Ecotoxicology
DOI 10.1007/s10646-017-1801-8

Tributyltin bioaccumulation and toxic effects in freshwater gastropods *Pomacea canaliculata* after a chronic exposure: field and laboratory studies

María L. Martínez[1,2] · María N. Piol[2] · Norma Sbarbati Nudelman[1] · Noemí R. Verrengia Guerrero[2]

Accepted: 1 April 2017

Abstract Freshwater samples and gastropod mollusks (*Pomacea canaliculata*) were collected at 5 sampling stations located along the lower Río de la Plata basin, Argentina, to assess the extent of tributyltin (TBT) contamination. Determined data revealed the presence of TBT and some of its breakdown products (dibutyltin: DBT, and monobutyltin: MBT) in all freshwater samples and also in soft tissues of *P. canaliculata* gastropods. Chronic bioassays (6 months) were performed using female gastropods that had been reared under laboratory conditions and exposed to a similar TBT concentration than the value determined in freshwater samples (1 μg L^{-1}). The aims of this study were to evaluate the extent of TBT accumulation, the tissue distribution, and the effects on selected biomarkers (activity of superoxide dismutasa: SOD, activity of catalase: CAT, levels of total glutathione: t-GSH, lipid peroxidation, and activity of acetylcholinesterase: AChE). Gonads presented the highest accumulation, followed by the cephalopedal region, albumin gland, and finally hepatopancreas. Both metabolites, DBT and MBT, were also found. All exposed female animals presented development of a penis reflecting the potential of TBT as an endocrine disrupting chemical for this gastropod species. Results on the selected biomarkers confirmed additional adverse effects induced by TBT. An increase in CAT activity and changes in t-GSH levels are indicative of alterations on the cellular redox status. The inhibition of AChE could reflect signs of neurotoxicity. Altogether, these results reveal a negative impact on the health of this gastropod population.

Keywords *Pomacea canaliculata* · TBT · Freshwater systems · Biomarkers · Female gastropods

Introduction

Tributyltin (TBT) is an acronym for a group of organotins (OTs) widely used as biocides in the formulation of antifouling paints. It is considered as one of the most toxic pollutants delivered to the environment by man (Jagtap et al. 2011). The ecotoxicological effects of TBT are well documented, including gastropod *imposex*, mussel larvae mortality and oyster malformation, even at TBT exposure concentrations as low as 1 ng L^{-1} (Liu et al. 2006; Leung et al. 2007; Higuera-Ruiz and Elorza 2011). Concentrations in the range of 1–2 ng TBT L^{-1} can cause chronic and acute poisoning to sensitive non-target species, including algae, zooplankton, mollusks and the larval stage of some fish (Hoch 2001). The banning of TBT by the International Maritime Organization (IMO) covers only its use in antifouling paints. However, this compound is also used as fungicide, wood preservative, PVC stabilizer, catalysts, etc., constituting additional sources of TBT contamination (Fent 1996; Hoch 2001; Wang et al. 2010). TBT and its principal breakdown products, dibutyltin (DBT) and monobutyltin (MBT), are widely found in marine waters and sediments throughout the world (Axiak et al. 2000; Buggy and Tobin 2006; Wang et al. 2008; Waisbaum et al. 2010). OTs can

✉ Noemí R. Verrengia Guerrero
noev@qb.fcen.uba.ar

[1] Facultad de Ciencias Exactas y Naturales, Dpto. de Química Orgánica, Universidad de Buenos Aires, 3° piso, Pabellón II, Buenos Aires, Argentina

[2] Facultad de Ciencias Exactas y Naturales, Dpto. de Química Biológica, Toxicología y Química Legal, Universidad de Buenos Aires, 4° piso, Pabellón II, Buenos Aires, Argentina

Published online: 13 April 2017

also be found in surface waters and sediments of rivers and lakes, especially near areas with high vessel traffic (Fent 1996). Degradation processes of TBT are controlled by several environmental conditions (especially temperature, sunlight radiation, and dissolved organic carbon content) and microorganism diversity (Tessier et al. 2007). In aquatic systems, TBT may be degraded by chemical and sunlight radiation and its half-life usually varies between 6 and 126 days (Fent et al. 2006). The half-life for the biotic degradation in both seawater and freshwater varies between 6 days to several weeks (Rüdel 2003). In sediments, degradation rates may be much lower especially in anoxic sediments (from months up to 20 years) (Dowson et al. 1996). While undesirable consequences at the ecosystem level have been convincingly shown for the marine environment, the impact of TBT in freshwater ecosystems has received much less attention.

The basin called Río de la Plata is one of the most important in South America. The Rio de la Plata river is formed by the confluence of the Paraná and Uruguay rivers and flows into the Río de la Plata estuary, ultimately draining into the Atlantic Ocean. Many well-established commercial and recreational ports are found along the coastal areas and channels are constantly dredged to permit the navigation of ships transporting, predominantly, agricultural products from the upper basin to the Atlantic Ocean.

Pomacea canaliculata belongs to the phylum mollusca, class gastropoda, family ampullaridae and genus Pomacea. They are amphibious and dioecious organisms that are internally fertilizing and oviparous (Cazzaniga 1990). *P. canaliculata* is widely distributed in South America and found southern Guyana to the Río de la Plata river. This organism is ecologically relevant as a prey for several fish and bird species. There are still not standardized protocols using freshwater gastropods in environmental risk assessment programs. However, this situation is progressively changing. Some species, including those of *Pomacea* genus, are under investigation in order to include them as bioindicator organisms for toxicity tests (Tallarico 2016). Besides, it is considered that frequently native species are more sensitive to stressors than non-autochthonous ones (Martins and Bianchini 2011). Therefore, its selection would be particular useful for assessing the impact of contaminants in South America freshwater systems (Tallarico 2016).

TBT is not only a well known endocrine disrupting chemical but also it can increase the generation of reactive oxygen species (ROS) (Bernanke and Köhler 2009; Zhou et al. 2010). Organisms have developed a complex system of antioxidant defenses to balance the redox cellular status. However, when these defenses are overcome by an excess of ROS, oxidative stress damages may occur, such as lipid peroxidation (Valavanidis et al. 2006; Lushchak 2011). Superoxide dismutase (SOD, EC. 1.15.1.1) and catalase (CAT, EC.1.11.1.6) play crucial roles as enzymatic defenses. The content of reduced glutathione (GSH) is one of the most important non enzymatic antioxidant agents (Hermes-Lima et al. 1998; Lesser 2006). Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation and it is considered as a good marker of free radical-mediated damage. Besides, TBT can induce neurotoxic effects in several vertebrate species by disrupting the metabolism of various neurotransmitters (Tsunoda et al. 2004; Nakayama et al. 2007; Mitra et al. 2013). Thus, the activity of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7), which catalyses the degradation of the neurotransmitter acetylcholine, may result affected.

