EPA Public Access

Author manuscript

Environ Toxicol Chem. Author manuscript; available in PMC 2019 September 01.

About author manuscripts

Submit a manuscript

Published in final edited form as:

Environ Toxicol Chem. 2018 September ; 37(9): 2350–2360. doi:10.1002/etc.4180.

Bioaccumulation and Effects of Dietary Exposure to the Alternative Flame Retardant, Bis(2-ethylhexyl) tetrabromophthalate (TBPH), in the Atlantic killifish, *Fundulus heteroclitus*

Diane Nacci,

US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI, USA 02882

Bryan Clark,

Oak Ridge Institute for Science and Education (ORISE), at the US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI, USA 02882

Mark J. La Guardia,

Aquatic Health Sciences, Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA, USA 23062-1246

Ken Miller,

CSC Government Solutions LLC, A CSRA Company, 6361 Walker Lane, Suite 300, Alexandria, VA, USA 22310

Denise Champlin,

US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI, USA 02882

Ian Kirby,

Student Services Contractor to US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI, USA 02882

Ashley Bertrand, and

Corresponding author: Diane Nacci, Nacci.diane@epa.gov.

Publisher's Disclaimer: Disclaimer

Publisher's Disclaimer: Mention of trade names, products, or services does not convey, and should not be interpreted as conveying official US EPA approval, endorsement, or recommendation. The findings and conclusions in this manuscript have not been formally disseminated by the US EPA, and should not be construed to represent any agency determination or policy.

Data accessibility

Data files supporting this publication are available through Supplemental Information, which includes details concerning diet preparation, and all chemical and biological data supporting this manuscript.

Student Services Contractor to US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI, USA 02882

Saro Jayaraman

US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI, USA 02882

Abstract

Bis(2-ethylhexyl) tetrabromophthalate (TBPH), a high production volume flame retardant (FR) chemical used as a replacement for banned FRs, has been detected in media and human and wildlife tissues globally. Here we describe bioaccumulation and biological effects from dietary exposure of TBPH to an estuarine fish, Atlantic killifish, *Fundulus heteroclitus*. Briefly, adult fish were fed exposure diets for 28 d, followed by control diet for 14 d. Exposure diets were amended with carrier, TBPH (TBPH_LO diet, 139 µg/g dry weight, dw, or TBPH_HI diet, 4360 µg/g dw) or a polychlorinated biphenyl congener (PCB153 diet, 13 µg/g dw), which was included as a positive control for bioaccumulation. Only a small proportion of TBPH offered (<0.5 % total TBPH) was bioaccumulated; in contrast, 24.5 % of the PCB153 offered was accounted for in 28 d PCB-treated fish. Although 28 d TBPH concentrations differed by sex and treatment, sexes did not differ in their rates of TBPH bioaccumulation, and the time to achieve 50% of the 28 d concentrations ($T_{1/2}$) was estimated as 13 days. Depuration rates of TBPH did not differ by sex or treatment, and $T_{1/2}$ was estimated as 22 days after exposure ceased. Independent of treatment, male fish grew faster than female fish, but for both sexes reproductive condition (gonadal somatic index) declined unexpectedly over the experimental period. Across treatments, only TBPH_LO treatment affected growth, reducing male but increasing female growth rates by small amounts relative to respective controls. In summary, our study used very high concentrations of dietary TBPH to contaminate fish tissues above the highest levels reported to date in wild biota, yet we observed few adverse biological effects.

Keywords

Brominated Flame Retardants; PCBs; Bioaccumulation

Introduction

Alternative halogenated flame retardants (FRs) were introduced into consumer products in the early 2000s as replacement products for ‘legacy’ FRs such as polybrominated diphenyl ethers (PBDEs) (Stockholm Convention 2008), many of which were withdrawn from global markets due to environmental and human health concerns (e.g., Venkatesan and Halden 2015, Knudsen et al. 2017). However, concerns have been raised that these alternative FRs may have similar potentials as the FRs they replace for persistence and long-range atmospheric transport, bioaccumulation and endocrine-disrupting effects (as reviewed in Saunders et al. 2013).

Bis(2-ethylhexyl) tetrabromophthalate (TBPH used here, but referred to elsewhere as BEH-TEBP, BEHTBP, and other acronyms) is a brominated phthalate that has been shown to be metabolized biologically to TBMEHP (mono -(2-ethylhexyl) tetrabromophthalate) (Roberts et al. 2012) (Fig. 1). TBPH (CAS number = 26040-51-7; Log K_{ow} = 11.95, estimated via EpiSuite, as described in (Knudsen et al. 2017) is listed as a high production volume chemical, with US production volume of 1 – 10 million lb/year (Covaci et al. 2011, Lee et al. 2016). TBPH is a component of commonly used FRs such as formulations of FireMaster® (FM), i.e., 550, 600, BZ-54 (Stapleton et al. 2012, Phillips et al. 2017), and is used in plasticizers such as DP-45 (Great Lakes Solutions 2010). The usage of mixtures containing TBPH in many household products that are disposed in the environment has resulted in the occurrence of TBPH worldwide in environmental media and biota (Klosterhaus et al. 2012, La Guardia et al. 2012, La Guardia et al. 2013, Schreder and La Guardia 2014). Most experimental studies characterizing the effects of TBPH have used commercial formulations that represent how TBPH is often encountered in the environment. For example, the commercial FR FM 550 is composed of brominated and 2 aryl phosphate ester components: 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB, 30%) and TBPH (8%); triphenyl phosphate (TPP, 17%) and triaryl phosphate isomers (45%) (Stapleton et al. 2008, Stapleton et al. 2012, McGee et al. 2013, Scanlan et al. 2015); FM BZ-54 is composed of TBB (70%) and TBPH (30%) (Barr et al. 2012); DP-45 is composed of TBPH, only (Barr et al. 2012). However, when tested as a mixture, results do not explicitly describe or quantify the role of TBPH in these outcomes, and therefore are not directly interpretable in risk assessments of this compound.

This study was designed to provide information supporting assessments of the ecological risks of TBPH. Specifically, this study provides novel information quantifying the bioaccumulation and biological effects of dietary exposure to TBPH using an ecological model fish species, and interprets this information within the context of existing literature about this compound. The species selected for study is the Atlantic killifish (*Fundulus heteroclitus*, referred to hereafter as killifish), an ecologically important resident to estuaries along the US East Coast that has been used for many laboratory and field assessments of human-mediated stressors (Burnett et al. 2007). Study was conducted during the normal reproductive temperature and season for this species locally, e.g., (Dibble and Meyerson 2016), and design was consistent with OECD dietary bioaccumulation test guidelines (OECD 2012). Briefly, fish were fed reference or contaminated food for 28 days (d) then allowed to depurate while being fed uncontaminated food for an additional 14 d. We used relatively high TBPH dietary concentrations, similar to those that produced adverse biological effects when TBPH was tested as part of a mixture on Japanese medaka, *Oryzias latipes*, (Saunders et al. 2015). An additional treatment, resembling an earlier study where killifish bioaccumulated dietary polychlorinated biphenyls (PCBs) (Gutjahr-Gobell et al. 1999), was included to compare bioaccumulation between chemicals. Throughout the experiment, individually-identified fish were measured repeatedly for growth, and at sampling points when fish were sacrificed, selected organs were also weighed to assess condition and a subset of whole fish from every treatment was archived for later contaminant and lipid analyses.

