Original Article

Hypothyroidism protects di(n-butyl) phthalate-induced reproductive organs damage in Sprague-Dawley male rats

Ena Lee1, Hee Jin Kim2, Ji Young Im1, Jeonga Kim1, Hyeyoung Park1, Ju Young Ryu1, Jaewon Lee1, Keun Aee Shim2, Kee Kyung Jung3, Soon Young Han3, Byung Mu Lee3, Seung Hee Kim1 and Hyung Sik Kim1

1College of Pharmacy, Pusan National University, San 30, Jangjeon-dong, Geujeong-gu, Busan 609-735, Korea
2Division of Toxicology, College of Pharmacy, Sungkyunkwan University, Chunchun-Dong 300, Changan-Ku, Kyunggi-Do, Suwon, 440-746, Korea
3National Institute of Toxicological Research, Korean Food and Drug Administration, 5 Nokbun-dong, Eunpyung-ku, Seoul 122-704, Korea

(Received February 18, 2008; Accepted April 3, 2008)

ABSTRACT — This study examined the deleterious effects of di(n-butyl) phthalate (DBP) on the male reproductive organs in hypothyroid rats. Hypothyroidism was induced in prepubertal male rats (28 days of age) by an intraperitoneal (i.p.) injection of 10 mg/kg/day propylthiouracil (PTU) for 30 days. DBP (100 and 500 mg/kg/day) were administered by oral gavages to the intact or hypothyroid rats for 30 days. The body weight of the PTU-treated rats was significantly lower than the control group. The total triiodothyronine (T3) and thyroxine (T4) serum level was lower, and the thyroid-stimulating hormone (TSH) level was higher in the hypothyroid rats than in the control rats. The DBP treatment rats showed significantly lower testes, epididymides, seminal vesicles, and ventral prostate weights than the untreated rats. The hypothyroid rats had significantly higher thyroid weights and lower adrenal glands weights than the control rats. The histomorphological examination showed diffused Leydig cells hyperplasias and germ cells loss in the DBP (500 mg/kg)-treated rats, whereas these effects were mild in the DBP-treated hypothyroid rats. The serum levels of monobutyl phthalate (MBP) were significantly lower in PTU-induced hypothyroid rats than in the DBP-treated rats. This data suggests that the hypothyroid status might offer some protection from male reproductive organ toxicity caused by a disturbance in the metabolic activation of the parent compound, DBP.

Key words: Di(n-butyl) phthalate, Monobutyl phthalate, Hypothyroidism, Testis, Thyroid hormone, Metabolic activation

INTRODUCTION

The thyroid hormone plays an important role in male reproductive organ growth and development because a deficiency occurring before puberty leads to testicular atrophy, a delay in sexual maturation, and altered testosterone production (Cooke, 1991; Cooke and Meisami, 1991). Many studies have shown that a thyroid hormone deficiency leads to decreases in sperm count and sperm motility resulting in male infertility (Cooke, 1991; Jannini et al., 1995). In particular, male reproductive organ development is sensitive to the thyroid hormones during fetal or neonatal periods (Choksi et al., 2003). It was reported that transient neonatal hypothyroidism induced by a treatment with 6-N-propyl-2-thiouracil (PTU) increases the testicular size, Sertoli cell number, and daily sperm production in adult rats and mice (Hess et al., 1993; Joyce et al., 1993; Kirby et al., 1992). However, the precise mechanisms of hypothyroidism on the growth of the male reproductive organs during the prepubertal period are unclear.

There is increasing evidence suggesting that various environmental chemicals can cause goiter through a thyroid hormone imbalance (Sikka and Wang, 2008; Yamauchi and Ishihara, 2006). Nevertheless, the effects of these chemicals on thyroid dysfunction in humans have
received little attention because only mild or moderate effects have been observed in clinical studies. However, humans are commonly exposed to low doses of environmental pollutants with endocrine disrupting effect, which represent major health concerns. The thyroid is essential for mammalian reproductive organ development both before and after birth, and recent evidence strongly suggests that reproductive organ growth and development is much more sensitive to the thyroid hormone than other organs (Price et al., 1988).