This study had two objectives. First, a field study was performed to assess the levels of TBT and its principal breakdown products (DBT and MBT), in freshwater samples and in biological tissues of *P. canaliculata* collected at different sampling stations located along the lower basin of the Río de la Plata, Argentina. Then, female organisms of *P. canaliculata* reared under laboratory conditions were chronically exposed to TBT (6 months) to assess levels of bioaccumulation, tissue distribution, and effects on a selection of biomarkers related to the cellular redox status (activities of SOD and CAT, t-GSH content, lipid peroxidation), and to neurotoxic effects (activity of AChE). The TBT exposure level was selected according to the values found in the freshwater samples collected from the field.

Materials and methods

Test substances

Tributyltin chloride (TBTCl) (purity >97%) and Florisil were obtained from Merck Schuchard. The breakdown products (DBT and MBT), $NaBH_4$, Dureno (1,2,4,5-tetramethylbenzene), acetylthiocholine iodide, 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), glutathione reductase, NADPH, $NADP^+$, epinephrine, and thiobarbituric acid (TBA) were purchased from Sigma–Aldrich of Argentina S.A. All other reagents and chemicals were of analytical grade.

Water and gastropod samples

Water and gastropod samples were collected from 5 sampling stations along coastal areas of the lower Río de la Plata basin, Argentina (Fig. 1). From north to south they were: Escobar and Tigre (both stations located along the delta of the Paraná river); San Fernando (along the coast of a minor tributary river); San Isidro and Olivos (both stations

Fig. 1 Map of the lower the Río de la Plata basin showing the 5 sampling stations

located along the Río de la Plata river). Samples were collected during January and March, (summer season). Water samples were placed in 1 L amber bottles and kept at 4 °C. Once in the laboratory samples were filtered through a 0.45 µm pore size filter.

Gastropods were placed in 10 L containers with about 5 L of water from the same sampling station where animals had been collected and transported alive to the laboratory.

For gastropods analyses, animals were frozen at −20 °C and then sacrificed. The whole body soft tissues were isolated and lyophilized. For this field study, no sex selection was performed.

Analyses of TBT ant its breakdown products

OTs in the water samples were analyzed according to the method previously described (Waisbaum et al. 2010). Water samples (250 mL) were acidified with 5 mL of HCl 1 M, shaked for 30 min and then an aliquot of 10 mL of NaCl 10% (w/v) was added. Organotins were extracted 3 times with 50 mL of a mixture of n-hexane and ethyl acetate (70:30). The organic phase was dried with $NaSO_4$ and then evaporated at 35 °C to a final volume of 5 mL. The extract was treated with 85 mg of $NaBH_4$ diluted in 5 mL of absolute ethanol for 60 min. Finally, 10 mL of NaCl 10% (w/v) were added and the ethanol phase was discarded. The other organic phase was purified by filtering through a glass column (0.50 mm diameter, 23 mm length) containing Florisil. A solution containing 2.71 µg Dureno mL^{-1} was added to the eluate as internal standard.

Soft tissues of gastropods were analyzed similarly. About 0.5–1.0 g of soft tissues were acidified with 5 mL of HCl 1 M and the extraction was performed using 15 mL of the binary mixture n-hexane-ethyl acetate (70:30).

The analyses were carried out using a Hewlett Packard 5890 Plus GC chromatograph (Agilent Technologies, Avondale, PA) equipped with a capillary injector and a flame ionization detector (FID). OTs compounds were separated using a capillary column DB-5 (Agilent Technologies) (diameter = 0.32 mm, length = 30 m). Temperature conditions for the running were as follows: injector = 280 °C, detector = 300 °C. The column was initially stabilized at 40 °C for 3 min and then the following gradients were applied: 15 °C per min until the temperature reached 140 °C; 20 °C per min until it reached 230 °C, and finally 25 °C per min until it reached 290 °C which was kept constant for 14 min.

Quantification of the different OTs compounds was performed using Dureno as internal standard. Several parameters that describe the goodness of the method are presented in Table 1.

P. canaliculata cultures

A laboratory culture was established using gastropods collected at the sampling station located in Escobar (Fig. 1). Once in the laboratory animals were placed in 20 L aquaria containing approximately 12–15 of potable tap water that had previously been dechlorinated by letting it sit at least 24 h and by filtering through a carbon column. The

Table 1 Detection and quantification limits, calibration curve and recovery of the test methods

	Detection limit (DL)[a]	Quantification limit (QL)[b]	Correlation coefficient (r)	Calibration curve	Lower and upper confidence intervals of the calibration curve[c]	Recovery (%)
Water samples (n = 27)[d]						
TBT	208 ng L^{-1}	694 ng L^{-1}	0.9748	y = 0.14x + 0.11	y = 0.13x + 0.03 and y = 0.16x + 0.16	80–125
DBT	247 ng L^{-1}	822 ng L^{-1}	0.9862	y = 0.15x + 0.18	y = 0.14x + 0.06 and y = 0.17x + 0.15	71–115
MBT	237 ng L^{-1}	789 ng L^{-1}	0.9801	y = 0.15x + 0.17	y = 0.14x + 0.05 and y = 0.18x + 0.17	72–130
Biological tissues (n = 13)[d]						
TBT	211 ng g^{-1}	702 ng g^{-1}	0.9713	y = 0.36x–0.23	y = 0.29x–0.44 and y = 0.39x + 0.24	76–110
DBT	217 ng g^{-1}	722 ng g^{-1}	0.9635	y = 0.36x–0,44	y = 0.27x–0.37 and y = 0.35x + 0.21	70–98
MBT	355 ng g^{-1}	1183 ng g^{-1}	0.9832	y = 0.28x–0,10	y = 0.24x–0.11 and y = 0,30x + 0,21	65–79

[a] DL was calculated as the ratio of 3 × SD of the blank and the slope of the calibration curve

[b] QL was calculated as the ratio between 10 × SD of the blank and the slope of the calibration curve

[c] Confidence intervals of the regression curves were calculated at $\alpha = 0.05$

[d] Number of replicates

following physicochemical parameters were determined: total hardness = 48 ± 3 mg $CaCO_3$ L^{-1}; alkalinity = 29 ± 2 mg.$CaCO_3L^{-1}$; pH = 7.0 ± 0.2 and conductivity = 250 ± 17 µS. The aqueous media were completely renewed once a week.

The cultures were maintained at T = 23 ± 2 °C, with constant aeration and under a photoperiod of 14:10 h light-dark. Animals were fed lettuce leaves *ad libitum*. Only animals born under laboratory conditions from the second generation were used for the experiments.

