NeuroToxicology 31 (2010) 747–751

Contents lists available at ScienceDirect

NeuroToxicology

# Impact of gestational and lactational phthalate exposure on hypothalamic content of amino acid neurotransmitters and FSH secretion in peripubertal male rats

Silvia Carbone [a,b], Berta Szwarcfarb [b], Osvaldo Ponzo [a,*], Roxana Reynoso [a], Nancy Cardoso [a], Laura Deguiz [a], Jaime A. Moguilevsky [a,c], Pablo Scacchi [a,b]

[a] Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 7° P (C1121 CABG), Buenos Aires, Argentina
[b] CONICET, Buenos Aires, Argentina
[c] Facultad de Postgrado, Universidad Favaloro, Buenos Aires, Argentina

ARTICLE INFO

*Article history:*
Received 18 February 2010
Accepted 14 June 2010
Available online 1 July 2010

*Keywords:*
Phtalate
Rats
Gestational
Lactational
Gonadotropin
Aspartate
GABA

ABSTRACT

This study investigated the effect of the pre- and perinatal exposure to di-(2-ethylhexyl) phthalate (DEHP) on the neuroendocrine parameters that regulate reproduction in peripubertal male rats. DEHP at dose of 3 and 30 mg/kg bw/day was administered orally to female rat since pregnancy onset until weaning. The male litters were sacrificed at 30 days of age to determine gonadotropin serum level and the hypothalamic contents of the amino acids aspartate and gamma-aminobutyric acid. No changes in gonadotropin, aspartate and gamma-aminobutyric acid levels were detected at the low dose. DEHP 30 mg/kg bw/day reduced testis weight and serum FSH, in correlation with a significant increase in the inhibitory GABAergic tone and a reduction in the stimulatory effect of aspartate on gonadotropin level. This study provides unknown data regarding changes in the hypothalamic contents of the amino acid neurotransmitters, which are involved in the neuroendocrine regulation of reproductive axis, in peripubertal male rat offspring from dams exposed to DEHP during gestational and lactational periods. This could be related with the gonadotropin modifications also here described.



## 1. Introduction

Di-(2-ethylhexyl) phthalate (DEHP) is the most widely used phthalate to convey flexibility and transparency to numerous plastic products made of polyvinyl chloride (PVC) (Latini et al., 2003; NTP-CERHR, 2006). Humans are daily exposed to this chemical through ingestion, inhalation and by dermal contact (Moore et al., 2001; NTP-CERHR, 2000). DEHP is not covalently bound to the polymer and therefore it can leach from plastic products into foods, beverages or directly into body fluids (Moore et al., 2001; Lovekamp-Swan and Davis, 2003). Moreover, also occupational and medical exposure (e.g., tubing, catheters and intravenous delivery sets) increase body burden levels (Latini et al., 2003; Doull et al., 1999; Calafat et al., 2004; Faouzi et al., 1999; Calafat and McKee, 2006; Koch et al., 2006), reaching much higher concentrations in this population.

Pre- and postnatal oral exposure to DEHP may be associated in animals with male reproductive development and function alteration. DEHP in rats is rapidly hydrolyzed in the gut to mono-(2-ethylhexyl) phthalate (MEHP), which pass into breast milk and cross the placental barrier (Latini et al., 2003; Stroheker et al., 2005). MEHP interact with fetal sexual steroids and produce testicular atrophy (Albro, 1987; Parks et al., 2000; Borch et al., 2005; Andrade et al., 2006), alteration in Leydig cell development and reduce serum testosterone levels in rats (Akingbemi et al., 2001). In uterus DEHP exposure produces abnormalities in androgen-dependent processes (e.g., undescended testis, retained nipples), malformations (e.g., ventral prostate, seminal vesicle, gubernacular cord and the epididymis) and sexual behavior alterations in male rat offspring (Andrade et al., 2006; Gray et al., 2000; Dalsenter et al., 2006). These alterations could be directly associated with epidemiological evidence indicating that boys born from women exposed to phthalates during pregnancy have an increased incidence of inborn genital malformations and spermatogenic dysfunction (Hu et al., 2009; Ge et al., 2007; Main et al., 2006).

