

## Decreased Sperm Number and Motile Activity on the F1 Offspring Maternally Exposed to Butyl *p*-Hydroxybenzoic Acid (Butyl Paraben)

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**ABSTRACT.** Butyl *p*-hydroxybenzoic acid (butyl paraben, BP) is widely used as a preservative in food and cosmetic products. Routledge *et al* showed that BP is weakly estrogenic in both *in vitro* and *in vivo* (rat uterotrophic) analyses. We investigated whether maternal exposures to BP during gestation and lactation periods affected the development of the reproductive organs of the F1 offspring. Pregnant Sprague-Dawley rats were injected subcutaneously with 100 or 200 mg/kg of BP from gestation day (GD) 6 to postnatal day (PND) 20. In the group exposed to 200 mg/kg of BP, the proportion of pups born alive and the proportion of pups surviving to weaning were decreased. The body weights of female offspring were significantly decreased at PND 49. The weights of testes, seminal vesicles and prostate glands were significantly decreased in rats exposed to 100 mg/kg of BP on PND 49. In contrast, the weights of female reproductive organs were not affected by BP. The sperm count and the sperm motile activity in the epididymis were significantly decreased at doses of 100 and 200 mg/kg of BP. In accordance with the sperm count in the epididymis, the number of round spermatids and elongated spermatids in the seminiferous tubule (stage VII) were significantly decreased by BP. Testicular expression of estrogen receptor (ER)- $\alpha$  and ER- $\beta$  mRNA was significantly increased in 200 mg/kg of BP treated group at PND 90. Taken together, these results indicated that maternal exposure of BP might have adverse effects on the F1 male offspring.

**KEY WORDS:** butyl *p*-hydroxybenzoic acid, male reproductive organ, maternal exposure, sperm.

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Recently, many synthetic or natural chemicals (environmental estrogens) have been implicated in a number of human reproductive deficits. These deficits include decreased sperm production and an increased incidence of hypospadias and cryptorchidism in human populations [36, 46]. It is now realized that certain synthetic compounds, included in a variety of products, can mimic the effects of the main natural estrogen, 17 $\beta$ -estradiol (E2), by binding to the estrogen receptor (ER) and influencing the expression of estrogen-dependent genes [28, 30]. This finding has coincided with epidemiological reports showing a progressive decline in human male reproductive health and fertility [3, 4].

*p*-Alkylphenols, breakdown products of surfactants, detergents, toiletries, and herbicides, have been found in sewage effluent. They competitively bind to the estrogenic receptor, and enhance mammalian breast cancer cell proliferation [23, 49]. They induce an estrogenic response in fish and rats as does E2, but these chemicals are 1000 times less potent than E2 [2].

Parabens, compounds with anti-bacterial and anti-fungal properties, are extensively used as food preservative and cosmetics. In 1981, the Food and Drug Administration reported the use of methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), and butyl paraben (BP) in over 13,200 formulations. Parabens are frequently used as preservative in cosmetics and toiletries due to their low toxicity, inertness, broad spectrum of activity, worldwide

legislative acceptance, biodegradability, and cost (inexpensive), safety [7]. Parabens are structurally similar to alkylphenols which are known to be weakly estrogenic [13, 30, 31, 40]. Routledge *et al.* reported that the four most widely used parabens (namely MP, EP, PP, and BP) were all found to be weakly estrogenic and the most potent compound among them was BP as shown in *in vitro* yeast-based estrogen assay [28, 30].

Therefore, we hypothesized that BP exposure during pregnancy and lactation might cause adverse effects on the reproductive organs of the F1 offspring.

### MATERIALS AND METHODS

**Animals:** This study was conducted under the U.S. federal guidelines for the care and use of laboratory animals [26] and was approved by the SNU Animal Care and Use Committee. Twenty-two female Sprague-Dawley (SD) rats (9 weeks of age) were purchased from Samyuk Laboratory Animal Institute (Osan, Korea). The female SD rats were then allowed to mate with male SD rats. Insemination was identified by the presence of sperm-plug in the vagina, and the day following the overnight mating was designated as gestation day (GD) 1. After mating, animals were randomly assigned to experimental group ( $n=6-8$  dams per group), and were housed in the laboratory animal facility that was maintained on a 12-hr light-dark cycle at approximately 19–25°C with relative humidity of 30–70%. Pregnant rats were housed individually and pups were housed in polycarbonate cages. Animals had free access to rodent purified diet (Purina Co. Ltd., Korea) and tap water.

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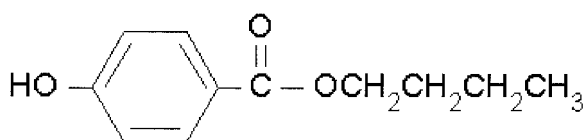


Fig. 1. Chemical structure of butyl paraben.