Methods

Dietary exposure system

Adult wild caught killifish (2–3 g wet weight, ww) were collected from Narragansett, RI (41°22'58.4"N 71°31'09.6"W), as previously described (Nacci et al. 2010). This site has been used historically as a source of uncontaminated reference killifish (e.g., Black et al. 1998, Gutjahr-Gobell et al. 1999). This site is undeveloped, with few residences nearby and no industrial activity, and sediment PCB concentrations here are low (McMillan et al. 2006) relative to many sites along the Atlantic Coast of the US (Nacci et al. 2010). Immediately following capture in the fall of 2014, fish were returned to the US EPA Office of Research and Development, Atlantic Ecology Division, Narragansett, RI, and held in 300–600 L tanks receiving flowing uncontaminated sea water at a rate of 6 L/min, and fed *ad libitum*, as described (Nacci et al. 2002) until their use in this study (> six months after collection).

Prior to the initiation of the experiment (Spring 2015), fish were measured and marked for individual identification using MiniHpt8 passive integrated transponder (PIT) tags (Biomark, Boise, ID). At the initiation of this study (day 0), 7 male and 8 female fish were sacrificed to characterize fish prior to treatment, the remaining fish (~144) were distributed into 38 L (10 gal) tanks receiving 21° C flowing sea water at a rate of 1.3 L/min. Each treatment consisted of 10 biological replicate tanks, except for the PCB treatment, where 6 tanks were sufficient because there was no depuration component. Each tank contained 4 fish (2 males and 2 females).

During the experimental period, fish were fed acetone-amended control or contaminated diets from 0–28 d (bioaccumulation period), followed by depuration period, 29 – 42 d during which time fish were fed control diet. Gel-based diets were composed of flaked fish food and other ingredients, amended with small volumes of carrier (acetone) or chemical stocks, as described in detail in Supplemental Information (SI). Stocks were made from TBPH purchased (AK111508, neat, >95% purity) from Ark Pharm, Inc (Libertyville, IL), or PCB congener 2,2',4,4',5,5' hexachlorobiphenyl (PCB153), purchased from AccuStandard, Inc (New Haven, CT), at 2 mg/ml in acetone. Dietary amendment was based on Gutjahr-Gobell and co-authors (Gutjahr-Gobell et al. 1999) for PCB153, and Saunders and co-authors (Saunders et al. 2015) for TBPH, and is described in detail in SI. In brief, we translated their dosing into amount per g fish dry dw, and created duplicate batches of dosed diets that fed all of the fish in a given treatment for two weeks (see SI). Feeding occurred twice daily, while flowing sea water was turned off. Individual daily rations were randomly assigned to a feeding day and kept at –20° C (in aluminum pans wrapped in foil), and thawed on the day of feeding or retained frozen for chemical analysis. The daily feeding rate was about 15.7% based on dry weight (dw), calculated based on an average initial fish weight (2.35 g wet weight, ww = 0.58 g dw).

Biological endpoints

Two tanks of fish per treatment were sacrificed on days 14, 35, and 42, and 4 tanks of fish per treatment were sampled at 28 d. On sampling days, all fish were subjected to 'live measurements', and 'terminal measurements' were conducted on sacrificed fish (detailed

below), prior to archiving (-80°C) for future chemical analysis. Live measurements taken repeatedly and non-invasively on tagged individual fish during the course of the experiment, and consisted of whole fish ww (g) and standard length (sl, mm). Terminal measurements consisted of ww for gonads, liver and abdominal fat. Health indices are ratios normalizing body attributes to body size, and were calculated as: condition index ($\text{CI} = 100 * (\text{ww}/\text{sl})$); gonadosomatic index ($\text{GSI} = 100 * (\text{gonad ww}/\text{body ww})$); hepatosomatic index ($\text{HSI} = 100 * (\text{liver ww}/\text{body ww})$); abdominal fat somatic index ($\text{AFSI} = 100 * (\text{abdominal fat ww}/\text{body ww})$).

All activities were conducted under strict compliance with the Atlantic Ecology Division Animal Care and Use Guidelines.

Statistical analyses: Fish held together in a single tank were sacrificed on a single sampling day, and analyzed statistically as a group (one exposure unit); statistical analyses were also conducted using individual fish data because tagging also permitted measurements to be assigned to a single fish within each tank. Summary statistics, mean (mn) and standard deviation (sd), were sometimes used to describe fish contamination or condition. To assess effects of treatments and factors, multiple regression models were fit with one term for the independent continuous variable (e.g., day or concentration), a separate binary variable for sex, and an interaction term between sex and day/concentration. This was used to test whether the relationship between the independent and dependent variables varied significantly between sexes, i.e., a significant binary sex difference indicates that the intercept differs significantly between sexes, while a significant interacting term indicates that the slopes differ significantly between sexes. Sex-specific model equations were generated by substituting 1 or 0 into the sex and interaction term values; all models were fit using sex-specific tank means due to tank being the experimental unit. Dependent variables that are ratios (i.e., normalized body size reflected as day_x to day_0 ratios, and the $\text{ww}:\text{sl}$ (condition) or organ:body weight ratios) were natural log (ln)-transformed prior to fitting the models; the tank means were the log ratio rather than the log of the tank means. SAS 9.2 (SAS Institute Inc. 2009) was used to analyze all data.

Chemical analysis

TBPH: Samples of whole fish and diet were analyzed at Virginia Institute of Marine Science as described previously (La Guardia et al. 2012, Dodder et al. 2014) (La Guardia et al. 2012, La Guardia et al. 2013). Briefly, samples (~300 mg dw whole fish and ~100 mg dw diet) were freeze-dried and then subjected to accelerated solvent extraction with methylene chloride; a surrogate standard was added to each sample prior to extraction. An aliquot (10%) of each post-ASE extract was removed for lipids determination. The remaining extracts were then purified by size exclusion chromatography (SEC), and each post-SEC extract was reduced in volume, added to the top of a solid phase 2-g silica glass extraction column and eluted with 3.5-mL hexane (fraction one), followed by 6.5 mL of 60:40 hexane/DCM and then 8 mL DCM (fraction two). The second fraction, containing the compounds of interest, was reduced in volume and solvent exchanged to methanol. Decachlorodiphenyl ether was added as the internal standard; identification and quantitation was done by ultra-performance liquid chromatography (UPLC)/atmospheric pressure

photoionization (APPI) tandem mass spectrometry (MS/MS). Samples were analyzed in batches of 20 plus a blank and a matrix-spike (1000 ng sample⁻¹). Each blank (n=5) did not contain TBHP above the quantitation limit (QL) of 1 ng/g dw. Matrix-spiked (n=4, one spiked sample was compromised and not reported) recoveries averaged 96% (range 82 – 126%, 21% RSD), SI Table 1. Also see additional information in SI.

PCB153: Fish diet and tissue samples were analyzed using methods described previously (Jayaraman et al. 2009, Nacci et al. 2016) at the US Environmental Protection Agency, Office of Research and Development, Atlantic Ecology Division, Narragansett, RI. To calculate recovery of PCB 153, known amounts of PCB 153 and PCB198 (internal standard) were added to untreated liver samples and taken through the extraction procedure. A spike standard (with same amounts as the sample) and matrix spike sample were analyzed. The percent recovery was calculated by dividing the concentration in the spiked sample by the spiked amount multiplied by 100; for PCB153, recovery efficiency averaged 97%. A portion (1/10th) of the hexane extract was dried and weighed (dw), and used to calculate the percent lipid per dw (lipid weight, lw) for each sample.