It has been proposed that DEHP acts in an anti-androgenic manner, which appears to result from an androgen receptor-independent mechanism of anti-androgenicity (Latini et al., 2003; Parks et al., 2000; Gray et al., 2000; Akingbemi et al., 2004; Foster et al., 2001; Bonefeld-Jorgensen et al., 2001). Others mechanisms as reduction in the expression of steroidogenesis related factors and in nuclear receptors that regulate cholesterol transport could explain the suppressive effect of DEHP on testosterone levels (Howdeshell et al., 2007; Borch et al., 2006; Wilson et al., 2008).

* Corresponding author. Tel.: +54 011 5950 9500x2147.
*E-mail address:* oponzo@fmed.uba.ar (O. Ponzo).

0161-813X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2010.06.006

The hypothalamic amino acid system appears to play an important role in the neurotransmission pathway that regulates gonadotropin releasing hormone (GnRH) neurons and gonadotropin secretion during sexual development in rats (Moguilevsky and Wutke, 2001). The excitatory amino acid aspartate (ASP) stimulates GnRH release (Brann and Mahesh, 1994). Previously we have demonstrated that *N*-methyl-D-aspartate and non-*N*-methyl-D-aspartate receptors of the excitatory amino acids system stimulate the release of Gn-RH from hypothalamic fragments in vitro and also increase serum gonadotropin levels during maturation in male rats (Carbone et al., 1996). Conversely, gamma-aminobutyric acid (GABA) has an important role in the gonadotropin inhibitory control in adult and prepubertal male rats (Feleder et al., 1996). Testosterone exerts a negative feed back effect on gonadotropin release in male rats (Ojeda and Urbanski, 1994). It is also demonstrated that the regulatory effects of amino acid neurotransmitters system on GnRH neurons are modulated by sexual hormones acting on different receptor-subtypes of these neurotransmitters in the anterior preoptic and medial basal areas (APOA-MBH) of the hypothalamus (Carbone et al., 1996; Szwarfarb et al., 1994).

Taking into account that the amino acid neurotransmitters are involved in the neuroendocrine regulation of the reproductive function during sexual maturation, this work was designed to study the impact of DEHP on gonadotropin levels and on the hypothalamic content of the amino acid neurotransmitters ASP and GABA, in 30-day-old immature male rats offspring from DEHP-exposed dams during gestational and nursing periods. An oral route of DEHP administration was chosen for this study to mimic the most likely route of exposure to the compound in humans and wildlife.

## 2. Materials and methods

### 2.1. Animals and drug

Wistar rats used for this work were provided by the Department of Physiology, School of Medicine, University of Buenos Aires. They were allowed at minimum 7-day acclimatization period and observed for signs of illness before experimental procedures took place. Animals were raised under light (lights on from 7 am to 7 pm), temperature (22–24 °C) and humidity controlled conditions. Rats were fed with balanced food and water ad libitum until time of sacrifice and were weighed daily. Adequate measures were taken to minimize pain or discomfort in accordance with protocols of the National Institute of Health Guide for the Care and Use of Laboratory Animals. Approval to conduct the study was granted by the Animal Care and Ethics Committee of the University of Buenos Aires.

DEHP (99% pure, Cat D 20,115-4, Aldrich Chemical Company, Inc. Milwaukee, WI, USA) were used.

