GESTATIONAL AND LACTATIONAL 2,2',3,3'4,4',5,5',6,6'-DECABROMODIPHENYL ETHER (BDE-209) EXPOSURE IN MICE: DEVELOPMENT AND TESTICULAR FUNCTION AT F_2 GENERATION

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Introduction

Decabrominated diphenyl ether (BDE-209), one of 209 congerners of polybrominated diphenyl ethers (PBDEs), has many applications in products such as electronic enclosures and upholstery textiles and foams[1]. PBDEs may leach into the environment when the products are manufactured, used or disposed[2]. PBDEs are persistent and bioaccumulative compounds[3] and have been found not only in the environment[4] , but also in blood of pregnant women[5] and breast milk[6]. In a study of the reproductive effects of penta-BDE on mice, Kuriyama et al. (2005) reported that *in utero* exposure of a single low dose (60 μg/kg) resulted in significant decreased sperm counts in adult offspring[7]. Another study further indicated that prenatal exposure to penta-BDE reduced testosterone and anogenital distance (AGD) in adult male rat offspring[8]. PBDEs show an even closer structural relationship to serum thyroxine (T_4) than polychlorinated biphenyls (PCBs), allowing them to bind competitively to thyroid hormone transfer proteins and act as endocrine disrupters[9]. Serum triiodothyronine (T_3) was found to have decreased significantly in offspring, but not T_4 in F_1 offspring prenatally exposed to BDE-209[10]. Not only do PCBs and other environmental endocrine disrupting chemicals directly affect the exposed individual, but they might exert alterations on subsequent generations that differ from those associated with primary exposure[11, 12]. To our knowledge, the specific effects of fetal exposure of the F_1 females on the reproductive physiology of their adult male F_2 offspring has never been examined in this context, and the objective of this study is to investigate the effects of prenatal and lactational exposure to BDE-209 on development and testicular DNA content of the F_2 generations.

Materials and methods

Animals and treatment. The pregnant CD-1 mice were gavaged with corn oil and 500 mg/kg BDE-209 in corn oil per day throughout pregnancy and lactation until their offspring were at postnatal day (PND) 20. This dose levels was used to cause reproductive adverse affects in the male offspring without maternal toxicity or fetal death[13]. The F_1 male offspring were mated with F_1 generation females from different litters on PND 70. Male adult F_2 offspring were divided to four groups as follows: CC group (control F_1 fathers + control F_1 mothers), CE group (control F_1 fathers + exposed F_1 mothers), EC group (exposed F_1 fathers + control F_1 mothers), and EE group (exposed F_1 fathers + exposed F_1 mothers). Before wearning, the sex ratio, total numbers of offspring, developmental landmarks including pinnae detachment, body hair fuzz appearance, incisor eruption, ear opening, and eyes opening as well as the offspring weight and anogenital distance (AGD) were recorded in F_2 offspring. After weaning, the body weight and AGD were measured once every three days in male offspring. F_2 male offspring were sacrificed at PND 70 to evaluate the developmental landmarks and DNA content of testis cells.

DNA content analysis of testis cells

Testis monocellular suspensions were prepared according to the procedure described by Span`o et al. (1996) [14]. The fluorescence intensity of DNA content of the testis cells was measured using a FACScan flow cytometry. PI

fluorescent emissions were monitored using a 620 nm band-pass filter. A total of 2×10^4 cells were collected for each sample. Based on the DNA content, main germ cell peaks can be classified into four categories: (1) mature haploid (elongated spermatids; stages XXIV), (2) immature haploid (round and elongating spermatids; stages I-IX), (3) diploid (spermatogonia, secondary spermatocytes, tissue somatic cells), and (4) tetraploid (mostly primary spermatocytes). The region between the diploid and tetraploid peaks, called the S-phase, is comprised of cells that actively synthesize DNA. The relative proportions of haploid, diploid, S-phase and tetraploid cell types were calculated.

Data analysis. Data were expressed as means ± standard error of the mean (SEM). For the analysis of F_2 offspring, the litter was considered the experimental unit. Body weight, developmental landmarks, and the relative proportions of testicular cell types were compared using one-way analysis of variance (ANOVA) with post hoc comparisons among each group followed by the Tukey–Kramer honestly significant difference (HSD) and a p value of <0.05 was considered statistically significant.

Results and discussion:

Reproductive parameters and developmental landmarks

As shown in Table 1, no significant differences in numbers of live pups, male sex rate, and developmental landmarks for the F_2 offspring were observed between the control and BDE-209-treated groups.

Body weight and adjusted AGD of maleF_2 offspring.

We found no significant differences between the BDE-209-exposed group and the controls in male F_2 offspring from PND 21 to PND 70 with regard to body weight and adjusted AGD (Figure 1).

DNA content analysis of testis cells

The cell frequencies of various testis subpopulations were expressed as percentage of total histogram area (Figure 3). The percentage of round spermatids was significantly decreased and the percentage of S-phase cells was significantly increased in the EE group as compared with CC group ($P < 0.5$) (Figure 3). However, there were no significant changes in testis DNA content of the percentage of elongated spermatids, diploid, and tetraploid cells.

