EXAMINATION OF BDE AND OH-BDE IN VITRO EXPOSURE ON ANDROGEN PRODUCTION IN MALE BROWN TROUT (SALMO TRUTTA) TESTES USING LC/MS/MS

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Introduction

Polybrominated diphenyl ether (PBDE) flame-retardants are lipophilic persistent organic compounds used in manufacturing of plastics, electronic equipment, polyurethane foam and textile materials. PBDEs have become an increasingly important environmental problem due to their ability to bioaccumulate and biomagnify. Since the late 1970's, substantial increases of these compounds have been measured in wildlife and human adipose tissue, with 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2´,4,4´,5-pentabromodiphenyl ether (BDE-99), and 2,2´,4,4´,6-pentabromodiphenyl ether (BDE-100) as the leading PBDE congeners found. The fully brominated, 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-209), is the predominant congener of the DecaBDE commercial formulation. Recent studies have focused on the potential endocrine disrupting properties of PBDEs, which include estrogenic/antiestrogen activity and effects on thyroid hormones, but very little work has been done to explore possible effects on androgen biosysthesis and activity. A study by Gregoraszczuk et al. (1) showed that BDE-47, 99, 100 and 209 increased testosterone (T) production in a porcine thecal cell *in vitro* assay; suggesting induction of aromatase (CYP17) and 17β-hydroxy steroid dehydrogenase activity. It has also been suggested that BDE-47, 99 and 100 can act as androgen receptor (AR) inhibitors by competing for binding sites (2, 3). The hydoxylated metabolite 6OH-BDE-47 also demonstrated antiandrogenic activity in the Harju et al. study. Other work with the hydroxylated metabolites of BDE-47 found that 3OH, 5OH and 6OH showed significant antiandrogenic activity by reducing the DHT-induced response in the yeast human androgen receptor (hAR) assay (4).

Very little is known about the action of BDEs on fish reproductive systems, particularly in male fish. To address this deficiency, the effects of environmentally relevant concentrations of BDE-47, 99, 100 and 209, as well as the 3OH, 5OH and 6OH hydroxylated metabolites of BDE-47, on the *in vitro* steroidogenic capacity of brown trout (*Salmo trutta*) testicular tissue were examined.

Material and Methods

Chemicals. Native and D_8 mass labelled hormones were purchased from Cambridge Laboratories and Steraloids. BDEs 47, 99, 100, 209 and 3OH-, 5OH-, OH6-BDE-47 were purchased from Wellington Laboratories. OmniSolv methanol and water were from VWR and chemicals for the Cortland's incubation medium were from Sigma-Aldrich Canada.

Fish. Pre-spawning brown trout (*Salmo trutta*) were obtained from Westhawk Fish Hatchery, Westhawk, Manitoba, Canada. Six males (1.33 ± 0.1 kg and 45.2 ± 0.5 cm) were sacrificed, weighed and measured. Gonadal tissues were removed, weighed and placed in Cortland's incubation medium (pH 7.6) on ice.

In vitro incubations. In vitro incubations were performed as outlined by McMaster et al. (1996). Briefly, 20 mg of testicular tissue were placed in each well of the 24-well culture plates with 1 mL of Cortland's medium. BDE-47, 100 and 209 were added to the wells in 5 µL of acetone carrier at the following concentrations: 0, 100, 500, 1000, 3000, and 5000 pg/µL. The hydroxylated compounds were each added at concentrations of 10, 50, 100, 500 and 1000 pg, also in 5 µL acetone carrier. Each treatment was duplicated under basal and stimulated conditions using 100 IU human chorionic gonadotropin (hCG), and acetone only solvent controls were added for each individual fish. The plates were incubated at 12.0 ± 0.5 °C for 24 hours. The incubation medium from each well was collected and stored separately at -80 °C until analysis.

Extraction and LC/MS/MS analysis. Samples were first thawed, and vortexed to ensure homogeneity. 850 µ L of sample was spiked with labeled recovery internal standards; D8-Progesterone (positive ion mode) and d4-

Estrone (negative ion mode). Hormones were extracted using a hexane:ethyl acetate, liquid:liquid method developed in our lab. Final extracts were spiked with d4-Estradiol as the internal precision standard. Testicular tissue incubations were analyzed for Testosterone (T), T-sulfate (T-S), T-Glucuronide (T-G), and 11-Ketotestosterone (11-KT).

An Agilent 1100 HPLC was coupled to an API 2000 triple quadrapole mass spectrometer (Sciex), and separations were achieved using a Grace (50 x 2.1 mm), 4µm C_{18} column. The mass spectrometer was operated in the ESI –ve ion mode for T-S analysis, while Testosterone, 11-KT and T-G were analyzed in +ve ion mode.