Many environmental chemicals such as pesticides, Polychlorinated Biphenyl (PCB), and plasticizers, are suspected of being thyroid disruptors (Sikka and Wang, 2008). Although phthalates do not have direct anti-thyroid activity, it has been proposed that phthalates might be goitrogenic (Rana and Allen, 2006). Several studies have suggested that synthetic chemicals, including plasticizers, can lead to an imbalance in the thyroid function (Brucker-Davis, 1998) and inhibits triiodothyronine (T3) binding to transthyretin (Ishihara et al., 2003). Similar changes in hormones were also observed in the blood stored in di(2-ethylhexyl) phthalate (DEHP) plasticized blood bags. Hinton et al. (1986) reported that DEHP induces hyperactivity and changes in the thyroid metabolism and morphology in rats. This indicates that the administration of low dose levels of DEHP to rats causes thyroid and adrenal cortical dysfunction. Phthalates are widely used as plasticizers as well as in certain personal care products, such as a solvent for perfumes and a fixative for hair spray (Ge et al., 2007). It was demonstrated that in utero or gestational exposure to di(n-butyl) phthalate (DBP) can disrupt male reproductive organ growth during the prepubertal period (Gray et al., 2001; Kavlock et al., 2002; Zhang et al., 2004). Although DBP does not have direct effects on thyroid hormone disturbance, a certain phthalate is biodegraded by gram-negative bacteria with the production of intermediate metabolites with anti-thyroid activity (Gaitan, 1983). However, it is unclear if DBP causes changes in reproductive organ growth through its anti-thyroid activity.

This study evaluated DBP-induced male reproductive organ growth and development in hypothyroid rats. Using this model, it was found that a change in the thyroid hormone level can affect the male reproductive organ toxicity through a disturbance in the metabolic activation of the parent compound, DBP.

MATERIALS AND METHODS

Animals and chemical treatments

Sprague-Dawley male rats (3 weeks of age, 60-70 g) were obtained from Charles River Laboratories (Orient, Seoul, Korea) and housed in controlled temperature (22 ± 2°C) and lighting (12 hr light and dark cycle) conditions. The animals were maintained in accordance with the Korea Food and Drug Administration Guide for the Care and Use of Laboratory Animals. All rats were given access to animal diet and tap water ad libitum. The animals were divided into six groups (6 animals/group), i.e. vehicle control (corn oil), DBP (100 and 500 mg/kg), 6-N-propyl-2-thiouracil (PTU, 10 mg/kg), PTU (10 mg/kg) plus DBP (100 and 500 mg/kg). Hypothyroidism was induced in prepubertal male rats (28 days of age) by an i.p. injection of 10 mg/kg PTU. DBP (100 and 500 mg/kg) was administered to the prepubertal rats (28 days of age) only by oral gavage daily for 30 days. The control groups were administered corn oil in the same manner. The dosages used in this study were based on a previous study showing the adverse effects on male reproductive development in adult male rats (Ryu et al., 2008).

Body and organ weight changes

The body weights were recorded daily before dosing. At the end of 30 days (approximately 24 hr after the last administration), all of the animals were anesthetized with diethyl ether. Blood was obtained from the abdominal aorta and plasma samples were stored at −80°C until needed for hormone analysis. Liver, kidney, spleen, adrenal glands, thyroid glands, testes, ventral prostate, seminal vesicles and epididymides were excised, weighed, frozen in liquid nitrogen, and stored at −80°C until use. The relative organ weight refers to the absolute organ weight/100 g body weight. Throughout the study period, each animal was observed at least once daily for any clinical signs of toxicity related to the DBP treatment. On working days, all the cages were checked in the morning and afternoon for the presence of dead or moribund animals.

Hormonal analyses

The serum T3, T4 and the thyroid-stimulating hormone (TSH) levels were measured using Radioimmunoassay (RIA) kits (Diagnostic Systems Laboratories, Inc., TX). The RIA assays were performed according to the manufacturer’s protocols, with the modification that the amount of serum used for T4 RIA assay was increased to 100 μl, and the incubation time was prolonged to 1.5 hr. All serum samples were analyzed in duplicate.