Laboratory bioassays

Chronic bioassays (6 months) were performed for determining OTs bioaccumulation and biomarker parameters in different soft tissues of female *P. canaliculata*. The selected exposure period ensured that all animals could reach the reproductive maturity under laboratory conditions. In the natural environment, the reproductive maturity may be reached in a broad range period (3–24 months) depending on the ambient temperature while longevity is up to 4 years (GISD 2016). Organisms of similar size (4.2 ± 0.5 cm) and about 4–5 months old were randomly selected from the cultures. Ten organisms were placed in 20 L aquaria containing 8 L and exposed to 1 µg TBT L^{-1} using a stock solution prepared in methanol. Controls were exposed to dechlorinated tap water and the same proportion of methanol. The nominal exposure level was selected accordingly to the results obtained from the field analyses of freshwaters (details in Results section). A week before the end of bioassays animals were allowed to depurate in dechlorinated tap water. Six animals were used for bioaccumulation studies and 4 animals for biomarker determinations. Four replicates were performed.

According to TBT water analyses, experimental concentration values did not change more than 20% than the nominal value when the test solutions were completely renewed twice a week. These were acceptable variations according to the criterion recommended for many regulatory aquatic toxicity tests (Rand 1995; US EPA 2002). Water physicochemical parameters were checked every time the aqueous medium was renewed. During the renewal of the test solution animals were allowed to feed *ad libitum* lettuce leaves for 2 h.

At the end of the exposure period, animals were frozen, sacrificed and the following anatomical regions were isolated at 4 °C: cephalopedal, gonads, hepatopancreas, and albumin gland. Tissues were lyophilized until analyses.

Biomarker determinations

SOD activity determination

Tissues were homogenized (1:5, w/v) in 0.05 M phosphate buffer, pH 7.0 containing 0.01 mM EDTA. The homogenates were centrifuged at 3000 *g* for 15 min at 4 °C and the supernatants were immediately used as enzymatic source. SOD activity was determined by the rate of autoxidation of epinephrine to adenochrome (Misra and Fridovich 1972). The reaction volume was 1 ml and contained 10 mM epinephrine, 50 mM sodium carbonate buffer pH 10.2, 10 mM EDTA. The results are given in units of SOD activity per milligram of protein (U mg^{-1}), where 1 U of SOD was defined as the amount of sample causing 50% of inhibition of epinephrine autoxidation.

CAT activity determination

Tissues were homogenized (1:5, w/v) in 0.05 M phosphate buffer, pH 7.0, 0.01 mM EDTA, and then centrifuged at 3000 *g* for 15 min at 4 °C. The resulting supernatants were diluted 1:2 with 0.05 M phosphate buffer, pH 7.0, 0.1% Triton X100, sonicated for 5 min in a bath sonicator and immediately used as enzymatic source. CAT activity was

determined by monitoring the decomposition of hydrogen peroxide using a spectrophotometric assay as described previously (Claiborne 1985). One unit of CAT activity was defined as the amount of catalase to degrade 1 µmol of hydrogen peroxide in 1 min at pH 7.0 at 25 °C, while the hydrogen peroxide concentration falls from 10.3 to 9.2 mM, measured by the rate of decrease of $A^{240\ nm}$ (extinction coefficient: 40 $M^{-1}cm^{-1}$).

Total GSH levels (t-GSH)

Tissues were homogenized (1:5, w/v) in 125 mM phosphate buffer, pH 7.5 containing 6.3 mM EDTA. To 900 µL of the homogenate, 100 µL of trichloroacetic acid (30%) were added and incubated in ice for 15 min. Following centrifugation at 11,000 *g* for 5 min, the clear supernatant was assayed for t-GSH by using the Tietze recycling assay which involves the sequential oxidation of GSH by 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) and reduction by NADPH in the presence of glutathione reductase (Tietze 1969).

Lipid peroxidation assay

Lipid peroxidation was determined by measuring the amount of thiobarbituric acid reactive substances (TBARS). Tissue homogenates were prepared in KCl 150 mM (1:10 w/v) and then centrifuged at 1000 *g* for 5 min prior to the reaction in order to eliminate the interference of any insoluble pigments. To 1 mL of homogenate it was added 0.5 mL of 20% trichloroacetic acid and 1 mL of 0.67% (w/v) thiobarbituric acid. After heating for 10 min at 100 °C, the precipitated proteins were centrifuged and the absorbance of the solution was measured at $\lambda = 530$ nm. A molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ was used. Tissue blanks were performed in all determinations.

AChE activity determination

Tissues were homogenized in 20 mM Tris/HCl buffer, pH 7.5, plus 0.5 mM EDTA. Homogenates were centrifuged at 11,000 *g* for 20 min at 4 °C. AChE activity was measured, in duplicate, in 100 mM phosphate buffer, pH 8, 0.2 mM DTNB, acetylthiocholine iodide 0.75 mM according to the method of Ellman et al. (1961). Activity was recorded continuously at 412 nm. Rates were corrected for spontaneous hydrolysis of the substrate and non-specific reduction of the chromogen by tissue extracts. The enzymatic activity was expressed as µmol min^{-1} mg $protein^{-1}$.

Protein content

Proteins were determined according to the method of Lowry et al. (1951), using bovine serum albumin as standard.

Statistical analysis

Results were expressed as mean ± S.D. Data were analyzed by one-way ANOVA followed by Tukey HSD post-test by using VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html). The level of significance used was 0.05. Prior to ANOVA, data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively by using OriginPro 7.5.

Results

OT concentrations in freshwater and gastropod samples from different sampling stations

Levels of OTs in freshwater and gastropod samples collected from different sampling stations located at the lower Río de la Plata basin are presented in Table 2. The three OTs were found in all freshwater samples excepting in Olivos Port, where MBT was below the detection limit. Mean values of TBT varied between 0.77 and 1.35 µg L^{-1}. In general, the breakdown products DBT and MBT were present at lower concentrations than TBT.

Gastropods collected at Tigre sampling station presented the highest TBT accumulation, followed by animals collected at Escobar. In two sampling stations (San Fernando and Olivos) no gastropods could be found, while the TBT concentration in *P. canaliculata* from San Isidro was below the detection limit. However, in these animals both DBT and MBT were found. These results were in contrast with the pattern observed in animals collected from Escobar and Tigre since levels of DBT were lower than those of TBT, and MBT was not detected.

