



## Acute toxicity in two edible marine gastropods with different sensitivity to Tributyltin

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### Abstract

*Buccinanops globulosus* and *Trophon geversianus* are high and low sensitive to TBT. LD50 was 5.41 and 6.21 µgTBT.g<sup>-1</sup>bw for *B. globulosus* and *T. geversianus* respectively, denoting high resistance to TBT acute exposure. These results can be used for future imposex induction experiments.

**Keywords:** mollusks, toxicity assays, TBT, environmental risk, imposex incidence, Patagonia.

**Resumen:** Toxicidad aguda del Tributilestano (TBT) en dos gasterópodos marinos comestibles. *Buccinanops globulosus* y *Trophon geversianus* son indicadores de alta y baja sensibilidad al TBT. La DL<sub>50</sub> fue de 5,41 y 6,21 µg TBT.g<sup>-1</sup>ph para *B. globulosus* y *T. geversianus* respectivamente, denotando elevada resistencia a la exposición aguda de TBT. Estos resultados pueden ser útiles en futuros experimentos de inducción.

**Palabras clave:** moluscos, ensayos de toxicidad, TBT, riesgo ambiental, imposex, Patagonia.

Biological effects of chemicals and measurements of toxicity in impacted environments can be assessed by bioassays (Lee *et al.* 2005). Tributyltin (TBT) is a highly toxic compound to organisms (Thain *et al.* 1987, de Mora & Pelletier 1997, Evans 1999, Meador 2000, among others) and the analysis of this contaminant has been focused on the chronic effects caused by long time of exposure to polluted environments (Moore *et al.* 1991, Foale 1993, Ruiz *et al.* 1994, Pessoa *et al.* 2001, Antizar-Ladislao 2008). In marine invertebrates, particularly mollusks, sublethal effects such as imposex development (Gibbs & Bryan 1986) or

shell thickening in gastropods and bivalves for commercial purpose (Ruiz *et al.* 1995, Horiguchi *et al.* 1998, Badow & Weltje 2012, among others) have been reported as consequence of TBT exposure.

The caenogastropod *Buccinanops globulosus* (Kiener, 1834) belonging to the family Nassariidae is an endemic species of the South Western Atlantic Ocean inhabiting sandy or muddy bottoms of shallow waters (Pastorino 1993). The genus has been affected by imposex phenomenon in all harbors and high maritime traffic areas from Argentina (Penchaszadeh *et al.* 2001, Bigatti *et al.* 2009,

Averbuj & Penchaszadeh 2010). On the other hand, the species *Trophon geversianus* (Pallas, 1774) belonging to Muricidae is widely distributed along the Atlantic and Pacific coasts of South America (Castellanos & Landoni 1993, Griffin & Pastorino 2005, Pastorino 2005), inhabiting hard bottoms. Muricids are the most used caenogastropods worldwide in monitoring studies due its high sensitivity to TBT (Gibbs & Bryan 1987, Axiak *et al.* 1995, Oehlmann *et al.* 1996). However, imposex development is very low in this species along the Argentinean coasts, even in harbor areas with TBT contamination (Bigatti *et al.* 2009). In contrast, *B. globulosus* has been classified as a good indicator of TBT contamination due to its high imposex levels even at low environmental concentrations (Bigatti *et al.* 2009). This edible species is also an expanding resource for artisanal fisheries in northern Patagonia, where it is locally consumed (Narvarte 2006, Averbuj *et al.* 2014) while *T. geversianus* is not consumed yet in the region (Bigatti *et al.* in press). However in Chile, *T. geversianus* is well consumed and commercialized (Leiva & Castilla 2002, González *et al.* 2007). Tributyltin concentrations up to 174.81 ngSn.g<sup>-1</sup>dw were recently measured in coastal sediments of Nuevo gulf, Patagonia (Bigatti *et al.* 2009, del Brío [unpublished data]). At the same time, Bigatti *et al.* (2009) and then Primost (unpublished data) found incidence of imposex and negative reproductive effects on the affected populations. Despite of the TBT worldwide prohibition since year 2008 (IMO 2002), the contaminant is even found in marine environments (Antizar-Ladislao 2008, Zhang *et al.* 2013) and the populations of marine gastropods and other species could be at risk of physiological failures or local extermination.