Statistical analyses: Prior to statistical analysis, chemical concentrations were ln-transformed due to the skewed distribution of the measured results, and the mathematical property of ratios (i.e., $\log(A/B) = \log(A) - \log(B)$), such that one can model ratios directly when A and B are ln normalized). Differences in chemical concentrations of whole fish, as per dw or lw, were determined using data for individual fish, including tank as a nested effect (i.e., the denominator in F-tests) to handle the random effect and experimental unit adjustment. Sex comparisons were made using two-factor models for each treatment, and the TBPH_LO vs. TBPH_HI comparisons were fitted using a three-factor model. Both unadjusted (dw) and lipid-normalized values were analyzed. Differences in concentrations of TBPH in fish were compared using multi-factor ANOVA with ln-transformed concentrations/ratios; results for individual fish were used, and tank was included as a random effect nested within treatment, with treatment and treatment x sex F-tests run with sex/tank interaction term in denominator. SAS 9.2 (SAS Institute Inc. 2009) was used to analyze all data.

Results

Contaminant uptake (PCB153 and TBPH) and depuration (TBPH)

As expected, the concentrations of TBPH and PCB153 in the control diet and fish were low, and concentrations of fish consuming treated diets reflected dietary exposures to PCB153 or increasing concentrations of TBPH, respectively (Table 1). Food consumption was not quantified but qualitative data indicating daily unconsumed food showed no treatment bias (data not shown). However, dietary bioaccumulation of PCB153 accounted for ~24% of the total amount of chemical provided in 28 days of feeding (averaging male and female fish values, Table 1), very consistent with a prior diet study using killifish (Gutjahr-Gobell et al. 1999). In contrast, the TBPH measured in fish tissues accounted for a much smaller proportion of the chemical provided, < 0.5% (Table 1). The difference between the two chemicals is also reflected in the bioaccumulation factor (BAF), defined as the ratio of tissue

to dietary concentrations (Arnot and Gobas 2006), where the BAF for PCB153 ~ 1, and the BAF for TBPH < 0.02 (Table 1).

Bioaccumulated concentrations of TBPH by 28 d differed from controls, and varied significantly between TBPH_LO and TBPH_HI treatments ($p = 0.0273$) in a manner consistent with total amounts of TBPH offered to fish in each treatment group. Sexes bioaccumulated treatments differently by 28 d ($p < 0.0001$): male concentrations were lower than females for TBPH_HI, but higher than female concentrations for TBPH_LO (Table 1). In fish measured for TBPH, lipids were higher ($p = 0.01$) for females (17.9% tank mn, 2.9% sd) than males (14.3% mn by tank, 3.5% sd), but did not vary with TBPH concentration ($p = 0.753$). Regression models generated to compare ln-transformed total concentrations per fish to fish body weight (tank means of dw or lw) showed that intercepts differed significantly by treatment ($p < 0.0092$), consistent with differences in dosing; however, neither slopes nor intercepts differed by sex (Fig. 2).

Simple linear regression models of ln-transformed concentrations were used to estimate rates of bioaccumulation (slopes), which did not differ between sexes ($p = 0.9417$) and differed marginally by treatment ($p = 0.0712$). Since 14 d TBPH exceeded 50% of 28 d concentrations ($T_{1/2}$), only 0 d and 14 d data were used to estimate time (d) to $T_{1/2}$ as 13 d for both TBPH_LO and TBPH_HI, where $\ln(\text{TBPH_LO}) = 0.545 \times d$, $r^2 = 0.98$; $\ln(\text{TBPH_HI}) = 0.689 \times d$, $r^2 = 0.99$ (Fig. 3). Depuration (28 d – 42 d) intercepts differed by sex ($p = 0.0373$) and treatment ($p = 0.041$), although depuration rates (slopes) did not ($p = 0.1934$) (Fig. 3). Time to depurate to 50% of the 28 d TBPH concentration ($T_{1/2}$) was about 22d after the TBPH exposure period ended, and was calculated using these models: $\ln(\text{TBPH}) = ((-0.0313 \times d) + \text{intercept}, i)$, where TBPH_LO (female) $i = 8.453$ ($r^2 = 0.261$), TBPH_LO (male) $i = 9.748$ ($r^2 = 0.471$), TBPH_HI (female) $i = 10.506$ ($r^2 = 0.143$), TBPH_HI (male) $i = 10.575$ ($r^2 = 0.142$). Analyses based on lipid normalized concentrations produced similar results (not shown).

Growth and condition under control treatment

Control (acetone-amended, only) diet treatment responses over time were used to estimate body condition and growth rates (for sl and ww) by sex under these experimental conditions for study duration (28 + 14 = 42 d total experimental period) (Fig. 4). To take advantage of individual fish data, size measurements during the experimental period were normalized to individual initial fish size. For this purpose, a single model was fit for each measure (using tank means of the log-transformed day_x to day_0 ratio), with a separate indicator (binary) variable for sex, and a sex x day interaction term, to test for differences in the intercept and slope of sex-specific models, respectively. For this normalized weight, the model intercept was significantly greater for females ($p = 0.0269$), and the growth rate slope was also larger by > 4-fold for males ($m = 0.51\% / d$ v. $f = 0.11\% / d$, $p < 0.0001$). For sl, neither was significantly different. Terminally-measured ratios of condition calculated for gonads-, livers-, and abdominal fat, i.e., GSI, HIS, AFSI, respectively, were analyzed similarly. The GSI, which is a measure of reproductive status, significantly decreased over time ($p = 0.0152$). However, other ratios showed no differences in either the initial values (intercept) or temporal rate of change (slope), or between sexes (see SI, as described below).

Treatment effects on growth and condition

There were several significant model terms describing 28 d exposure effects on growth as ww, but none for length. The normalized ww model was fit based on $\log(\text{day X measure} / \text{day 0 measure})$, where the ratio was calculated on the individual fish, from which sex-specific tank means of the ratios were calculated. (Because this value will always be $\log(1) = 0$ for day 0, the line was forced through the origin rather than estimating an intercept. Slopes for normalized ww differed significantly between sexes ($p < 0.0001$) (Fig. 5). For example, during 28 d exposures under control diets, males gained 0.39% ww/day, which was significantly more ($p < 0.0001$) than females (0.13% ww/day). Only TBPH treatment significantly affected normalized ww growth rate ($p = 0.0132$), and the effect differed by sex ($p = 0.0007$). Modeled estimates suggested that by 28 d, control male fish gained 11% ww, while gains for males consuming treated diets ranged from 6% (TBPH_LO and TBPH_HI) to 14% (PCB153) ww. Similarly, by 28 d control female fish increased by 4% ww, and gains for females consuming treated diets ranged from 5% (PCB153, TBPH_HI) to 8% (TBPH_LO). Thus, TBPH_LO reduced male growth and increased female growth by relatively small amounts in comparison to respective controls. (Fig. 5).

There were no treatment effects on specific organ:body ww ratios, however males and female fish sometimes differed in these factors over the course of the experiment. Therefore, a single model was fit for each ratio indicative of condition, i.e., GSI, HSI and AFSI, using tank means of the log-transformed organ-specific weight to total ww ratio, with a separate indicator (binary) variable for sex, and a sex x concentration interaction term, to test for differences in the intercept and slope of sex-specific models, respectively. GSI declined over the course of the experiment for fish of both sexes, independent of treatment ($p < 0.0001$) (see SI and Fig. S1). HSI differed over time by sex ($p < 0.0176$); temporal slopes for males were positive, but slopes for females declined over time (Fig. S2). The AFSI increased over time ($p < 0.0001$), but differed between sexes (where male slopes were greater than females), although both were positive (Fig. S3).

