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Toxicity of tributyltin (TBT) to the freshwater planarian *Schmidtea mediterranea*

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HIGHLIGHTS

- TBT is still a common pollutant in freshwaters and ecotoxicological data is needed.
- The planarian *Schmidtea mediterranea* showed sensitivity to low TBT concentrations.
- TBT exposure impaired locomotion and feeding activity of *S. mediterranea*.
- Delayed regeneration and genotoxicity were observed in planarians exposed to TBT.
- Freshwater planarians are suitable for a wide range of ecotoxicological tests.

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ABSTRACT

The freshwater planarian *Schmidtea mediterranea*, one of the best characterized animal models for regeneration research and developmental biology, is being recognised as a useful species for ecotoxicological studies. Sensitive endpoints related to planarians' behaviour and regeneration can be easily evaluated after exposure to environmental stressors. In this work the sensitivity of *S. mediterranea* to a gradient of environmentally relevant concentrations of TBT was studied using multiple endpoints like survival, locomotion, head regeneration and DNA damage. In addition, a feeding assay based on planarian's predatory behaviour was performed. Results indicated that TBT is toxic to planarians with $LC_{50's}$ of 1.87 μg L^{-1} Sn and 1.31 μg L^{-1} Sn at 48 h and 96 h of exposure respectively. Sub-lethal exposures to TBT significantly reduced locomotion and feeding, delayed head regeneration and caused DNA damage in planarians. The behavioural endpoints (feeding and locomotion) and head regeneration were the most sensitive parameters followed by DNA damage. Similar to other aquatic model organisms, *S. mediterranea* showed high sensitivity towards TBT exposure. Based on our results, and though further research is required concerning their sensitivity to other pollutants, the use of freshwater planarians as a model species in ecotoxicology is discussed.

1. Introduction

Tributyltin (TBT) is an organometallic compound, mainly used as biocide in antifouling paints applied to boat and ship hulls and submerged static structures to discourage attachment and growth of organisms (Turner, 2010). Also, TBT is used as molluscicide to control snail vectors of schistosomiasis, for wood preservation, slime control in paper mills and industrial disinfectant (Snoeij et al., 1987). Many countries have regulated the use of TBT (Abbott et al., 2000; IMO, 2001), but given its persistence in sediments (Dowson et al., 1996) and ability to diffuse into water column (Unger et al., 1988), sediments can act as long-term sources. Moreover, ineffectiveness of alternative products led to illegal use of TBT and is still permitted in International Maritime Organization (IMO) non-member countries (Barroso and Moreira, 2002; Okoro et al., 2011). Thus, environmental concentrations of TBT remain high enough to motivate concern.

The majority of studies on toxicity of TBT have focused on marine species and imposex in marine and freshwater molluscs

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leading to reproductive failure and population decline has been shown as one of the most deleterious effects of TBT exposure (Oehlmann et al., 1996; Barroso et al., 2000). However, TBT concentrations in freshwaters and freshwater sediments have been estimated to reach 7.1 μg L^{-1} and 3700 μg kg^{-1}, respectively (IPCS, 1990), indicating its potential impact on freshwater biota and the need for more ecotoxicological studies.

In non-target organisms TBT toxicity has been studied using reproduction, mortality and growth as endpoints. Few reports deal with behavioural and regeneration endpoints. Behavioural responses are usually sensitive and suitable parameters to evaluate effects of low levels of contaminants (Pestana et al., 2007; Alonso and Camargo, 2011; Rodrigues et al., 2015). Similarly, regeneration occurs in many metazoans, and involves re-creation of lost parts (Sánchez-Alvarado, 2000). Since many contaminants are known to have cytotoxic and teratogenic effects (Mizuhashi et al., 2000; Hagger et al., 2002; Velma et al., 2009), they may alter the process of regeneration. Therefore, regeneration can also be useful in studying effects of toxicants on differentiation and growth (Weis and Weis, 1987).

Freshwater planarians are benthic invertebrates that occupy an important position in the food chain as abundant predators (Thorp and Covich, 2001). Planarians undergo blastemal regeneration, a process that could be used to understand regeneration in other studied model systems and metazoans, given the important position occupied by planarians in Metazoan evolution (Sánchez-Alvarado and Newmark, 1998).

Freshwater planarians have been suggested as useful indicators for water quality and pollution (Kent, 1974) and are sensitive to low concentrations of environmental toxins (Nano et al., 2002; Rodrigues et al., 2015). Although freshwater planarians are not model organisms in ecotoxicology, they have been used in various studies and simple protocols exist to measure locomotor behaviour and regeneration (Pagan et al., 2009; Knakievicz, 2014). Additionally, feeding bioassays based on planarians' predatory behaviour may be useful in understanding the sub-lethal effects of contaminants (Rodrigues et al., 2015).