Histological evaluations

After sacrifice, the right testis was fixed overnight in Bouin’s fixative and dehydrated with 70% ethanol. The tissue was embedded in paraffin and 5 μm sections were
cut and mounted on slides. The slide sections were stained with hematoxylin and eosin (H&E). The histopathological findings of the testis were determined by an optical microscopy observation.

**Serum MBP and DBP analysis by HPLC**

HPLC was performed using high-performance liquid chromatography (model L-7100, Tokyo) equipped with a Hitachi model L-7200 autosampler and a Hitachi pump [21]. Separation was achieved using a 5 μm SUPELCOSIL LC-18 column (250 × 4.6 mm) (Tokyo) operating at 20 ± 2°C. Elution was performed isocratically using a mobile phase consisting of an acetonitrile-aqueous buffer (0.08% triethylamine adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at a flow rate of 0.7 ml/min. The mobile phase was prefilled through a 0.45 μm membrane and degassed. The run time was 50 min. The analytical reproducibility for the individual phthalates at 10, 25, 50, 100, 150, 200, 250, 300, 350, and 400 ppm was assessed using 5 sample replicates. The calibration curves were prepared as peak-area ratios versus the d(n-hexyl) phthalate (DNP) internal standard. monobutyl phthalate (MBP), DNHP, and DBP were eluted at 11.5, 16.8, and 18.4 min, respectively (Shimadzu). The serum levels were calculated using the peak ratio (MBP or DBP/DNHP peak area) of the calibration curves obtained during validation of the methods. The limit of detection for MBP and DBP was 100 ng/ml. All experimental quality controls were carried out to avoid phthalate contamination.

**Statistical analysis**

All values are expressed as mean ± S.D. (n=6 animals). The data for the mean initial or necropsy body weights, organ weights, and hormone levels were analyzed statistically for the homogeneity of variance using Bartlett’s test. Nonparametric analysis of variance was applied when the samples were proven to be homogeneous. The organ weights were analyzed using an analysis of covariance (ANCOVA) with the body weight at necropsy used as the covariate. When a significant treatment effect was observed, a Dunnett’s test (control vs. treatment groups) was used to compare the treatment groups.

**RESULTS**

**Body and reproductive organs weights change**

The adverse effects of DBP on male reproductive organ growth between the normal and hypothyroid rats were compared after administering DBP orally to the normal or hypothyroid (10 mg/kg PTU) rats over 30 day periods. In this study, prepubertal rats were used because young animals are more sensitive to phthalate-induced toxicity than adults. During the study period, there were no significant differences in clinical signs observed between the treatment groups (Data not shown). The changes in body weight in PTU-treated hypothyroid or DBP-treated hypothyroid rats (PTU + DBP) were consistently lower than the controls from days 35 to 58 (Fig. 1). At 58 days, the lowest body weights were detected in the hypothyroid rats (192.5 ± 4.8 g), which was only 56% of the weight of the controls (308.3 ± 23.5 g). Moreover, hypothyroid rats maintained lower body weights than the controls (Fig. 1). Prepubertal exposure to DBP did not affect the body weight until the end of the experiment. DBP had significant effects on male reproductive organ growth. The weight of the testes was significantly lower in the high-dose DBP (500 mg/kg) treatment groups (Table 1) but PTU did not affect the testes weight. In addition, the weights of the accessory sex organs (epididymides, ventral prostate, and seminal vesicles) were also significantly lower in the 500 mg/kg DBP groups than the control (Table 1). In the case of the relative weights, DBP (500 mg/kg) significantly reduced the relative weight of the testes, epididymides, ventral prostate, and seminal vesicles (Table 2). As expected, the thyroid weights were significantly higher in all PTU-treated groups than the control, whereas the adrenal glands were markedly lower in the PTU-treated groups (Table 1).

