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Imposex and organotin compounds in marine gastropods and sediments from the Mar del Plata coast, Argentina

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The occurrence of imposex in *Adelomelon brasiliana* during two consecutive years near to Mar del Plata (the principal fishery port of Argentina) and its relation to tributyltin (TBT) and dibutyltin (DBT) content in muscle tissue and digestive gland was investigated using gas chromatography. Percentage of imposex affected females increased to over 80% in spring and summer of both years. However, its intensity, given by the relative penis size index (RPSI), fluctuated around low values. *Adelomelon brasiliana* digestive gland samples showed a very low concentration of TBT (36–46 ng/g dry weight). Egg capsules presented normal viable embryos, which had TBT concentrations similar to the capsule wall (15 ng/g) while the intracapsular liquid was only 2 ng/g, similar to the sediment concentration. The proportion and distribution of butyltin compounds (BTs) in sediments indicate a major contamination event.

INTRODUCTION

The application of paint with organotins (OTs) to ships' hulls has been banned in many countries and its use on vessels of less than 25 m in length is forbidden. In Argentina, the ordinance 4/98 of the Environment Protection Secretary (DPMA, 1998) prohibits the application of tributylin (TBT) on sports boats.

Penchaszadeh et al. (2001) reported the occurrence of imposex on the coast of Buenos Aires province, Argentina for two gastropod species, *Adelomelon brasiliana* (Lamarck, 1811) and *Buccinanops monilifer* (Kiener, 1834) but until this date no studies determined OT concentrations in these gastropods. The control of TBT level in marine invertebrates is relevant because of the reproductive impairment that TBT can cause.

Here were investigated the OT levels in sediments, *A. brasiliana* tissues and their egg capsules and their relation to the occurrence of imposex, along 50 km of coast from Mar del Plata port, which has the highest landings in the country, up to a protected area, in order to control pollution events. Imposex shown by these commercial gastropods was related to the OTs content in their tissues.

MATERIALS AND METHODS

Sampling

About ten females of the black snail *Adelomelon brasiliana* were captured monthly over a 24 month period at approximately 1000 m northwards from the mouth of Mar del Plata port, Station 1 (Figure 1) in order to study the degree of OT pollution and its effect on the population. The sampled area was of 15,000 m^2. The relative penis size index (RPSI) and imposex percentage of these specimens was recorded. As controls, black snail seasonal samples (one sample every three months) were taken near the Mar Chiquita coastal reserve, where neither imposex nor TBT was recorded before (Goldberg et al., 2004).

Monthly percentages of imposex-affected females were calculated as the number of females with penis and/or vas deferens with respect to all females sampled during this month.

Monthly RPSI was calculated according to the following equation (Gibbs & Bryan, 1994):

$$\mathrm{RPSI} = \frac{(\text{mean length of female penis size})^3}{(\text{mean length of male penis size})^3} \times 100 \quad (1)$$

TBT in gastropods and sediments

In January, July and December 2002, *A. brasiliana* samples were taken near the port. Tributylin and dibutyltin (DBT) content in the foot and digestive gland of the captured animals were determined by gas chromatography (GC). The analytical procedure was carried out under DIN-Norm 38407-F13 (modified) for water, sewage and mud.

For each month, ten grams of foot tissue of five individuals (total=50 g), from Station 1 was ground and mixed with each other to obtain a single foot mix. The same was done with digestive gland tissue. For each of the three months, 1.5 g foot mix and 1.5 g digestive gland mix were separately homogenized and 250 ng (62.5 ng each compound) of an internal standard consisting of tetrapropyltin (TePrT), tripropyltin (TPrT), monoheptyltin (MHT), and diheptyltin (DHT) 100 ng/ml was added to each sample in order to control the effectiveness of the recovery of OTs.

Each sample was extracted with 20 ml methanol, 40 g of a 10% NaCl solution, 40 ml sodium acetate/acetic buffer (pH approximately 4.6) and finally 100 ml hexane.