OTs in female *P. canaliculata* chronically exposed to TBT under laboratory conditions

Table 3 shows the levels of TBT and its breakdown products, DBT and MBT, found in female gastropods exposed to 1 µg TBT L^{-1} for 6 months. After the chronic exposure, there was no mortality in any treatment. Gonads presented the highest levels of TBT, followed by the cephalopedal region, albumin gland and finally hepatopancreas ($p < 0.05$). Both metabolites, DBT and MBT, were found in all tissues of gastropods but at comparatively lower concentrations than TBT ($p < 0.05$). Values of OTs in tissues from control organisms were below the detection limits. In

Table 2 Levels of TBT and its breakdown products, DBT and MBT, in freshwater and gastropod samples collected from coastal areas of the lower Rio de la Plata basin (mean values and SD)

	Escobar	Tigre	San Fernando	San Isidro	Olivos
Freshwater samples ($\mu g\ L^{-1}$)					
n[a]	6	4	6	4	5
TBT	1.35 ± 0.08	1.15 ± 0.05	1.07 ± 0.22	0.98 ± 0.17	0.77 ± 0.15
DBT	0.51 ± 0.09	0.42 ± 0.11	0.75 ± 0.15	0.44 ± 0.15	0.62 ± 0.07
MBT	0.78 ± 0.12	0.39 ± 0.15	0.29 ± 0.12	0.47 ± 0.19	< 0.24
Gastropod samples[b] ($\mu g\ g^{-1}$ dw)					
n[a]	6	6	0	7	0
TBT	0.59 ± 0.16	1.20 ± 0.20	–	< 0.16	–
DBT	0.31 ± 0.08	0.36 ± 0.06	–	0.41 ± 0.12	–
MBT	<0.36	<0.36	–	0.31 ± 0.14	–

n[a]: Number of samples

[b] Whole body soft tissues

Table 3 Levels of TBT and its breakdown products (DBT and MBT) in different anatomical regions of female *P. canaliculata* exposed to 1 μg TBT L^{-1} for six months (mean values ± SD) under laboratory conditions

	TBT ($\mu g\ g^{-1}$ dw)	DBT ($\mu g\ g^{-1}$ dw)	MBT ($\mu g\ g^{-1}$ dw)
Cephalo-pedal	0.91 ± 0.22	0.13 ± 0.03	0.12 ± 0.05
Gonads	2.44 ± 0.77	0.39 ± 0.07	0.55 ± 0.11
Hepatopancreas	0.30 ± 0.09	0.24 ± 0.11	0.18 ± 0.04
Albumin gland	0.58 ± 0.06	0.22 ± 0.02	0.11 ± 0.04

Mean values of 4 replicates of 6 gastropods each (n = 24).

addition, all females *P. canaliculata* exposed to TBT showed imposex (Fig. 2).

Biomarkers related to the generation of ROS

The biomarkers related to the generation of ROS selected for this work are shown in Fig. 3. In comparison with control organisms, SOD activity did not show any significant difference in any tissue of TBT exposed gastropods ($p > 0.05$). On the contrary, the CAT activity was increased in all tissues analyzed ($p < 0.05$). The highest CAT activity was observed in gonads, followed by hepatopancreas, and finally cephalopedal and albumin gland ($p < 0.05$). Between these last two tissues, no significant differences were observed ($p > 0.05$).

Significant increases in t-GSH levels were observed in the cephalo-pedal, hepatopancreas and albumin gland of exposed female *P. canaliculata* in comparison with control organisms ($p < 0.05$) (Fig. 3c). On the contrary, levels of t-GSH were diminished in gonads of TBT exposed female organisms ($p < 0.05$).

Figure 3d shows the levels of lipid peroxidation, measured as thiobarbituric acid reactive species (TBARS) in different tissues of female *P. canaliculata* chronically exposed to TBT. In all anatomical regions, values were similar in both control and exposed organisms ($p > 0.05$).

Activity of AChE

Female *P. canaliculata* chronically exposed to TBT presented significant decreases in the AChE activity in the cephalo-pedal and gonadal regions respect to control organisms ($p < 0.05$) (Fig. 4). However, the activity in the albumin gland remained unchanged ($p > 0.05$). The hepatopancreas tissue was not analyzed since many esterase enzymes other than AChE may be present interfering with the results.

Discussion

TBT and its principal breakdown products were detected in all freshwater samples collected at the different sampling stations located at the lower Río de la Plata basin, excepting Olivos. In this area, only the parent compound and DBT were found. The concentrations of the breakdown products were always lower than the values of the parent compound. These results could reflect that those areas have not only been subjected to a historical release of TBT but also to present sources of contamination. In samples from Olivos, where MBT was not detected, it could reflect a comparatively more recent contamination. In Escobar sampling station there is a relatively small recreational port for fishing and sporting activities. Among all stations, Tigre is undoubtedly the main city in the delta area, attracting both local and foreign visitors. It is located at about 32 km at the

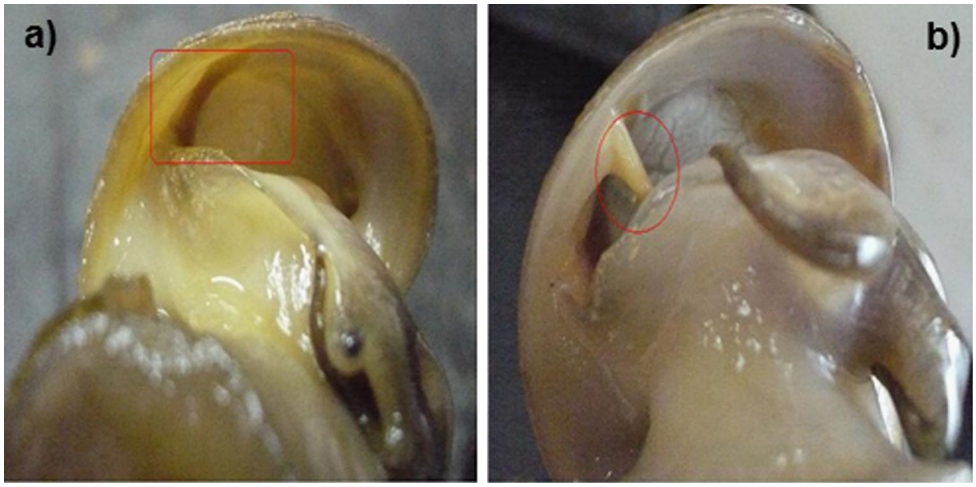

Fig. 2 External view of the reproductive system of: (**a**) control female *P. canaliculata*, (**b**) female organisms exposed to 1 µg TBT L^{-1} for 6 months, where the area in *red* color shows the presence of imposex

north of Buenos Aires, capital city of Argentina. The Tigre Port has three inner docks. One is dedicated for tourist excursions. Another concentrates the grocer's boats that supply the demands of the inhabitants. The last one is the place to unload the timbered boats transporting wood from the forested islands of the Paraná delta. In San Fernando recreational port there is also a pier for ships transporting sand. The port located in San Isidro has been abandoned during the last years and it has been subjected to multiple sources of anthropogenic contamination. Olivos is principally a recreational port located along the big estuary of the Río de la Plata River.

