liver samples from five fish species were analysed: *Mugil curema*, n=10; *Micropogonias furnieri*, n=24; *Centropomus undecimalis*, n=10; *Aspistor luniscutis*, n=7; and *Trichiurus lepturus*, n=8. Sampling occurred in February and March 2009. The samples were dried at 60°C for 3 days and then macerated. Aliquots were solubilized by acid digestion. All fish were sampled at the body length on which the dolphins prey[14].

Liver samples were solubilized in a closed vessel under microwave irradiation in a CEM microwave digester, MARS 6. After cooling, the solutions were made up to a known volume with high purity deionised water (18.2 MΩ cm) from a Milli-Q system. Quality control (QC) was carried out through the use of blanks, which were proceeded through in the same way as the samples, as well as through the use of the standard addition method. Regarding the later QC procedure, recoveries from fortified extracts were always above 90%. The temperature program used and additional methodology details can be found elsewhere[7]. Total tin concentrations were determined by electrothermal atomic absorption spectrometry (ET-AAS), using an Analytic Jena spectrometer ZEEnit 60 equipped with Zeeman-effect background correction. Palladium nitrate was used as a matrix modifier and hence added to each solution to be analysed.

For statistics, depending on data normality (Shapiro-Wilk's W test), parametric (Student's t-test and Pearson's correlation test) or non-parametric (Mann-Whitney U test and Spearman correlation test) tests were used. The level of significance was set at $p \leq 0.05$. The biomagnification factor (BMF) was calculated between each prey (fish) species and the predator marine mammal (Guiana dolphin), in accordance with the study of Connell[16] in which the BMF is determined by dividing the concentration in the consumer by the concentration in the diet. For these calculations, literature data on hepatic ΣSn concentrations of dolphins from SB[15] were used. It was also possible to calculate the Trophic Magnification Factor (TMF). TMF was calculated using the antilog slope of linear regression between the log of the contaminant and the trophic position of organisms, represented by the value of $\delta^{15}N$, where TMF>1 indicates biomagnification and TMF<1 indicates a trophic dilution of contaminant.

Results and discussion

Hepatic ΣSn concentrations of *M. furnieri, C. undecimalis, A. luniscutis* and *T. lepturus* are reported in Table 1. Concentrations were below the detection limit (DL, 9.0 ng.g-1 d.w.) for seven out of ten analysed liver samples from *M. curema*. This finding corroborates previous investigations about the species in SB. A recent study[14] has found low values of delta notation of stable isotopes of nitrogen ($\delta^{15}N$) in *M. curema* from Sepetiba Bay, which denotes a low trophic position[14]. Significant differences were found between prey (fish) and predator (dolphin) in all teleost species analysed, with higher levels in dolphins ($p<0.0004$, in all cases). Significantly higher hepatic ΣSn concentrations were found in *A. luniscutis* than in *T. lepturus* (Mann-Whitney test, p=0.017). Literature data indicate higher $\delta^{15}N$ values in *T. lepturus* than *A. luniscutis* in SB[14]. At first, this would indicate a trend opposite to that of biomagnification, *i.e.*, trophic dilution. The largehead hairtail (*T. lepturus*) is a pelagic predator; however, benthic invertebrates constitute an important part of the diet of *A. luniscutis*. Therefore, the strong association between OTs and bottom sediments would help to explain this finding. In addition, a recent study has shown that fish scales constitute important feeding itens for *A. luniscutis* in Sepetiba Bay[17]. Therefore, the possibility that this cutaneous structure acts as an excretion route for OTs in fish should not be ruled out as a contributor to the hepatic ΣSn concentrations found in *A. luniscutis*. Integumentary structures have already been demonstrated to constitute excretion routes for OTs in birds (feathers)[18-20] and mammals (hair)[21]. Consequently, the possibility that fish scales play a similar role in OT toxicokinetics than do the integumentary structures of other vertebrates deserves further investigation.

M. curema, the fish species that presented the lowest $\delta^{15}N$ values, *i.e.* the species who occupies the lowest trophic position, exhibited the lowest hepatic ΣSn concentrations. In fact, the samples from this species, in majority, presented ΣSn levels below DL.

Regarding the BMFs between each fish species and Guiana dolphins, the values found for *M. curema, M. furnieri, C. undecimalis, A. luniscutis* and *T. lepturus* were 66.0, 9.7, 14.2, 6.8 and 17.6, respectively. These calculations suggest *A. luniscutis* as a potentially important contributor for the high OT levels found in Guiana dolphins. Nevertheless, additional studies should investigate not only the importance of each of this fish species as a vector of OTs for the dolphin, but also the possible role of the fish scales as excretion for these POP-like compounds.