Discussion

TBPH environmental exposure and bioaccumulation

There are a limited number of reports of TBPH in environmental media and biota samples collected from global locations, some of which reflect known point sources. For example, TBPH in sediment was first reported in a Chinese harbor with values as high as 0.74 ng/g dw, which was described as “moderate compared to the global range” for legacy flame retardants despite the relatively recent usage of TBPH (Liu et al. 2014). However, downstream from a textile manufacturing outfall in North Carolina, USA, sediment concentrations were as high as 19,200 ng TBPH/g total organic carbon, and the maximal concentration found in indigenous estuarine bivalves was 1370 ng/g lipid weight (La Guardia et al. 2012). These data suggest that TBPH persists in sediment, but the log biota-sediment accumulation factor (BSAF) calculated from these data (La Guardia et al. 2012) indicated that TBPH has low bioaccumulation potential. A limited number of recent reports describe TBPH in whole biota as ranging from about 2 – 1400 ng/g dw, with higher concentrations likely in lipid-rich tissues (Table 2). Furthermore, environmental exposures

may be widespread, e.g., 89% of gull livers in Montreal contained detectable concentrations of TBPH, with a maximum concentration estimated as 76 ng/g dw (Gentes et al. 2012).

In this study, TBPH dietary exposures to killifish resulted (on average) from 2319 to 24738 ng TBPH/g dw (Table 1), exceeding TBPH concentrations in the limited number of environmental biota sampled (Table 2). However, these concentrations were achieved using very high TBPH dietary concentrations. Specifically, TBPH in fish accounted for < 0.5% total contaminant provided by diet, suggesting a BAF for TBPH < 0.02% (Table 1). Thus, TBPH bioaccumulation was very low in TBPH_LO and TBPH_HI dietary exposures. In contrast, bioaccumulation of dietary PCB153 was high and similar to a prior dietary study using killifish (Gutjahr-Gobell et al. 1999). Furthermore, similar growth responses and similar size-based bioaccumulation across treatments (Fig. 2) argue against poor consumption of the TBPH-amended diets as a confounding factor when considering bioaccumulation.

The low bioaccumulation of TBPH observed in our study is consistent with limited studies in mammals (Knudsen et al. 2017, La Guardia et al. 2017) and in fish where TBPH was tested as a mixture. We cannot compare our bioaccumulation results to a fish dietary study that showed adverse reproductive effects of a TBPH mixture because TBPH bioaccumulation was not measured in that study (Saunders et al. 2015). However, fathead minnows exposed to FM 550 or BZ-54 (1 mg/fish/d), bioaccumulated only a very small proportion of the TBPH provided in these mixtures into fish tissues (0.6% of daily feeding, estimated < 0.01% of total TBPH supplied) (Barr et al. 2010). In another study, fathead minnows were exposed in outdoor mesocosms to BZ-54 -loaded sediment at a concentration of 500 ng/g (reflective of San Francisco, CA, US, Bay sludge), and assessed for bioaccumulation and transformation of TBPH (and other alternative FRs). Relative to environmental fate, their results showed that TBPH is persistent under environmental conditions: median dissipation time, DT₅₀, for TBPH was estimated as 25 days in particulate matter and >200 days in sediment, while TBB dissipated more quickly than TBPH in particulate phase and TBB could not be detected in sediment (de Jourdan et al. 2013). TBPH bioaccumulation was detected in only 1 fish in one replicate enclosure on 1 day during the uptake phase, suggesting minimal bioaccumulation. Moreover, the appearance of brominated products in tissues suggested that some metabolism of BZ-54 had occurred.

Biotransformation of TBPH

Low tissue concentrations of the parent compound, TBPH, in fish tissue from our study could also be accounted for by rapid metabolism of TBPH to TBMEHP (Fig. 1). Although we did not measure metabolite concentrations, rapid metabolism is not consistent with the slow decay rate we observed (Fig. 3). Furthermore, laboratory evaluations of TBPH metabolism in a variety of systems support the likelihood that TBPH is not extensively metabolized in our fish system. For example, the hydrolysis of TBPH has not been observed in *in vitro* systems (Roberts et al. 2012, Fang and Stapleton 2014). Furthermore, unlike the non-brominated analogue of TBPH, DEHP, which is metabolized quickly *in vitro*, no TBPH metabolites were detected in subcellular fractions; however, TBMEHP (Fig. 1) was produced relatively slowly in the presence of TBPH and purified carboxylesterase. These

results suggest that TBPH is relatively recalcitrant to metabolism, and therefore relatively persistent *in vivo* (Roberts et al. 2012). In mice and rats dosed by gavage or IV administered radioactively-labeled TBPH, TBPH was largely excreted in feces (i.e., poorly absorbed) within 24 – 72 hours of exposure; the primary metabolite was identified as TBMEHP (Knudsen et al. 2017). Consistent with mammalian systems, an *in vitro* study using carp (*Cyprinus carpio*) liver microsomes showed little metabolism of TBPH, although the other component of BZ-54, TBB, was rapidly metabolized (Barr et al. 2010). Species differences in metabolism of BZ-54 components were also examined using fathead minnow (*Pimephales promelas*) and common carp (*Cyprinus carpio*), mouse (*Mus musculus*) and snapping turtle (*Chelydra serpentina serpentina*) *in vitro* systems, which indicated that TBPH was relatively poorly metabolized in comparison to TBB (Barr et al. 2012).

Toxic effects and mechanisms

While our study showed very limited adverse effects of TBPH dietary exposures to killifish, expectations concerning the toxicity of TBPH were based in part on the toxicity of structurally similar compounds. Specifically, TBPH is the brominated analogue of the known carcinogen, teratogen and reproductive toxicant, bis(2-ethylhexyl)phthalate, DEHP (Caldwell 2012), and on this basis is predicted to be moderately toxic for these endpoints (USEPA 2015). Furthermore, various endpoints have been measured using *in vitro* and *in vivo* systems to infer the mechanisms such as endocrine active properties by which TBPH may produce adverse biological outcomes, such as abnormal development, or reduced growth and reproduction, as detailed below.

Mammalian species/*in vitro* systems

It has been suggested that TBPH may act as a metabolic disrupting agent or ‘environmental obesogen’, i.e., stimulating adipogenesis (as reviewed in Capita et al. 2017). For example, both FM 550 and TBPH alone induced lipid accumulation in a dose-dependent manner in mammalian cultured cells (Tung et al. 2017). In another study, adult female rodents were exposed to FM 550 via diet at 0.1 or 1 mg/day, resulting in liver concentrations of ~ 322 or 2384 ng/g dw, respectively. Pups of exposed dams had increased serum thyroxine (T4), weight gain, and impaired glucose tolerance, suggesting that FM 550 components might interfere with the action of nuclear receptors associated with regulating energy (Patisaul et al. 2013). Consistent with this experimental study, a human epidemiological study also found TBPH concentrations in house dust were correlated with elevated thyroid hormone (T3) concentrations in men (Johnson et al. 2013), which suggested a potential mechanistic relationship between this FR and thyroid hormone system. However, TBPH and its metabolite TBMEHP have also been characterized as anti-thyroidal, anti-androgenic and anti-glucocorticoid agents in *in vitro* promoter-reporter gene assays using mammalian cell lines, and *in silico* methods identified TBPH as directly antagonizing androgenic receptor binding (Klopčič et al. 2016). Furthermore, dosing rats with TBMEHP produced hypothyroidism (low serum T3), and *in vitro* exposures to TBMEHP inhibited deiodinase activity (Springer et al. 2012). Other pathways regulating metabolism have been reportedly affected by exposure to FR chemicals. For example, *in vitro* tests using rat tissues showed that FM activated that peroxisome proliferator-activated receptor (PPAR γ) pathway, however, this activity was attributed to FM component triaryl phosphate (TPP) (Belcher et

al. 2014). In addition, TBPH did not exhibit ligand activity in response to PPAR α or PPAR γ (Skledar et al. 2016, Tung et al. 2017). However, both TBPH and TBMEHP acted as potent agonists for the pregnane X receptor (PXR), and the subsequent upregulation of CYP3A4 (Skledar et al. 2016). PXR activation can enhance metabolism of xenobiotics and endogenous hormones, such as T3, and has been associated with metabolic diseases like diabetes (Skledar et al. 2016). A recent report also suggests that hydroxylated metabolites of TBPH that have been detected in dust are potent PPAR α agonists and weak estrogen receptor agonists (Peng et al. 2017); however, we don't know whether TBPH metabolites are present in our dietary exposure system.

