Recent Progress in the Study of Adverse Effects of Phthalate Esters to the Male Reproductive Organ : A Short Review

Takao Ichimura, Ph.D.*

Abstract :

Recent studies on adverse effects of phthalate esters to the male reproductive system were reviewed to provide a perspective view of this field of research for further studies. Research papers were collected from the MEDLINE database by a keyword search protocol. The scope of the collected papers covers sciences of reproductive health, urology, andrology, toxicology, endocrinology, pathology, and cell biology. The key words used for the MEDLINE search include phthalate, testis, apoptosis, and their arbitrary combinations. 34 papers were selected out of the collected 120 papers, mostly of experimental animal studies. The species of animals, choice of phthalate esters, and schedule of exposure varied from author to author. Almost all animal studies used mice or rats as the model animal, and DEHP, MEHP, DBP or BBP as the phthalate ester to expose animals. The amount of phthalates used for exposure ranged from 1mg/kg to 1000mg/kg. The phthalates were administered once, several times, or daily for weeks.

Histopathological changes observed after phthalate exposure were malformations in male reproductive organs accompanied by altered weight of testis, liver and kidney. These toxic effects were more obvious in the pups exposed to phthalates in utero. Examination of phthalate exposed testis at cellular or subcellular levels indicated that the action of DEHP or MEHP was targeted to Sertoli cell in the seminiferous tubules. The stimulated Sertoli cell triggers apoptotic cell death in germ cells through Fas-ligand-Fas signaling system. This apoptotic effect of phthalates was observed after a single dose of 100mg/kg DEHP. Although the site of action of phthalates at the developmental stage is still unclear, recent observations of transgenerational effects of DEHP or MEHP at low doses suggest that the action took place below the proposed no observed adverse effect level (NOAEL). Vitamin C, E or B12 were reported to act as a preventive supplement for phthalate ester intoxication, but its evaluation is still immature.

Key words : phthalate ester, testis, sperm, apoptosis, trans-generational effect

Abbreviations

DEHP	di-(2-ethylhexyl) phthalate
MEHP	mono-(2-ethylhexyl) phthalate, the metabolite of DEHP
DBP	di-n-butyl phthalate
MBP	mono-n-butyl-phthalate
BBP	butyl benzyl phthalate
DINP	di-isononyl phthalate
DDE	1,1'-dichloroethylidene bis 4-chlorobenzene
PCB	poly-chlorinated biphenyls
FSH	follicle stimulating hormone
FasL	Fas ligand
Fas	Fas ligand receptor, Apo-1
Fap	Fas-asssociated phosphatase
TNFR	tumor necrotic factor receptor
FADD	Fas associated death domain

Introduction

During the last decade, hundreds of research reports on the effect of plasticizers on reproductive health have been published. Now it is widely accepted that some members of phthalate ester groups especially DEHP induce pathological altera-

* Division of Life and Health Sciences, Graduate School of Health and Welfare, Yamaguchi Prefectural University

tions in both developing and maturing male reproductive system. However, researchers still worry about the lack of evidences that show the direct link of phthalates to pathological changes in human reproductive system. They say "Most of the results in this area, especially those related to di-ethylhexyl phthalate, are collected from animal studies and the extrapolation to humans is still controversial and difficult." (Yakubovich et al., 2000)

Since normal testis formation and testicular hormone production are essential for a full development of phenotypic male, recent increase in human testicular cancer might point to disturbed development of the fetal testis. By comparison of testicular dysgenesis in humans and phthalate-exposed rats, Sharpe (2001) pointed that similarities in outcomes and testicular cell-cell disruption lead us to suppose the pathway via which estrogenic and anti-androgenic environmental chemicals might act to induce such changes. Although no direct evidence links human exposure to environmental chemicals and male reproductive disorders, it is believed simply because of the lack of information. The author emphasized that "until the appropriate in vivo studies are undertaken, the safety of hormonally active environmental chemicals, especially in mixtures, will continue to give cause for concern as far as testicular development is concerned".