**Animal treatment:** The chemical structure of BP (Sigma, St. Louis, MO) is shown in Fig. 1. Based on the report that 200 mg/kg of BP induced a significant uterotrophic response in immature rats [4], we selected doses of 100 and 200 mg/kg of BP. Pregnant rats were treated with BP in dimethylsulfoxide (DMSO) by subcutaneous injection of 100 or 200 mg/kg/day. The control group received DMSO only. The total treatment period through dams exposed to BP was from gestation day (GD) 6 to postnatal day (PND) 20, with a 2-day interruption at parturition.

**Necropsy of the dams:** Dams were examined for clinical signs of toxicity and weighed daily before treatment. Dams were sacrificed by ether when their litters were weaned (PND 21) and their body and organ weights were recorded. The numbers of implantation sites of each dam were determined by staining the uterine lumen (exposed by cutting the uterus longitudinally) with 6% ammonium sulfide (v/v). Non-pregnant rats were killed 5 days after the expected date of parturition, and the uteri were examined for implantation sites.

**Examination of the F1 offspring:** Following delivery of the entire litter (PND 1), live pups were counted and examined for clinical signs of toxicity. The length of the perineum from the base of the sex papilla to the proximal end of the anal opening (anogenital distance, AGD) was measured with caliper (0.05-mm accuracy), and one investigator performed all measurements. Pups were grouped by sex according to AGD, and weighed. During the lactation period, pups were weighed weekly in group (by sex and litter) and examined for gross morphological abnormalities. At weaning (PND 21), pups were housed in groups of three to five animals according to treatment and sex under the conditions described above for the dams. Individual pup body weights were recorded weekly.

Vaginal opening was monitored daily from PND 29 until each animal acquired the developmental landmark or PND 49, whichever came first.

**Necropsy of the F1 offspring:** After culling of live pups, postmortem examinations were conducted on F1 offspring (n=5–7 per sex, group and time) at PND 21, 49, 70, and 90. Body and reproductive organ weights (testes, seminal vesicles [with coagulating glands and seminal fluids], prostate gland, uterus, ovaries), and gross morphology of the internal and external genitalia were examined at necropsy. Histopathology of the testes, prostate glands, seminal vesicles, uteri, and ovaries were conducted on all rats. All the tissues, except the testes prepared in Bouin's fixative solution, were placed in 10% neutral buffered formalin, and embedded in paraffin. Tissue sections were cut at 5  $\mu$ m and stained with

hematoxylin and eosin.

**Sperm count in the caudal epididymides:** For the sperm count in the caudal epididymides, the distal sections of the left caudal epididymides of 5 male offspring of each group at PND 90 were weighted, and then minced with shears into 5 ml of physiological salt solution and a 0.1 ml portion diluted with 4.9 ml physiological salt solution. Each sample was observed with an optical microscope using a hemocytometer with a cover glass of 0.1 mm thickness. The total numbers of sperm heads were counted and the means were calculated from 3 counts per animal or per gram of each caudal epididymis.

**Examination of sperm motile activity:** The distal sections of the right caudal epididymides from 5 male offspring of each group at PND 90 were cut with shears, nicked and immersed in Hank's Balanced Salt Solution containing 0.5% BSA, 20 mM HEPES to diffuse the sperm. They were then incubated in a glass chamber at 37°C for 5 min. Ten  $\mu$ l of samples were dropped onto the plate of an objective micrometer (Fujihira Industrial Co., Ltd., Japan), which had been warmed to 37.5°C and examined with an optical microscope. Sperm motile activity was calculated as (No. of motile sperm/No. of total sperm)  $\times$  100.

**Spermatogenesis in the seminiferous tubules:** The right testes of 5 rats of each group on PND 90 were fixed in Bouin's solution, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The numbers of spermatogonia (S), preleptotene spermatocytes (PLS), pachytene spermatocytes (PCS), round spermatid (RS) and elongated spermatids (ES) were counted at stage VII. Spermatogenesis was classified into 14 stages, based primarily upon changes of the acrosome and head morphology of spermatids. Among these stages, stage VII is a lengthy stage in which elongated spermatids move to the luminal aspect of the seminiferous epithelium and line the lumen [19]. This stage was chosen to examine how the sperm numbers and motility were decreased. Spermatogenic staging of the testis was performed according to the method described by Takahashi and Matsui [42] and Creasy [5].