Some evidence suggests that TBPH may affect reproduction: in a yeast estrogen/androgen screening assay, TBPH and TBB, components of the FR FM 550, acted antagonistically towards the human estrogen/androgen receptors; yet both compounds (separately) increased testosterone (T) and estr(E2) in mammalian cells, albeit at a high concentration: 15 mg TBPH/L (Saunders et al. 2013). Similarly, TBPH exposure (≥ 0.15 mg/L) to porcine testicular cells *in vitro* increased the production of T and E2, which was consistent with increased expression of steroidogenesis genes, CYP11A and CYP19A (aromatase, which catalyzes T $\rightarrow$ E2 conversion), at higher tested concentration (15 mg/L) (Mankidy et al. 2014).

Aquatic species

Although there was some expectation of effects based on mechanistic studies using other biological systems (discussed above), TBPH treatment did not affect killifish lipogenesis as measured by HSI (a fat storage organ in fish) or gross abdominal fat deposition (AFSI). However, TBPH_LO treatment had a minor but opposite effect on body growth (as ww) in males and females. Specifically, TBPH depressed male growth rate but enhanced female growth rate (for TBPH_LO, only), relative to sex-specific controls (Fig. 5). Thus, the only adverse outcome observed in our study from TBPH exposures was a non-dose responsive reduction in male growth rate. Consistent with our study, dietary exposure of fathead minnows for 56 days to TBPH as a component of FM 550 or BZ-54 via diet showed low bioaccumulation and few health effects (Barr et al. 2010). Specifically, treatments resulting in fish concentrations of 360 or 4500 ng TBPH/g dw, similar to or lower than the TBPH_LO bioaccumulation in our study (Table 1), produced no effects on health endpoints to adult fish (Barr et al. 2010). Subtle effects described in the fathead minnow dietary study of FR mixtures included some evidence of DNA damage in blood cells and higher lipid content at the end of the uptake period, for some treatments (Barr et al. 2010). Another FR exposure of fathead minnows (i.e., to sediments amended with BZ-54 at 500 ng/g reflective of San Francisco, CA, US, Bay sludge) also resulted in minimal measurable bioaccumulation (see above). Those exposures increased condition factor and oxidative damage in immature fish and increased liver:somatic indices in male fish (de Jourdan et al. 2013, de Jourdan et al. 2014), but, similar to our study of TBPH, growth rates for whole bodies and livers and lipid content (%) were unaffected (de Jourdan et al. 2014).

While in our study, TBPH had small, sex-specific effects on killifish growth rates. However, fish were not reproductively active, which was unexpected given concurrence with

reproductive season in the source population, e.g., (Dibble and Meyerson 2016), and in other the lab killifish (not shown), so we produced no new information on TBPH as reproductive toxicant. In other studies, TBPH has been tested as a 1:1 mixture with another component of FM 550, TBB, in a 21-day study using Japanese medaka (Saunders et al. 2015). This high dose dietary exposure (nominal food concentrations, 1500 µg/g TBB and 1500 µg/g TBPH; fish tissue concentrations were not reported) resulted in reduced fecundity and down-regulation of genes related to hypothalamus-pituitary-liver-gonadal (HPLG) reproductive axis; however, to what extent the TBPH component of this mixture contributed to these effects is not known. Similarly, the contribution of TBB to the adverse effects observed in the Saunders et al. study cannot be inferred, although much lower level dietary exposure to TBB (~ 5 µg/g) by juvenile rainbow trout did not result in adverse biological effects or alterations gene expression (Giraud et al. 2017).

Concerns over the reproductive toxicity of TBPH to aquatic species have also been based on the toxic effects of TBPH's non-brominated analogue, DEHP. For example, DEHP exposure to Chinese rare minnow (*Gobiocypris rarus*) resulted in increased T, E2 and vitellogenin, the egg yolk precursor complex (Wang et al. 2013). Similarly, DEHP exposure reduced vitellogenin and resulted in fewer oocytes to Japanese medaka (Kim et al. 2002) and zebrafish (*Danio rerio*) (Carnevali et al. 2010), which also responded to DEHP with reduced sperm quality (Golshan et al. 2015). Given structural similarities to DEHP, oxidative and DNA damage were predicted from exposures to TBPH in fathead minnow, which were observed following 56 d oral exposures but were undetectable after 22 d depuration (Barr et al. 2010).

Mechanistic insight on the effects of TBPH alone and in mixtures as FM 550 and BZ-54 is also provided by *Daphnia* exposures and transcriptomic, metabolomic and lipidomic analyses (Scanlan et al. 2015). Here, TBPH was shown to be highly toxic to this freshwater invertebrate (LC50 ≤ 1 mg/L, nominally), and when tested at 10% of the LC50, transcriptomic changes suggested that TBPH contributed significantly to the activity of BZ-54, affecting glycosphingolipid synthesis (Scanlan et al. 2015). *Daphnia* results suggest the potential of TBPH to affect early development, which was not explored in our study.

Other studies support the potential of FRs to affect early life stages of fishes. For example, while some components of FM 550 cause cardiotoxicity in zebrafish larvae, TBPH did not exhibit embryotoxicity nor the cardiotoxicity characteristic of dioxin-like compounds (i.e., those mediated via the aryl hydrocarbon receptor) when tested as at concentrations as high as ~ 7 mg/L (McGee et al. 2012, McGee et al. 2013). However, zebrafish larvae exposed to lower concentrations of FM 550 (0.01 – 1 mg/L) demonstrated reduced social behavior and hypoactivity (Bailey and Levin 2015). Therefore, it may be useful to consider to what extent TBPH alone may contribute to behavioral effects from FR exposures.

Summary

Our results are consistent with prior studies (Barr et al. 2010, Knudsen et al. 2017) showing limited availability and bioaccumulation of TBPH. In fact, our study showed that extremely high concentrations of dietary TBPH are required to contaminate fish tissues above the

highest levels observed to date in wild biota, and that these exposures produced few adverse biological effects on the health of adult fish. This contrasts with some experimental studies, often testing TBPH as a mixture, whose results suggest that the parent compound or its primary metabolite, TBMEHP, may affect growth through hormone dysregulation. In our system, high exposures to TBPH had little effect on growth and no effect on fat deposition. Since *in vivo* and *in vitro* systems have shown that TBPH is poorly metabolized biologically, it is unlikely that killifish tissue concentrations of TBMEHP, a potentially active obesogenic agent, was high enough to elicit adverse effects; however, better quantitative information on TBPH metabolism in fish is required to address this issue definitively. Furthermore, while mixtures containing TBPH have sometimes been toxic to the early development and reproductive function of fish, our study did not evaluate these outcomes, which may be important in considering the ecological risks associated with this compound. Studies to address some of these gaps in our understanding of the ecological risks of TBPH exposures are ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This is tracking # ORD-023049 of the US EPA, Office of Research and Development (ORD), and Contribution No. 3750 of the Virginia Institute of Marine Science, College of William & Mary. This research was also supported in part by a fellowship appointment at the US EPA (to BC) administered by the Oak Ridge Institute for Science and Education (ORISE) through Interagency Agreement No. DW92429801 between the US Department of Energy and the US EPA, and student services contracts (to AB, US EPA Student Services Contract EP-D-13-000145, and to IK, US EPA Student Services Contract EPD-12-000400). TBPH chemical analysis was conducted through EPA contract # EP-15-W-000309 to VIMS (ML). Statistical support provided by CSRA (KM) under EPA Contract # HHSN3162012000013W, Task Order # EP-G16H-01256. An early version of the manuscript benefitted greatly by reviews from US EPA ORD Atlantic Ecology Division scientists, Dr. Mark Cantwell, Dr. Glen Thursby, Dr. Lesley Mills, and Mr. Joseph LiVolsi. Helpful discussions concerning study design and the interpretation of results were also provided by Dr. Lawrence Burkhart (US EPA ORD) and Dr. Adam Biales (US EPA ORD).