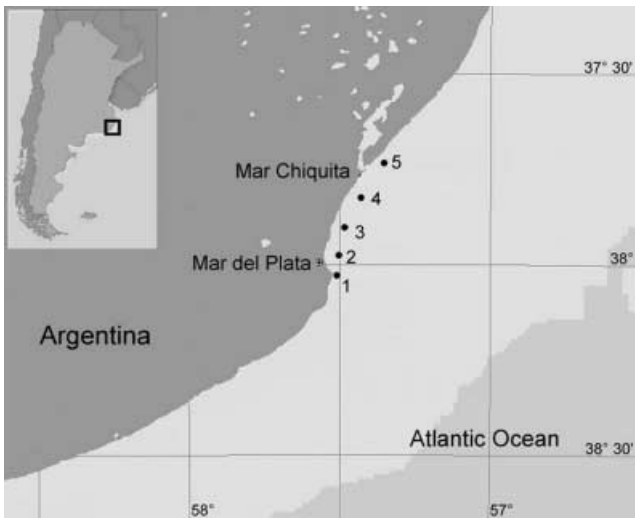

Figure 1. Sampling stations for sediment along the coast of Buenos Aires, Argentina. Monthly samples of *Adelomelon brasiliana* were taken from the southern location (1) and seasonal samples from Station 5.

The ethylation was carried out by adding 3×2 ml of 10% Na tetraethylborate to each extract, with 30 min between each addition. Fifty ml of supernatant hexane solution were taken from the solution with a 10 ml pipette. Five g of Na sulphate were added to this 50 ml hexane solution to dry it during 60 min. This dry solution was concentrated to 2–3 ml in a rotary evaporator.

A clean-up was made in a silica gel column with 15 ml of hexane:acetate 92:8 eluate. The resultant was concentrated again to 1.5 ml.

The OT content in *A. brasiliana* egg capsules was also measured. Egg capsules were divided into egg capsule wall, liquid phase and embryos in order to study the differential accumulation of OTs.

The measurement of OT content in extracts was with the GC/AED-System. The GC used was a HP6890 gas chromatograph equipped with an atomic-emission-detector HP G2350A with ICP plasma. The separation was carried out on a GC-column 30 m HP-5MS 5% phenyl-methyl-siloxane, 0.25 mm ID coated with 0.25 μm film phase. Injection was performed with a Gerstel Cold-System at 40°C, separation with a temperature programme of 40–320°C, within 35 min. Helium was used as the carrier gas at a flow-rate of 2.7 ml×min^{-1}. The ICP was maintained with H_2, O_2 and Ar/CH_4 and N_2 as make-up gas (2000°C). AED-signals were measured at 303 and 301 nm wavelength.

Because TBT deposited in marine sediments can be bioavailable for years (Strand et al., 2003), samples of sediments were collected with a dredge in January 2001 and December 2002 in five places on a transect parallel to the coast (Figure 1), limited by the port to the south and the Mar Chiquita coastal reserve to the north. Fifteen g of wet sediments were analysed following the same procedure described above.

As a control for the chemical procedure the reference material BCR 646 freshwater sediments (TBT, DBT, MBT, TPhT, DPhT, MPhT certified) and BCR 477 mussel tissue were used. The detection limits of OTs were in the order of 0.2 ng×g^{-1} dry weight. The bioconcentration factor (BCF) was calculated as: TBT in tissue×TBT in sediments^{-1} (Takeuchi et al., 2001) as a way of comparison with other molluscan species affected by TBT in the world.

Concentrations are reported for each OT, as mass per gram of sediments.

For total organic carbon (TOC) determination, 30 mg dry samples were weighted and placed in stannous cups for determination of nitrogen and total carbon. Thereafter, samples were placed in silver cups with 200 μl 1 M HCl overnight at 40°C for determination of organic carbon. Samples were burned in an Elementaranalysator under constant helium current.