**RNA Extraction and RT-PCR:** Total RNA was extracted from 50 mg of frozen samples of testes of 3 male offspring of each group at PND 21, 49, 70 and 90 using Trizol reagent (GibcoBRL, U.S.A.) according to the method described by the manufacturer, and then the RNA samples were stored at –80°C until use. Total RNA (5  $\mu$ g) was used for a first strand cDNA synthesis using a SuperScript™ Preamplification System according to the method described by the manufacturer (Gibco, U.S.A.) and PCR was performed using a Touchdown™ temperature cycling system (Hybaid, U.K.). After an initial denaturing step at 95°C for 10 min, amplification of the  $\beta$ -actin mRNA was performed with 23 cycles at 95°C for 1 min (denaturing), 60°C for 1 min (annealing), 72°C for 1.5 min (extension), and further extension at 72°C for 10 min (enzyme inactivation). For the ER- $\alpha$  mRNA, 33 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1.5 min, and further extension at 72°C for 10 min were performed. For the ER- $\beta$  mRNA, 37 cycles at 95°C for 1 min, 66°C for

1 min, 72°C for 1.5 min, and further extension at 72°C for 10 min were performed. For amplification of the target genes, the following primer pairs were used:  $\beta$ -actin forward primer 5'-CAGCCTTCCTTCCTGGGTATG-3' and reverse primer 5'-TAGAGCCACCAATCCACACAG-3', for amplification of a 246-bp fragment of rat  $\beta$ -actin cDNA; ER- $\alpha$  forward primer 5'-AATTCTGACAATCGACGC-CAG-3' and reverse primer 5'-GTGCTTCAACATTCTC-CCTCCTC-3', for amplification of a 344-bp fragment of rat ER- $\alpha$  cDNA; ER- $\beta$  forward primer 5'-AAAGCCAA-GAGAAACGGTGGGCAT-3' and reverse primer 5'-GCCAATCATGTGCACCAGTTCCTT-3', for amplification of a 203-bp fragment of rat ER- $\beta$  cDNA [27, 44, 48]. Electrophoresis for the PCR products was performed in a 1.5% agarose gel and the densitometry was carried out using an inverted scan image analyzer (Biorad, Gel-doc., U.S.A.).

**Statistical analysis:** Statistical analyses were conducted using SAS systems (v 6.12, SAS Institute, U.S.A.).

Significant differences between groups were determined by two-way ANOVA ( $p < 0.05$ ).

## RESULTS

**Reproductive parameters:** There were no indications of clinical signs of toxicity or significant effects on body weight or food consumption in the pregnant and lactating dams at any BP dose level during the study (data not shown). The number of implantation sites, the number of total pups and sex ratio were not affected by BP treatment. However, the proportion of pups born alive was significantly decreased at both doses of BP. The proportion of pups surviving to weaning was significantly decreased in 200 mg/kg/day group (Table 1). The anogenital distance in the F1 offspring on PND 1 was not altered by BP treatment. Vaginal opening occurred several days earlier in the 100 mg/kg group compared with the DMSO control group but a similar effect was not seen in the 200 mg/kg group (Fig. 2).

**Body weights and reproductive organ weights in male F1**

**offspring:** Daily administration of BP to dams during gestation and lactation significantly decreased body weight in the male F1 offspring of the 100 mg/kg treatment group on PND 49 but not in the 200 mg/kg group (Fig. 3A). Testicular weight in the 100 mg/kg group was significantly increased at PND 21, but was significantly decreased at PND 49. In the 200 mg/kg/day group, testicular weight was significantly increased at PND 90 (Fig. 4A). The weight of the prostate gland and seminal vesicles were significantly decreased in the 100 mg group at both PND 49 and 90 or only PND 49, respectively (Figs. 4B and 4C). Histopathologic examination of male reproductive organs was performed on the F1 offspring at PND 21, 49, 70 and 90. No BP-related abnormalities were detected.

**Body weights and reproductive organ weights in female F1 offspring:** The body weights of the female F1 offspring were significantly decreased at both doses of BP at PND 49 to PND 90, compared with the DMSO treatment group (Fig. 3B). However, the weight of the female reproductive organs (uterus, ovary) were not altered by BP treatment during the studies (Figs. 4D and 4E). Histopathologic examination of reproductive organs was performed on the female F1 offspring at PND 21, 49, 70 and 90. No BP-related abnormalities were detected.

**Sperm count and motile activity in caudal epididymis:** To evaluate spermatogenesis in the F1 offspring maternally exposed to BP, we examined the number of sperm in the caudal epididymis and sperm motile activity. The number of sperm in caudal epididymis was significantly decreased to 50% of control levels at all doses of BP treatments (Fig. 5A). Sperm motile activity was significantly decreased in both BP treated groups (Fig. 5B).

**Spermatogenic cell count in seminiferous tubule:** Each cell type in the seminiferous tubules (stage VII) was counted. The total cell numbers of round spermatid (RS) and elongated spermatid (ES) in the seminiferous tubules at stage VII were significantly decreased (Fig. 6).