**Fig. 1.** Comparison of the body weight changes in Sprague-Dawley rats from 28 to 58 days of age. The data is reported as the mean ± S.D. of 6 animals/group. Asterisks indicate a significant difference from control group (*p < 0.05*).
Table 1. Comparisons of absolute organ weights in Sprague-Dawley rats treated with di(n-butyl) phthalate and propylthiouracil.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vehicle Control b</th>
<th>DBP 100 mg/kg</th>
<th>DBP 500 mg/kg</th>
<th>PTU 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ weight</td>
<td>0 mg/kg</td>
<td>100 mg/kg</td>
<td>500 mg/kg</td>
<td>0 mg/kg</td>
</tr>
<tr>
<td>Initial B.W. (g)</td>
<td>115.53 ± 3.60&lt;1</td>
<td>114.97 ± 3.57</td>
<td>117.38 ± 2.95</td>
<td>114.81 ± 6.32</td>
</tr>
<tr>
<td>Final B.W. (g)</td>
<td>325.74 ± 20.53</td>
<td>327.82 ± 29.6</td>
<td>328.26 ± 18.7</td>
<td>177.69 ± 9.23*</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>9.49 ± 0.86</td>
<td>10.14 ± 1.22</td>
<td>13.78 ± 1.05*</td>
<td>6.31 ± 0.38*</td>
</tr>
<tr>
<td>Testis (g)</td>
<td>2.98 ± 0.15</td>
<td>2.81 ± 0.17</td>
<td>1.14 ± 0.19*</td>
<td>2.94 ± 0.14</td>
</tr>
<tr>
<td>Epididymis (g)</td>
<td>0.75 ± 0.07</td>
<td>0.71 ± 0.09</td>
<td>0.48 ± 0.05*</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>Seminal vesicles (g)</td>
<td>1.15 ± 0.07</td>
<td>1.06 ± 0.09</td>
<td>0.61 ± 0.12*</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>Ventral prostate (g)</td>
<td>0.47 ± 0.08</td>
<td>0.44 ± 0.05</td>
<td>0.36 ± 0.04*</td>
<td>0.40 ± 0.05*</td>
</tr>
<tr>
<td>Adrenal glands (mg)</td>
<td>54.70 ± 8.91</td>
<td>49.01 ± 43.6</td>
<td>52.14 ± 45.2</td>
<td>17.82 ± 30.8*</td>
</tr>
<tr>
<td>Thyroid glands (mg)</td>
<td>10.12 ± 2.76</td>
<td>12.54 ± 3.72</td>
<td>11.78 ± 2.84</td>
<td>43.52 ± 11.24*</td>
</tr>
</tbody>
</table>

* Male rats were administered with di(n-butyl) phthalate (100 and 500 mg/kg/day) by oral gavage for 30 days. Hypothyroidism was induced in Sprague-Dawley rats by an i.p. injection of 10 mg/kg/day propylthiouracil.

* Vehicle control received corn oil containing 0.25% NaOH.

* Data are presented as mean ± S.D. (n=6)

Significantly different from vehicle control (*p < 0.05); significantly different from di(n-butyl) phthalate alone (**p < 0.05)

Table 2. Comparisons of relative organ weights in Sprague-Dawley rats treated with di(n-butyl) phthalate and propylthiouracil.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vehicle Control b</th>
<th>DBP 100 mg/kg</th>
<th>DBP 500 mg/kg</th>
<th>PTU 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ weight</td>
<td>0 mg/kg</td>
<td>100 mg/kg</td>
<td>500 mg/kg</td>
<td>0 mg/kg</td>
</tr>
<tr>
<td>Initial B.W. (g)</td>
<td>115.53 ± 3.60&lt;1</td>
<td>114.97 ± 3.57</td>
<td>117.38 ± 2.95</td>
<td>114.81 ± 6.32</td>
</tr>
<tr>
<td>Final B.W. (g)</td>
<td>325.74 ± 20.53</td>
<td>327.82 ± 29.6</td>
<td>328.26 ± 18.7</td>
<td>177.69 ± 9.23*</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>2.91 ± 0.35</td>
<td>3.09 ± 0.48</td>
<td>4.19 ± 1.21*</td>
<td>3.55 ± 0.97*</td>
</tr>
<tr>
<td>Testis (g)</td>
<td>0.91 ± 0.09</td>
<td>0.86 ± 0.17</td>
<td>0.37 ± 0.09*</td>
<td>1.66 ± 0.15*</td>
</tr>
<tr>
<td>Epididymis (g)</td>
<td>0.23 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.15 ± 0.25*</td>
<td>0.37 ± 0.03*</td>
</tr>
<tr>
<td>Seminal vesicles (g)</td>
<td>0.35 ± 0.05</td>
<td>0.32 ± 0.04</td>
<td>0.18 ± 0.02*</td>
<td>0.57 ± 0.07*</td>
</tr>
<tr>
<td>Ventral prostate (g)</td>
<td>0.14 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.11 ± 0.01*</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Adrenal glands (mg)</td>
<td>16.81 ± 1.54</td>
<td>14.95 ± 1.98</td>
<td>15.82 ± 1.24</td>
<td>10.07 ± 1.09*</td>
</tr>
<tr>
<td>Thyroid glands (mg)</td>
<td>3.11 ± 0.54</td>
<td>3.82 ± 0.64</td>
<td>3.57 ± 0.37</td>
<td>24.58 ± 2.51*</td>
</tr>
</tbody>
</table>