### 2.2. Verification of DEHP dose

The doses of exposure to dams were 3 and 30 mg/kg bw/day of DEHP in drinking water. The doses and the administration pathway were chosen based on those previously used by Arcadi et al. (1998). Also, a preliminary study was undertaken to confirm that the selected experimental doses of DEHP were not overtly toxic. For this purpose, four pregnant Wistar rats were exposed orally to DEHP at the low and high doses (two rats per group) during gestational and lactational periods. Two solutions of DEHP at the concentrations of 32.5 and 325 μl/l were made up fresh daily by sonicating for 30 min that ensure a permanent and homogenized solution. Each dam was weighed daily and the liquid ingested was measured to adjust the volume of DEHP solution that it was necessary to add in the corresponding glass bottle to reach the dose chosen. The assessments assume that all the DEHP solutions lost from the bottle were consumed by the animal. They do not account for possible leakage or evaporation of the solution or for potential loss of DEHP activity during the 24-h period. According to the study of Rubin et al. for bisphenol A exposure in drinking water, the level of DEHP affecting the foetuses during gestation or that was ingested postnatally by the offspring during the period of lactation, was not estimated in this work (Rubin et al., 2001). The doses of DEHP 3 and 30 mg/kg bw/day were used for the main part of the study as no overt signs of toxicity. These doses were less and greater than 3.7 and 14 mg/kg bw/day, respectively, which represent the range of the no-observed-adverse-effect level (NOAEL) determined by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR 2006) for testis/developmental effects of DEHP by oral route in rodents (NTP-CERHR, 2006).

### 2.3. Experimental design

Ten Female young adult rats (with weights from 240 to 250 g on the day after mating) received DEHP or vehicle during pregnancy and lactation. For that purpose, each female was put to mate with a male in individual metallic cages for 4 days (one estrous cycle). The animals were daily examined for mucous plug. The presence of copulatory plugs in the cage was taken as evidence of mating and designated as first day of gestation (GD1). At that time each mated rat was separated from the male. Pregnant rats ($n = 9$) were randomly assigned (3 per group) to one of the three experimental groups (Control; DEHP 3 mg/kg bw/day and DEHP 30 mg/kg bw/day). The control group received water without DEHP added. Dams exposed to DEHP with dosage of 3 or 30 mg/kg bw/day in drinking water every day from GD1 to weaning. During the treatment each dam was weighed and DEHP solution consumption was measured to determine the dosage to be given. The number of male litters was not altered by maternal DEHP treatment (4–5 males per dam in DEHP and control groups).

On 21th perinatal day pups of the three groups were removed from their mothers' cage and then housed by sex and treatment. The male litters that were born from each of the three groups of pregnant rats were assigned randomly to sacrifice at 30 days of age with a body weight from 70 to 90 g. Males with undescended testes were not used for hormone and amino acids assay. Ten males per group were used.

### 2.4. Tissue collection

When the target age was reached the male animals were weighed and sacrificed by decapitation at 16.00–17.00 h. Blood was collected from the trunks and the samples centrifuged for 10 min at 2500 rpm, the serum separated and stored at −20 °C until gonadotropin determination. Testes were also dissected and weighed immediately after sacrifice.

Brains were rapidly removed and hypothalami dissected out with a single razor blade and weighed. Hypothalamic samples containing the anterior preoptic and medial basal areas (APOA-MBH) were dissected with the help of a stereomicroscope. The hypothalamic samples were obtained at a depth of 3–4 mm and were bordered laterally by the hypothalamic sulci; rostrally 3 mm anterior to the optic chiasma and caudally by the mammillary bodies. The samples were less than 2 mm thick and no significant differences in weight were observed.

### 2.5. Hypothalamic homogenization and amino acid measurement

Each APOA-MBH was weighed and homogenized in $HClO_4$ acid 0.6 N, glass Potter homogenizer refrigerated with ice. The

**Table 1**
Peripubertal male rats offspring from dams exposed to low (3 mg/kg/day) and high (30 mg/kg/day) doses of DEHP.

| | Control | DEHP (3 mg/kg/bw day) | DEHP (30 mg/kg/bw day) |
|---|---|---|---|
| Body weight (g) | 89.1 ± 5.7 | 78.0 ± 22.2 | 85.7 ± 17.9 |
| Testes weight (mg) | 403 ± 28.5 | 398 ± 38.1 | 260.7 ± 32.2** |
| LH (ng/ml) | 13.8 ± 4.4 | 15.4 ± 5.1 | 18.7 ± 9.1 |
| FSH (ng/ml) | 403.3 ± 115.4 | 395.7 ± 87.5 | 270.1 ± 107.4* |
| Aspartate (pmol/mg tissue) | 998.2 ± 274.5 | 1046.5 ± 226.0 | 494.8 ± 186.1** |
| GABA (pmol/mg tissue) | 116.2 ± 35.8 | 12 7.9 ± 26.9 | 1.403.5 ± 222.9** |