Despite some findings that at first would suggest ΣSn biomagnification, the relationship between the log-transformed hepatic ΣSn concentrations and the $\delta^{15}N$ values of organisms from Sepetiba Bay (Figure 1) was not significant. Therefore, no significant relationship was found between hepatic tin concentrations and $\delta^{15}N$ values of specimens analyzed in this study (p=0.6).

Table 1. Hepatic ΣSn concentrations [ng.g^{-1} dry weight; Mean (Median) ± SD; Min - Max] in fish species from Sepetiba Bay, exhibiting the total number of analysed individuals (*n* analysed), as well as the number of fish that rendered results above de detection limit (*n*>DL). Literature data on $\delta^{15}N$ values for the same species and estuary is additionally shown.

	M. curema	*M. furnieri*	*C. undecimalis*	*A. luniscutis*	*T. lepturus*
Mean ± SD	16.2 ±7.3	199±185	137 ±75.1	284 ±255	111 ±28.1
Median	13.5	117.8	113	181	103
Min - Max	10.7 - 24.5	22.6-805.5	56.7 - 287	114 - 780	86.2 - 159
n analysed (n>DL)	10 (3)	24(22)	10 (10)	7 (6)	8 (5)
$\delta^{15}N$ (ref. [14])	10.0 ±0.8	13.4 ±1.1	15.1 ±0.9	14.5 ±0.7	16.6 ±0.4

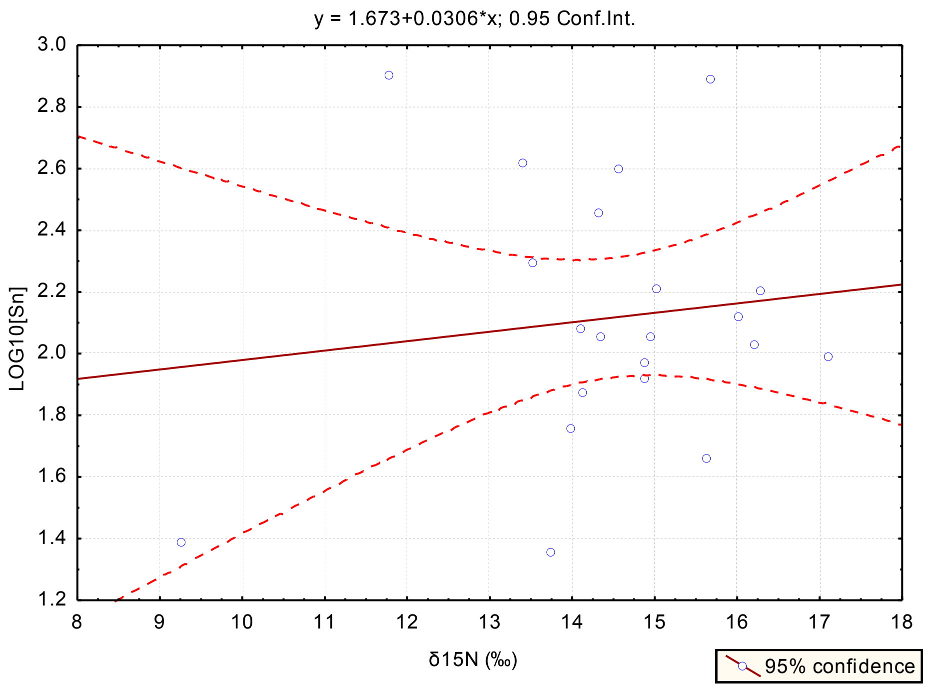

Figure 1. Relationship between log-transformed hepatic ΣSn concentrations (ng.g^{-1} dry weight) of five fish species from the Sepetiba Bay and the respective $\delta^{15}N$ values.

Tin biomagnification was not observed in the food chain investigated. Bisi et al. (2012) did not observe a significant linear relationship between log-transformed ΣHg concentrations and $\delta^{15}N$ values in SB, despite having found it in two other estuaries from RJ. The absence of ΣSn biomagnification together with the findings from Bisi et al. (2012) suggest that particular characteristics of each area may have great influence on occurrence or absence of biomagnification of a given analyte. However, it should be kept in mind that most of the fish species analyzed in the present study occupy a relatively high trophic position. Therefore, a wider range of fish species should be analyzed before ΣSn biomagnification can be dismissed. In addition, the biomagnification phenomenon seems to be more frequently observed for TPhT than for TBT[22] and RJ coastal waters are apparently more contaminated by butyltin than by phenyltin compounds[23]. Though other aspects might be influencing values of TMF obtained, the possibility of OT biomagnification should not be dismissed and consequently neglected, especially considering that this phenomenon is a potential threat to the conservation of species occupying the top of food chains, as well as a risk for human populations due to the consumption of fish.

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