The oxidation column was at 1050°C and filled with Cr_2O_3 for the combustion and with silvered Co_3O_4 for elimination of sulphur. The reduction column, at 650°C, was filled with pure copper for reduction of NO_x to N_2. Water was eliminated in a column at room temperature filled with warm-conductance by thermal conductivity. For determination of CO_2 and N_2, a GC was used with a

Table 1. *Organotin content in pared sediment samples from Mar del Plata to Mar Chiquita, Argentina in ng×ml^{-1} of extract. TePrT was an internal standard added as control for the organotin recovery. BCR 646 was the reference material used with certified content of organotins. Numbers 1 to 5 refer to the station position.*

	BCR646	1		2		3		4		5	
	Recovered	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MBT	154.92	13.97	0.18	12.20	0.1	16.5	0.56	15.46	0.35	13.7	0.41
TePrT(standard)	50.00	50.00	0.00	50.00	0.00	50.00	0.00	50.00	0.00	50.00	0
DBT	126.63	8.22	0.12	7.68	0.24	6.53	0.58	5.65	0.13	5.40	0.26
TBT	74.23	2.45	0.01	2.21	0.11	1.51	0.13	1.03	0.04	0.50	0.01
TeBT	1.22	0.19	0.001	0.22	0.003	0.27	0.03	0.26	0.04	0.21	0.001
MPhT	13.8	0	0	0	0	0	0	0	0	0	0
DPhT	8.09	0	0	0	0	0	0	0	0	0	0
TPhT	4.04	0	0	0	0	0	0	0	0	0	0
Sample wet weight (g)	1.038	10.1	0.05	10.05	0.07	15.15	0.04	15.05	0.06	15.1	0.01

SD, standard deviation; MBT, monobutyltin; TePrT, tetrapropyltin; DBT, dibutyltin; TBT, tributyltin; TeBT, tetrabutyltin; MPhT, monophenyltin; DPhT, diphenyltin; TPhT, triphenyltin.

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Table 2. *Results in ng/g sediment dry weight as cation of tributyltin (TBT) and dibutyltin (DBT) and total organic carbon (TOC) in each station.*

	BCR646		1		2		3		4		5	
	Certified	Recovered	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TBT	480	330	1.4	0.01	1.3	0.08	0.56	0.04	0.38	0.02	0.19	0.006
DBT	770	496	2.6	0.06	2.5	0.02	1.4	0.14	1.3	0.03	1.2	0.02
TOC	–	–	0.1	0.007	0.105	0.007	0.095	0.007	0.08	0.01	0.1	0.007
TOCerr	–	–	0.08	0.003	0.07	0.008	0.08	0.007	0.07	0.008	0.07	0.005

SD, standard deviation.

Table 3. *Organotin content in tissue samples ($ng \times g^{-1}$ wet weight) of* Adelomelon brasiliana *females from Mar del Plata, Argentina, in three separate months of 2002.*

	BCR477 certified	BCR 477 recovered	H 01-2002	M 01-2002	H 06-2002	M 06-2002	H 12-2002	M 12-2002
TBT	2200	1427	46	14	46	21	36	21
DBT	1540	1063	38	18	35	9.1	39	17
MBT	1500	1678	29	35	31	10	133	26
DPhT	–	103	19	4.3	33	30	8.9	10

H, digestive gland; M, muscle; TBT, tributyltin; DBT, dibutyltin; MBT, monobutyltin; DPhT, diphenyltin.

column at 40°C; a warm-conductance detector and resulting peaks were integrated with a computer.

RESULTS

The efficiency of the OT recovery was 80% for the sediment samples, this can be seen in the recovery of 50 ng of the original 62.5 ng of tetrapropyltin (Table 1). The analysis of sediment samples indicated the presence of a gradient of BTs in the environment with a maximum of TBT, DBT and MBT at the port entrance decreasing towards Mar Chiquita (Table 1). There was a clear dominance of MBT and a low presence of TBT in all sediment samples.

Organotins presented a low value when standardized to gm of dry weight, as well as the TOC available for adsorption of these OTs (Table 2).

The digestive gland of the analysed animals always presented a higher TBT and DBT content than the foot (Table 3). The TBT content gradually decreased in the digestive gland while MBT increased indicating degradation and accumulation in this gland. The presence of TBT in muscle indicates that the analysed animals were still incorporating OTs from the environment, because TBT is rapidly transformed into DBT in the digestive gland.