To determine acute toxicity by median lethal dose (LD<sub>50</sub> 96h) assay, we injected both *Buccinanops globulosus* and *Trophon geversianus* with different doses of TBT. These results will be used as reference doses in future imposex induction experiments. Seawater and marine gastropods were collected from Cerro Avanzado beach (42°49'37.66"S; 64°51'29.19"W), a zone with scarce maritime traffic and very low imposex incidence for *B. globulosus* and *T. geversianus* (Bigatti *et al.* 2009, Primost [unpublished data]). Tests were performed with mature specimens (sizes over 18 mm for males and 30 mm for females [unpublished data]). They were identified and classified by observing the ventral pedal gland in females crawling over glass aquaria. Glass aquaria (20x30x20 cm) with 18 liters

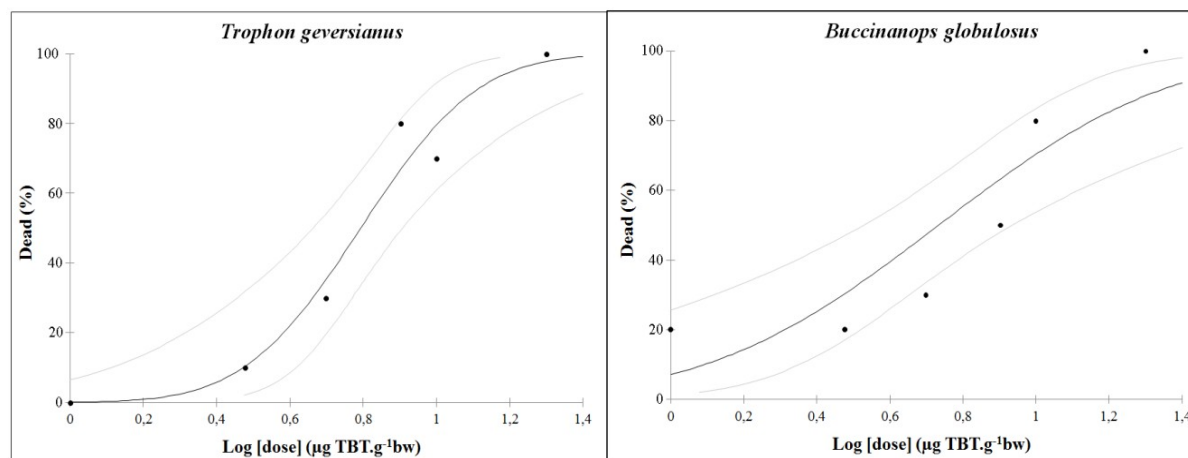
of filtered seawater, also collected from Cerro Avanzado beach, were used in the tests. Water temperature (Collin & Spangler 2012) and salinity (35 mg/L) were controlled during the experiments using an OAKTON 600 series waterproof portable meter kit. A photoperiod of 12 h light/dark was regulated with a timer. Internal circulation inside each aquarium and constant aeration were provided to ensure organisms survival in laboratory conditions, but without recirculation among aquaria. Gastropods were acclimated for one week in each aquarium and starved before and during the experiment. Total body weight (0.1 g precision) of each gastropod was determined before injection in the laboratory. Exposure to TBT was performed by injecting liquid doses in the foot with a Hamilton micro syringe of 10 µl. Doses to be injected (µg of TBTCI per gram of wet body weight) were selected following previously published reports (Oberdörster & McClellan-Green 2000, Oberdörster & McClellan-Green 2002, Santos *et al.* 2005, Horiguchi *et al.* 2007, Garaventa *et al.* 2008) as well as own preliminary assays (0.1 and 0.5 µgTBTCI.g<sup>-1</sup>bw) using the same species (unpublished data). They were prepared from a stock solution of TBT chloride (Sigma Aldrich®) in absolute ethanol (960 µg of TBTCI per µl<sup>-1</sup>). The relationship between total weight and wet body mass weight (bw) (Primost unpublished data) was used to calculate the injection volume of TBT per organism and are shown as supplementary data. For the test, eight groups of ten individuals each were conformed. Six treatments (1; 3; 5; 8; 10 and 20 µgTBTCI.g<sup>-1</sup>bw) and two controls (without injection and with injection of 7 µl of absolute ethanol) were performed for both gastropod species. Size and weight parameters for gastropods in each treatment are shown in Table 1.