**Testicular expression of the estrogen receptor  $\alpha$  and  $\beta$ :** In

Table 1. Reproductive parameters in rats subcutaneously treated with butyl paraben during gestation and lactation

	Treatment <sup>a)</sup>		
	DMSO	BP 100	BP 200
No. of dams	6	8	8
No. of sperm positive	6	8	8
No. of pregnant	5	7	6
No. of littering	5	7	5
Implantation sites per litter at PND 21	9.4 $\pm$ 1.8 <sup>b)</sup>	13.7 $\pm$ 0.5	12.6 $\pm$ 1.1
Total pups per litter	8.8 $\pm$ 2.7 <sup>b)</sup>	9.6 $\pm$ 4.1	9.6 $\pm$ 4.2
Proportion of pups born alive <sup>c)</sup>	0.93 $\pm$ 0.02	0.69 $\pm$ 0.30*	0.77 $\pm$ 0.33*
Proportion of pups surviving to weaning <sup>d)</sup>	1.00 $\pm$ 0.00	0.98 $\pm$ 0.06	0.65 $\pm$ 0.49*
Sex ratio of live pups <sup>e)</sup>	0.49 $\pm$ 0.20	0.60 $\pm$ 0.09	0.50 $\pm$ 0.09

a), Sperm-positive female rats were administered DMSO or butyl paraben (100 mg/kg or 200 mg/kg) daily by subcutaneous injection from GD6 until the offspring were at PND 20, with a 2-day interruption at parturition (PND 1–2); b), Values are presented as litter means  $\pm$  S.D.; c), No. of pups born alive/No. of total pups per litter; d), No. of live pups at weaning/No. of pups born alive; e), No. of male pups/No. of pups born alive. \*, Significantly different from DMSO treatment group

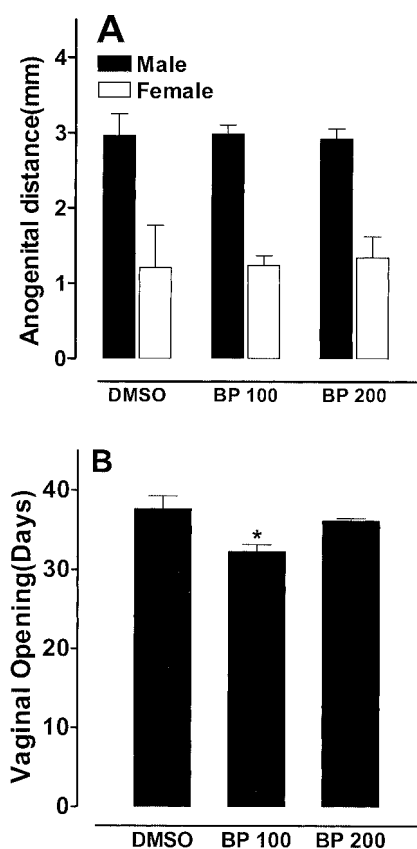


Fig. 2. Effect of butyl paraben treatment during gestation and lactation on anogenital distance and vaginal opening day. Values are presented mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

order to study how BP induced spermatogenesis dysfunction, testicular expressions of ER- $\alpha$  and ER- $\beta$  mRNAs were measured by RT-PCR. Representative expression patterns of ER- $\alpha$  and ER- $\beta$  mRNAs at different age from the weaning until adult stage (days 21–90) are presented in Figs. 7 and 8. Expression of both ER- $\alpha$  and ER- $\beta$  mRNAs was dose-dependently decreased in BP-treated testis at PND 21. In contrast, expression of both estrogen receptor mRNAs was significantly increased in the testis from the 200 mg/kg of BP-treated animals at PND 90. However, expression patterns of both receptor mRNAs were different at PND 49 and 70. These results indicated those testicular expressions of ER- $\alpha$  and ER- $\beta$  mRNAs were altered by BP treatment in the testis during development.

## DISCUSSION

It has been reported that several synthetic and natural chemicals through either maternal or neonatal exposure induced developmental disorder of the male and female offspring in rats [1, 9, 15, 24, 25, 41]. However, the effect on