Citations

- Arnot JA and Gobas F (2006). "A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms." *Environmental Reviews* 14(4): 257–297.
- Bailey JM and Levin ED (2015). "Neurotoxicity of Fire Master 550 (R) in zebrafish (*Danio rerio*): Chronic developmental and acute adolescent exposures." *Neurotoxicology and Teratology* 52: 210–219. [PubMed: 26239867]
- Bearr JS, Mitchelmore CL, Roberts SC and Stapleton HM (2012). "Species specific differences in the *in vitro* metabolism of the flame retardant mixture, Firemaster (R) BZ-54." *Aquatic Toxicology* 124: 41–47. [PubMed: 22889877]
- Bearr JS, Stapleton HM and Mitchelmore CL (2010). "Accumulation and DNA damage in fathead minnows (*Pimephales promelas*) exposed to 2 brominated flame retardant mixtures, Firemaster(R) 550 and Firemaster(R) BZ-54 " *Environmental Toxicology and Chemistry* 29(3): 722–729. [PubMed: 20821500]
- Belcher SM, Cookman CJ, Patisaul HB and Stapleton HM (2014). "In vitro assessment of human nuclear hormone receptor activity and cytotoxicity of the flame retardant mixture FM 550 and its triarylphosphate and brominated components." *Toxicology Letters* 228(2): 93–102. [PubMed: 24786373]

- Black DE, Gutjahr-Gobell R, Pruell RJ, Bergen B and McElroy AE (1998). "Effects of a mixture of non-ortho-and mono-ortho-polychlorinated biphenyls on reproduction in *Fundulus heteroclitus* (Linnaeus)." *Environmental Toxicology and Chemistry* 17(7): 1396–1404.
- Burnett KG, Bain LJ, Baldwin WS, Callard GV, Cohen S, Di Giulio RT, Evans DH, Gomez-Chiarri M, Hahn ME, Hoover CA, Karchner SI, Katoh F, MacLatchy DL, Marshall WS, Meyer JN, Nacci DE, Oleksiak MF, Rees BB, Singer TD, Stegeman JJ, Towle DW, Van Veld PA, Vogelbein WK, Whitehead A, Winn RN and Crawford DL (2007). "Fundulus as the premier teleost model in environmental biology: Opportunities for new insights using genomics." *Comparative Biochemistry and Physiology D-Genomics & Proteomics* 2(4): 257–286.
- Caldwell JC (2012). "DEHP: Genotoxicity and potential carcinogenic mechanisms-A review." *Mutation Research-Reviews in Mutation Research* 751(2): 82–157. [PubMed: 22484601]
- Capitao A, Lyssimachou A, Castro LFC and Santos MM (2017). "Obesogens in the aquatic environment: an evolutionary and toxicological perspective." *Environment International* 106: 153–169. [PubMed: 28662399]
- Carnevali O, Tosti L, Speciale C, Peng C, Zhu Y and Maradonna F (2010). "DEHP impairs zebrafish reproduction by affecting critical factors in oogenesis." *Plos One* 5(4).
- Covaci A, Harrad S, Abdallah MAE, Ali N, Law RJ, Herzke D and de Wit CA (2011). "Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour." *Environment International* 37(2): 532–556. [PubMed: 21168217]
- de Jourdan BP, Hanson ML, Muir DCG and Solomon KR (2013). "Environmental fate of three novel brominated flame retardants in aquatic mesocosms." *Environmental Toxicology and Chemistry* 32(5): 1060–1068. [PubMed: 23400845]
- de Jourdan BP, Hanson ML, Muir DCG and Solomon KR (2014). "Fathead minnow (*Pimephales promelas* Rafinesque) exposure to three novel brominated flame retardants in outdoor mesocosms: Bioaccumulation and biotransformation." *Environmental Toxicology and Chemistry* 33(5): 1148–1155. [PubMed: 24477969]
- Depew DC, Basu N, Burgess NM, Campbell LM, Devlin EW, Drevnick PE, Hammerschmidt CR, Murphy CA, Sandheinrich MB and Wiener JG (2012). "Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations." *Environmental Toxicology and Chemistry* 31(7): 1536–1547. [PubMed: 22549893]
- Dibble KL and Meyerson LA (2016). "Detection of decreased quantities of actively spawning female *Fundulus heteroclitus* in tidally restricted marshes relative to restored and reference sites." *Biological Invasions* 18(9): 2679–2687.
- Dodder NG, Maruya KA, Ferguson PL, Grace R, Kiosterhaus S, La Guardia MJ, Lauenstein GG and Ramirez J (2014). "Occurrence of contaminants of emerging concern in mussels (*Mytilus* spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent." *Marine Pollution Bulletin* 81(2): 340–346. [PubMed: 23849955]
- Fang M and Stapleton HM (2014). "Evaluating the bioaccessibility of flame retardants in house dust using an *in vitro* tenax bead-assisted sorptive physiologically based method." *Environmental Science & Technology* 48(22): 13323–13330. [PubMed: 25330458]
- Gentes ML, Letcher RJ, Caron-Beaudoin E and Verreault J (2012). "Novel flame retardants in urban-feeding ring-billed gulls from the St. Lawrence River, Canada." *Environmental Science & Technology* 46(17): 9735–9744. [PubMed: 22845168]
- Giraud M, Douville M, Letcher RJ and Houde M (2017). "Effects of food-borne exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to emerging brominated flame retardants 1,2-bis(2,4,6-tribromophenoxy)ethane and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate." *Aquatic Toxicology* 186: 40–49. [PubMed: 28249227]
- Golshan M, Hatef A, Socha M, Milla S, Butts IAE, Carnevali O, Rodina M, Sokolowska-Mikolajczyk M, Fontaine P, Linhart O and Alavi SMH (2015). "Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish." *Aquatic Toxicology* 163: 16–26. [PubMed: 25827748]
- Great Lakes Solutions. (2010). "Technical Information DP-45™", from <http://greatlakes.com/deployedfiles/ChemturaV8/GreatLakes/Flame%20Retardants/FR%20Products/DP-45%20TDS.pdf>.