Recently, a series of intensive studies by Richburg, Boekelheide and their colleagues provided us with evidences suggesting a germ cell apoptosis in the testis induced by a Sertoli cell cytotoxic action of phthalate esters DEHP and MEHP (Richgurg and Boekelheide, 1996 ; Lee et al., 1997 ; Boekelheide et al., 1998 ; Lee et al., 1999 ; Richburg et al., 1999 ; Richburg et al., 2000). In their studies, evidences were obtained mostly from animals exposed to a single dose of phthalates as high as the order of 1000mg/kg. Probably because of this high dosage, the phthalate exposure resulted in an increased incidence of apoptotic germ cell death in the testis. This acute cell death was explained by an amplified cell to cell interaction of Fas-ligand positive Sertoli cell and Fas-positive germ cell. It is quite natural to ask whether this acute cell death of germ cells may be induced by an exposure to physiological levels of phthalates.

In this short review, we surveyed and collected information available from recently published papers of experimental studies on testicular adverse effects of phthalate esters, especially of DEHP and MEHP, and to provide a perspective view of the research trends in this field of research for further studies.

testicular toxicity

Phthalate esters are chemical compounds commonly used as plasticizers for polyvinyl chloride and are known to be hormone-disrupting chemicals. Among them, DEHP, an outstanding high production volume plasticizers used in vinyl products, has long been suspected to cause testicular toxicity. Especially, mono-(2-ethylhexyl) phthalate (MEHP), the metabolite of di-(2-ethylhexyl) phthalate (DEHP), has been an alleged Sertoli cell toxicant. However, despite their widespread and long term use, most phthalate esters have not been adequately tested for reproductive or trans-generational toxicity. This is the cause for concern. Several investigations have shown that DEHP, BBP, DBP, and DINP disrupt reproductive tract development of the male rat in an anti-androgenic manner.

Recently, an extensive study of toxicity of DEHP to male and female mice was conducted by David et al. (2000). They treated B6C3F1 mice with DEHP (100～6000ppm in the diet) for up tp 104 weeks. Clinical chemistry, hematology, urinalysis, weight of body, kidney, testis, liver, uterus, as well as their histopathology were examined. For 500～6000 ppm groups, increased liver weight and decreased testis weight were seen in male mice. Hepatic tumors with increased peroxisome proliferations were also observed (David et al., 1999). For 6000ppm group, increased liver weight and decreased uterine weight were also seen in female. In addition, this high dose group showed hepatocyte pigmentation, cytoplasmic eosinophilia, and chronic inflammation in the liver, and chronic progressive nephropathy in the kidney. Immature or abnormal epididymal sperm

and bilateral hypospermia were also observed for this dose group. They identified the dose level of 500 ppm (98.5-116.8mg/kg/day) as a no-observed-adverse effect level (NOAEL) for noncarcinogenic effects.

An intensive study by Gray et al. (2000) showed adverse effects of phthalates including DEHP, BBP, and DINP on the reproductive system of male rat. Their aims were to determine which phthalate esters altered sexual differentiation in an antiandrogenic manner. They observed that DEHP, BBP, and DINP (0.75g/kg from 14 gestation day to 3 postnatal day) induced reproductive malformations including reduced anogenital distance, retained nipples, cleft phallus with hypospadias, undescended testes, a vaginal pouch, epididymal agenesis in pups of about 80% for DEHP or BBP and about 8% for DINP.

Although the site of action of DEHP was once thought to be the cAMP second messenger system in the Sertoli cell, Grasso et al. (1993) characterized the action of MEHP might be at the level of GTP-binding protein that couples the FSH receptor to the adenylate cyclase catalytic subunit. However, Gray et al. argued that high incidence of testicular and epidydimal alterations associated with toxic effects of phthalate esters were distinct from those of antiandrogenic chemicals such as flutamide, an androgen receptor antagonist, or finasteride, an inhibitor of 5-alpha-dihydrotestosterone synthesis. This distinction suggested a mechanism of action of the phthalate in altering reproductive development distinct from the antiandrogenic chemicals (Gray et al., 1999). Mylchreest et al. (1999) also recognized the different pattern of antiandrogenic effects of flutamide and DBP in disrupting the androgen signaling necessary for male sexual differentiation.

Recently, Parks et al. (2000) further examined whether the antiandrogenic action of DEHP occured by inhibiting testosterone production or by inhibiting androgen action by binding to the androgen receptor. From their observation of malformations and undetectable affinity to human androgen receptor, they indicated that DEHP disrupts male sexual differentiation by reducing testosterone to female levels in the fetal male rat during a critical stage of reproductive tract differentiation.