This short survey establishes a baseline of OT concentrations in freshwater courses affected by commercial and recreational ports in Argentina. The values found in this survey were considerable higher than the concentration of TBT recommended to protect the freshwater aquatic life from chronic toxic effects (0.072 µg L^{-1}) (USEPA 2003).

Measurable concentrations of TBT were detected in the whole body soft tissue of *P. canaliculata* gastropods collected from Escobar and Tigre. Assuming that in the environment a steady state could be reached, bioaccumulation factors can be calculated as the ratio between TBT concentrations in gastropods and the concentration in waters. By this method, bioaccumulation factors of 437 and 1043 were observed in samples from Escobar and Tigre, respectively. These values were expected because Tigre is subjected to a higher traffic of ships and boats than Escobar. In gastropods collected from San Isidro only DBT and MBT were detected, possibly due to the abandoned conditions of the port. No animals could be found in San Fernando and Olivos sampling stations.

Since 2008 the use of TBT as antifouling in ship paints has been banned by the International Marine Organization (IMO). Several reports have documented a general trend of decreasing TBT concentrations in marine waters, sediments and biota from several ecosystems (Kim et al. 2011; Arp et al. 2014). Some systems have shown a relatively rapid recovery but others are still struggling (Matthiessen 2013). In Argentina, the product was banned in 1998 (PNA 1998). However, regular monitoring programs were not performed to assess temporal variations. In addition, the available data are mostly based on analyses of surface sediment samples collected before 2008. In marine coastal areas of Argentina, values of TBT from 1.9 up to 1370 ng Sn g^{-1} (dw) have been found (Bigatti et al. 2014). For other South America regions, the following ranges of TBT have been reported: <2.0–2796 ng Sn g^{-1} (dw) in ports located at the South of Brazil (Bigatti et al. 2014); 84.1–1929 ng Sn g^{-1} (dw) in coastal areas of the Venezuelan Caribbean Sea (Paz-Villarraga et al. 2015); 14–1560 ng Sn g^{-1} (dw) in coastal areas of Chile (Pinochet et al. 2009), and 12.7–99.5 ng Sn g^{-1} (dw) in coastal areas of Ecuador (Castro et al. 2012). According to surveys performed after 2008, decline trends have been observed in sediments from the same coastal areas of Chile (range = 0.8–122.3 ng Sn g^{-1}) (Batista et al. 2016) and along the coast of Bahía Blanca Bay (Argentina) (Quintas et al. 2016). In coastal areas of Ecuador, the incidence of imposex has reduced from 2009 to 2012, however, the phenomenon is still present (Rodríguez Grimón et al. 2016). The banning of TBT covers its use in commercial paint formulations but the compound is still manufactured and available for other purposes. Therefore, it can be present in handcrafted formulated paints. Besides, the lack of strict controls and analyses to certify the banning of TBT in paint formulations may contribute to the OTs contamination.

For this short survey, analyses were performed on freshwater samples to establish an environmental relevant exposure level (1 µg L^{-1}) to conduct the laboratory chronic study. Several authors have found that OTs bioaccumulation in whole body soft tissues of gastropods collected at

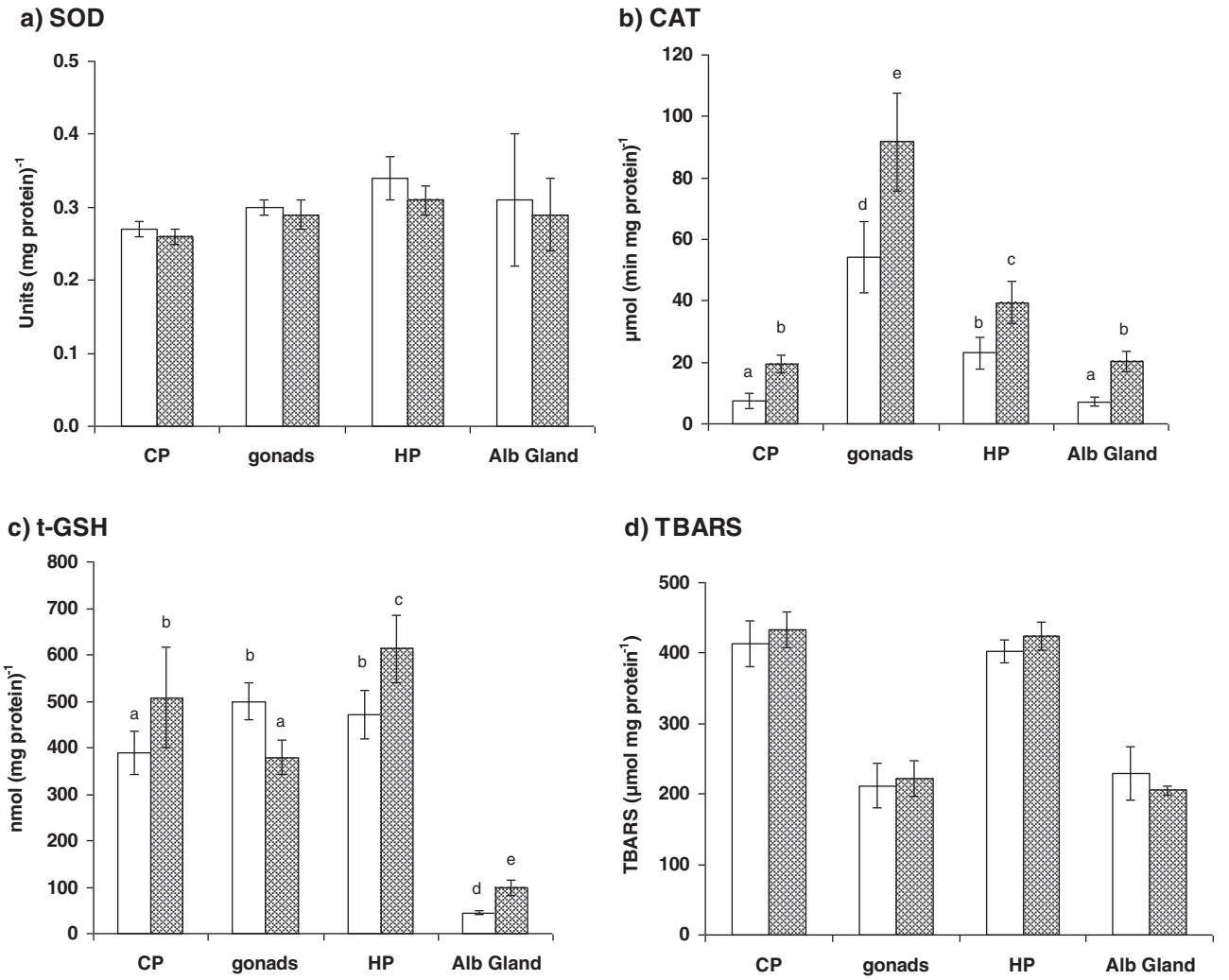

Fig. 3 Biomarkers related to the generation of ROS in different anatomical regions of control female *P. canaliculata* (*white box*) and those exposed to 1 µg TBT L^{-1} for 6 months (*gray box*) (**a**) SOD activity; (**b**) CAT activity; (**c**) total glutathione (t-GSH); (**d**) thiobarbituric acid reactive species (TBARS). *CP* cephalopedal region, *HP* hepatopancreas, *Alb gland* albumin gland. Mean values (±SD) of 4 replicates of 4 gastropods each ($n = 16$). Different letters indicate significant differences at $p < 0.05$

