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# Inhibition of metamorphosis in tadpoles of *Xenopus laevis* exposed to polybrominated diphenyl ethers (PBDEs)

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## Abstract

Tadpoles of the African clawed frog, *Xenopus laevis* were exposed, beginning at stage 50, to a commercial pentabromodiphenyl ether mixture (DE-71) through the diet. Subsequent experiments were conducted using a single intraperitoneal injection at stage 58 with limited quantities of two purified brominated diphenyl ether (BDE) congeners, BDE47 and BDE99 and DE-71 to determine the relative potency of these BDE congeners within the commercial mixture. Significant inhibition of tail resorption, delayed metamorphosis and impacts on skin pigmentation were observed in *Xenopus* exposed to DE-71 in the diet at nominal doses of 1000 and 5000 μg $g^{-1}$ of food. The estimated time required for 50% of the tadpoles to complete metamorphosis was significantly lengthened in *Xenopus* exposed to a dietary concentration of 1 μg DE-71 per gram of food. Analysis of PBDEs (sum of 32 congeners) in *Xenopus* from the treatment with 5000 μg $g^{-1}$ of DE-71 indicated that the frogs accumulated an average of 1030 μg $g^{-1}$ (wet weight) of PBDEs. In the intraperitoneal injection trials, similar inhibitory responses were observed in *Xenopus* injected with DE-71 at a nominal dose of 60 μg per tadpole, or injected with BDE47 at a nominal dose of 100 μg per tadpole. No responses were observed in *Xenopus* injected with BDE99 at doses up to 100 μg per tadpole. Complete inhibition of metamorphosis was observed only in the highest DE-71 dietary treatment. The results of this study are consistent with a mechanism of action of PBDEs involving competitive inhibition of binding of thyroid hormones to transporter proteins, although the mechanism cannot be definitively determined from this study. The observed effects may have occurred through other mechanisms, including sublethal toxicity. The doses used in this study are greater than the levels of PBDEs to which anurans are exposed in the environment, so further studies are required to determine whether exposure to PBDEs at environmentally relevant concentrations can affect frog metamorphosis.


*Keywords:* *Xenopus*; Tadpole; Brominated flame retardant; Metamorphosis; Thyroid hormones

## 1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been detected in most environmental compartments, including sediments, invertebrates, amphibians, fish, birds, mammals and human breast milk, and are the subject of several recent reviews (de Wit, 2002; Alaee et al., 2003; Hakk and Letcher, 2003; Law et al., 2003; Sjödin et al., 2003; D'Silva, 2004; Law et al., 2004). The concentrations of this class of brominated flame retardants (BFRs) continue to

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rise in the environment, particularly within marine biota of the Canadian arctic (de Wit, 2002; Law et al., 2003). However, the recent decision by the European Union (EU) to cease production and use of the pentaBDE commercial formulations (Kemmlein et al., 2003) may mean that concentrations of these compounds will decline with time. Apart from decreasing PBDE concentrations in Guillemot eggs from the Baltic Sea (Sellström et al., 2003) and sea bass and grey mullet from Osaka Bay, Japan (Ohta et al., 2001) a trend towards declining concentrations is not yet widely evident in environmental samples (Law et al., 2003; Law et al., 2004). In North America, the only manufacturer of the pentaBDE and octaBDE commercial formulations, Great Lakes Chemical Corporation, has announced a voluntarily phase-out of both products by the end of 2004 (Moss, 2004). In addition, both the USA and Canada are making efforts to regulate the manufacturing, importation and use of these products in North America (Gutzman et al., 2004; Moss, 2004). However, there is still concern that PBDE levels in the environment will continue to rise as they are released from unregulated sources such as through disposal of electronic equipment (Watanabe and Sakai, 2003).

While there are data on the concentrations of PBDEs in the environment, much remains to be determined regarding the biological effects of these chemicals. A recent review by Hakk and Letcher (2003) summarized the current understanding regarding the metabolism of different classes of BFRs, including PBDEs and the influence that metabolism has on the toxicokinetics and fate of these compounds. Studies with mammalian models have shown that PBDEs are biologically transformed through the activities of CYP enzymes to generate hydroxy-metabolites that appear to have a greater potential for toxicity than do the parent congeners (Meerts et al., 2000; Zhou et al., 2001; Hallgren and Darnerud, 2002; Zhou et al., 2002; Chen and Bunce, 2003; Stoker et al., 2004).