Li et al. (2000) studied the toxic effect of a single dose of DEHP (20-500mg/kg) on neonatal rats. They observed a morphological change in germ cells after 24hours of exposure. The sertoli cell proliferation rate assessed by the labeling index of the cell with bromodeoxy uridine was obviously diminished in the pup testicular sections. Thus, they characterized extreme sensitivity of the neonatal testis to injury by DEHP and MEHP.

Piersma et al. (2000) examined the developmental toxicity of BBP in rat exposed to 2100mg/kg/day of BBP from gestation day 5 through 20. BBP exposure induced increase in weight of liver and kidney, resorption, skeletal anomalies, retarded testicular descent, and reduction in the weight of testis and of fetuses themselves. As these fetotoxicity was found at relatively lower dosages compared to observed maternal toxicity, it appeared to the authors that BBP was specifically a embryotoxic compound.

Shono et al. (2000.) determined the time-specific effects of MBP on the transabdominal migration of the testis in fetal rats. Pregnant rats were administered MBP at 7-10 days, 11-14 days, or 15-18 days of gestation, and fetuses were examined at gestation day 20. The third group showed the maximum inhibition of transabdominal testicular descent. The epididymis showed a few small ducti deferentia. Thus, they indicated that even a brief exposure to MBP could inhibit the transabdominal migration of the testis.

transgenerational effects

To test the reproductive toxicity of DBP, the National Toxicology Program's Reproductive Assesment by Continuous Breeding protocol was conducted by Wine et al. (1997). For F0 rats 0.1～1.0% DBP was dosed daily in diet, and F1 and F2 rats were served for examination. Total number of live pups per litter was reduced by 8-17%, and live pup weights by less than 13%. Weight of pups from treated females were decreased and weight of pups

from treated males were unchanged. In the F1 mating trial, mating, pregnancy, and fertility in the highest-dose group (1%) were all sharply decreased. This group of F1 males showed decreased number of sperm counts and testicular spermatid head counts. 80% of them had degenerated seminiferous tubules, and 50% underdeveloped or otherwise defective epididymides. Thus, their results showed a reproductive /developmental toxicity of DBP in S-D rats exposed as adult or during development. Since the only observed effects on F0 rats were 14% reduced body weight (female), 10-15% increased kidney and liver to weight ratio (both sex), their results further indicated that the adverse reproductive /developmental effects of DBP on the second generation were greater than on the first generation.

Transgenerational or multigenerational effects of BBP were stuied by Nagao et al. (2000) in SD rat. The effect in parent (F0) animals were decreased body weight gain, increased kidney and (male) liver weight. Serum concentration of testosterone was increased and that of FSH was decreased. The effect in the next generation (F1) included decreased body weight at birth, decreased or increased anogenital distance at birth in male pups or female pups, respectively. Macroscopic and microscopic changes of the testis, and decreased serum testosterone level were observed at highest dosage group. From these data, the NOAEL for reproductive effects on parent animal and the next generation appeared to be 20mg/kg.

Alterations in the male reproductive development by exposure to DBP in late gestation period were examined by Mylchreest et al. (2000). Pregnant rats were given DBP from gestation day 12 to 21, and the reproductive development was examined in male offsprings. For the highest dosage group, most of the male offsprings showed reduced anogenital distance, retained areolas or nipples. At this dosage, absent or partially developed epididymis, vas deferens, seminal vesicles, and ventral prostate occurred. The weight of the testis, epididymis, dorsolateral and ventral prostate, seminal vesicles, and levator ani-bulbocavernosus muscle were all decreased. From these observations they described the NOAEL and OAEL (observed adverse effect level) for the toxicity of DBP to be 50 and 100mg/kg/day, respectively.

Imajima et al. (2001) investigated the biological effect of MBP, rather than DEHP, on the transmembrane migration of the testis in prenatal rats by comparing this with the prenatal effect of the antiandrogen flutamide on testicular descent. By comparing the position of the testis on the gestation day 19, they suggested that maternal MBP prevented transabdominal migration of the testis in prenatal rats due to a direct toxic effect of MBP on the testis.

Recently, Dees et al showed an evidence suggesting MEHP might act as a mitochondrial toxicant and lipid metabolism disrupter (Dees et al., 2001). Muhlenkamp and Gill (1998.) performed mRNA differential display using total liver RNA from male mice treated with 2% DEHP in diet for 7 days. Their Northern hybridization analysis showed downregulation of transcript for grp58 in the liver after DEHP exposure. Immunoblot analysis confirmed a correlated decrease of grp58 protein levels in DEHP-treated mice. Jones et al. (1993) suggested that MEHP intoxication might adversely alter structural and functional integrity of the Leydig cell as determined by testosterone output.