Discussion. Developmental exposure to BDE-209 might induce sperm-head abnormality, oxidative stress, chromatin DNA damage, and testicular histopathological changes in F_1 male offspring[13]. Prenatal exposure to BDE-99 from GD 10 to GD 18 is reported to result in a reduction of AGD and testosterone levels in adult male offspring[8]. In mice and rats, *in utero* exposure to cytotoxic drugs or toxic substances induces permanent reductions in the number of germ cells in the offspring without affecting phenotypic sex differentiation. F_1 offspring prenatally exposed to PCBs might induce the decrease of sperm count[15, 16]. Our experiments extended analyses to the F_2 offspring in order to assess possible initiator of transgenerational effects, which have been reported for several species[17], with other environmental toxicants[18], and for the pharmaceutical, diethylstilbestrol (DES). Genotoxic effects on the germ line might explain the trend for decreased numbers of male offspring in the F_2 generation, because male fetuses are more susceptible to detrimental genomic errors[19]. Thus, altered the distribution of testicular cell types in F_2 generation-exposed male are most likely due to epigenetic patterning, either by gene promoter alteration transmitted to F_2 offspring.

Conclusion. This is the first study to demonstrate the effects of BDE-209 on DNA content of testis cells in male F_2 offspring. Thus, BDE-209, while banned in some countries, should continue to be scrutinized for their persistent and latent risks to reproductive health in humans and wildlife.

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Table 1. Numbers of live pups, male sex rate, and developmental landmarks for the F_2 offspring.

Parameters	CC group (N = 6)	CE group (N = 6)	EC group (N = 6)	EE group (N = 6)
Number of live pups per litter	11.0 ± 2.0	12.8 ± 2.8	12.7 ± 1.4	10.5 ± 2.1
Male sex rate (%)	47.5 ± 16.5	47.5 ± 16.5	47.5 ± 16.5	47.5 ± 16.5
Pinna detachment (day)	3.3 ± 0.2	3.3 ± 0.2	3.6 ± 0.2	3.5 ± 0.2
Body hair fuzz appearance (day)	1.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Incisor eruption (day)	9.5 ± 0.1	10.1 ± 0.1	9.7 ± 0.1	10.0 ± 0.1
Eye opening (day)	13.4 ± 0.3	13.3 ± 0.3	14.2 ± 0.3	13.4 ± 0.3
Ear opening (day)	11.0 ± 0.4	12.8 ± 0.4	12.7 ± 0.4	13.5 ± 0.4

N: Numbers of litter; all data are shown as mean ± SEM.

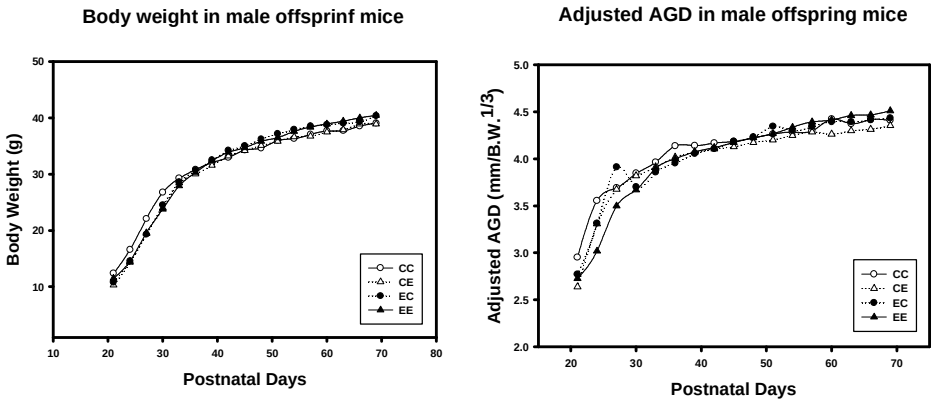

Figure 1. Body weight and adjusted AGD in the male F_2 offspring. Data was presented as group means.

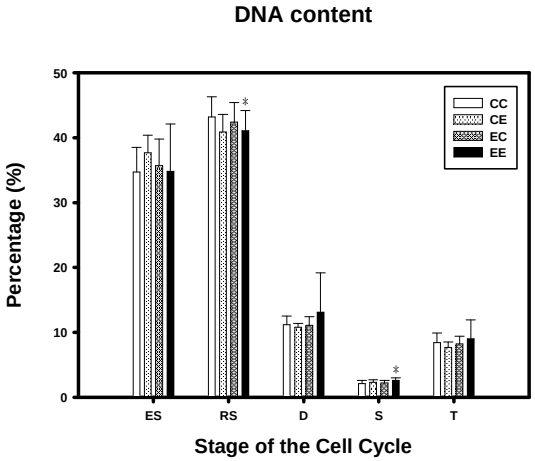

Figure 2. Relative fractions (means ± SEM) of the various testis subpopulations, calculated as percentage of the histogram area in the male F_2 offspring. Data was presented as group means. *: $P < 0.05$ as compared with CC group; ES: elongated spermatids; RS: round spermatids; D: diploid; S: S-phase; T: Tetraploid.