Parameters were measured at 30 days of age. Each value represents the mean ± SD of 10 animals. No statistically significant differences between control and DEHP (3 mg/kg bw/day) groups were present.
* $p < 0.05$ DEHP (30 mg/kg bw/day) vs control.
** $p < 0.001$ DEHP (30 mg/kg bw/day) vs control.

homogenate was centrifuged at 13000 rpm for 2 min, and the supernatant obtained frozen at −80 °C until amino acids were determined. ASP and GABA concentrations were determined by high performance liquid chromatography after derivatization with phenylisothiocyanate and UV detection at 254 nm (Jarry et al., 1992). The drugs used did not interfere in the derivatization process. Mean inter- and intra-assay coefficients of variability were 5% (ASP) and 8% (GABA). The detection limit was 20 pmol for ASP and 5 pmol for GABA. The mobile phase consisted of 0.57 M sodium acetate buffer (pH 6.5) containing 10% acetonitrile (Sintorgan, Buenos Aires, Argentina). Amino acid standards were ASP and GABA (Sigma Chemical Co., St Louis, Mo, USA). The results are expressed as pmol/mg of tissue.

### 2.6. LH and FSH determination

Serum LH and FSH was determined in duplicate by using the double antibody radio immunoassay technique (Niswender et al., 1968). The material for this assay was provided by the National Hormone and Peptide Program of the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) (Harbor-UCLA Medical Center, Torrance, California, USA). Results were expressed in serum ng/ml, in accordance with the referenced preparation (rat LH RP-1, rat FSH RP-1). All samples were analyzed in the same assay and intra-assay coefficient of variation was 6%.

### 2.7. Statistical analysis

Results were expressed as the mean ± S.E. Statistical analysis was conducted using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, CA, USA. Analysis was performed non-parametrically with Kruskal–Wallis Test followed by Dunn's Multiple Comparisons Test. Differences were considered to be statistically significant at a probability level of 5% ($p < 0.05$)

## 3. Results

### 3.1. Exposure to low dose (3 mg/kg bw/day) of DEHP

In 30-day-old male rats offspring from DEHP-exposed dams, the body and testis weights, LH and FSH levels and the hypothalamic ASP and GABA concentrations were not altered (Table 1).

### 3.2. Exposure to high dose (30 mg/kg bw/day) of DEHP

The body weight gain was not altered in 30-day-old male rats that were born from females exposed to DEHP at high dose in comparison with the control group. However, the absolute testicular weight showed a significant decrease. Undescended testes were found in about 25% of the males (these animals were not used for hormone or amino acids assay). No changes in plasmatic LH level were detected in peripubertal male rats at 30 days of age. In these animals similar treatments produced a significant decrease in FSH level. The hypothalamic content of the excitatory amino acid ASP decreased significantly in 30-day-old male rats. Also a very important increase in the concentration of the inhibitory neurotransmitter GABA was observed in these peripubertal rats (Table 1).

## 4. Discussion

The widely used plasticizer DEHP has shown to have the potential to alter the development of the reproductive tract in male rats. Several investigations have demonstrated that DEHP inhibits testicular testosterone production. It has been suggested that this effect reflects the direct action of DEHP or its metabolite MEHP on the testis.

In this work we analyzed the in vivo effect of DEHP at different doses applied to female rats during pregnancy and lactation, on the neuroendocrine regulation of the reproductive axis of immature male litter. The results show that exposure to low dose of DEHP was unable to produce adverse effects on litter size, pup weight and viability. No external macroscopic abnormalities were seen in newborns. No significant changes in the testicular axis of 30-day-old peripubertal male rats were detected, not only in the absolute testicular weight but also in the gonadotropin serum levels and the hypothalamic content of amino acid neurotransmitters.