Enhanced toxicity of DEHP in developing animals was examined in 25-day old rats which received 250-500mg DEHP for consective 30 days (Parmar et al., 1995). Dose-dependent increase or decrease of enzymes was observed in lactate dehydrogenase in testes or in cytochrome P-450, respectively. Together with the observed destructive changes in the germ cell layers, the authors suggested that impaired metabolism of DEHP could lead to higher amounts of DEHP or its metaboliotes reaching the testes, and resulting in enhanced vulnerability of the testes to DEHP in developing animals.

In addition, Arcadi et al. (1998) observed an increase in time needed to perform a learned avoidance test "beam walking" in 1-month-old female suckling pups when exposed to DEHP from gestation day 1 to day 21 after delivery.

Induction of germ cell apoptosis

In 1996, Richburg, Boekelheide and their coleagues provided a comprehensive evidences showing a pivotal role of FasL-Fas interaction in initiating the germ cell apoptosis in testis exposed to MEHP. After 3 to 12 hours of MEHP exposure, Sertoli cell vimentin filaments were found collapsed and progressively condensed in the perinuclear region of the cell. As the consequence of this change in Sertoli cells, fragmentation of DNA in germ cells decreased after 3 hours, and then developed progressively from 6 to 12 hours (Richburg & Boekelheide, 1996).

Lee et al. (1997 ; 2000), with using the immunohistochemistry, localized the Fas protein to germ cells and FasL protein to Sertoli cells. Furthermore, the expression of their genes were found dramatically upregulated after exposure to MEHP or 2,5 Hexanedione.

Boekelheide et al. (1998) further revealed the postnatal expression of FasL/Fas genes in rat testis with peak expression coincident with the high levels of germ cell apoptosis during the first wave of spermatogenesis. Expression of Fas-related genes RIP and Fap-1 increased after exposure to MEHP. Since additional apoptosis inducing genes including TNFR and FADD was also detected in mammalian testis, Sertoli-germ cell interactions are not only important in controlling the germ cell apoptosis, but also Fas system and other paracrine systems are important in the homeostasis of the testis.

A possible effect of low levels of MEHP on co-cultured gonocytes and Sertoli cells was explored by Li et al. (1998). They observed a dose-dependent increase of detachment of gonocytes from Sertoli cells in culture. Furthermore, MEHP added to the culture medium inhibited FSH-stimulated Sertoli cell proliferation in a dose-dependent manner. These observations suggested that a relatively low levels of MEHP disrupted the cell-to-cell physical contact between gonocytes and the supportive Sertoli cells and suppress Sertoli cell proliferation.

Richburg et al. (1999) examined alterations in the expression of Fas and FasL in the testis after the Seltori cell and germ cell association was disrupted by exposure to MEHP. Significant increase of Fas -positive germ cells and FasL-positive Sertoli cells was observed. With time after MEHP exposure, soluble form of FasL and membrane-bound form of Fas increased. Their observation suggested that Fas-FasL paracrine signaling mechanism was involved in triggering apoptosis of germ cells in the absence of close physical association of germ cells and Sertoli cells. Using gld mice that express a nonfunctional form of Fas ligand and cell-specific toxicant-exposure paradigm, Richburg et al. (2000) indicated that the Fas signaling pathway was 1) involved in regulating the numbers of germ cells, 2) crutial for the initiation of germ cell apoptosis, and 3) acting differentially in the cell-specific regulation of germ cell apoptosis.

Dalgaad et al. (2001) investigated the testis toxic effects of mono (2-ethylhexyl) phthalate (MEHP) orally administered by a single dose of 400mg/kg body weight. They observed detachment and sloughing of germ cells and collapsed intermediate vimentin filaments in the Sertoli cells. Distribution of the androgen receptor was unchanged, while caspase-3 activity was found increased after 3 and 12 hours after MEHP exposure. The increased activity of caspase-3 was not correlated to an increase in DNA fragmentation or increase in apoptotic numbers of germ cells. Thus, they concluded that vimentin localization to Sertoli cells and the increased activity of caspase-3 might be early markers of MEHP testis toxicity, while MEHP did not affect androgen receptor.