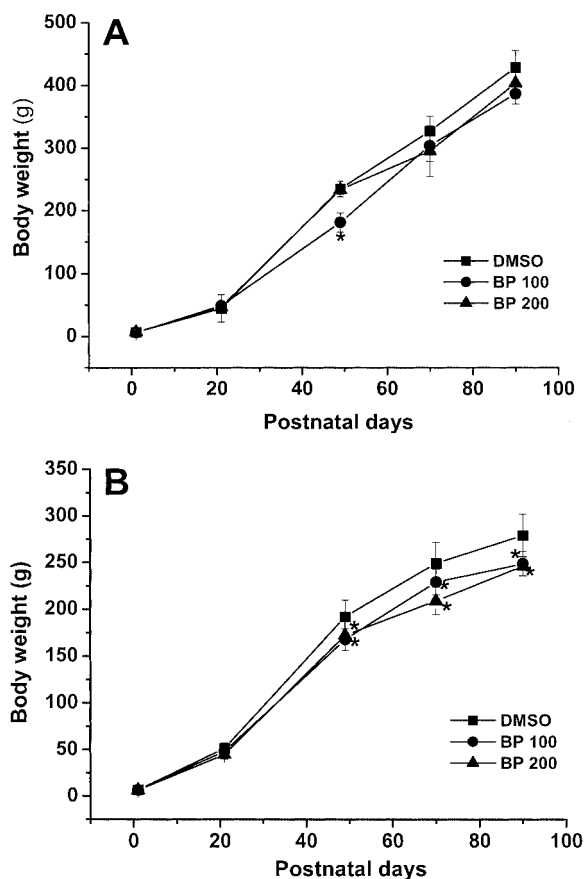


Fig. 3. Body weights of male (A) and female (B) offspring. Values are presented as mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

the male and female reproductive organs of the offspring maternally exposed to BP was not yet reported and poorly understood. This study demonstrated that daily exposures of BP throughout gestation and lactation in rats altered proportion of pups born alive, proportion of pups surviving to weaning, body weight of offspring, vaginal opening, and reproductive organ weights of the male offspring. These results suggest that maternal exposure of BP might have adverse effects on the offspring.

*p*-Hydroxybenzoic acids (paraben) were firstly used as preservatives in pharmaceutical agents in the mid 1920s [32]. It is used widely as an antimicrobial preservative in pharmaceuticals, cosmetic products, processed foods, and beverages. Because of their widespread use, the potential toxicity of parabens has been investigated *in vivo* and *in vitro* to assess various toxicological properties [12, 14, 20–22, 29, 37].

Several papers reported that parabens have estrogenic properties using *in vivo* and *in vitro* systems [13, 18, 31]. Four days after paraben (0.5, 5, 50, and 500  $\mu$ g/100 g) treatment, a dose-dependent response on vaginal cornification and uterotrophic activity were induced in both immature and

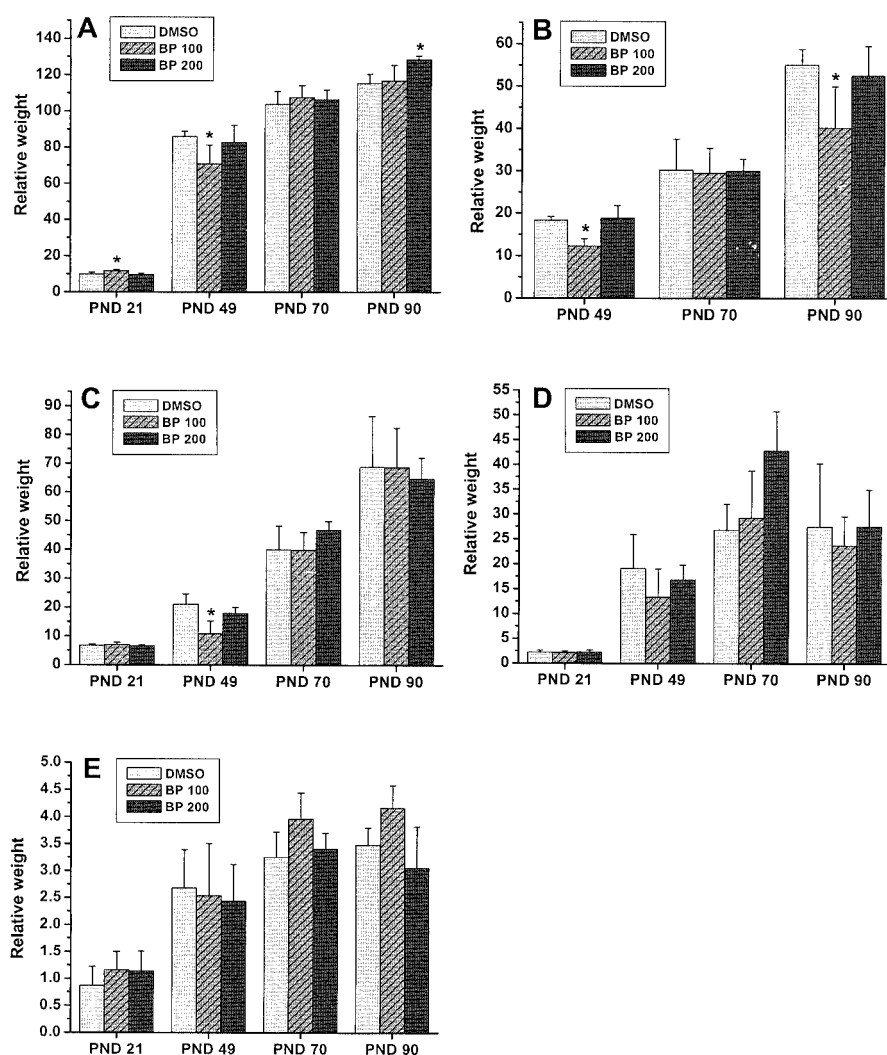


Fig. 4. Reproductive organ weights in male and female offspring. A, Testis; B, Prostate; C, Seminal vesicle; D, Uterus; E, Ovary. Data are shown as absolute organ weights/brain weights. Values are presented as mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