* Male rats were administered with di(n-butyl) phthalate (100 and 500 mg/kg/day) by oral gavage for 30 days. Hypothyroidism was induced in Sprague-Dawley rats by an i.p. injection of 10 mg/kg/day propylthiouracil.

* Vehicle control received corn oil containing 0.25% NaOH.

* Data are presented as mean ± S.D. (n=6)

Significantly different from vehicle control (*p < 0.05); significantly different from di(n-butyl) phthalate alone (**p < 0.05)

Serum T3, T4 and TSH levels

The effects of DBP on the serum levels of thyroid hormones (T3, T4 and TSH) were measured in both the hypothyroid and normal rats. As shown in Fig. 2, the PTU-treated rats showed significant decreases in the serum T3 (27% of control) and T4 (35% of control) levels, respectively. In contrast, the serum TSH levels were significantly higher in the hypothyroid rats than the con-
Hyperthyroidism protects DBP toxicity

Fig. 2. Comparisons of the serum T3, T4 and TSH level in the different treatment groups. The results are represented as the mean ± S.D. of 6 animals/group. The asterisks indicate a significant difference from the control group (p < 0.05).

The Japanese Society of Toxicology

Testicular morphological analysis

The histological evaluation of the testicular tissue revealed the initiation of apparently normal spermatogenesis in the normal rats. The seminiferous tubules in the control rats were filled with spermatogonia, spermatocytes, and spermatids and the lumen development was also observed. In the high dose (500 mg/kg) DBP-treated rats, significantly decreases in the size of the seminiferous tubules was observed in the testes (Fig. 3). Lumen formation and interstitial cells or Sertoli cells were not detected in the 500 mg/kg DBP-treated rats. The hypothyroid rats showed delayed lumen formation and significant decreases in the number of Leydig cells (Fig. 3). The diameter of the seminiferous tubules in the hypothyroid rats was far lower than that of the controls. In contrast, there were fewer histological malformations observed in the hypothyroid group than in the DBP group. However, the number of Leydig cells was not recovered and a few multinuclear germ cells were also observed in hypothyroid rats treated with DBP (Fig. 3).

Analysis of serum MBP and DBP levels by HPLC

The serum MBP and DBP levels were measured by HPLC after a chronic 30-day DBP treatment. The serum levels of MBP were significantly lower in the PTU + DBP

trols. This shows that the hypothyroid rat models induced by PTU were well established in this study design. However, there was no significant difference in the thyroid hormone levels observed in the hypothyroid and normal rats treated with DBP (500 mg/kg).