- Gutjahr-Gobell RE, Black DE, Mills LJ, Pruell RJ, Taplin BK and Jayaraman S (1999). "Feeding the mummichog (*Fundulus heteroclitus*) a diet spiked with non-ortho- and mono-ortho-substituted polychlorinated biphenyls: Accumulation and effects." *Environmental Toxicology and Chemistry* 18(4): 699–707.
- Jayaraman S, Nacci DE, Champlin DM, Pruell RJ, Rocha KJ, Custer CM, Custer TW and Cantwell M (2009). "PCBs and DDE in tree swallow (*Tachycineta bicolor*) eggs and nestlings from an estuarine PCB Superfund Site, New Bedford Harbor, MA, USA." *Environmental Science & Technology* 43(21): 8387–8392. [PubMed: 19924974]
- Johnson PI, Stapleton HM, Mukherjee B, Hauser R and Meeker JD (2013). "Associations between brominated flame retardants in house dust and hormone levels in men." *Science of the Total Environment* 445: 177–184. [PubMed: 23333513]
- Kim EJ, Kim JW and Lee SK (2002). "Inhibition of oocyte development in Japanese medaka (*Oryzias latipes*) exposed to di-2-ethylhexyl phthalate." *Environment International* 28(5): 359–365. [PubMed: 12437285]
- Klopčič I, Skledar G, Masić LP and Dolenc MS (2016). "Comparison of in vitro hormone activities of novel flame retardants TBB, TBPH and their metabolites TBBA and TBMEPH using reporter gene assays." *Chemosphere* 160: 244–251. [PubMed: 27380226]
- Klosterhaus SL, Stapleton HM, La Guardia MJ and Greig DJ (2012). "Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife." *Environment International* 47: 56–65. [PubMed: 22766500]
- Knudsen GA, Sanders JM and Birnbaum LS (2017). "Disposition of the emerging brominated flame retardant, bis(2-ethylhexyl) tetrabromophthalate, in female Sprague Dawley rats: effects of dose, route and repeated administration." *Xenobiotica* 47(3): 245–254. [PubMed: 27098498]
- La Guardia MJ, Hale RC, Harvey E, Mainor TM and Ciparis S (2012). "In situ accumulation of HBCD, PBDEs, and several alternative flame-retardants in the bivalve (*Corbicula fluminea*) and gastropod (*Elimia proxima*)." *Environmental Science & Technology* 46(11): 5798–5805. [PubMed: 22571713]
- La Guardia MJ, Hale RC and Newman B (2013). "Brominated flame-retardants in Sub-Saharan Africa: Burdens in inland and coastal sediments in the eThekweni Metropolitan Municipality, South Africa." *Environmental Science & Technology* 47(17): 9643–9650. [PubMed: 23899189]
- La Guardia MJ, Schreder ED, Uding N and Hale RC (2017). "Human indoor exposure to airborne halogenated flame retardants: Influence of airborne particle size." *International Journal of Environmental Research and Public Health* 14(5).
- Lam JCW, Lau RKF, Murphy MB and Lam PKS (2009). "Temporal trends of hexabromocyclododecanes (HBCDs) and polybrominated diphenyl ethers (PBDEs) and detection of two novel flame retardants in marine mammals from Hong Kong, South China." *Environmental Science & Technology* 43(18): 6944–6949. [PubMed: 19806725]
- Lee SC, Sverko E, Harner T, Pozo K, Barresi E, Schachtschneider J, Zaruk D, DeJong M and Narayan J (2016). "Retrospective analysis of "new" flame retardants in the global atmosphere under the GAPS Network." *Environmental Pollution* 217: 62–69. [PubMed: 26857525]
- Liu HH, Hu YJ, Luo P, Bao LJ, Qiu JW, Leung KMY and Zeng EY (2014). "Occurrence of halogenated flame retardants in sediment off an urbanized coastal zone: Association with urbanization and industrialization." *Environmental Science & Technology* 48(15): 8465–8473. [PubMed: 24988362]
- Mankidy R, Ranjan B, Honaramooz A and Giesy JP (2014). "Effects of novel brominated flame retardants on steroidogenesis in primary porcine testicular cells." *Toxicology Letters* 224(1): 141–146. [PubMed: 24466573]
- McGee SP, Cooper EM, Stapleton HM and Volz DC (2012). "Early zebrafish embryogenesis is susceptible to developmental TDCPP exposure." *Environmental Health Perspectives* 120(11): 1585–1591. [PubMed: 23017583]
- McGee SP, Konstantinov A, Stapleton HM and Volz DC (2013). "Aryl phosphate esters within a major pentaBDE replacement product induce cardiotoxicity in developing zebrafish embryos: Potential role of the aryl hydrocarbon receptor." *Toxicological Sciences* 133(1): 144–156. [PubMed: 23377616]

- McMillan AM, Bagley MJ, Jackson SA and Nacci DE (2006). "Genetic diversity and structure of an estuarine fish (*Fundulus heteroclitus*) indigenous to sites associated with a highly contaminated urban harbor." *Ecotoxicology* 15(6): 539–548. [PubMed: 16988885]
- Nacci D, Champlin D and Jayaraman S (2010). "Adaptation of the estuarine fish *Fundulus heteroclitus* (Atlantic killifish) to polychlorinated biphenyls (PCBs)." *Estuaries and Coasts* 33(4): 853–864.
- Nacci DE, Champlin D, Coiro L, McKinney R and Jayaraman S (2002). "Predicting the occurrence of genetic adaptation to dioxinlike compounds in populations of the estuarine fish *Fundulus heteroclitus*." *Environmental Toxicology and Chemistry* 21(7): 1525–1532. [PubMed: 12109755]
- Nacci DE, Hahn ME, Karchner SI, Jayaraman S, Mostello C, Miller KM, Blackwell CG and Nisbet ICT (2016). "Integrating monitoring and genetic methods to infer historical risks of PCBs and DDE to common and roseate terns nesting near the New Bedford Harbor Superfund site (Massachusetts, USA)." *Environmental Science & Technology* 50(18): 10226–10235. [PubMed: 27564328]
- OECD (2012). OECD Guidelines for testing chemicals, Guideline 305, Bioaccumulation in fish: Aqueous and dietary exposure. Paris, France, Organization for Economic Cooperation and Development: 72.
- Patisaul HB, Roberts SC, Mabrey N, McCaffrey KA, Gear RB, Braun J, Belcher SM and Stapleton HM (2013). "Accumulation and endocrine disrupting effects of the flame retardant mixture firemaster (R) 550 in rats: An exploratory assessment." *Journal of Biochemical and Molecular Toxicology* 27(2): 124–136. [PubMed: 23139171]
- Peng H, Sun JX, Saunders DMV, Codling G, Wiseman S, Jones PD and Giesy JP (2017). "Hydroxylated 2-Ethylhexyl tetrabromobenzoate isomers in house dust and their agonistic potencies with several nuclear receptors." *Environmental Pollution* 227: 578–586. [PubMed: 28505588]
- Phillips AL, Hammel SC, Konstantinov A and Stapleton HM (2017). "Characterization of Individual Isopropylated and tert-Butylated Triarylphosphate (ITP and TBPP) Isomers in Several Commercial Flame Retardant Mixtures and House Dust Standard Reference Material SRM 2585." *Environmental Science & Technology* 51(22): 13443–13449. [PubMed: 29076339]
- Roberts SC, Macaulay LJ and Stapleton HM (2012). "In vitro metabolism of the brominated flame retardants 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl) 2,3,4,5-tetrabromophthalate (TBPH) in human and rat tissues." *Chemical Research in Toxicology* 25(7): 1435–1441. [PubMed: 22575079]
- SAS Institute Inc. (2009). SAS Statistical Software Version 9.2 Cary, North Carolina, USA, SAS Institute Inc.
- Saunders DMV, Higley EB, Hecker M, Mankidy R and Giesy JP (2013). "*In vitro* endocrine disruption and TCDD-like effects of three novel brominated flame retardants: TBPH, TBB, & TBCO." *Toxicology Letters* 223(2): 252–259. [PubMed: 24064184]
- Saunders DMV, Podaima M, Codling G, Giesy JP and Wiseman S (2015). "A mixture of the novel brominated flame retardants TBPH and TBB affects fecundity and transcript profiles of the HPGL-axis in Japanese medaka." *Aquatic Toxicology* 158: 14–21. [PubMed: 25461741]
- Scanlan LD, Loguinov AV, Teng Q, Antczak P, Dailey KP, Nowinski DT, Kornbluh J, Lin XX, Lachenauer E, Arai A, Douglas NK, Falciani F, Stapleton HM and Vulpe CD (2015). "Gene transcription, metabolite and lipid profiling in eco-indicator *Daphnia magna* indicate diverse mechanisms of toxicity by legacy and emerging flame retardants." *Environmental Science & Technology* 49(12): 7400–7410. [PubMed: 25985095]
- Schreder ED and La Guardia MJ (2014). "Flame retardant transfers from U.S. households (dust and laundry wastewater) to the aquatic environment." *Environmental Science & Technology* 48(19): 11575–11583. [PubMed: 25288150]
- Skledar DG, Tomasic T, Carino A, Distrutti E, Fiorucci S and Masic LP (2016). "New brominated flame retardants and their metabolites as activators of the pregnane X receptor." *Toxicology Letters* 259: 116–123. [PubMed: 27506419]
- Springer C, Dere E, Hall SJ, McDonnell EV, Roberts SC, Butt CM, Stapleton HM, Watkins DJ, McClean MD, Webster TF, Schlezinger JJ and Boekelheide K (2012). "Rodent thyroid, liver, and fetal testis toxicity of the monoester metabolite of bis-(2-ethylhexyl) tetrabromophthalate (TBPH),