their natural habitats did not differ between males and females (Couceiro et al. 2009; Batista et al. 2016). This consideration facilitates field monitoring programs but it cannot be applied to investigate subcellular responses.

Assuming that during the chronic exposure a steady state could be reached, bioaccumulation factors were 2444 for gonads, 910 for the cephalopedal region, 380 for the albumin gland and 300 for the hepatopancreas. Wang et al. (2010) have also found the highest level of TBT in the reproductive organs of marine whelks exposed to the same concentration of TBT for 45 days under laboratory conditions. The breakdowns products DBT and MBT were detected in all tissues from female organisms, indicating their ability to metabolize the parent compound. However, both metabolites were present at comparatively lower proportions than TBT.

Although it was not the main purpose of this work to investigate the reproductive success in *P. canaliculata*, imposex was observed in all female gastropods after the chronic exposure. This result is in agreement with a previous report of Giraud-Billoud et al. (2013) who used the same freshwater gastropod species exposed to 6 µg TBT L^{-1} for 28 days. The mechanism involved in TBT induced imposex still remains elusive. Several hypotheses have been proposed: (a) TBT increases free testosterone levels in female organisms; (b) TBT induces the secretion of neurohormones that contribute to male sexual differentiation; (c) TBT acts as an agonist of the retinoid X receptor, an important regulator of male sex characteristics (Sternberg et al. 2009).

TBT may also elicit several toxic responses other than endocrine disruption. In vitro studies have revealed that this

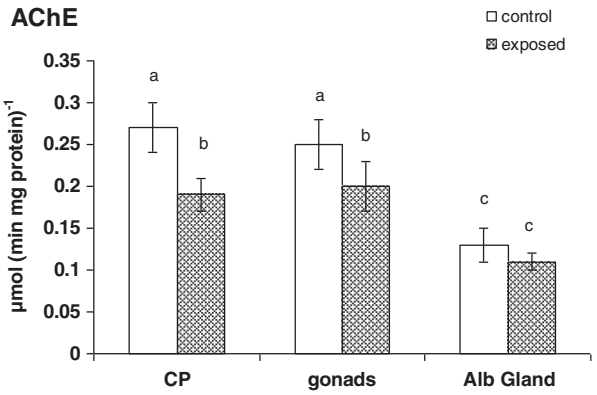

Fig. 4 AChE activity in different anatomical regions of female *P. canaliculata* exposed to 1 µg TBT L^{-1} for 6 months. *CP* cephalopedal region, *HP* hepatopancreas, *Alb gland* albumin gland. Mean values (±SD) of 4 replicates of 4 gastropods each ($n = 16$). Different letters indicate significant differences at $p < 0.05$

compound is able to induce perturbations on lipid homeostasis, favoring the accumulation of fat; it causes immunotoxicity, and it negatively affects both cell and mitochondria membrane functions (Pagliarani et al. 2013). In vivo and in vitro studies have shown an increased generation of ROS that perturbs the cell antioxidant status, leading to lipid peroxidation and protein carbonylation in cerebral rat cortex (Mitra et al. 2013).

In all tissues of exposed female *P. canaliculata* only the CAT activity showed higher values than controls, suggesting an increase in the generation of ROS. Since lipid peroxidation did not occur in any tissue, it could be hypothesized that the excess of ROS could be efficiently neutralized by the enhanced levels of glutathione. Glutathione plays a central role in maintaining the cellular redox status and protecting cells from oxidative injury (Dickinson and Forman 2002). This hypothesis cannot be applied to gonads since a decrease in t-GSH was observed in comparison to control gastropods. However, cells have many other antioxidant defenses to neutralize ROS. It is generally accepted that after a chronic exposure to sublethal concentrations of contaminants it may result in an up-regulation of the antioxidant defenses (Abele et al. 2012).

Depletion of antioxidant defenses may lead to oxidative stress damage. On the contrary, their increases reflect the ability of the organism to cope with the stressor. In agreement with our results, Kim et al. (2011) did not found a significant change in SOD activity in a marine copepod species (*Tigriopus japonicus*) after an acute exposure (96 h) to 1 µg TBT L^{-1}. On the contrary, a significant decrease in SOD activity was reported in the haemolymph of the abalone (*Haliotis diversicolor supertexta*) exposed to 10 and 30 µg TBT L^{-1} after a 30 days exposure (Zhou et al. 2010). On the other hand, Wang et al. (2005) have reported significant increases in SOD and CAT activities in the hepatic tissue of the marine fish *Sebastiscus marmoratus* after short term exposures to TBT via i.p. injections. These authors have also found increased levels of hepatic GSH (Wang et al. 2005). No change in CAT activity was observed in the bivalve mollusk *Mytilus edulis* exposed to TBT (10 and 1000 ng Sn L^{-1}) for 96 h (Devier et al. 2003). However, direct comparisons with data reported in the literature are difficult due to differences in the tissues analyzed, the selected animal species, the route of administration, the exposure time and the dose or concentration used.

The decrease in AChE activity found in gonads and in the cephalopedal region could indicate that TBT and/or its breakdown products (DBT and MBT) could induce neurotoxic effects in female *P. canaliculata*. Since long, the activity of AChE has been recognized as a sensitive biomarker of both exposure and effect to organophosphate and carbamate pesticides. However, many other metal and organic pollutants are also able to inhibit this enzyme activity (Labrot et al. 1996; Láng et al. 1997; Alves Costa et al. 2007). Usually, the inhibition caused by these agents is restored by transferring the organisms to clean water for about 24 h. Depuration periods much longer than 24 h are required when the enzyme is inhibited by organophosphate pesticides. The inhibition caused by TBT in tissues of female *P. canaliculata* was observed in animals that had been depurated during a week. However, all tissues still showed measurable levels of OT compounds as a consequence of the bioaccumulation process that had taken place during the chronic exposure. Therefore, it could be expected an enzyme inhibition. Opposite to our results, AChE activity was not modified in mussels (*Mytilus edulis*) after an acute exposure to TBT (Devier et al. 2003) or in tissues (muscle and brain) of the freshwater fish *Hoplias malabaricus* after a subchronic dietary exposure (Rabitto et al. 2005).

