Ecotoxicology and Environmental Safety 139 (2017) 308–315

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Polybrominated diphenyl ethers (PBDEs) effects on *Chironomus sancticaroli* larvae after short-term exposure

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ARTICLE INFO

Keywords:
Midge
Acetyl cholinesterase
DNA

ABSTRACT

In-*vivo* effects of polybrominated diphenyl ethers (PBDEs) containing 3, 4 and 5 bromine atoms were tested on fourth-instar larvae of *Chironomus sancticaroli* (Diptera: Chironomidae) after 48 h of exposure, by measuring the activity of the acetyl cholinesterase, alpha and beta esterases and glutathione S-transferase. The PBDE congeners 2,2′,4-triBDE (BDE-17), 2,2′,4,4′-tetraBDE (BDE-47) and 2,2′,4,4′,5-pentaBDE (BDE-99) were evaluated at 0.5, 1.0, 2.0 and 3.0 ng mL^{-1}. Acetyl cholinesterase activity decreased significantly ($p \leq 0.05$) at all evaluated concentrations of the three PBDE congeners, except for larvae exposed to BDE-17 at 1.0 and 2.0 ng mL^{-1}. The significant inhibition of acetyl cholinesterase activity ranged from 18% (BDE-47 at 0.5 ng mL^{-1}) to 72% (BDE-47 at 2.0 ng mL^{-1}). The enzymes alpha and beta esterase were also affected by the three congeners, reducing their activity from 14% (BDE-99 at 1.0 ng mL^{-1}) to 52% (BDE-47 at 2.0 ng mL^{-1}) and from 7% (BDE-99 at 2.0 ng mL^{-1}) to 34% (BDE-47 at 3.0 ng mL^{-1}) respectively. Substantial increments in glutathione S-transferase activity were similarly observed, varying from 138% (BDE-99 2.0 at ng mL^{-1}) to 346% (BDE-17 at 1.0 ng mL^{-1}). DNA strand breaks were detected exclusively in larvae exposed to BDE-99 at 2.0 and 3.0 ng mL^{-1} ($H = 11.7$, $p = 0.019$). These results showed that *C. sancticaroli* larvae were sensitive to the PBDEs treatments under the experimental conditions.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that are added in household and commercial polymer-based products, including textiles, electronic devices and furniture, to increase the fire resistance of these products (Alaee et al., 2003; WHO, 1997). There are 209 congeners, which are commercialized according to their degree of bromination as penta-BDE, octa-BDE and deca-BDE (de Wit, 2002). These compounds are released into the environment during the manufacturing process of the aforementioned products, or leach from them into wastewaters as well as the atmosphere. In addition, they undergo global transport, are persistent and can bioaccumulate in aquatic and terrestrial organisms (de Wit et al., 2010; Hale et al., 2003, 2002; Law et al., 2006; North, 2004). The concerns about the effects of some congeners on humans and the environment are related to their effects on the reproductive, developmental systems, neurotoxic, carcinogenic and endocrine disruption, and their impact on the immune system of organisms (Dufault et al., 2005; Fernie et al., 2005; Man et al., 2011; Metcalfe et al., 2013; Ward et al., 2014).

Even though some of the formulations of PBDEs were withdrawn from the market and banned by the Stockholm Convention, they are still present in environmental compartments (European Union, 2004; Great Lakes Chemical Corporation 2003; Stockholm Convention, 2009). PBDEs can even be detected at concentrations as low as picograms per litre the water column, sediments and soils (Lohmann et al., 2013; Richman et al., 2013; Shaw and Kannan, 2009). The most abundant congeners identified in environmental samples are 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and the 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), which are components of the penta-BDE commercial mixture (Dornbos et al., 2015; La Guardia et al., 2006). However, 2,2′,4-Tribromodiphenyl ether (BDE-17) has also been reported in environment compartments (Olukunle et al., 2015; Wong et al., 2016). Previous studies on Brazil displayed the presence of BDE-47 and BDE-99 in mussels, fish and the adipose breast tissue of humans;

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http://dx.doi.org/10.1016/j.ecoenv.2017.01.052
Received 27 September 2016; Received in revised form 20 December 2016; Accepted 31 January 2017
Available online 07 February 2017
0147-6513/

the BDE-17 congener was also reported in those samples (da Silva et al., 2013; Kalantzi et al., 2009).