adult ovariectomized mice [18]. In a receptor-binding assay, BP was able to bind rat estrogen receptors with 20% affinity compare to diethylstilbestrol. In *in vitro* yeast-based estrogen assay, the four most widely used parabens (namely methyl-, ethyl-, propyl-, and butylparaben) were all found to be weakly estrogenic with the most potent (butylparaben) being 10,000-fold less potent than  $17\beta$ -estradiol. Subcutaneous administration of butylparaben produced a positive uterotrophic response *in vivo*, although it was approximately 100,000 times less potent than  $17\beta$ -estradiol [30]. In immature Wistar rats, subcutaneous administration of butylparaben produced a weak estrogenic response at 600 mg/kg body weight per day [13].

Our results demonstrated that vaginal opening of the female offspring was significantly decreased at 100 mg/kg BP treatment, compared with vehicle treatment or 200 mg/

kg BP treatment. Concurrently, reproductive organ weights (testis, ventral prostate, and seminal vesicle) of the male offspring were significantly decreased at PND 49. These results suggested that BP might have hormonal action in male and female offspring at 100 mg/kg dose level. In addition, critical points of the BP treatment were found to be post-pubertal stage (PND 49).

Another report suggested inhibition of the proteolytic activity of acrosin in human spermatozoa by BP. The death of spermatozoa caused by BP is probably due to impairment of sperm membrane function. Both the inhibitory effect on acrosin and the adverse effect on membrane function suggest that BP can be developed as a new vaginal contraceptive [38, 39]. However, maternal exposure to BP during gestation and lactation reduced the number of sperm in the epididymis and the sperm motile activity in male offspring.

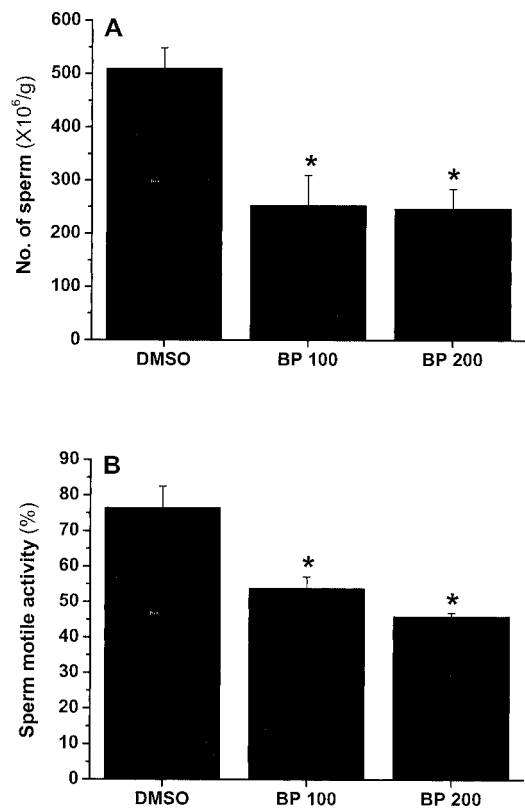


Fig. 5. Sperm count in caudal epididymis (A) and sperm motile activity (B) in F1 offspring. Values are presented as mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

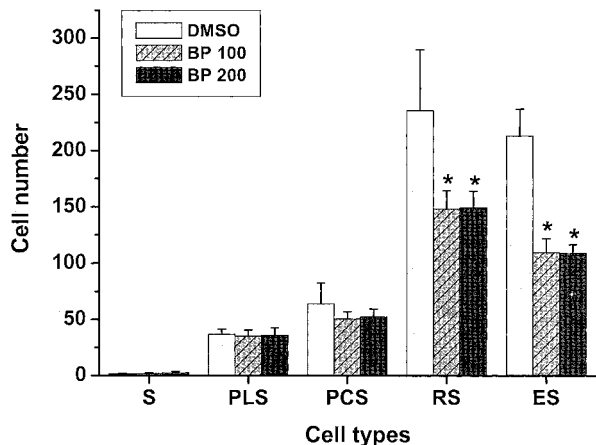


Fig. 6. Cell types in the seminiferous tubules (stage VII) in the F1 offspring. S, spermatogonia; PLS, preleptotene spermatocytes; PCS, pachytene spermatocyte; RS, round spermatid; ES, elongated spermatid. Values are presented as mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