Contrary to what was described in the case of low dose, in 30-day-old peripubertal male rats that were exposed to high dose (30 mg/kg bw/day) of DEHP an alteration on the testicular axis was observed. This effect was reflected not only in a significant reduction in absolute testes weight but also in the changes in gonadotropin balance and in the ASP and GABA hypothalamic contents. The reduction in fetal Leydig cell testosterone and insulin-like growth factor 3 productions, hormones which induce scrotal descent of testis (Howdeshell et al., 2007), might explain the cryptorchidism seen in the 25% of the animals at high dose exposure. The data reported here are in accordance with results previously published, which described a decrease in gonadal weight, cryptorchidism and sex steroids alterations when DEHP was administered through similar or different pathways and doses to the one used in this work (Stroheker et al., 2005; Parks et al., 2000; Akingbemi et al., 2001; Howdeshell et al., 2007; Borch et al., 2006; Arcadi et al., 1998; Shinji et al., 2005; Wilson et al., 2004).

In peripubertal male rats born to pregnant dams treated with DEHP at high doses during pregnancy and lactation a reduction in serum FSH level was detected. We also found a relationship between the decrease in FSH and changes in the hypothalamic content of amino acid neurotransmitters (a decrease in the excitatory amino acid ASP and an increase in the inhibitory tone of GABA), which regulate gonadotropin secretion in immature male rats (see Fig. 1). Previously we have demonstrated that the activation of different receptors-subtypes of the excitatory amino acids and GABA systems is capable to stimulate or inhibit,

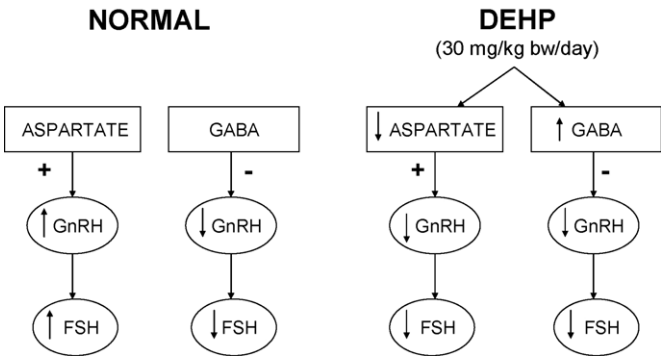


**Fig. 1.** Scheme summarizing DEHP effects on hypothalamic–pituitary axis in peripupertal male rats offspring from dams exposed to the phthalate during pregnancy and lactation.

respectively, the plasmatic FSH levels (Carbone et al., 1996; Feleder et al., 1996). Also, it is well known that amino acidergic neurons which regulate Gn-RH neurons in the APOA-MBH of the hypothalamus are modulated by sexual steroids, which can modulate different receptor-subtypes of excitatory or inhibitory amino acid neurotransmitters, showing ontogenic changes through the development (Szwarfarb et al., 1994).

On the other hand, there were no differences in plasmatic LH concentration between controls and immature rats exposed to DEHP at 30 mg/kg bw/day. Normally, when androgen biosynthesis is depressed, the lowered serum testosterone concentration produces a reduction in the negative feedback mechanisms, inducing an increase in LH output from pituitary. LH stimulates Leydig cells to secrete more testosterone, which acting via the same pathway restores pituitary LH secretion to normal levels. At hypothalmic level excitatory and inhibitory neurotransmitters regulate Gn-RH releasing, which stimulates LH. Therefore, serum LH is the result of neuroendocrine mechanisms that involved the balance between the inhibition by testosterone negative feedback mechanism and the stimulation by hypothalamic Gn-RH which is also regulated by neurotransmitter systems. Our results show a significant decrease in hypothalamic content of the excitatory amino acid ASP and an increase in the inhibitory neurotransmitter GABA. It is therefore possible that in this case the unchanged LH levels are the consequence of a simultaneous decrease of stimulatory hypothalamic signal on gonadotroph cells and the reduction of negative feedback produced by testosterone fall in DEHP exposed male rats, described by other authors (Parks et al., 2000; Akingbemi et al., 2001).

Therefore, our study provides new findings regarding changes in the hypothalamic contents of the amino acid neurotransmitters, which are involved in the neuroendocrine regulation of reproductive axis, in peripubertal male rat offspring from dams exposed to DEHP at dose of 30 mg/kg bw/day during gestational and lactational periods. This could be related with the FSH modifications also here described.

