Aquatic Toxicology 168 (2015) 38–47

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Developmental toxicity of the PBDE metabolite 6-OH-BDE-47 in zebrafish and the potential role of thyroid receptor β

Laura J. Macaulay, Albert Chen, Kylie D. Rock, Laura V. Dishaw, Wu Dong, David E. Hinton, Heather M. Stapleton*

Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

ARTICLE INFO

Article history:
Received 4 May 2015
Received in revised form
14 September 2015
Accepted 15 September 2015
Available online 24 September 2015

Keywords:
PBDE
OH-BDE
Zebrafish
Metabolite
Development
Thyroid receptor

ABSTRACT

6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47) is both a polybrominated diphenyl ether (PBDE) flame retardant metabolite and a marine natural product. It has been identified both as a neurotoxicant in cell-based studies and as a developmental toxicant in zebrafish. However, hydroxylated PBDE metabolites are also considered thyroid hormone disruptors due to their structural similarity to endogenous thyroid hormones. The purpose of this study was to evaluate the effects of 6-OH-BDE-47 on a developmental pathway regulated by thyroid hormones in zebrafish. Morphological measurements of development (head trunk angle, otic vesicle length, and eye pigmentation) were recorded in embryos at 30 h post fertilization (hpf) and detailed craniofacial morphology was examined in 4 day old larvae using cartilage staining. Exposure to 6-OH-BDE-47 resulted in severe developmental delays. A 100 nM concentration resulted in a 26% decrease in head trunk angle, a 54% increase in otic vesicle length, and a 42% decrease in eye pigmentation. Similarly, altered developmental morphology was observed following thyroid receptor β morpholino knockdown, exposure to the thyroid hormone triiodothyronine (T_3) or to thyroid disrupting chemicals (TDC; iopanoic acid and propylthiouracil). The threshold for lower jaw deformities and craniofacial cartilage malformations was at doses greater than 50 nM. Of interest, these developmental delays and effects were rescued by microinjection of TRβ mRNA during the 1–2 cell stage. These data indicate that OH-BDEs can adversely affect early life development of zebrafish and suggest they may be impacting thyroid hormone regulation *in vivo* through downregulation of the thyroid hormone receptor.

1. Introduction

Hydroxylated polybrominated diphenyl ethers (OH-BDEs) may be produced from either natural (*e.g.*, marine algae) or anthropogenic sources (Nomiyama et al., 2011; Wan et al., 2009). In mammals, OH-BDEs are formed by oxidative metabolism of polybrominated diphenyl ether (PBDE) flame retardants by cytochrome p450s, particularly CYP2B6 (Erratico et al., 2011; Feo et al., 2013). Both PBDEs and OH-BDEs are persistent and bioaccumulative chemicals which are widely detected in environmental media and human tissues (Chen et al., 2013; Kelly et al., 2008; Sun et al., 2013).

PBDEs affect estrogen, androgen, and thyroid hormone regulation *in vitro* (Kojima et al., 2009; Meerts et al., 2001; Ren et al., 2013), and *in vivo*. For example, rodents showed reduced circulating thyroid hormone levels, as well as altered reproductive and metabolic functioning following exposure to specific BDE congeners or the commercial mixtures (Stoker et al., 2004; Szabo et al., 2009; Zhou et al., 2002). Some investigators have hypothesized that endocrine effects of PBDEs observed in vivo result from exposure to the OH-metabolites, rather than the parent compounds (Dingemans et al., 2008, 2011). OH-BDEs share a strong structural resemblance to endogenous thyroid hormones and in vitro studies show disruption of thyroid hormone signaling by competitive binding to serum thyroid transporter proteins and nuclear receptors (Hamers et al., 2008; Meerts et al., 2000; Ren et al., 2013). In addition, OH-BDEs inhibit the activity of thyroid sulfotransferase and deiodinase enzymes, which are critical for maintaining thyroid hormone levels in peripheral tissues (Butt and Stapleton, 2013; Butt et al., 2011).