In accordance with the number of sperm in caudal epididymis, round and elongated spermatids in seminiferous tubule at stage VII were significantly decreased. These decreased

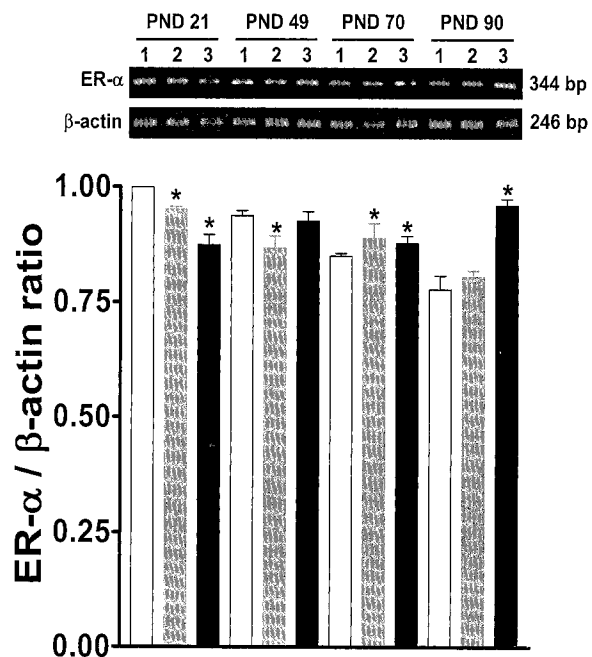


Fig. 7. Effect of butyl paraben on the testicular expression of the ER- $\alpha$  mRNA. Lane 1, DMSO; Lane 2, BP 100 mg/kg; Lane 3, BP 200 mg/kg. Values are presented as mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

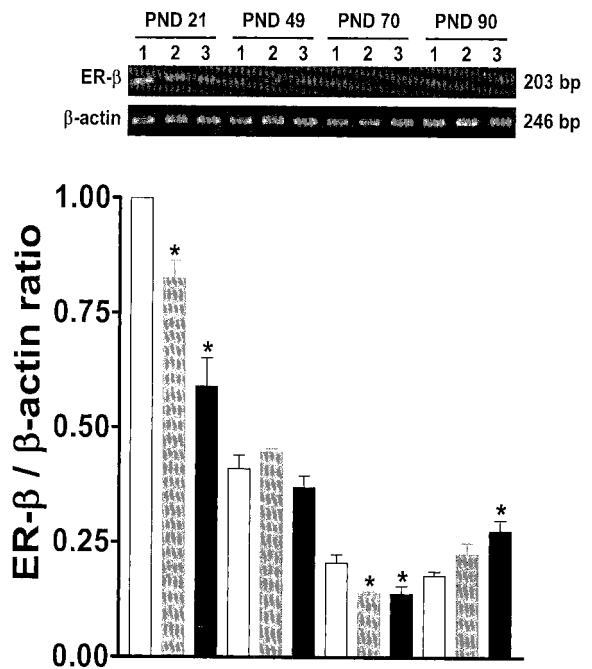


Fig. 8. Effect of butyl paraben on the testicular expression of the ER- $\beta$  mRNA. Lane 1, DMSO; Lane 2, BP 100 mg/kg; Lane 3, BP 200 mg/kg. Values are presented as mean  $\pm$  S.D. \*, Significantly different from DMSO treatment group ( $p < 0.05$ ).