Finally, we think that more experiments are need to evaluated the impact of DEHP at hypothalamic level and the consequences that would result from cumulative effect of the exposure to this substance.

## Conflict of Interest statement

The authors declare that there are no conflicts of interest.

## Acknowledgments

This work was supported by Grants from Agencia de Promoción Científica y Técnica BID 1728 PIC 2003 and the Universidad Buenos Aires (UBACYT M816 and UBACYT M006). We thanks Angela Ciocca for the manuscript revision.

## Disclaimer

The findings and conclusions in this report are those of the authors.

## References

Akingbemi BT, Youker RT, Sottas CM, Ge R, Katz E, Klinefelter GR, et al. Modulation of rat Leydig cell steroidogenic function by di (2-ethylhexyl) phthalate. Biol Reprod 2001;65:1252–9.
Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP. Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc Natl Acad Sci USA 2004;101:775–80.
Albro PW. The biochemical toxicology of di-(2-ethylhexyl) and related phthalates: Testicular atrophy and hepatocarcinogenesis. Rev Biochem Toxicol 1987;8: 73-19.
Andrade AJ, Grande SW, Talsness CE, Grote K, Golombiewski A, Sterner-Koch A, et al. A dose response study following in utero and lactational exposure to di-(2-ethylheyl) phatalate (DEHP): effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 2006;225:64–74.
Arcadi FA, Costa C, Imperatore C, Marchese A, Repisarda A, Salemi M. Oral toxicity of bis-(2-ethylhexyl) phthalate during pregnancy and sucking into de Long-Evans rats. Food Chem Toxicol 1998;36:963–70.
Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TR, Vingaard AM. Effects of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 2001;158:141–53.
Borch J, Dalgaard M, Ladefoged O. Early testicular effects in rats perinatally exposed to DEHP in combination with DEHA—apoptosis assessment and immunohistochemical studies. Reprod Toxicol 2005;19:517–25.
Borch J, Metzdorff SB, Vinggaard AM, Brokken L, Dalgaard M. Mechanisms underlying the anti-androgenic effect of diethylhexyl phthalate in fetal testis. Toxicology 2006;223:144–55.
Brann WD, Mahesh VB. Excitatory amino acids: functiona and significance in reproduction and neuroendocrine regulation. Front Neuroendocrinol 1994;15:3–49.
Calafat AM, McKee RH. Integrating biomonitoring exposure data into the risk assessment process: phthalates [diethyl phthalate and di(2-ethylhexyl) phthalate] as a case study. Environ Health Perspect 2006;114:783–9.
Calafat AM, Needham LL, Silva MJ, Lambert G. Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. Pediatrics 2004;113:429–31.
Carbone S, Szwarcfarb B, Rondina D, Feleder C, Moguilevsky JA. Differential effects of N-methyl-D-aspartate and non-*N*-methyl-D-aspartate receptors of the excitatory amino acids system on LH and FSH secretion. Its effects on the hypothalamic luteinizing hormone releasing during maturation in male rats. Brain Res 1996;707:139–45.
Dalsenter PR, Santana GM, Grande SW, Andrade AJ, Araújo SL. Phthalate affect the reproductive function and sexual behavior of male Wistar rats. Hum Exp Toxicol 2006;25:297–303.
Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, et al. A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S. EPA Risk Assessment Guidelines. Regul Toxicol Pharmacol 1999;29:327–57.
Faouzi MA, Dine T, Gressier B, Kambia K, Luyckx M, Pagniez D, et al. Exposure of hemodialysis patients to di-2-ethylhexyl phthalate. Int J Pharm 1999;80:113–21.
Feleder C, Jarry H, Leonhart S, Wutke W, Moguilevsky JA. The GABAergic control of gonadotropin-realising hormone secretion in male rats during sexual maturation involves effects on hypothalamic excitatory and inhibitory amino acids systems. Neuroendocrinology 1996;64:305–12.
Foster PM, Mylchreest E, Gaido KW, Sar M. Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 2001;7:231–5.
Ge RS, Chen GR, Tanrikut C, Hardy MP. Phthalate ester toxicity in Leydig cells: developmental timing and dosage considerations. Reprod Toxicol 2007;23: 366–73.
Gray LE Jr, Ostby J, Fur J, Price M, Veeramachanemi DN, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 2000;58:350–65.
Howdeshell KL, Furr J, Lambright CR, Rider CV, Wilson VS, Gray LE Jr. Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. Toxicol Sci 2007;99:190–202.
Hu GS, Lian QQ, Ge RS, Hardy DO, Li XK. Phthalate-induceded testicular dysgenesis syndrome: Leydig cell influence. Trends Endocrinol Metab 2009;20:139–45.
Jarry H, Hirsch B, Leonhart S, Wutke W. Amino acid neurotransmitter release in the preoptic area of rats during the positive feedback actions of estradiol on LH release. Neuroendocrinology 1992;56:133–40.
Koch HM, Preuss R, Angerer J, Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure– an update and latest results. J Androl 2006;29:155–65.
Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 2003;111:1783–5.
Lovekamp-Swan T, Davis BJ. Mechanisms of phthalate ester toxicity in the female reproductive system. Environ Health Perspect 2003;111:139–45.
Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, et al. Human breast milk contamination with phthalates and alterations of endogenous