- a novel brominated flame retardant present in indoor dust.” *Environmental Health Perspectives* 120(12): 1711–1719. [PubMed: 23014847]
- Stapleton HM, Allen JG, Kelly SM, Konstantinov A, Klosterhaus S, Watkins D, McClean MD and Webster TF (2008). “Alternate and new brominated flame retardants detected in US house dust.” *Environmental Science & Technology* 42(18): 6910–6916. [PubMed: 18853808]
- Stapleton HM, Sharma S, Getzinger G, Ferguson PL, Gabriel M, Webster TF and Blum A (2012). “Novel and high volume use flame retardants in US couches reflective of the 2005 pentaBDE phase out.” *Environmental Science & Technology* 46(24): 13432–13439. [PubMed: 23186002]
- Stockholm Convention. (2008). “Decabromodiphenylether (commercial mixture, c-decaBDE” from <http://chm.pops.int/Implementation/Alternatives/AlternativestoPOPs/ChemicalslistedinAnnexA/cdecaBDE/tabid/5985/Default.aspx>.
- Tung EWY, Ahmed S, Peshdary V and Atlas E (2017). “Firemaster (R) 550 and its components isopropylated triphenyl phosphate and triphenyl phosphate enhance adipogenesis and transcriptional activity of peroxisome proliferator activated receptor (Ppar gamma) on the adipocyte protein 2 (aP2) promoter.” *Plos One* 12(4).
- USEPA. (2015). “Flame Retardants used in Flexible Polyurethane Foam: An Alternatives Assessment Update.” Retrieved EPA 744-R-15-002, from https://www.epa.gov/sites/production/files/2015-08/documents/ffr_final.pdf.
- Venkatesan AK and Halden RU (2015). “Effective Strategies for Monitoring and Regulating Chemical Mixtures and Contaminants Sharing Pathways of Toxicity.” *International Journal of Environmental Research and Public Health* 12(9): 10549–10557. [PubMed: 26343697]
- Wang X, Yang Y, Zhang L, Ma Y, Han J, Yang L and Zhou B (2013). “Endocrine disruption by di-(2-ethylhexyl)-phthalate in Chinese rare minnow (*Gobiocypris rarus*).” *Environmental Toxicology and Chemistry* 32(8): 1846–1854. [PubMed: 23625782]

Fig. 1.
: Parent compound, bis(2-ethylhexyl) tetrabromophthalate, TBPH and primary metabolite, mono-(2-ethylhexyl) tetrabromophthalate, TBMEHP (Roberts et al. 2012).

Fig. 2.

: After 28 days of feeding, the total amount of contaminant (ng) bioaccumulated into individual male (triangles) and female (circles) fish was proportional to fish size (dry weight), and independent of sex for diets amended with PCB153 (13 $\mu\text{g/g}$ dw, orange) or one of two concentrations of TBPH (139 $\mu\text{g/g}$ dw, TBPH_LO, light blue; 4360 $\mu\text{g/g}$ dw, TBPH_HI, dark blue).

Fig. 3.

: Statistical models for rates of TBPH initial bioaccumulation using sex-tank averages (ng/g dry weight) for female (circles, dotted lines), male fish (triangles, dashed lines) or both sexes (mixed dash line) during uptake phase (0 to 28 d) of TBPH_LO (light blue) or TBPH_HI (dark blue) from contaminated diet, followed by depuration during uncontaminated, depuration dietary phase (28 – 42 d).

Fig. 4.

: Growth trajectories of control tank averages by sex for wet weight (ww) normalized by initial ww of individual fish for male (triangles, dashed line) and females (circles, dotted line) over the 42-day experimental period.

Fig. 5.

: Growth trajectories over 28 d exposure period as tank averages by sex and treatment for wet weight (ww) normalized to initial ww of individual fish for male (upper figure, triangles) and female fish (lower figure, circles), showing control (open symbols and black dashed lines), PCB153 (orange symbols and dotted lines), TBPH LO (light blue symbols and dotted lines) TBPH HI (dark blue symbols and dotted lines).

Table 1.

Chemical contaminant (CHEM) supplied by diet for 28 days and bioaccumulated into male and female fish (ng/g dry weight, dw, or ng/g lipid) held together in replicate tanks per treatment for treatments PCB153, TBP_H_LO or TBP_H_HI, as described in methods, where bioaccumulation factor = concentration in tissues/ concentration in diet (Arnot and Gobas 2006).

Treatment CHEM	Diet concentration, ng CHEM/g dw	Dietary CHEM/day, µg offered to each tank of four fish	Dietary CHEM/28 day, µg offered to each tank of four fish	Dietary CHEM/28 day, ng offered per g dw fish	Sex	Fish concentration, ng CHEM/g dw	Bioaccumulation Factor	CHEM accounted for in fish tissue, %	Fish concentration, ng CHEM/g lipid
PCB153_control	13				f	24			
					m	34			
PCB153	12993	5	133	57192	f	13115	1.01	22.93%	66677
					m	14883	1.15	26.02%	102372
TBP _H _control	25				f	ND ¹			
					m	ND			
TBP _H _LO	139000	51	1422	611825	f	2319	0.017	0.38%	7748
					m	3314	0.024	0.54%	14089
TBP _H _HI	4360000	1593	44604	19191601	f	24738	0.006	0.13%	90145
					m	16174	0.004	0.08%	72245

¹/ND = Not Detected, where the limit of detection was 1 ng/g dw; of 8 female fish were measured, TBP_H was not detected in 7 fish and 1 fish contained 97 ng/g TBP_H.

Table 2:

Concentrations of TBPH reported in wild-caught biota and, for comparability, estimated in common concentration units (ng/g, body dry weight) as per Depew and coauthors (Depew et al. 2012).

Species	Tissue	Location	TBPH	Original concentration units ^{citation}	TBPH concentration, estimated ng/g body dw
Indo-Pacific humpback dolphin (<i>Sousa chinensis</i>)	Blubber	China	0.51	ng/g lipid weight ¹	2
Ring-billed gull (<i>Larus delawarensis</i>)	Liver	Canada	17.6	ng/g wet weight ²	70
Gastropod (<i>Elimea proxima</i>)	Whole	USA	380	ng/g wet weight ³	76
Bivalve (<i>Corbicula fluminea</i>)	Whole	USA	1370	ng/g lipid weight ³	274
Finless porpoise (<i>Neophocaena phocaenoides</i>)	Blubber	China	374	ng/g lipid weight ¹	1368

^{1.}(Lam et al. 2009);

^{2.}(Gentes et al. 2012);

^{3.}(La Guardia et al. 2012)