Considering the aquatic systems as destination of diverse environmental pollutants, habitat organisms can be affected. The non-biting midge *Chironomus sancticaroli* Strixino & Strixino, 1981 is a freshwater benthic insect, endemic from Latin America whose larvae are an important recycler of organic matter (Armitage et al., 1995; Trivinho-Strixino, 2011) and are an essential food source for predaceous organisms. Chironomid food chain position, short lifecycles, their easy identification and maintenance in the laboratory, and their physiological tolerance to different environmental conditions during the larval stages are features that contribute to their use as models to monitor the risk assessment of freshwater and sediment toxicity (Callisto et al., 2002; EPA, 1996; Lee and Choi, 2009; OECD/OCDE, 2010; Osmulski and Leyko, 1986; Qi et al., 2015a).

The potentially harmful effects caused by environmental contaminants can be measured by biochemical and molecular markers that give early signals of the presence of those compounds in an ecosystem (McCarthy and Shugart, 1990; Walker, 2014). Cholinesterases and non-specific esterases can change their activity when the organism is exposed to environmental contaminants. For instance, the AChE is inhibited by a wide range of compounds such as phosphates, carbamates, metal species, and surfactants, among other mixtures (de Lima et al., 2013; Domingues et al., 2010; Guilhermino et al., 2000). Its inhibition produces neurotoxicity in the organisms due to the accumulation of acetylcholine in the synapse area, leading to the over stimulation of the nervous system, which leads to the mortality (Walker et al., 2001). Chronic and acute tests on several *Chironomus* species have documented the AChE inhibition after exposure to organophosphorated and organochlorinated compounds (Printes et al., 2011; Rebechi et al., 2014), pyrethroids and polycyclic aromatic hydrocarbons (Ibrahim et al., 1998; Qi et al., 2015a,b).

The non-specific esterases, α-esterase (EST-α) and β-esterase (EST-β) are associated with the detoxification process of organisms and are also involved in the metabolic resistance process of insects (Hemingway and Ranson, 2000). EST-α and EST-β inhibition have been observed in larvae of *C. sancticaroli* and *C. tentans* Fabricius, 1805, after exposure to organophosphorated compounds (Rakotondravelo et al., 2006; Rebechi et al., 2014). Likewise, the antioxidant defence enzyme glutathione-S-transferase (GST) helps to protect the organism from contaminants and endogenous compounds throughout their conjugation with the tripeptide glutathione (GSH), which makes them more hydrophilic, promoting their excretion from the organism. Moreover, the GST forms covalent bonds with the electrophilic compounds produced by phase I enzymes, protecting the DNA and other cellular macromolecules of the activated species (Boelsterli, 2007; Enayati et al., 2005). It has also been suggested that the GST is involved in the formation of BDE-glutathione metabolites in rodents (Hakk and Letcher, 2003) and avian species (Fernie et al., 2005), being also responsible for the metabolism of PBDEs via debromination in fish species (Noyes et al., 2010; Roberts et al., 2011). This enzyme has been used to identify physical and chemical stress in *Chironomus riparius* (Choi et al., 2000; Nair and Choi, 2011; Nair et al., 2013) and *Chironomus tepperi* Skuse, 1889 (Jeppe et al., 2014).

The DNA damage, in terms of strand breaks, caused by contaminants can be evaluated using the comet assay, that detects genotoxicity in cells even after a few hours of exposure to toxicants at low concentrations (Frenzilli et al., 2009; Labieniec et al., 2007). Some studies have documented that the DNA of Chironomidae larvae is vulnerable to the effect of xenobiotics like metal species, bisphenol A, nonylphenol, pentachlorophenol, phenanthrene, tributyltin and triclosan, suggesting the use of *Chironomus* larvae as a model in genotoxic studies (Al-Shami et al., 2013; Martínez-Paz et al., 2013; Morais et al., 2014; Park and Choi, 2009).