Conclusions

The field study confirmed the hypothesis that TBT and its breakdown products were present in freshwater samples collected from 5 sampling stations located along coastal areas at the lower Río de la Plata basin. Concentration levels were considerably higher than the value recommended by the US EPA to protect freshwater ecosystems. *P. canaliculata* gastropods were only found in 3 of the 5 sampling stations. They also showed a high bioaccumulation of OT compounds.

The laboratory study demonstrated that female gastropods chronically exposed to a concentration of TBT similar to the values found in natural freshwaters accumulated OT compounds and all of them presented imposex. According to this study, gonads were the most affected tissue since

they exhibited the highest TBT accumulation, an increase in CAT activity, a decrease in t-GSH content, and an inhibition of the AChE enzyme. All these parameters could be used as useful biomarkers of TBT exposure and effects. In decreasing order, the cephalopedal region presented also a great ability to accumulate OT compounds, followed by the albumin gland and finally the hepatopancreas. In all these tissues the CAT activity and levels of t-GSH were increased in exposed gastropods. In addition, the activity of the AChE enzyme was inhibited in the cephalopedal region. According to the TBARS values, lipid peroxidation did not occur in any tissue. Therefore, TBT and/or its breakdown products were able to affect the cellular redox status and also to induce neurotoxic effects in tissues of female *P. canaliculata*. In this way, organisms may become susceptible in the presence of an extra environmental challenger.

Acknowledgements The authors are indebted to the Universidad de Buenos Aires and the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). MLM acknowledges fellowship from the United Nations Environmental Program-Argentina. The authors also thank to anonymous reviewers for helpful comments on a previous version of the manuscript. This study was funded by grants to the Project N° 02/018/Subproject B-C-53 from the United Nations Environmental Program-Argentina (UNEP-ARG), and N° 20020130100454BA from the Universidad de Buenos Aires.

Compliance with ethical standards

Conflict of interest The authors declare that they have no Conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Abele D, Vazquez-Medina JP, Zenteno-Savin T (2012) Oxidative stress in aquatic ecosystems. Wiley-Blackwell, Chichester, West Sussex

Alves Costa JRM, Mela M, da Silva de Assis HC, Pelletier E, Ferreira Randi MA, Oliveira Ribeiro CA (2007) Enzymatic inhibition and morphological changes in *Hoplias malabaricus* from dietary exposure to lead(II) or methylmercury. Ecotox Environ Safe 67:82–88

Arp HPH, Eek E, Nybakk AW, Glette T, Møskeland T, Pettersen A (2014) When will the TBT go away? Integrating monitoring and modelling to address TBT's delayed disappearance in the Drammensfjord, Norway. Water Res 65:213–223

Axiak V, Vella AJ, Agius D, Bonnici P, Cassar G, Cassoe R, Chircop P, Micallef D, Mintoff B, Sammut M (2000) Evaluation of environmental levels and biological impact of TBT in Malta (central Mediterranean). Sci Total Environ 258:89–97

Batista RM, Castro IB, Fillmann G (2016) Imposex and butyltin contamination still evident in Chile after TBT global ban. Sci Total Environ 566-567:446–453

Bernanke J, Köhler HR (2009) The impact of environmental chemicals on wildlife vertebrates. Rev Environ Contam Toxicol 198:1–47

Bigatti G, Averbuj A, Braga Castro I, Primost M, Carranza A, Commendatore M, Fillmann G, Delgado E, Scarabino F, del Bríó F, Franco M, Letamendía M, Martínez G, Sebastián Sauco S, Penchaszadeh PE (2014) Situación de contaminación en las costas del sur de Brazil, Uruguay y Argentina luego de la prohibición mundial de TBT. In: Goso C (ed) Nuevas miradas a la problemática de los ambientes costeros. Difusión, Relaciones y Actividades Culturales (DIRAC) – Facultad de Ciencias, Montevideo, Uruguay. 277–301

Buggy CJ, Tobin JM (2006) Seasonal and spatial distributions of tributyltin in surface sediment of the Tolka Estuary, Dublin, Ireland. Environ Pollut 143:294–303

Castro IB, Arroyo M, Costa P, Fillmann G (2012) Butyltin compounds and imposex levels in Ecuador. Arch Environ Contam Toxicol 62:68–77

Cazzaniga NJ (1990) Sexual dimorphism in *Pomacea canaliculata*. Veliger 33:390–394

Claiborne A (1985) Catalase activity. In: Greenwald R (ed) CRC handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL, p 283–284

Couceiro L, Díaz J, Albaina N, Barreiro R, Irabien JA, Ruiz JM (2009) Imposex and gender-independent butyltin accumulation in the gastropod *Nassarius reticulatus* from the Cantabrian coast (N Atlantic Spain). Chemosphere 76:424–427

Devier MH, Augagneur S, Budzinski H, Mora P, Narbonne JF, Garrigues P (2003) Microcosm tributyltin bioaccumulation and multibiomarker assessment in the blue mussel *Mytilus edulis*. Environ Toxicol Chem 22:2679–2687

Dickinson DA, Forman HJ (2002) Cellular glutathione and thiols metabolism. Biochem Pharmacol 64:1019–1026

Dowson PH, Bubb JM, LesterJN (1996) Persistence and degradation pathways of tributyltin in freshwater and estuarine sediments. Estuar Coast Shelf S 42:551–562

Ellman GL, Courtney KD, Andres Jr V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Fent K (1996) Ecotoxicology of organotin compounds. Crit Rev Toxicol 26:1–117

Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 73:122–159

Giraud-Billoud M, Vega IA, Wuilloud RG, Clément ME, Castro-Vazquez A (2013) Imposex and novel mechanisms of reproductive failure induced by tributyltin (TBT) in the freshwater snail *Pomacea canaliculata*. Environ Toxicol Chem 32:2365–2371

GISD: Global Invasive Species Database (2016) http://www.iucngisd.org/gisd/species.php?sc=135. Accessed 22 October 2016.