reproductive hormones in infants three months of age. Environ Health Perspect 2006;114:270–6.
Moguilevsky JA, Wutke W. Changes in the control of gonadotrophin secretion by neurotransmitters during sexual development in rats. Exp Clin Endocrinol Diabetes 2001;109:188–95.
Moore RW, Rudy TA, Lin TM, Ko K, Peterson RE. Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. Environ Health Perspect 2001;109:229–37.
Niswender GH, Midgley AT, Monroe SE, Reichert LE Jr. Radioimmunoassay for rat luteinizing hormone with antiovine serum and ovine LH-1 311. Proc Soc Exp Biol Med 1968;128:807–11.
NTP-CERHR (2000). NTP-CERHR. Expert Panel Report on di(2-ethylhexyl) phthalate. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction, NTP-CERHR-DEHP-00. Available: http://cerhr.niehs.nih.gov/chemicals/dehp/DEHP-final.pdf.
NTP-CERHR (2006). NTP-CERHR Monograph on the Potential Human reproductive and Developmente Effects of di(2-ethylhexyl) phthalate (DEHP). National Toxicology Program Center for the Evaluation of Risks to Human Reproduction, NIH Publication N° 06-4476. Available: http://cerhr.niehs.nih.gov/chemicals/dehp/DEHP-Monograph.pdf.
Ojeda SR, Urbanski HF. Puberty in the rat. In: Knobill E, Neill JD, editors. The physiology of reproduction, vol II. New York: Raven Press; 1994. p. 363–409.
Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, et al. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 2000;58:339–49.
Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect 2001;109:675–80.
Shinji T, Mitsuru L, Satoshi K, Kazuo J, Tadashi M, Hiroyuki K. Differential effects of phthalate esters receptors α and β and androgen receptor. Toxicology 2005;210:223–33.
Stroheker T, Cabaton N, Nourdin G, Régnier JF, Lhuguenot JC, Chagnon MC. Evaluation of anti-androgenic activity of di-(2-ethylhexyl)phthalate. Toxicology 2005; 208:115–21.
Szwarfarb B, Carbone S, Stein M, Medina J, Moguilevsky JA. Sexual differences in the effect of the GABAergic system on LH secretion and in the hypothalamic ontogenesis of GABA-A receptors in peripubertal rats. Brain Res 1994;646:351–6.
Wilson VS, Lambright C, Furr J, Ostby J, Wood C, Held G, et al. Phthalate ester-induced gubernacular lesions are associated with reduced insl 3 gene expression in fetal rat testis. Toxicology Lett 2004;146:2107–215.
Wilson VS, Blystone CR, Hotchkiss AK, Rider CV, Gray LE. Diverse mechanisms of anti-androgen action: impact on male rat reproductive tract development. Jr Int J Androl 2008;31:178–87.