Abbreviations: HPC, halogenated phenolic compound; dpf, days post fertilization; CNC, cranial neural crest; DI, deiodinase enzyme; DMSO, dimethyl sulfoxide; FR, flame retardant; hpf, hours post fertilization; IOP, iopanoic acid; MO, morpholino; OH-BDE, hydroxylated polybrominated diphenyl ether; PBDE, polybrominated diphenyl ether; TH, thyroid hormone; TDC, thyroid disrupting chemical; PTU, propylthiouracil; T_4, thyroxine; T_3, triiodothyronine; TR, thyroid receptor.

* Corresponding author at: Nicholas School of the Environment, Duke University, Box 90328 LSRC A220, Durham, NC 27708, USA. Fax: +1 919 684 8741.
E-mail address: heather.stapleton@duke.edu (H.M. Stapleton).

http://dx.doi.org/10.1016/j.aquatox.2015.09.007
0166-445X/

However, epidemiological studies in humans have observed conflicting associations between PBDE and thyroid hormone levels in serum (Abdelouahab et al., 2013; Chevrier et al., 2011; Stapleton et al., 2011; Zota et al., 2011). Sources of such differences may be related to the specific population characteristics (*i.e.*, age and pregnancy), methods used to measure thyroid hormone levels, or differences in metabolism. Alternatively, PBDE metabolites may be responsible for driving some of the observed associations; but metabolites are infrequently measured in epidemiological studies.

The PBDE metabolite, 6-OH-BDE-47, is both a naturally produced chemical and a result of *in vivo* metabolism of PBDEs. 6-OH-BDE-47 disrupts thyroid homeostasis and causes developmental toxicity in zebrafish (Liu et al., 2015; Usenko et al., 2012; Van Boxtel et al., 2008). When the relative acute toxicity of various BDE-47 isomers was assessed in zebrafish, 6-OH-BDE-47 was the most potent isomer tested (Usenko et al., 2012). In our study, we also evaluated overt toxicity of eleven halogenated phenolic compounds (HPC), including chlorinated and brominated phenols, and also found 6-OH-BDE-47 to be the most acutely toxic compound in zebrafish embryos (see Supporting information Table S1 and Fig. S1).

Because 6-OH-BDE-47 has been detected in maternal serum and umbilical cord blood, concern for human developmental exposures has followed (Chen et al., 2013; Stapleton et al., 2011; Zhao et al., 2013; Zota et al., 2011). Fetuses and infants undergoing rapid development may be more sensitive to chemical exposures. Furthermore, the maintenance of thyroid homeostasis during pregnancy and early neurodevelopmental periods is of critical importance (Howdeshell, 2002), underscoring the need for assessing developmental impacts of OH-BDEs.

Based on previous work from our laboratory providing evidence of altered deiodinase and thyroid receptor expression after exposure to 6-OH-BDE-47 (Dong et al., 2014, 2013), we sought to further study these pathways by determining their role in developmental morphology, including larval cartilage formation. The objectives of the present study were to examine how early-life exposure to 6-OH-BDE-47 affects developmental morphology relative to native thyroid hormones and thyroid disrupting chemicals in embryo-larval zebrafish. Secondly, we sought to determine whether co-exposure with thyroid hormones or overexpression of the thyroid receptor would recover the observed developmental delays and adverse effects from 6-OH-BDE-47 exposures.