Once injected, gastropods were daily monitored. Total number of dead gastropods was recorded 96 hours later in each treatment. Lethal median dose LD<sub>50</sub> 96h and confidence intervals (95%) were calculated by XLStat 2012®-XLSTAT-Dose software for each species. Probit method (Finney 1971) with 100 iterations and 0,000001 of convergence was used.

Gastropods mortality was not observed in the two settled controls during the experiment for each species. No mortality was registered in *Trophon geversianus* in the dose of 1 µg while 10 % mortality occurred for the 3 µg TBTCI.g<sup>-1</sup>bw dose. For doses higher than 5 µg TBTCI.g<sup>-1</sup>bw, the proportion of dead gastropod increased for both species. Mortality was increased to 100% in the higher dose (20 µg

TBT.g<sup>-1</sup>bw) for both *B. globulosus* and *T. geversianus* (Fig.1). Imposex development at the end

of the experiment (96 h) was analyzed and didn't find imposex induction in all treatments (Table 1).



**Figure 1.** Dose-response curves for *Trophon geversianus* and *Buccinanops globulosus*.

The lethal median dose calculated for 96h was 5.41 µg TBT.g<sup>-1</sup>bw with 3.37 and 8.60 µg TBT.g<sup>-1</sup>b as 95% confidence intervals in *B. globulosus*, and 6.21 µg TBT.g<sup>-1</sup>bw with 4.61 and 8.08 µg TBT.g<sup>-1</sup>bw as 95% confidence intervals in *T. geversianus*.

Reports of TBT lethal doses in marine gastropods and bivalves are scarce although acute effects using water dilutions of TBT have been reported (Morris 1987, Thain *et al.* 1987, Waldock *et al.* 1987, Bushong *et al.* 1988). The results obtained in our study with adult specimens indicated that TBT presented low acute toxicity for *Buccinanops globulosus* and *Trophon geversianus* compared with doses used to imposex induction (sublethal effects) in other species (Oberdörster & McClellan-Green 2000, Santos *et al.* 2005). Only for the highest dose (20 µgTBT.g<sup>-1</sup>bw), 100% of mortality was recorded. Despite *T. geversianus* showed a higher value of LD<sub>50</sub> than *B. globulosus*, the overlapped confidence intervals do not allow ensuring a difference that can explain the different sensitivity to imposex development between muricids and nassarid (Bigatti *et al.* 2009). However, at low dose of 1 µg TBT.g<sup>-1</sup>bw, also used by others authors in imposex induction experiments (Castro *et al.* 2007, Horiguchi *et al.* 2008), *B. globulosus* presented 20% of mortality while no *T. geversianus* died. Probably the differences in imposex incidence could be triggered by low concentrations of TBT in *B. globulosus*, and 1 µg TBT.g<sup>-1</sup>bw could be the best starting dose in future imposex induction experiments in *T. geversianus*. Studies of imposex induction in caenogastropods have been conducted by injecting TBT into the foot region (Oberdörster &

