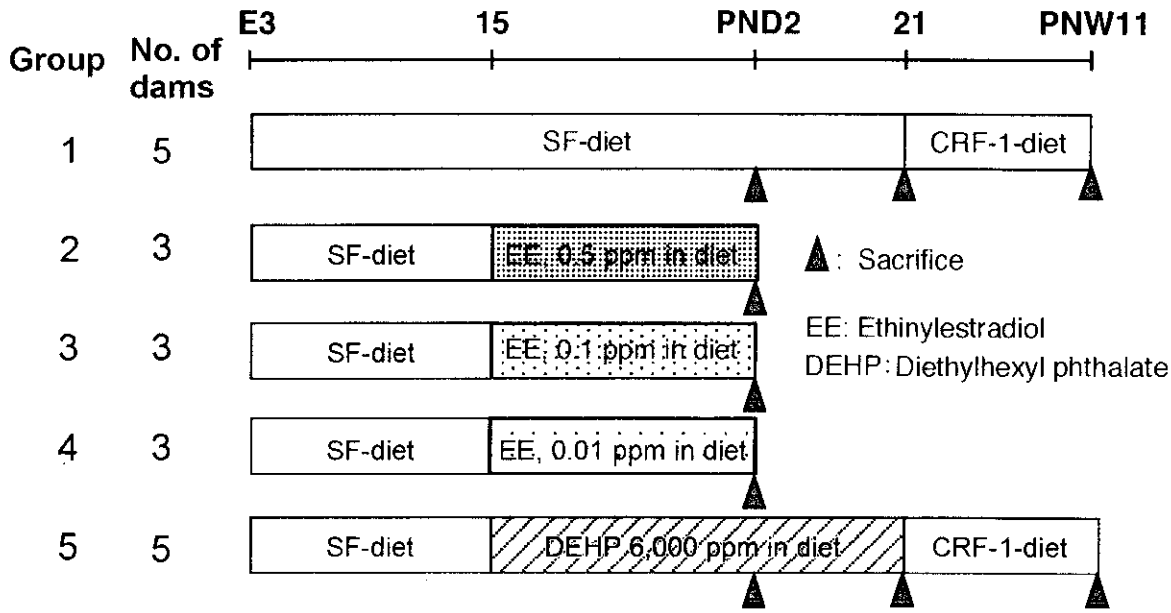

Fig. 3
Animal studies for global gene expression profiling for dissruption of the brain sexual differentiation.

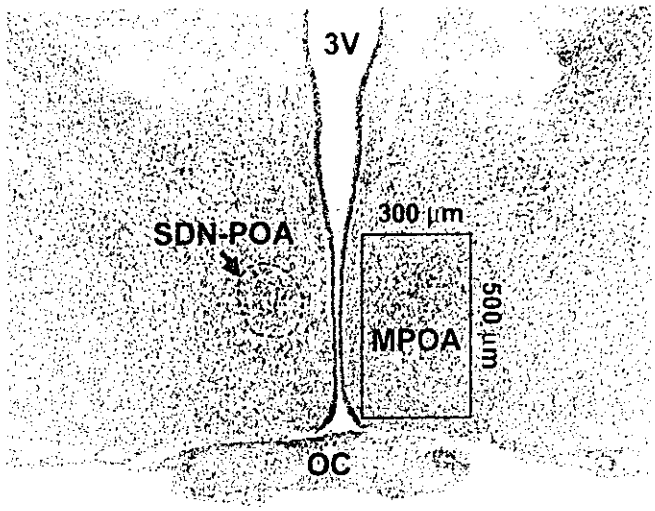

Fig. 4

Schematic view of the hypothalamic MPOA at PND 9.

The enclosed area shown in this figure was microdissected from sections of methacarn-fixed paraffin-embedded brain slices for gene expression analysis. Optic chiasm was manually removed before paraffin-embedding. Abbreviations: 3V: 3rd ventricle; SDN-POA: sexually dimorphic nucleus of the preoptic area; MPOA: medial preoptic area.