Despite the frequent detection of the PBDEs and their persistence in aquatic environments, there are no data on the biochemical and genotoxic effects of PBDE congeners on aquatic insects endemic to South America. Even though some Chironomidae species have been used as a model to assess the bioaccumulation of PBDEs (Bartrons et al., 2012, 2007), there are no data on the biochemical or molecular responses of this organism to brominated flame retardants.

Taking all of the aforementioned aspects into account, the aim of the present study was to investigate the effects of BDE-17, BDE-47 and BDE-99 on the activity of the enzymes acetyl cholinesterase, alpha and beta esterase and glutathione S-transferase. In addition, DNA damage was also assessed using *Chironomus sancticaroli* larvae as a model organism under *in vivo* exposure conditions.

2. Materials and methods

2.1. Exposure of chironomids

Chironomus sancticaroli larvae were obtained from the colony of the Laboratory of Medical Entomology and Ecotoxicology maintained at the Federal University of Paraná. The colony was kept in aerated aquaria following the protocol of Maier et al. (1990), under 25 ± 2 °C, 80% relative humidity and photophase: scotophase (12:12). Larvae were fed three times a week with Dog Chow®. Voucher specimens of this colony are in the Entomology Museum Padre Jesus Santiago Moure of the Zoology Department at the Federal University of Parana (DZUP) with accession numbers from 249269 to 249276.

Freshly laid egg masses from the colony were transferred to trays containing reconstituted water with 1.2 mg L^{-1} hydrated $CaSO_4$, 0.08 mg L^{-1} KCl, 2.44 mg L^{-1} $MgSO_4 \cdot 7H_2O$, and 1.92 mg L^{-1} Na_2CO_3, conductivity of 160 μS cm^{-1}, pH 7.2 and hardness 16 mg L^{-1}. Larvae were fed TetraMin® fish three times per week and constant aeration was maintained until they reached the fourth-instar, when they were exposed to PDBE.

Bioassays were carried out in glass vessels containing ten larvae, 50 mL of reconstituted water and 13 g of sand 50–70 mesh Sigma®. The effects of individual PBDE congeners were tested at 0.5, 1.0, 2.0 and 3.0 ng mL^{-1} after 48 h of exposure. The control groups were exposed to acetone and water. Temperature, conductivity, pH and dissolved oxygen concentrations were determined at 0 h and 48 h of the exposure. Bioassays were conducted in a BOD chamber under 25 ± 2 °C, 80% relative humidity and photophase: scotophase (12:12).

2.2. Experimental solutions

Analytical grade standards of BDE-17 (CAS No. 147217-75), BDE-47 (CAS No 5436-43-1) and BDE-99 (CAS No 60348-60-9) in isooctane (50 μ mL^{-1}) were purchased from Accustandard®. Stock solutions at 1000 μg L^{-1} for dosing were prepared in acetone and stored in amber glass vials at −20 °C until the bioassays started.

2.3. Enzyme activities

The biochemical effects of PBDE were studied in 13 pools, each composed of five larvae. This totalled 65 individuals per each of the seven replicates of each concentration. A total of 910 larvae were then necessary to carry out this portion of the study. Exposed larvae were kept in eppendorf tubes and stored at −80 °C before enzyme activity measurements. Each pool was homogenized in 620 μL of Milli-Q type water and centrifuged at 12.000*g* for 1 min at 4 °C. 96 well microplates (Greiner bio-one) and a BioTek® microplate reader (BioTek Instruments, Inc.) were used to carry out the analyses.

Total protein concentration was determined according Bradford's method (1976), using bovine serum albumin as the standard. A volume of 10 μL of supernatant and 250 μL of Bradford reagent (Sigma®) was placed in a microplate and the absorbance measured at 595 nm. All enzyme activities were run in triplicate wells.

Cholinesterase (ChE) activity tests were performed in microplates