Fig. 3. Histological changes of the rat testis in the different treatment groups. The photomicrographs were taken at x100 magnification by hematoxylin and eosin staining. (A) control group, (B) 100 mg/kg di(n-butyl) phthalate (DBP), (C) 500 mg/kg di(n-butyl) phthalate (DBP), (D) 10 mg/kg 6-N-propyl-2-thiouracil (PTU), (E) PTU (10 mg/kg) + DBP (100 mg/kg), and (F) PTU (10 mg/kg) + DBP (500 mg/kg) treated group. Note the slight residual decrease in diameter of seminiferous tubules of the DBP-treated animals (C) and diffuse Leydig cell hyperplasia can be seen (D).
group (1.91 ± 0.45) than in the DBP group (3.74 ± 1.27). However, there was no significant difference in the serum levels of DBP between PTU + DBP and DBP groups (Fig. 4). These measurements suggest that effects of DBP on testicular abnormalities are mainly due to the action of MBP or its metabolites in testicular cells.

**DISCUSSION**

It was previously reported that an altered thyroid hormone status might show changes in the biosynthesis of steroid hormones as well as impair male reproductive organ growth and development (Ryu et al., 2008). Previous report indicated that PTU protects against arsenic-induced toxicity through the formation of reactive oxygen species (ROS) in the liver and kidney of arsenic-treated rats (Rana and Allen, 2006). Therefore, it was suggested that DBP might also have anti-thyroid activity under specific circumstances. Although the protective effects of antioxidants such as curcumin, vitamin C and E on phthalate-induced male reproductive organs toxicity has been studied (Ishihara et al., 2000; Kim et al., 2002), the effect of the thyroid hormone on DBP toxicity is unknown.

In this study, DBP was administered to hypothyroid rats over a 30 day period. It was found that DBP significantly affected the development of the male reproductive tracts, whereas DBP-induced male reproductive toxicity was only slightly decreased in the hypothyroid rats. These effects clearly showed that the relative weights of the reproductive organs are not markedly affected in the DBP-treated hypothyroid rats compared with that observed with DBP alone. The most significant effects were observed in the testes and epididymides. This study is the first to report that hypothyroid rats might be somewhat protected from DBP-induced male reproductive organ damage. It is believed that the protective effects on DBP-induced male reproductive organ toxicity in hypothyroid rats are the result of the inhibition of metabolic activation. Therefore, the serum MBP and DBP levels were measured to determine if hypothyroid status inhibits the DBP metabolism. It was clearly demonstrated that the serum levels of MBP were significantly lower in the hypothyroid rats than in the DBP group alone. This suggests that the effects of DBP on testicular abnormalities were mainly due to the action of MBP or its metabolites in testicular cells. Similar to this study, several reports show that a pre-treatment with PTU offers some protection against CCl4 (Orrego et al., 1976) and acetaminophen (Linscheer et al., 1980) toxicity.

There is no report showing the influence of the hypothyroid status on the testicular morphological changes. Therefore, this study examined the testicular histomorphological changes to determine the cause of the hypothyroid effects on the protection of DBP-induced testicular

![Fig. 4](image-url) Comparisons of serum DBP and MBP levels in different treatment groups. The results are reported as the means ± S.D. of duplicate experiments of 6 animals/group. Asterisks indicate a significant difference from control group (p < 0.05).

Vol. 33 No. 3
damage in hypothyroid rats. The seminiferous tubules in
testes were significantly smaller in size in the high dose (500 mg/kg) DBP-treated rats. In particular, there was no
lumen formation, interstitial cells or Sertoli cells detect-
ed in the 500 mg/kg DBP-treated rats. The hypothyroid
rats showed delayed lumen formation and a significant
decrease in the number of Leydig cells. The diameter of
seminiferous tubules was far smaller in the hypothyroid
rats than the controls. In contrast, there were fewer histo-
logical malformations observed in the hypothyroid group
than in the DBP group. However, the number of Leydig
cells had not recovered and a few multinuclear germ cells
were also observed in the hypothyroid rats treated with
DBP. Previously, it was reported that the PTU-induced
hypothyroid rats showed slightly lower levels of testicu-
lar lipid peroxidation or oxidative DNA damage; but this
was not statistically significant (Rya et al., 2008). Ot-
ners have described similar results in various rat tissues
(Venditti et al., 1997; Yilmaz et al., 2003). It was sug-
gested that hypothyroidism reduces the level of oxidative
damage in the cerebral, hepatic and cardiac tissues of rats
(Mogulkoc et al., 2005). In earlier studies, it was dem-
strated that thyroid hormones increase the activity of
the drug-metabolizing enzymes (Goudonnet et al., 1990;
Mehrotra et al., 1997). Moreover, significant decreases in
the cytochrome P450 content and monooxygenase activity
were observed in the testes of the hypophysectomy rats
(Lee et al., 1980).

