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Physiological effects of polybrominated diphenyl ether (PBDE-47) on pregnant gartersnakes and resulting offspring

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants and are persistent contaminants found in virtually every environment and organism sampled to date, including humans. There is growing evidence that PBDEs are the source of thyroid, neurodevelopmental, and reproductive toxicity. Yet little work has focused on how this pervasive contaminant may influence the reproduction and physiology of non-traditional model species. This is especially critical because in many cases non-model species, such as reptiles, are most likely to come into contact with PBDEs in nature. We tested how short-term, repeated exposure to the PBDE congener BDE-47 during pregnancy affected physiological processes in pregnant female gartersnakes (thyroid follicular height, bactericidal ability, stress responsiveness, reproductive output, and tendency to terminate pregnancy) and their resulting offspring (levels of corticosterone, bactericidal ability, and size differences). We found potential effects of BDE-47 on both the mother, such as increased size and higher thyroid follicular height, and her offspring (increased size), suggesting the effects on physiological function of PBDEs do indeed extend beyond the traditional rodent models.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are compounds used as flame retardants for industrial, commercial, and residential purposes (Birnbaum and Staskal, 2004). Concentrations of PDBEs have increased in human tissue by a factor of 100 since their first widespread use in the 1970s (Hites, 2004). Although companies began to phase out use of PBDEs in 2004, they are still released into the environment at a high rate through dissociation, disposal, and incineration (Gullett et al., 2009; Hale et al., 2003; Xu et al., 2009).

PBDEs have been found in high concentrations in nearly every environment surveyed, including atmosphere (Strandberg et al., 2001; Su et al., 2007), water (Hale et al., 2003; Qiu et al., 2010), and substrates such as soil and sediment, where they remain stable and long-lasting (Hale et al., 2003; Hites, 2004). Great attention has focused on PBDEs because of their prevalence in human blood and breast milk (Bi et al., 2006; Hooper and McDonald, 2000; Lorber, 2008; She et al., 2007). Numerous studies have documented presence and bioaccumulation of PBDEs in a variety of animals, including seals, sea birds, trout, frogs, invertebrates, raptors, and turtle eggs (Hale et al., 2003; Hall et al., 2003; reviewed in Law et al., 2003; Venier et al., 2010; Weijs et al., 2010) and potential for long-range atmospheric transport, especially in the lower brominated class of PBDEs, such as 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47; Boon et al., 2002; Gouin and Harner, 2003).

Although presence of PBDEs in wildlife is evident, relatively little research has been conducted on functional effects of PBDE exposure. There is evidence that the thyroid gland and its hormonal products are impacted by exposure to PBDEs (Costa et al., 2014; Hallgren and Darnerud, 2002; McDonald, 2002) which is likely due to the structural similarity of PBDEs to thyroxine (T_4; Meerts et al., 2000). Lower brominated PBDEs compete with and often displace T_4 when binding to the critical thyroid transport protein transthyretin (TTR; Meerts et al., 2000). This competitive binding may result in hypothyroidism because when target cells do not receive thyroid hormones, hypertrophy of the thyroid follicular cells can occur (Capen and Martin, 1989). Contaminant-induced hypothyroidism can affect a suite of characters, including metabolism, brain development, somatic growth, and development of reproductive organs (Cooke et al., 2004; Parrott et al., 1961; Rivera and Lock, 2008) in mammals (de Wit, 2002; Hallgren et al., 2001; Zhou et al., 2001), birds (Fernie et al., 2005b), fish (reviewed in Brown et al., 2004 and Carr and Patiño, 2011), amphibians (reviewed in Carr and Patiño, 2011) and likely

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other vertebrates, as thyroid hormones are highly conserved across vertebrate taxa (Hulbert and Else, 1981).

Due to the known effects on the thyroid, PBDEs may potentially affect other physiological processes. One key response in vertebrates to environmental challenges including contaminants is energy metabolism and secretion of glucocorticoids (GCs) through activation of the hypothalamic–pituitary–adrenal-axis (HPA; Franceschini et al., 2008; Gunderson et al., 2003; Hopkins et al., 1997; Lorenzen et al., 1999; Pruett et al., 2003; Romero and Wikelski, 2002; Sanders et al., 1974; Wingfield and Romero, 2001). Glucocorticoids, such as corticosterone (CORT), are important mediators in mobilization of energy during stress (Wingfield and Romero, 2001). Given that wild animals have limited energy stores, investing energy into coping with a stressor requires trade-offs with other physiological processes, such as reproduction and immune function (Bremner and Vermetten, 2001; Cooke et al., 2004; French et al., 2007; Suter and Schwartz, 1985; Tilbrook et al., 1999, 2000). Therefore, GCs may serve as an important link between of contamination and the health of the individual (Wingfield and Sapolsky, 2003).