The percentage of imposex affected females (I%) and the RPSI fluctuated following a similar pattern. The I% always reached 100 at the end of summer (Figure 2). Almost half of the population showed no signs of imposex at the end of the sampling (spring 2002), but this decrease during spring was already observed in 2001 and 2000. The RPSI presents a maximum value in January 2002. These two indices suggest intermittent

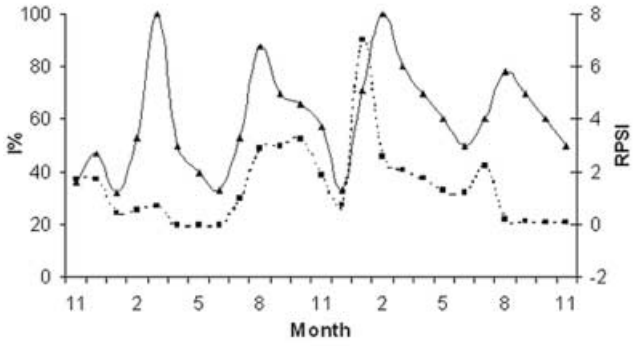

Figure 2. Relative penis size index (RPSI) and percentage of imposex affected females in *Adelomelon brasiliana* of Mar del Plata, Argentina, from November 2000 to November 2002. Dashed, RPSI; continuous, % of females with imposex.

Table 4. *Organotin concentration in ng $g \times^{-1}$ wet weight in the three parts of an egg capsule in late development stage and in intracapsular liquid of an egg capsule in early stage of development.*

Capsule stage	Capsule part	TBT	DBT	MBT	TPhT	MPhT
Late	Intracapsular liquid	2	1.3	3.0	0.4	0
Late	Capsule wall	18	10	21	82	2.3
Late	Embryos in late stage	15	4.4	10	8.9	0
Early	Intracapsular liquid	2.8	2.2	2.0	0.9	0

TBT, tributyltin; DBT, dibutyltin; MBT, monobutyltin; TPhT, triphenyltin; MPhT, monophenyltin.

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Table 5. *Bioconcentration factors (BCFs) reported for different molluscan species.*

Species	BCF	Reference
Gastropoda		
Adelomelon brasiliana	7.7–32.8	Present study
Thais clavigera	5000–10,000	Horiguchi et al., 1995
Nucella lapillus	29,000	Bryan & Gibbs, 1991
Bivalvia		
Dreissena polimorpha	20–45	Stäb et al., 1996
Nuculana pernula	138–404	Strand et al., 2003
Mytilus edulis	7400–19,000	Zoulian & Jensen, 1989

pulses of OT presence in the environment. The animals collected in the Mar Chiquita area showed no signs of imposex.

The analysed egg capsules contained early and late stage embryos and the TBT content in embryos was higher than in intracapsular liquid (Table 4).

DISCUSSION

Low concentration of TBT in sediments from the study area is the result of its natural degradation process and small new inputs. The high level of MBT indicates an old contamination event reported by Penchaszadeh et al. (2001).

It is important to underline that a low content of organic carbon in sediments, results in poor sorption of OTs. So the amount of pollutant in the water can be much higher, which is difficult to register because OTs are probably released in pulses and washed away by currents. The analyses of sediments, tissue and imposex indices indicate not only a unique major OT contamination episode, but also minor episodes, which apparently occur at the end of summer.

The amount of the contaminant in sediments from Mar del Plata in 2002 was already lower than in other port areas of the world such as in Osaka, which was estimated as 10 ng/g (Harino et al., 1998), 6–520 ng/g in Westeinder Lake, Netherlands (Stäb et al., 1996) and 2.4–18.8 ng/g in Øresund and Kattegat/Skagerrak (Strand et al., 2003).

Tributyltin produces imposex in *Nucella lapillus* (Linnaeus, 1758) and *Hinia reticulata* (Risso, 1826) at concentrations of 1 ng Sn/l water (Gibbs et al., 1987; Stroben et al., 1992) but in *Buccinum undatum* Linnaeus, 1758 only at 7 ng/g of sediments (Mensink et al., 2002). In the studied area, BT content in the sediments changed from 240 $ng \times g^{-1}$ in 2001 (Goldberg et al., 2004) to 1.4 $ng \times g^{-1}$ at the end of 2002 in Mar del Plata and from 0 $ng \times g^{-1}$ in 2001 (Goldberg et al., 2004) to 0.2 $ng \times g^{-1}$ in 2002 in Mar Chiquita, suggesting a current transport of the pollutant northwards. The small amounts of organic carbon in sediments made the adsorption of OTs difficult. It is clear that the pollution level was higher than the recorded level in the sediments, since the low organic content of the sediments makes OT adsorption difficult. These BT concentrations induced the development of small penises in females captured near the port, but did not further affect the reproduction since the functional reproductive organs were not modified and gonads of these animals were histologically similar to those of specimens captured in Mar Chiquita (Cledón et al., 2005), which did not present any signs of imposex.