In conclusion, these results suggest that DBP-induced
testicular damage might be closely related to the meta-
bulic activation of DBP. Moreover, hypothyroidism can
protect against the testicular damage caused by chronic
exposure to DBP. However, further research on the activ-
ities of xenobiotic-metabolizing enzymes will be needed
to clarify the molecular mechanism of the DBP-induced
reproductive organ toxicity.

ACKNOWLEDGMENT

This work was supported by the fund for 2006 En-
docrine Disruptors Research (06152EDS314) from the
National Institute of Toxicological Research/Korea Food
& Drug Administration.

REFERENCES

Bruckner-Davis, F. (1998): Effects of environmental synthetic chemi-
cals on thyroid function. Thyroid, 8, 827-856.
Choksi, N.Y., Jahnke, G.D., St Hilaire, C. and Shelby, M. (2003): Role of thyroid hormones in human and laboratory animal repro-
479-491.

Cooke, P.S. (1991): Thyroid hormones and testis development: a
model system for increasing testis growth and sperm production.
causes increased adult testis and reproductive organ size but does
2, 295-308.
Ge, R.S., Chen, G.R., Tantrikut, C. and Hardy, M.P. (2007): Phtha-
late ester toxicity in Leydig cells: developmental timing and dosage
Goudonnet, H., Magdalous, J., Mounic, J., Naounni, A., Virost, M.L.,
on the activity of UDP-glucuronosyltransferases and cytochrome
Gray, L.E.Jr., Ostby, J., Furr, J., Wolf, C.J., Lambright, C., Parks,
L., Veeramachaneni, D.N., Wilson, V., Price, M., Hotchkiss, A.,
Orlando, E. and Guillette, L. (2001): Effects of environmental antiandrogens on reproductive development in experimental ani-
testicular enlargement induced by neonatal hypothyroidism is accom-
panied by increased Sertoli and germ cell numbers. Endo-
crinology, 132, 2607-2613.
Hinton, R.H., Mitchell, F.E., Mann, A., Chescoe, D., Price, S.C.,
acid esters on the liver and thyroid. Environ. Health Perspect.,
70, 195-210.
The effect of endocrine disrupting chemicals on thyroid hor-
none binding to Japanese quail transthyretin and thyroid hor-
Ishihara, M., Itoh, M., Miyamoto, K., Sura, S., Takeuchi, Y.,
induced by di-(2-ethylhexyl) phthalate is significantly prevented by treatment with antioxidant vitamins in the rat. Int. J. Androl.,
23, 85-94.
treatment increases adult testis size and sperm production in the
mouse. J. Androl., 14, 448-455.
Kavlock, R., Boekelheide, K., Chapin, R., Cunningham, M.,
Faustman, E., Foster, P., Golub, M., Henderson, R., Hinberg, I.,
Little, R., Seed, J., Shek, K., Tabacora, S., Tyl, R., Williams, P.
and Zacharewski, T. (2002): NTP Center for the Evaluation of
Risks to Human Reproduction: phthalates expert panel report on
the reproductive and developmental toxicity of di-n-butyl phtha-
Kim, S.H., Kim, S.S., Kwon, O., Sohn, K.H., Kwack, S.J., Choi,
dibutyl phthalate and monobutyl phthalate on cytotoxicity and
differentiation in cultured rat embryonic limb bud cells; protes-
Kirby, J.D., Jetton, A.E., Cooke, P.S., Hess, R.A., Bunick, D.,
mental hormonal profiles accompanying the neonatal hypo-
thyroidism-induced increase in adult testicular size and sperm pro-
duction in the rat. Endocrinology, 131, 559-565.
Lee, I.P., Suzuki, K., Mukhtar, H. and Bend, J.R. (1980): Hormo-

Vol. 33 No. 3