The aim of this study was to determine the effects of TBT on locomotion, regeneration and DNA (comet assay) using the freshwater planarian *Schmidtea mediterranea* as model organism. In addition, we tried to devise a convenient quantitative endpoint of planarian feeding bioassay that can be used to complement the ecotoxicological evaluation of a wide range of environmental stressors with this planarian species.

2. Materials and methods

2.1. Tributyltin (TBT)

Tributyltin chloride (TBTCl; 97%) from Fluka, Switzerland was used to prepare a stock solution of 0.1 M dissolved in absolute ethanol. Experimental treatments and dilutions from this stock solution were prepared using ASTM hardwater (ASTM, 2004) keeping concentration of ethanol below 0.01%. Experimental solutions and water samples were kept from light at 4 °C to avoid degradation.

2.2. Organotin chemical analysis

The stock solution (100 μg L^{-1} TBTCl), and experimental treatments were analysed at the end of the exposure (after 48 h) to evaluate degradation of TBT. 10 mL of sample from each concentration was acidified with 5 mL of acetic acid (ultrapure grade) and then subjected to extraction using a microwave system (CEM Explorer, 3 min, 40 W) with tripropyltin as standard and a procedure blank. Extracts were kept at 4 °C before further analysis. Before derivatisation, extracts from each sample were pooled and appropriate volume of a standard solution containing TBT, DBT and MBT (1000 mg L^{-1} in methanol; LGC standards) added to help determine butyltin species. 5 mL of 1 M acetic acid/sodium acetate (ultrapure grade) was added to 1 mL of supernatant (extract + standard solution) and the pH was adjusted to 4.5 with ammonium hydroxide (ultrapure grade). To this solution was added 1 mL of isooctane (ultrapure grade) and 1 mL of freshly prepared 1% aqueous sodium tetraethyl borate (99.8%). The mixture was shaken at 300 rpm for 20 min for phase separation, the organic layer was transferred into amber Gas Chromatography (GC) auto-sampler vials. These were stored at −20 °C until analysis with gas chromatograph-inductively coupled mass spectrometry (GC-ICPMS).

2.3. Estimation of TBT degradation efficiency

TBT degradation efficiency was obtained using the butyltin degradation index (BDI) according to Díez et al. (2002) and butyltin degradation index percentage (BT_{deg}) according to Díez and Bayona (2009) with slight modification. BDI is the ratio of TBT main degradation products [monobutyltin (MBT), dibutyltin (DBT)] and TBT. $BDI = \frac{MBT + DBT}{TBT}$

Similarly,

$$BT_{deg} = \left(1 - \left[\frac{TBT}{TBT + DBT + MBT}\right]\right) \times 100$$

where MBT, DBT and TBT refer to the concentrations of the butyltins expressed as tin (Sn). BDI value less than 1 and BT_{deg} less than 50% signify poor or moderate TBT degradation while BDI greater than 1 and BT_{deg} higher than 50% imply efficient of high TBT degradation (Diez et al., 2002; Diez and Bayona, 2009).

2.4. Test organisms

S. mediterranea (asexual strain) cultures were maintained in plastic containers with ASTM hardwater (ASTM, 2004), under constant darkness and temperature of 20 ± 1 °C. Planarians were fed chicken liver once a week, with medium renewal after feeding and every 2 days.

Planarians used in experiments ranged from 0.5 cm to 1.0 cm in length. Before experiments, animals were starved for 1 week to prevent contamination due to food digestion (Wu and Persinger, 2011) and ensure uniformity in response to toxicant (Oviedo et al., 2008a). All exposures were performed in darkness at 20 ± 1 °C.

2.5. Acute toxicity

Based on range finding experiments, 7 TBT concentrations plus control (CTR) and solvent control treatments (SCTR, absolute ethanol) were chosen for the acute test. 10 replicates (1 planarian per 40 mL glass crystalizing dish) per concentration were used containing 20 mL of medium. Solutions were renewed after 48 h. Mortality was checked every 24 h up to 96 h and number of dead organisms recorded. Animals with degenerating body or without detectable movement under strong light were considered dead.

2.6. Behavioural parameters

Planarians were exposed to sub-lethal TBT nominal concentrations of 0.25, 1.0 and 4.0 μg L^{-1}, control and solvent control treatments for 48 h. Exposure was carried out in 40 mL glass vials with 20 mL of medium. 10 replicates (1 planarian per vial) per