McClellan-Green 2002, Santos *et al.* 2005) and even, it has been proposed that the pedal ganglia contains a penis morphogenic factor, which can interact directly to induce imposex development (Oberdörster & McClellan-Green 2000, Oberdörster *et al.* 2005). In this study, TBT was injected into the foot region in the same way that induction studies in other species of gastropods (Spooner *et al.* 1991, Oehlmann *et al.* 2000, McClellan-Green *et al.* 2006) to avoid the effect of adsorption of TBT on the walls of the aquarium (Laughlin *et al.* 1996, Panagoula *et al.* 2002). We assume that the whole TBT dose entered the muscular tissues of the gastropod. As the present study only focused on the short term (acute) effects of TBT, chronic and sublethal doses should be investigated on both *B. globulosus* and *T. geversianus* to better understand the chronic impacts and imposex development mediated by TBT. Acute toxicity tests conducted under controlled laboratory conditions are effective for exploring the worst situation of exposure (Matthiessen 2013) but provides no information about what actually occurs in the environment. In particular, interactions such as synergism or antagonism with other contaminants, as trace elements and polyaromatic hydrocarbons reported in Nuevo gulf (Gil *et al.* 2006, Commendatore & Esteves 2007, Giarratano *et al.* 2013, Giarratano *et al.* 2014, Gil *et al.* 2015, Primost *unpublished data*) may increase or reduce the tested effect. In this sense, experiments of imposex induction by TBT will be necessary in order to certainly corroborate the minimum dose to induce imposex in the species *B. globulosus* and *T. geversianus*.

**Table 1.** Size, weight (mean  $\pm$  standard deviation) and imposex parameters in *B. globulosus* and *T. geversianus* in each treatment

Treatment	Total size (mm)	Total weight (g)	Body mass weight (g)	Imposex % after 96 h
<i>Buccinanops globulosus</i>				
Without injection	36.99 $\pm$ 2.00	9.23 $\pm$ 1.65	6.32 $\pm$ 0.72	0
7 $\mu$ l ethanol	41.92 $\pm$ 0.83	12.04 $\pm$ 0.74	5.64 $\pm$ 0.36	0
1 $\mu$ g TBT.g <sup>-1</sup>	38.72 $\pm$ 2.09	10.59 $\pm$ 1.53	4.93 $\pm$ 0.75	0
3 $\mu$ g TBT.g <sup>-1</sup>	40.39 $\pm$ 1.28	11.99 $\pm$ 0.98	4.05 $\pm$ 0.48	0
5 $\mu$ g TBT.g <sup>-1</sup>	42.05 $\pm$ 1.30	12.47 $\pm$ 0.96	4.11 $\pm$ 0.47	0
8 $\mu$ g TBT.g <sup>-1</sup>	39.14 $\pm$ 1.73	10.79 $\pm$ 1.15	5.02 $\pm$ 0.57	0
10 $\mu$ g TBT.g <sup>-1</sup>	39.91 $\pm$ 2.25	11.49 $\pm$ 1.65	7.13 $\pm$ 0.81	0
20 $\mu$ g TBT.g <sup>-1</sup>	39.27 $\pm$ 2.33	10.99 $\pm$ 1.60	7.35 $\pm$ 0.79	0
<i>Trophon geversianus</i>				
Without injection	27.37 $\pm$ 0.94	3.62 $\pm$ 1.12	1.18 $\pm$ 0.12	0
7 $\mu$ l ethanol	27.95 $\pm$ 0.90	3.85 $\pm$ 1.06	1.32 $\pm$ 0.12	0
1 $\mu$ g TBT.g <sup>-1</sup>	28.15 $\pm$ 1.59	3.93 $\pm$ 1.88	1.35 $\pm$ 0.21	0
3 $\mu$ g TBT.g <sup>-1</sup>	27.69 $\pm$ 1.44	3.75 $\pm$ 1.70	1.29 $\pm$ 0.19	0
5 $\mu$ g TBT.g <sup>-1</sup>	27.34 $\pm$ 1.25	3.60 $\pm$ 1.48	1.24 $\pm$ 0.17	0
8 $\mu$ g TBT.g <sup>-1</sup>	27.44 $\pm$ 1.80	3.64 $\pm$ 2.13	1.25 $\pm$ 0.24	0
10 $\mu$ g TBT.g <sup>-1</sup>	26.66 $\pm$ 0.94	3.34 $\pm$ 1.11	1.15 $\pm$ 0.13	0
20 $\mu$ g TBT.g <sup>-1</sup>	28.89 $\pm$ 1.92	4.22 $\pm$ 2.27	1.45 $\pm$ 0.26	0

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