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Early Exposure to 2,2′,4,4′,5-Pentabromodiphenyl Ether (BDE-99) Affects Mating Behavior of Zebra Finches

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2,2′,4,4′,5-Pentabromodiphenyl ether (BDE-99) is a brominated flame retardant congener that has pervaded global food chains, being reported in avian egg and tissue samples throughout the world. Its effects on birds are not well known, but there is evidence in exposed mammals that it directly mediates and causes neurotoxicity, alters thyroid hormone homeostasis, and lowers sex steroid hormone concentrations. In birds, those processes

there is evidence that many of the neurons that project into the song-control nuclei have thyroxine (T4) receptors, and exposure to abnormally elevated T4 levels increases cell death in song-control nuclei of zebra finches (Tekumalla ., 2002). Disruption of thyroid hormone homeostasis by BDE-99 could therefore alter the cell number and volume of the song-control nuclei. The song-control nuclei could also be affected by any changes in sex steroid hormones caused by BDE-99, as several of the song-control nuclei contain androgen and estrogen receptors, and decreased levels of sex steroids can result in smaller nuclei (Ball ., 2002). Despite evidence that BDE-99 disrupts many of the mechanisms that can affect the song-control system, there have been no studies investigating possible PBDE-induced effects on the song-control system of birds.

Reduction in song-control nuclei volume as a result of BDE-99 exposure could lead to lowered song quality and/or song perception, as the volumes of key song-control nuclei correlate with male song characteristics, such as song complexity and duration (Garamszegi and Eens, 2004), and the song-control nuclei can also play a role in song perception in females (Brenowitz, 1991). Singing is an important aspect of reproduction in birds, serving to define territories and attract females (e.g., Krebs ., 1978; Kroodsma, 1976; Searcy, 1992). Learned features of song can act as an honest signal of male quality (Nowicki ., 2002), and females of many species prefer males with higher song rate (Collins ., 1994) and more complex and longer songs (Clayton and Prove, 1989). Developmental conditions that result in smaller song-control nuclei, and reduced song quality in males, or altered song perception in females, could ultimately disrupt pair formation and lower reproductive success.

If BDE-99 lowers sex steroid hormones in birds as it does in mammals, then we would also expect exposed male birds to exhibit decreased mating behavior as androgens and estrogens play an important role in mediating sexual behaviors in birds (Ball and Balthazart, 2004). There are reports suggesting that penta-BDE technical mixtures containing BDE-99 may reduce reproductive behavior and circulating testosterone in male birds (Fernie ., 2008; Marteinson ., 2011), but no studies have specifically looked at the BDE-99 congener.

We investigated the effect of early developmental exposure to environmentally relevant levels of BDE-99 in birds, using the zebra finch ( ) as a model songbird species. The zebra finch is a well-established model species that has been extensively studied in avian neuroscience and endocrinology (e.g., Ball ., 2002) and has been successfully used in toxicological dosing studies (e.g., Hoogesteijn ., 2008). Our

50.7 ng/g = 6, 173.8 ng/g = 6). Birds were orally dosed between day 1–21 posthatching. Once young were independent from parents (day 30), they were placed into cages (102 3 39 3 43 cm) as juvenile groups and separated by sex once adult plumage and bill color started to form. Two adult male song tutors were placed in each juvenile cage containing males, and birds were not visually or acoustically isolated from birds in adjacent cages. Blood samples were collected at 30 and 90 days of age.

Once birds reached sexual maturity (day 90), male mating trials were conducted. For each exposed male, two mating trials were conducted over two separate days. At the start of each mating trial, an experienced clean wild-type female was randomly chosen from a pool of 60 females and placed in a cage for 5 min to acclimate alone. Different females that were novel to the experimental male were chosen for each male and trial. The cage contained two perches, grit, a cuttlefish bone, but no water or food inside. A microphone was positioned in the upper right corner of the cage. For each trial, an experimental male was placed in the cage with the experienced female, and the behaviors of both the male and the female were recorded for 10 min by an observer blind to treatment. All of the courtship trials were performed between 09:00 and 12:00 h. The following typical male courtship behaviors (described in Zann, 1996) were recorded during the trial: invitation (Y or N), bill wiping (number of wipes against perch), head or tail twisting (scored per left to right cycle), following (number of times the male followed the female), number of copulation attempts, number of successful copulations, and time in seconds to initial copulation attempt. The female response to the male was also recorded (scored 1–5; 1 = no response, 5 = solicitation of copulation). All songs were digitally recorded during the male mating trials using a Sennheiser ME62 microphone with a K6 p (mating)-e

(Agilent technologies, Palo Alto, CA). The analytical column was a 15 m 3 0.25 mm 3 0.10 μm DB-5HT fused-silica column (J & W Scientific, Brockville, ON, Canada). Helium and methane were used as the carrier and reagent gases, respectively. A sample volume of 1 μl was introduced to the injector operating in pulsed splitless mode (injection pulse at 25.0 psi until 0.50 min; purge flow to split vent of 96.4 ml/min to 2.0 min; gas save flow of 20 ml/min at 2.0 min), with the injector held at 240°C. The GC oven ramping temperature program was as

repeatable ($F_{16,17} = 0.53$, $= 0.897$, 0%) and so was not considered in further analysis. BDE-99 dosing had no effect on phrase duration ($F_{4,22} = 0.75$, $= 0.568$) or repertoire size ($F_{4,22} = 1.06$, $= 0.398$).

Male behaviors that were repeatable across mating trials include the number of copulation attempts ($F_{47,48} = 3.46$, < 0.0001, 55.2%), the number of bill wipes ($F_{47,48} = 5.58$, < 0.0001, 69.6%), whether the male invited the female (F_{47}Td(p)Te1.0015 --5.4 (8w220.0r)-1.2007 Tdl.9 7220. (00)-4(0.)-4.226196 0.1 (t)-1.3 (he)-448.95Tc 9.962.0235 Tm0.

technical mixtures may reduce reproductive behavior and circulating testosterone levels (Fernie ., 2008; Marteinson ., 2011). In the present zebra finch study, male mating behavior was affected by BDE-99 exposure, but the male song-control system was not; this suggests that the neural circuits that underlie the expression of male sexual behavior may be more sensitive to changes in sex steroids than the song-control nuclei. Future work should examine the effects of early BDE-99 exposure on brain regions controlling sexual motivation and the motivation to sing, such as the medial preoptic area of the hypothalamus (Riters and Ball, 1999).

In mating trials, just the males had been exposed to BDE-99, but there were still effects of treatment on female behavior. Clean experienced female zebra finches paired with BDE-99-exposed males responded the least to the highest dose group, which is likely a consequence of the reduced singing and courtship behavior exhibited by males in the higher dose groups. The behavioral changes caused by BDE-99 could potentially lead reduced reproductive success. Observations in captive American kestrels (*F*) studies have shown that unexposed females lowered their investment in the number and size of eggs laid when paired with penta-BDE–exposed males that exhibited reduced reproductive behavior, such as fewer copulations and fewer mating calls (Marteinson ., 2010).

In conclusion, this study shows that early exposure to BDE-99 has adverse long-term effects on the behavior of zebra finches. Although previous studies in birds have looked at the effects of PBDEs on growth, physiology, and reproduction (e.g., Fernie ., 2005, 2006; Marteinson

Gauthier, L. T., Hebert, C. E., Weseloh, D. V. C., and Letcher, R. J. (2008). Dramatic changes in the temporal trends of polybrominated diphenyl ethers