A significant proportion of the research regarding the mechanisms of action of PBDEs has focused on the ability of hydroxylated metabolites of specific congeners, such as BDE47, to bind competitively to thyroid transport proteins, which results in reduced levels of total and free thyroid hormones in serum (Meerts et al., 2000; Hallgren et al., 2001; Zhou et al., 2001; Hallgren and Darnerud, 2002; Zhou et al., 2002). However, binding to thyroid transport proteins is only one of multiple mechanisms which can influence thyroid homeostasis. Other potential mechanisms of disruption to the thyroid system from chemical contaminants include effects on thyroid gland function and regulation, plus disruption to thyroid hormone metabolism such as sulfation, deiodination and glucuronidation (Legler and Brouwer, 2003). Recent reviews regarding the toxicology of PBDEs (Darnerud, 2003; Legler and Brouwer, 2003) have also indicated that some congeners have the potential to disrupt other endocrine and physiological systems besides the thyroid, resulting in potential impacts to reproduction, development and behaviour.

Toxicological studies are needed to assess the risks to environmental health posed by PBDE compounds (Darnerud, 2003). In this study, an experimental model with the African clawed frog (*Xenopus laevis*) was used to assess the potential of a commercial pentaBDE formulation (DE-71) and the two most prominent PBDE congeners present in this commercial mixture (BDE47 and BDE99) to disrupt tadpole development. *Xenopus* and other anurans have been used to assess the developmental effects of a variety of other xenobiotics, including bisphenol A (Iwamuro et al., 2003), PCBs (Gutleb et al., 2000), atrazine (Carr et al., 2003), diethylstilbestrol (Yamauchi et al., 2000), ammonium perchlorate (Goleman et al., 2002), and dieldrin (Schuytema et al., 1991; Suwalsky et al., 2002). In the tadpoles of anurans, tail resorption and metamorphosis are under the control of thyroid hormones (Kawahara et al., 1991; Brown et al., 1996; Tata, 1998; Huang et al., 2001). However, several of the compounds shown previously to have effects on tadpole development did not induce these effects through disruption of thyroid homeostasis. To our knowledge, this is the first study to assess the biological impacts of PBDEs in an anuran species.

## 2. Methods and materials

### 2.1. *Xenopus husbandry*

Tadpoles of the African clawed frog originated from breeding pairs of frogs maintained at Trent University, which were induced to spawn by subcutaneous injections of chorionic gonadotropin (NASCO, Fort Atkinson, WS, USA). Spawning was accomplished by injection of gonadotropin (150 IU) into a male on the morning of day one. On the morning of day two, the male was re-injected with an additional 150 IU and a female was injected with 250 IU, followed by a second injection into the female of 500 IU at the end of day two. Once the final set of injections was completed, the male and female frogs were placed together in a rectangular polyurethane tub fitted with a bottom screen and a lid to ensure seclusion. The tub contained dechlorinated tap water maintained at a temperature of $25 \pm 2$ °C. The next morning, fertilized eggs were collected from under the screen and separated into groups of approximately 100 eggs and placed into polyurethane tubs containing 4 l of gently aerated dechlorinated tap water ranging in pH between 7.4 and 7.8, alkalinity of 60–80 mg $CaCO_3$ and hardness of 80–100 mg $l^{-1}$ $CaCO_3$. The temperature was maintained at $23 \pm 2$ °C and the photoperiod was 16 h light: 8 h dark.

Tadpoles hatched approximately 2 days after fertilization and were maintained in dechlorinated tap water under the same environmental conditions for the remainder of the study. Food was introduced to the tadpoles approximately 2–3 days post-hatch, corresponding to the start of exogenous filter feeding. During the first week after hatch, tadpoles were fed Heinz™ strained peas by adding a 2–3 ml