Results and Discussion

Preliminary analyses shows that there was no significant differences from the controls in 11-Ketotestosterone production by male testicular tissues incubated with BDE-47 or 6OH-BDE-47. Basal testosterone glucuronide (T-Gluc) production increased with the addition of BDE-47, however it was not significantly different from the controls (p=0.142), and there were no differences under stimulated conditions (p=0.697).

hCG stimulated testosterone production was elevated with increasing BDE-47 concentrations, but was not significant (p=0.144) (Figure 1). The metabolite, 6OH-BDE-47, appears to have a greater androgenic effect than the parent compound as tissues treated with it under hCG stimulated conditions produced significantly (p=0.003) more testosterone than the controls (Figure 2). This suggests potential CYP 17 or 17β-hydroxy steroid dehydrogenase induction.

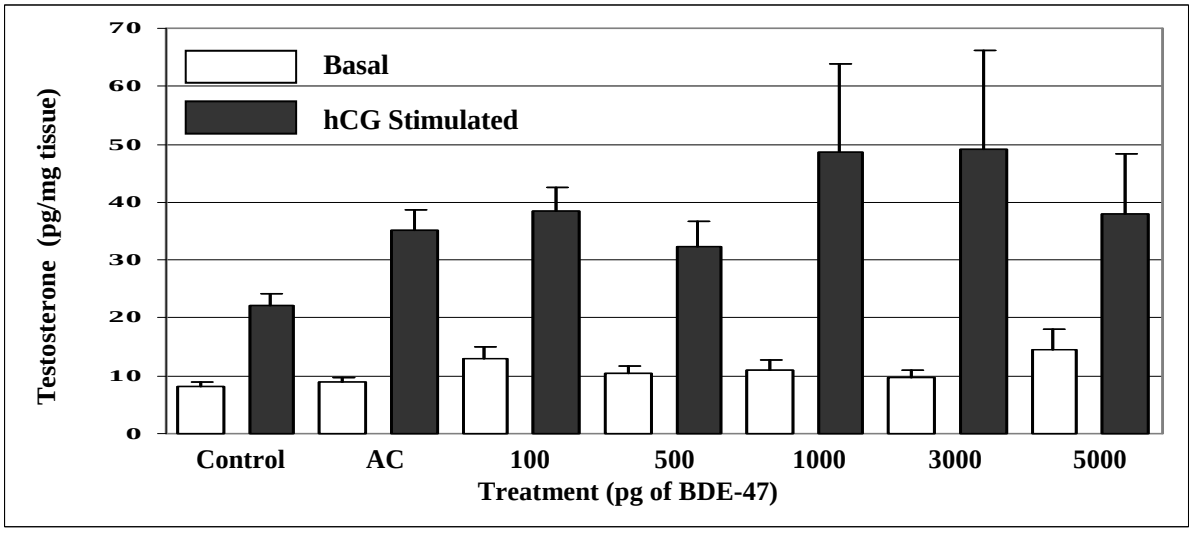

Figure 1: Testosterone production by male brown trout testicular tissue incubated with increasing concentrations of BDE-47.

The 6OH-BDE-47 metabolite also induced a significant increase in T-Gluc production under basal conditions (p=0.007), and an increase at the 10 pg concentration (p=0.058) for the hCG stimulated conditions (Figure 3). The increase in the glucuronidation of testosterone from testicular tissues incubated with 6OH-BDE-47 indicates the activation of UDP-glucuronosyltransferase (UDPGT or UGT) Phase II enzymes. It also suggests that 6OH-BDE-47 is not competing with testosterone as a substrate for UDP-glucuronosyltransferase (UDPGT or UGT). Stoker et al. (5) reported that *in vivo* exposure to BDE-71 resulted in an increase in UDPGT activity together with a decrease in plasma thyroid hormone (T4) levels. T4 is conjugated with glucuronic acid in a reaction catalyzed by UDPGT before being excreted into the bile. This suggests that 6OH-BDE-47 could have similar effects on plasma testosterone levels in exposed males.
Results from BDE-99, 100, 209 and the other BDE-47 hydroxylated metabolites on androgen steroidogenesis by exposed brown trout testicular tissue will also be discussed.