2. Materials & methods

2.1. Fish husbandry

Adult wild-type (Tropical 5D) zebrafish were used in this study. Fish were provided by Dr. David Volz, University of South Carolina, Columbia, SC, USA. Adult fish were housed at 28 ± 0.5 °C on a 14:10 light/dark photoperiod in a recirculating AHAB system (Aquatic Habitats) and fed brine shrimp and Ziegler's Adult Zebrafish Complete Diet (Aquatic Ecosystems, Apopka, FL). Embryos were collected from breeder tanks at 2 h post-fertilization (hpf) and maintained in embryo medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$) within incubators (at 28 °C) under identical

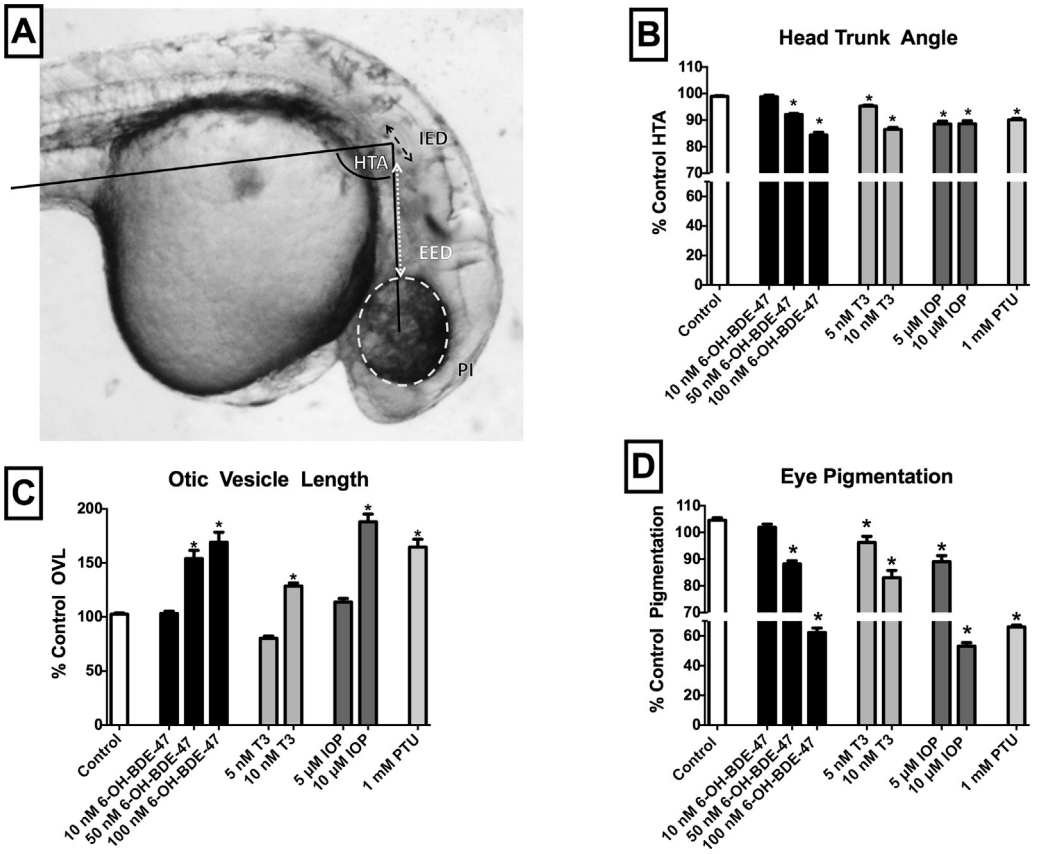

Fig. 1. Various anatomical features used to establish morphometrics are illustrated in embryo image in panel A. The HTA is formed by a line between the ear and the eye and by a line parallel to the notochord extending caudally to somite 5. The otic vesicle length was calculated using eye-ear-distance (EED—dashed white line) and inner ear diameter (IED—dashed black line) at widest point; OVL = EED/IED. The eye region is also highlighted to show area used for pigmentation measurement. Values for each parameter are shown for each experimental group (panel B—HTA, panel C—OVL, panel D—eye pigmentation). Increases in OVL, decreases in pigmentation, and decreases in HTA are all indicative of developmental delays. Data are normalized to control values and presented as mean ± SEM ($n > 30$/treatment) with statistical differences from controls denoted by an asterisk (One-way ANOVA, Dunnett's post-hoc $p < 0.05$).