Hermes-Lima M, Storey JM, Storey KB (1998) Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. Comp Biochem Phys Part B 120:437–448

Higuera-Ruiz R, Elorza J (2011) Shell thickening and chambering in the oyster *Crassostrea gigas*: natural and anthropogenic influence of tributyltin contamination. Environ Technol 32:583–591

Hoch M (2001) Organotin compounds in the environment-an overview. Appl Geochem 16:719–743

Jagtap JT, Shejule KB, Jaiswal DP (2011) Acute toxicity study of tributyltin chloride on the freshwater bivalve, *Lamellidens marginalis*. World J Fish Marine Sci 3:100–103

Kim BM, Rhee JS, Park GS, Lee J, Lee YM, Lee JS (2011) Cu/Zn- and Mn-superoxide dismutase (SOD) from the copepod *Tigriopus japonicus*: Molecular cloning and expression in response to environmental pollutants. Chemosphere 84:1467–1475

Labrot F, Ribera D, Saint Denis M, Narbonne JF (1996) *In vitro* and *in vivo* studies of potential biomarkers of lead and uranium contamination: lipid peroxidation, acetylcholinesterase, catalase

and glutathione peroxidase activities in three non-mammalian species. Biomarkers 1:21–28

Láng G, Kufcsák O, Szegletes T, Nemcsók J (1997) Quantitive distributions of different cholinesterases and inhibition of acetylcholinesterase by metidathion and paraquat in alimentary canal of common carp. Gen Pharmac 29:55–59

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu Rev Physiol 68:253–278

Leung K, Grist E, Morley N, Morritt D, Crane M (2007) Chronic toxicity of tributyltin to development and reproduction of the European freshwater snail *Lymnaea stagnalis* (L.). Chemosphere 66:1358–1366

Liu WH, Chiu YW, Huang DJ, Liu MY, Lee CC, Liu LL (2006) Imposex in the golden apple snail *Pomacea canaliculata* in Taiwan. Sci Total Environ 371:138–143

Lowry OH, Rosebrough A, Farr L, Randall RS (1951) Protein measurement with the folin phenol reagent. J Biol Chem 249:265–275

Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101:13–30

Martins SE, Bianchini A (2011) Toxicity tests aiming to protect Brazilian aquatic systems: current status and implications for management. J Environ Monitor 13:1866–1875

Matthiessen P (2013) Detection, monitoring, and control of tributyltin - an almost complete success story. Environ Toxicol Chem 32:487–489

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Mitra S, Gera R, Siddiquib WA, Khandelwal S (2013) Tributyltin induces oxidative damage, inflammation and apoptosis via disturbance in blood–brain barrier and metal homeostasis in cerebral cortex of rat brain: an in vivo and in vitro study. Toxicology 310:39–52

Nakayama K, OshimaY, Tachibana T, Furuse M, Honjo T (2007) Alteration of monoamine concentrations in the brain of medaka, *Oryzias latipes*, exposed to tributyltin. Environ Toxicol 22:53–57

Pagliarani A, Nesci S, Ventrella V (2013) Toxicity of organotin compounds: shared and unshared biochemical targets and mechanisms in animal cells. Toxicol in Vitro 27:978–990

Paz-Villarraga CA, Castro IB, Miloslavich P, Fillmann G (2015) Venezuelan Caribbean Sea under the threat of TBT. Chemosphere 119:704–710

Pinochet H, Tessini C, Bravo M, Quiroz W, De Gregori I (2009) Butyltin compounds and their relation with organic matter in marine sediments from San Vicente Bay, Chile. Environ Monit Assess 155:341–353

PNA: Prefectura Naval Argentina (1998) Régimen para la protección del medioambiente. Ordenanza No 4/98. Buenos Aires, Argentina

Quintas PY, Oliva AL, Arias A, Domini CE, Alvarez MB, Garrido M, Marcovecchio JE (2016) Seasonal changes in organotin compounds in sediments from the Bahía Blanca Estuary. Environ Earth Sci 75:659–671

Rabitto IS, Alves Costa JRM, Silva de Assis HC, Pelletier É, Akaishi FM, Anjos A, Randi MAF, Oliveira Ribeiroa CA (2005) Effects of dietary Pb(II) and tributyltin on neotropical fish, *Hoplias malabaricus*: histopathological and biochemical findings. Ecotox Environ Safe 60:147–156

Rand GM (1995) Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment, 2nd edn. Taylor & Francis, London

Rodríguez Grimón RO, Arroyo Osorio MF, de Freitas DM, Castro IB (2016) Tributyltin impacts in Galapagos Islands and Ecuadorian shore: marine protected areas under threat. Marine Policy 69:24–31

Rüdel H (2003) Case study: bioavailability of tin and tin compounds. Ecotox Environ Safe 56:180–189

Sternberg RM, Gooding MP, Hotchkiss AK, LeBlanc GA (2009) Environmental-endocrine control of reproductive maturation in gastropods: implications for the mechanism of tributyltin induced imposex in prosobranchs. Ecotoxicology 19:4–23

Tallarico LF (2016) Freshwater gastropods as a tool for ecotoxicology assessments in Latin America. Amer Malac Bull 33:330–336

Tessier E, Amouroux D, Morin A, Christian L, Thybaud E, Vindimian E, Donard OFX (2007) Tri)Butyltin biotic degradation rates and pathways in different compartments of a freshwater model ecosystem. Sci Total Environ 388:214–233

Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 27:502–522

Tsunoda M, Konno N, Nakano K, Liu Y (2004) Altered metabolism of dopamine in the midbrain of mice treated with tributyltin chloride via subacute oral exposure. Environ Sci 11:209–219

US EPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edition. EPA-821-R-02-012. United States Environmnental Protecion Agency, Washington, DC, USA.

US EPA (2003) Ambient aquatic life water quality criteria for tributyltin (TBT) – Final. EPA 822-R-03-031. United States Environmnental Protecion Agency, Washington, DC, USA.

Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotox Environ Safe 64:178–189

Waisbaum RG, Rodriguez C, Nudelman NS (2010) Determination of TBT in water and sediment samples along the Argentine Atlantic coast. Environ Technol 31:1335–1342

Wang CG, Chen YX, Li Y, Wei W, Yu Q (2005) Effects of low dose tributyltin on activities of hepatic antioxidant and phase II enzymes in *Sebastiscus marmoratus*. Bull Environ Contam Toxicol 74:114–119

Wang X, Fang C, Hong H, Wang WX (2010) Gender differences in TBT accumulation and transformation in *Thais clavigera* after aqueous and dietary exposure. Aquat Toxicol 99:413–422

Wang XH, Hong HS, Zhao DM, Hong LY (2008) Environmental behavior of organotin compounds in the costal environment of Xiamen, China. Mar Poll Bull 57:419–424

Zhou J, Zhu X, Cai Z (2010) Tributyltin toxicity in abalone (*Haliotis diversicolor supertexta*) assessed by antioxidant enzyme activity, metabolic response, and histopathology. J Hazard Mater 183:428–433