Moore et al. (2001) conducted experiments to determine how in utero and lactational exposure to DEHP affect the development of the male reproductive system and sexual behavior. Animals were dosed with DEHP from day 3 of gestation to postnatal day 21, and their offsprings were examined with the development of their reproductive system. Dose-related alterations were reduced anogenital distance, areola and nipple retension, undescended testes, and permanently incomplete preputial separation. Weight of testis, epididymis, glans penis, ventral prostates, dorsolateral prostate, anterior

prostate, and seminal vesicle was reduced postnataly. Prostste agenesis, reduced sperm count, and testicular, epididymal, and penile malformations were also seen. Many DEHP-exposed males were sexually inactive, but the sexual inactivity did not correlated well with abnormalities in male reproductive organs. These findings suggested a possible influence of in utero and lactational DEHP exposure to the development of sexual dimorphic nucleus in the hypothalamus. They suggested that these actions of DEHP are distinct from those of other antiandrogens.

The early study by Oishi (1994) showed a preventive effect of a vitamin on testicular atrophy. In his experimental condition, adenocyl cobalamin, one of the active vitamin B12s, was co-administered with 2g/kg of DEHP and fluctuations in testicular weight were examined. Severe testicular atrophy was prevented, although hepatic changes including hypertrophy, disturbed concentrations of several metals and serum biochemical parameters were not.

Since it has been known that significant amount of di (2-ethylhexyl) phthalate leach into blood stored in DEHP-plasticized polyvinyl carbonate bags, Manojkumar et al. (1998) studied whether DEHP at such low levels had any effect on the concentration of an antioxidant vitamin E. Concentration of vitamin E decreased in the liver and testes in DEHP -treated rats, in the blood stored in the glass bottles or DEHP-plasticized polyvinyl carbonate bags. This decrease in vitamin E was prevented by administration of vitamin E to the experimental animals. The protective effect of coadministration of vitamin-C/E in preventing testicular injury due to DEHP was suggested by Ishihara et al. (2000.).

For further studies

To summarize the present status of our knowledge about the testicular toxicity of phthalate esters from experimental approaches, materials, dosage, and observations described in the selected papers are listed in Table 1. The list provides (1) the name of the phthalate examined, (2) the amount of the phthalate given to animal, (3) the method used to expose animals to the phthalate, (4) the species of animals (or cell lines) used, (5) duration or timing of exposure, and (6) the tissue, organ or function examined.

All of the experiments were done according to an independent precisely designed protocol. For DEHP or MEHP, various colonies of rats (Wister, Fisher, SD, CD) and mice (B6C3F1, gld), both male and female, were used. They were exposed to varying amount of phthalates ranged from 2 mg/kg to 2 g/kg, once or daily for weeks or even for months, before or after birth. Alterations in the weight of liver, kidney, uterus, and testis, as well as their histopathology were examined. In addition, increased incidence of germ cell DNA-fragmentation was quantitated. Clearly, comparative evaluation of all these findings is too complicated and difficult.

One of the aims of this review is to find the hitherto smallest amount of phthalate that shows any sign of adverse effects at any level from DNA to tissue, from embryo to adult, from malformation to cancer, or from learning ability to reproductive behavior. As far as the noncarcinogenic effects of DEHP are concerned, David et al. (2000) identified about 100mg/kg/day as a NOAEL for B6C3F1 mouse. As to the cytotoxic effects that induce apoptosis to germ cells, Richgurg, Boekelheide and their colleagues found a single dose of 200-400mg/kg MEHP was more than enough for rat germ cells to cause apoptotic cell death (Richgurg and Boekelheide, 1996 ; Lee et al., 1997 ; Boekelheide et al., 1998 ; Lee et al., 1999 ; Richburg et al., 1999 ; Richburg et al., 2000). Recently, apoptosis-inducing effects of a single dose of 20mg/kg of DEHP for SD mice were suggested after amplifying fragmented DNA in germ cells by an augmented PCR technique (Ichimura et al., 2001). Accordingly, even a single dose of 20mg/kg DEHP or MEHP might be enough to find any sign of acute cytotoxic effects to germ cells in mouse or rat testis. This single dose of 20mg/kg is exactly the same with the NOAEL dose for reproductive effects on parent animal and the next generation as suggested by Nagao et al. (2000). However, the reproductive or developmental effects of phthalates on second generation are expected to

be greater than on the first generation. Taking together, the NOAEL for developmental effects on the next generation might be far below 20mg/kg.