The MBT contained in the digestive gland of the analysed gastropods increased during the last year of sampling reflecting the continuous incorporation and accumulation of the pollutant, which was over 30 times higher than the values for sediments. Such a high concentration in only few trophic levels was already reported in other organisms. Bioconcentration factors (BCFs), TBT tissue content×TBT sediments $content^{-1}$, are one way to compare this phenomenon among different organisms and can be as high as 5×10^4 (Takeuchi et al., 2001) depending on the BT content in the sediments and the species incorporation rate. Comparing all these facts with the reports on other molluscan species (Table 5) the studied population of *Adelomelon brasiliana* showed a low BCF.

The adult females of *B. undatum* are less sensitive to TBT than juveniles because they already have a developed genital system (Mensink et al., 2002). In the present study, no indication of such an effect was registered since affected and unaffected animals reached maturity at the same length and age (Cledón, 2006); and old individuals, already mature before pollution, also presented imposex. This observation does not mean a contradiction with Mensink et al. (2002), because the old imposexed females could have been exposed to OTs in previous occasions to this study.

It is still not clear how BTs are incorporated. Strand et al (2003) suggested that deposit feeders are ingesting the TBT rich fraction. *Adelomelon brasiliana* mainly feeds on *Amiantis purpurata* (Lamarck, 1835), a bivalve living in close relation with the sediments, so it is possible that *A. purpurata* individuals of the area incorporated and accumulated the pollutant. On the other hand Stäb et al. (1996) proposed that in molluscs, BTs are passed on primarily via water contact with the different epithelia. It seems possible that *A. brasiliana* incorporates BTs through both pathways and which epithelia that produces a stronger effect should be studied.

Mensink et al. (2002) suggested those egg capsules are a relatively clean environment for *B. undatum* embryos, since embryos developed normally in the capsule but died after hatching in heavily-contaminated water. The results of this study indicate that the liquid phase of the egg capsules of *A. brasiliana* depends on the TBT content of the surrounding water and sediments.

If TBT is incorporated in the egg capsule through the mother or during the development in the environment and how it occurs still needs to be investigated. Nevertheless the high TBT content of late embryos suggests that it is a dynamic process of incorporation from the environment, which occurs while egg capsules roll on the sediments. Otherwise TBT should decrease considerably in the liquid phase during embryo development and almost disappear before hatching.

Tributyltin-based antifoulants are still used on large vessels and are consequently found in ports. Due to the slow degradation of TBT in sediments, it is continuously accumulating. This may cause a problem if sediments are remobilized through events such as storms or dredging operations (de Mora et al., 1995). During 1998 an enlarge-

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ment of Mar del Plata beaches implied the mobilization of almost 2.5 million m^2 of sand (Marcomini & López, 1999) possibly causing the occurrence of imposex in the area. However, sand has a very low content of organic carbon and would hence capture low amounts of OT, facilitating the transport via currents. The present findings are opposite to many studies reporting virtually no imposex on open coasts (Smith & McVeagh, 1991; Evans et al., 1995). Bryan & Gibbs (1991) and Evans & Nicholson (2000) among others, proposed that open oceanic coasts are free of TBT pollution at biologically significant levels probably because of the dilution effect of the sea. Our findings agree with Evans & Nicholson (2000), who concluded that TBT pollution is localized in ports and disappears rapidly when distance from the port increases. Even after the 30 years in which it has been used as the major biocide in antifoulants, there are still healthy gastropod populations within a few kilometres from harbours all around the world (Smith & McVeagh, 1991).

Further studies are needed to control the evolution of this situation in Mar del Plata to clarify if intermittent pulses of OTs are currently released in the area. These kinds of studies should be done in all major ports in South America in order to set the basis for a control programme.

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