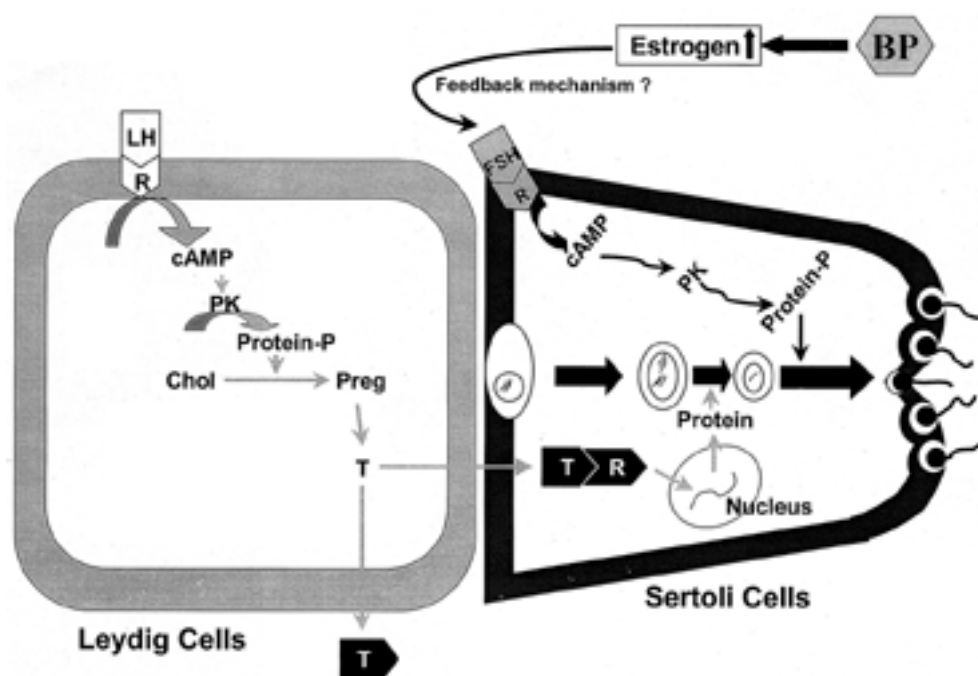


Fig. 9. Possible feedback mechanism of butyl paraben in testis. LH-R, leutenizing hormone receptor; FSH-R, follicular stimulating hormone receptor; T-R, testosterone receptor; Chol, cholesterol; Preg, pregnenolone.

later stages of spermatids might relate to the disruption of hormonal homeostasis both by BP, both FSH and testosterone feedback mechanism. Both FSH and testosterone act on Sertoli cells to control spermatogenesis. FSH is required for the later stages for spermatid maturation to spermatozoa. Once FSH and testosterone initiate spermatogenesis at puberty in the rat, testosterone alone is sufficient to maintain sperm production. Increased estrogen feed back from a variety of factors, especially estrogen-like acting chemicals such as BP, can decrease in follicle stimulating hormone, and finally affects late stages of spermatogenesis at puberty. Therefore, our results suggested that maternal exposure to high dose of BP delayed late stage of spermatogenesis in the hormonal regulation (Fig. 9).

Although changes in testicular morphology and function after neonatal exposure to estrogenic compounds have been well-characterized [10, 11, 35, 45], little attention has been paid to molecular events in the rat testis after prenatal estronization. We examined the changes of testicular expression of ER $\alpha$  and ER $\beta$  genes in this experimental model. First, the presence of ER $\alpha$  and ER $\beta$  in the developing testis has been well documented [8, 34, 47], yet little was known about the homologous regulation of their expression at the mRNA level. Secondly, the molecular phenotype and mechanisms of testicular damage in the neonatally estrogenized rat remained to be fully characterized, and no attempt had been made to evaluate the potential contribution of changes in the pattern of expression of ER subtypes. In addition, identification of specific responses in terms of

ER $\beta$  gene expression may help to elucidate its role. In this sense, the widespread expression of ER $\beta$  in the male gonad, as well as its distinct pattern of cellular distribution from ER $\alpha$  [8, 34, 47], strongly suggested a specific role of the  $\beta$  form of ER in the regulation of testicular function. However, as reported recently [16], targeted disruption of ER $\beta$  gene failed to cause overt abnormalities in the reproductive tract, and neither impaired male fertility. This is in contrast to the reproductive phenotype of knockout mice lacking the genes encoding ER $\alpha$  [6] or aromatase [29]. The possibility remains, however, that although not absolutely essential for reproduction in the male, the  $\beta$  form of ER may play a role in the fine-tuning of estrogen actions on testicular function. Interestingly, redundant regulatory pathways seem to operate within the testis to ensure fertility; as an example, the lack of biological actions of FSH, despite its well-proven role in the regulation of spermatogenesis, is associated with partially preserved male fertility, in both humans and rodents [17, 43]. Tena-Sempere *et al.* [44] reported neonatal exposure to estradiol benzoate differentially alters testicular expression of  $\alpha$  and  $\beta$  ER messages: ER  $\alpha$  mRNA levels, as well as androgen receptor (AR), were significantly decreased, whereas relative and total expression levels of ER  $\beta$  mRNA increased during postnatal/prepubertal development after neonatal estrogen exposure. In this study, assessment of the level of expression of ER  $\alpha$  and ER  $\beta$  mRNAs was carried out by means of RT-PCR. Both receptor mRNA expressions were significantly increased at adult stage. During developmental stage of testes, both ER sub-

types were differentially altered. These results indicated that ER  $\alpha$  and ER  $\beta$  mRNA expression in rat testis is differentially regulated by BP treatment.

Because parabens are widely used in the nutrition and cosmetic industry, a broad range of human population could be exposed to BP. The existence of hormonal activity in chemical agents widely and constantly used that can act as "endocrine disrupters" is a matter of concern for the possible impact on reproductive health in the next generations in human beings and wildlife populations. Therefore, we hope that the substitute materials for parabens will be come out in near future. However, what problem we confront is that we don't have any substitution of parabens like bisphenol A problem, even if there are varieties of usage of these chemicals in human life, now.

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