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Legend of the Table

Table. 1 Materials, dosage, and observations described in the references.

The columns from left to right represent (1) reference number, (2) the name of phthalate examined, (3) the amount of phthalate given to animals or cells, (4) method of phthalate exposure, (5) animal or cell line, (6) duration or timing of exposure, (7) tissue, organ or function examined.
oral : oral administration ; ip : intraperitoneal injection ; culture : addition to culture medium ; M : male ; F : female ; PND : postnatal day, GD : gestational day.

Table. 1 (Left)

ref #	phthalate	dosage	administration	animal(cell)/sex/age
1	DEHP	32.5-325ml/liter water	oral	rat/F
2	MEHP	2g/kg	oral	rat
3	MEHP	400mg/kg	oral	Wister rat/M/PND28
4	DEHP	100-6000ppm/day	oral	B6C3F1 mouse
5	DEHP	728-879mg/kg/day 1227-1408mg/kg/day	oral	Fischer rat B6C3F1 mouse
6	MEHP	1mM-1mM	culture medium	MA10 Leidig tumor cell
7	MEHP	(100mM)	culture medium	(Sertoli cell membrane)
9	DEHP, BBP, DINP	750mg/kg/day	oral	rat
10	DEHP	2mg-2mg/kg	oral	mouse
11	MBP	300mg/day		Wister King A rat/M/G19
12	DEHP	1-2% in diet	oral	rat
13	DEHP, DPP, DOP, DEP, MEHP, MPP, MOP, MEP	2g/kg/day, 1mM	oral, culture medium	rat
14	MEHP, hexanedione	2g/kg	oral	Fischer rat, PND28
15	MEHP, hexanedione	2g/kg	oral	Fischer rat, PND28
16	MEHP	0.01-1mM in culture medium	culture medium	(gonocyte-Sertoli cell)
17	DEHP, MEHP	20-500mg/kg	oral	PND3 rat
18	DEHP	1.5-7.5mg/kg	ip	rat
19	DEHP	375-1500mg/kg/day		SD rat//M/
20	DEHP	2% in diet	oral	C57B6 mouse
21	DBP	100-500mg/kg/day		CD rat
22	DBP	0.5-500mg/kg/day	oral	CD rat
23	BBP	20-500mg/kg/day	oral	SD rat/F&M/
24	DEHP	2g/kg		rat
25	DEHP	750mg/kg/day	oral	rat
26	DEHP	50-500mg/kg/day	oral	rat
27	BBP	270-2100mg/kg/day	oral	rat/M/
28	MEHP	2g/kg	oral	Fisher rat/M/PND28
29	MEHP	2g/kg	oral	Fisher rat/M/PND28
30	MEHP	1g/kg (5Gy radiation)		gld mouse
32	MBP		stomach-tube	GD20
33	DBP	0.1-1% in diet (52-509mg/kg)	oral	SD rat/ F&M/pup

Table. 1 (Right)

exposure term	structure and function examined
GD1-PND21	beam walking test
	genes expression of Fas, FasL, FAP, TNFR, FADD etc.
single	Sertoli, AR, Caspase3, apoptosis
-104weeks	weight, clinical chemistry, hematology, urinarlysis, sperm
78weeks	hematology, histopathology of liver, kidney, testis
24h	cell structure, progesterone, viability
3-24h	FSH binding
GD14-PND3	weight, development
12h, PND28	testis, apoptosis
GD15-GD18	testicular descent
	testis, sperm
2days	Leidig cell, Sertoli cell, testosterone histopathology,
0-12h	testis, apoptosis
0-12h	testis, apoptosis
48 h	cel-cell contact, Sertoli proliferation
24-48h	germ cell morphology, certoli cell proliferation, cyclin D2 mRNA
single	serum vitamin E
GD3-PND21	development, behavior
	GRP58 gene expression
GD12-21	development
	development
	development, weight, fertility, testosterone, FSH in F0/F1
	testis, liver, serum parameters
GD14-PND3	weight, development
30days	testicular enzyme, hepatic P-450
GD5-GD20	development, weight
0-12h	Fas, FasL
0-12h	Fas, FasL
	germ cell apoptosis, Sertoli cell
GD7-10, GD11-14, GD15-18	testis position, epididymis development
(GD1−)	weight, fertility, sperm