Reproduction is one of the most energetically-costly life history stages for an individual (Gittleman and Thompson, 1988). Therefore, required increases in energetic requirements needed to cope with other stressful events such as contaminant exposure may cause a decrease or cessation in reproductive (Wingfield and Sapolsky, 2003) through spontaneous abortion or resorption of follicles and embryos (Clark et al., 1993; Mendola et al., 2008). Studies on rodent models have also indicated direct deleterious effects of BDE-47 on reproduction (Lilienthal et al., 2006; Talsness et al., 2008) and developing embryos (reviewed in Costa et al., 2008); however, wildlife have not been well investigated, despite a growing body of evidence suggesting negative effects in fish (Arkoosh et al., 2010; Lema et al., 2008; Muirhead et al., 2005), mammals (Hall et al., 2003), birds (Fernie et al., 2008), and amphibians (Van Schmidt et al., 2012).

When an organism is undergoing chronic stress and has an even more limited pool of resources due to physiological processes such as reproduction, measuring immune function is a critical component in determining the consequences of this stress. PBDEs have been implicated in decreased immune function in both birds (Fernie et al., 2005a) and humans (Leijs et al., 2009), although some studies show no immune consequences of PBDE exposure (Fernlöf et al., 1997). Innate immune function is a highly effective measure of immunocompetence because it relies on a rapid response to virtually all foreign pathogens (Janeway et al., 2001). Further, vertebrates have similar innate immune function, while humoral and cell-mediated responses are differ among taxa (Zimmerman et al., 2010).

Populations of reptiles are declining rapidly around the world, yet we know little about how reptiles respond to contamination (Gibbons et al., 2000; Hopkins, 2000; Sparling et al., 2010). Ecotoxicological research involving squamates (snakes and lizards) is limited (Hopkins et al., 1999; Murray et al., 2010; Ohlendorf et al., 1988). Snakes are important as ecotoxicological models because they are found in a wide variety of ecosystems, they are large enough for use in physiological tests, exhibit high site fidelity, are long-lived, and many species exist in abundance in contaminated environments (Beaupre and Douglas, 2009; Hopkins et al., 2002, 1999; Hopkins, 2000; Neuman-Lee et al., 2014). We studied the Western Terrestrial Gartersnake (*Thamnophis elegans*), which is found throughout the western United States and Canada (Rossman et al., 1996) across a diversity of different landscapes, are viviparous (live-bearing), dietary generalists, and their basic physiology has been well-studied, providing an important knowledge base (Moore et al., 2000; Robert and Bronikowski, 2010; Rossman et al., 1996; Sparkman and Palacios, 2009).

To determine potential effects of chronic BDE-47 ingestion on snakes, we examined a suite of physiological endpoints that influence individual health and reproductive capabilities in adult females. We quantified effects of BDE-47 on mass, thyroid morphology, stress-reactivity, ability to maintain gravidity throughout the reproductive season, and innate immunocompetence in adult female gartersnakes and size at birth, sex ratio, CORT levels, and innate immunocompetence in their developing neonates. We hypothesized that female snakes exposed to BDE-47 would show thyroid hypertrophy and chronically elevated CORT, which would correlate with lowered stress-reactivity, reproductive investment, and immune function. We also hypothesized that neonates exposed to BDE-47 in utero would be smaller, have higher CORT levels, and decreased immunocompetence.

2. Methods

2.1. Animal collection and care

Female gartersnakes (*T. elegans*) were collected in Cache Valley, Utah in April and May of 2012. We transported snakes back to Utah State University in opaque cloth bags and housed them individually in 37.8 l glass aquaria. Each aquarium was lined with newspaper and had a plastic hide with moist sphagnum moss. We supplied deionized water in glass dishes. The room was kept at 26 °C on a 12:12 on:off light cycle. Snakes were weighed every two weeks and given 20% of their body weight in thawed mice, alternating with weeks in which they were dosed. The mass of food consumed was recorded and uneaten mice were removed after 24 h. We confirmed large, developing follicles in snakes in late May (directly prior to dosing) using an ultrasound (Sonosite, MicroMaxx) and palpitation. We determined termination of pregnancy (resorption of follicles) by the complete absence of birthing (stillborn, unfertilized, or live born).

Snakes were randomly divided into one of two treatment groups (BDE-47 and vehicle control). To simulate ingestion of contaminated food items, snakes were weighed then orally gavaged with either corn oil ($n = 10$; control) or 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47, Sigma Aldrich, St. Louis, MO) in corn oil ($n = 9$) every two weeks. Snakes receiving BDE-47 were given 50 ng/g body weight each dosing period such that each female received 300 ng/g body weight by the end of the study. The chosen dose fell midway within the range of doses found in field settings in wild organisms (Hale et al., 2003; Law et al., 2003). Because no studies have yet been published on concentrations of PBDEs in wild snakes, we selected this dose based on studies conducted by Fernie et al. (2005a, 2005b) in which physiological effects on kestrels were observed. We, however, modified the dosing schedule slightly to better accommodate snakes' less frequent feeding strategy.

2.2. Bi-weekly blood sampling and stress-reactivity test

We took a blood sample every other week throughout the reproductive season (beginning in June) until August, such that there were six samples for each female. For the stress-reactivity test at the end of the experiment, post-parturition (or after the termination of pregnancy was confirmed) in August, we obtained the first sample as described below within three minutes (baseline). We then placed the snake individually in an opaque bag, and took a second blood sample after 30 min (stress-induced).

For all blood sampling, we obtained approximately 200 μl of blood sample via the caudal vein using a sterile syringe within three minutes of removing the snake from its cage. Samples were