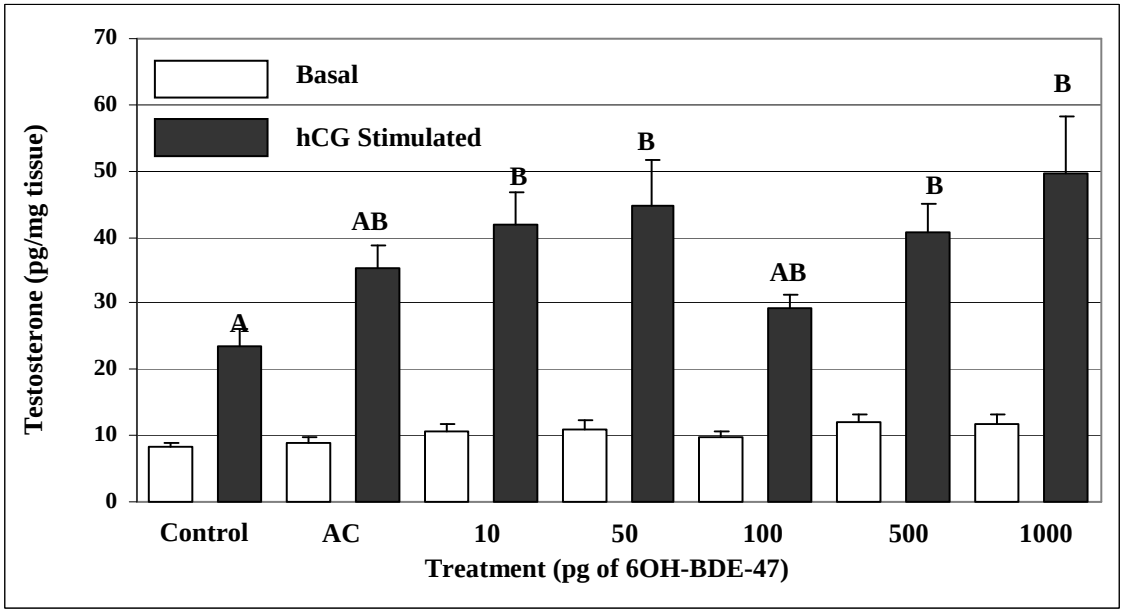

Figure 2: Testosterone production by brown trout testes incubated with 6OH-BDE-47

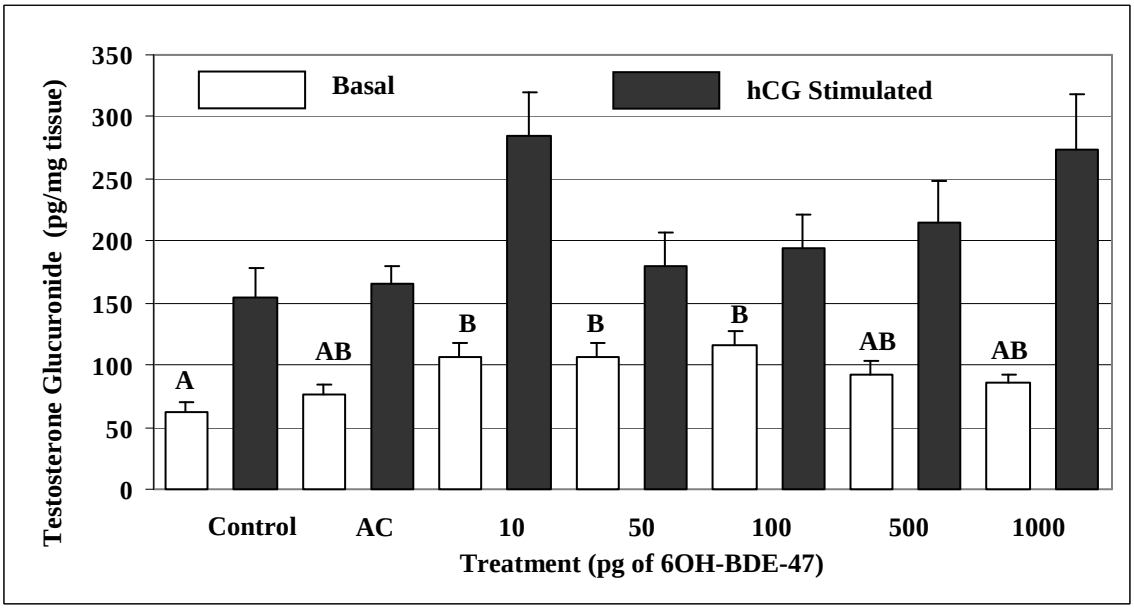

Figure 3: Testosterone glucuronide production by brown trout testicular tissues incubated with increasing concentrations of 6OH-BDE-47

References

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