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Rapid communication

Triphenyltin and tributyltin, single and in combination, promote imposex in the gastropod *Bolinus brandaris*

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Abstract

Specimens of *Bolinus brandaris* (neogastropod) were injected with a single dose of 500 ng/g body weight (b.w.) of tributyltin chloride (TBTCl) or triphenyltin chloride (TPTCl), or a mixture of both compounds (250 ng TBT/g b.w. + 250 ng TPT/g b.w.), for a period of up to 31 days. At the end of 4 weeks, significant increases in the female penis size of those gastropods injected with TBT ($P<0.05$), TPT ($P<0.05$), or the mixture TBT + TPT ($P<0.01$) were recorded. In parallel, a group of animals was injected with the neuropeptide APGWamide but this compound failed to promote imposex, suggesting that APGWamide is not involved in imposex promotion in *B. brandaris*. Acetylcholinesterase activity, a biomarker of neurotoxicity, was determined in the neuroganglia at the end of the experiment, but no significant differences among treatments were found. Overall, these results support the hypothesis that TPT also acts as an endocrine disrupter in this neogastropod species. Our observations also highlight, for the first time, synergistic effects of organotin mixtures having imposex promotion as an endpoint.

Keywords: Imposex; Triphenyltin; Tributyltin; Chemical mixture; Bioindicator; *Bolinus brandaris*; APGWamide; Acetylcholinesterase

1. Introduction

Organotins and tributyltin (TBT) in particular, have been extensively used since the late 1960s as biocides in antifouling paints for ship's hulls (Fent, 1996). In neo- and mesogastropods, TBT is responsible for the development of male sexual characteristics in females (a penis and vas deferens), a phenomenon termed imposex (Smith, 1971).

Many field studies have reported imposex in marine snails in association with the presence of TBT in the environment (Gibbs and Bryan, 1996; Santos et al., 2002a). Yet, few have demonstrated this induction under laboratory conditions and even fewer have tested the role of triphenyltin (TPT) having imposex as an endpoint. This is probably due to the lack of imposex induction/promotion by TPT in some highly TBT-sensitive snail species such as *Nucella lapillus* and *Hinia reticulata* (Bryan et al., 1988; Schulte-Oehlmann et al., 2000). For *H. reticulata*, although sediment-exposed females did not develop imposex (Schulte-Oehlmann et al., 2000), water exposure to relatively high concentrations of TPT (100–500 ngSn/L) was able to induce imposex in this species (Barroso et al., 2002). In addition to imposex, TPT has been shown to cause deleterious effects in several mollusk species (Schulte-Oehlmann et al., 2000; Horiguchi et al., 2002; Duft et al., 2003); in *N. lapillus* and *H. reticulata*, TPT leads to several

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adverse effects, such as a reduction of the pallial sex organs of up to 25% (*N. lapillus*) and impairment of spermatogenesis and oogenesis (*H. reticulata*). In addition to the former two species described, TPT induces imposex in *Thais clavigera* (Horiguchi et al., 1997), *Marisa cornuarietis* (Schulte-Oehlmann et al., 2000), and *Charonia lampas* (our own unpublished results). This is highly significant as the number of TPT-sensitive species can probably be extended as the number of studies increase.

Due to all the field and laboratory evidence of negative ecological consequences of organotin exposures, the use of these compounds as biocides in antifouling paints was initially forbidden, within the European Union (EU Directive (89/677/CEE)), for ships smaller than 25 m. Recently, the International Maritime Organization has adopted a new convention that bans the application of these compounds to all sizes of ships from the 1st of January 2003 and its presence on ship's hulls by 1 January 2008 (Anonymous, 2001). Whereas this measure is expected to lead to a substantial decrease in the levels of TBT in the marine environment, it is likely to have little effect on the loads of TPT, since TPT is mainly used as a fungicide in agriculture to fight several fungal diseases (Stäb et al., 1994).

From a regulatory/mandatory point of view it is of great interest to evaluate the effectiveness of the implementation of the new regulations. Imposex in highly sensitive marine snails is a well-accepted biomarker of TBT pollution and therefore will most likely be used to monitor trends of TBT in coastal areas and the open sea. Thus, it will be important to identify snail species that can be used as bioindicators of TBT contamination in both environments, specially in areas where *Buccinum undatum*, the species put forward by OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic) to monitor TBT contamination in open sea, is absent.

The commercial gastropod *Bolinus brandaris* has been used in several field studies as a bioindicator of TBT contamination, showing that imposex in this species is a widespread phenomena also in offshore Mediterranean waters (Solé et al., 1998; Ramón and Amor, 2001; Chiavarini et al., 2003). Thus, *B. brandaris* has great potential as a bioindicator of TBT contamination even in the open sea. Nevertheless, imposex induction by TBT has not yet been demonstrated under laboratory conditions. In one of these former field studies with *B. brandaris* a better correlation of imposex with TPT rather than with TBT body burden was observed (Solé et al., 1998). Moreover, although TBT and TPT are found together in the environment (Fent, 1996), their potential synergistic effects have never been reported before.

In a recent study, Oberdorster and McClellan-Green (2000) demonstrated the role of the neuropeptide APGWamide in inducing imposex in *Ilyanassa obsoleta*. They hypothesize that imposex may be initially induced by neuropeptides which would then modulate the biosynthesis of steroid hormones. This theory does not contradict previous findings and in fact integrates well with the fact that both TBT and TPT show a strong affinity to mollusk ganglia (Bryan et al., 1993; Mensink et al., 1997; Horiguchi et al., 2002). Thus, we included the neuropeptide APGWamide in our study to test the imposex promotion of APGWamide in an organotin-sensitive species and to determine whether this hypothesis could also apply to *B. brandaris*.

Since organotins may accumulate at high levels in mollusk ganglia, it is relevant to evaluate whether gastropod neurotransmission is affected by these compounds. As acetylcholinesterase (AChE) is known to be involved in neurotransmission in invertebrates (Fulton and Key, 2001), we will report the effects of organotins in this enzymatic activity.

The aims of the study were: (a) to test imposex promotion of TBT and TPT alone and in combination in the subtidal gastropod *B. brandaris*, (b) to test the role of the neuropeptide APGWamide in imposex promotion, and (c) to observe any effects of the tested compounds (organotins and neuropeptide) in females snail acethylcholinesterase activity.

2. Material and methods

2.1. Experimental design and dosing

Specimens of *B. brandaris* were fished in February 2003 by local fisherman off the Coast of Cadiz (SW Spain) in an area relatively clean from TBT pollution (Santos, 2002). Animals were transferred to the laboratory and divided into six groups: control organotins ($n = 27$), TBT ($n = 30$), TPT ($n = 29$), mixture TBT + TPT ($n = 28$), control APGWamide ($n = 26$), and APGWamide ($n = 28$), with a male:female ratio of 1:1. As this snail species crawls out of its shell when exposed to the air, the initial assessment of imposex and sex determination was done without the need to sacrifice them. Females were identified either by absence or by presence of a small penis and/or vas deferens. All animals were left to adapt for 7 days in separate tanks before being injected. After that period, the penis size of individuals from both sexes was recorded and the presence of a vas deferens annotated. Even in relatively clean areas, it is difficult to find *B. brandaris* populations without imposex (Solé et al., 1998; Ramón and Amor, 2001; Chiavarini et al., 2003); this was the reason that most of the females used in our experiment already had a tiny penis (86%). Once the initial data were recorded, animals were weighed, sized, and given a single injection dose of 500 ng/g body weight (b.w.) of TBTCl (96%