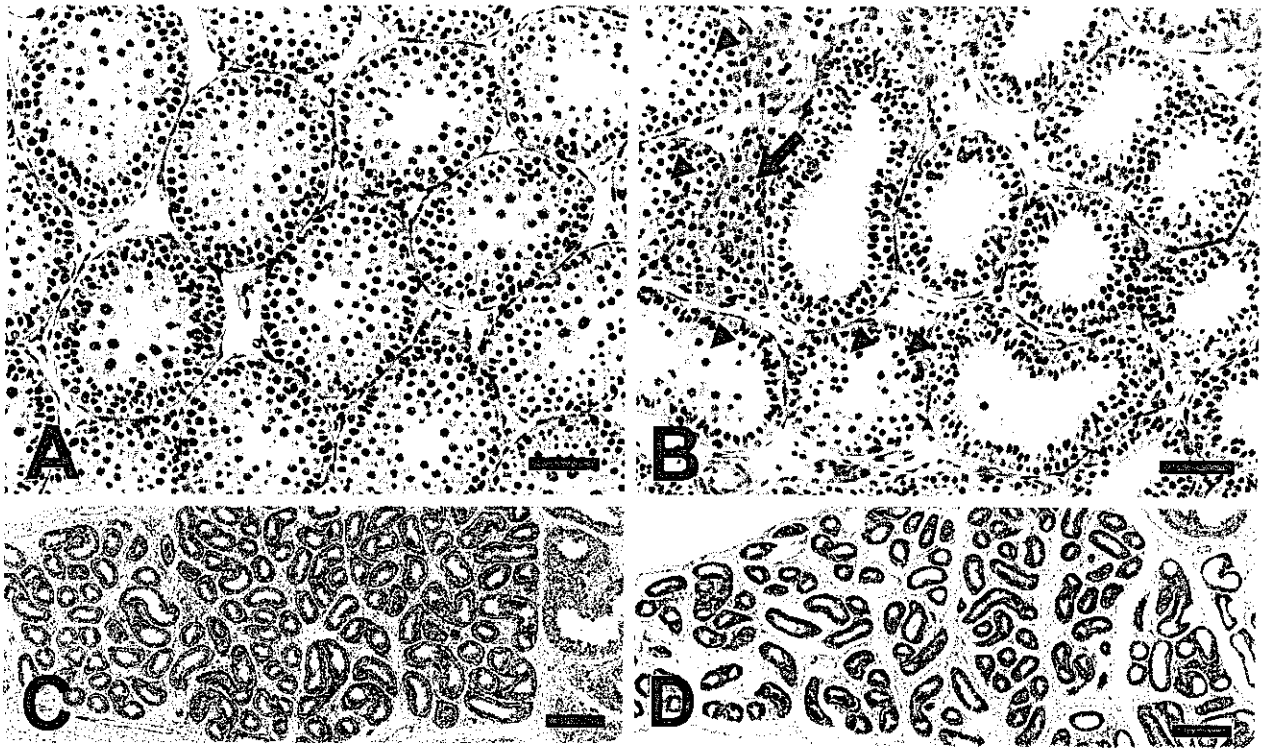

Fig. 5. Histopathological changes of the testis and epididymis in offspring at PND 21 following exposure to DBP during the late gestation and lactation periods (A–D). (A). Testis of a control male. (B). Reduction of spermatocyte development as manifested by decreased numbers in the seminiferous tubules in a male exposed to 10,000 ppm DBP. Note tubules lacking generation of spermatocytes and a focus of Leydig cell aggregation (arrow). Arrowheads indicate normally developing spermatocytes in some tubules. (C). Longitudinal section of the epididymal tail of a control male. (D). Decreased ductular cross sections of the epididymal ducts indicating reduced coiling in the corresponding portion to panel (C) in a male exposed to 10,000 ppm DBP. Bar = 50 μm (A and B); 200 μm (C and D).

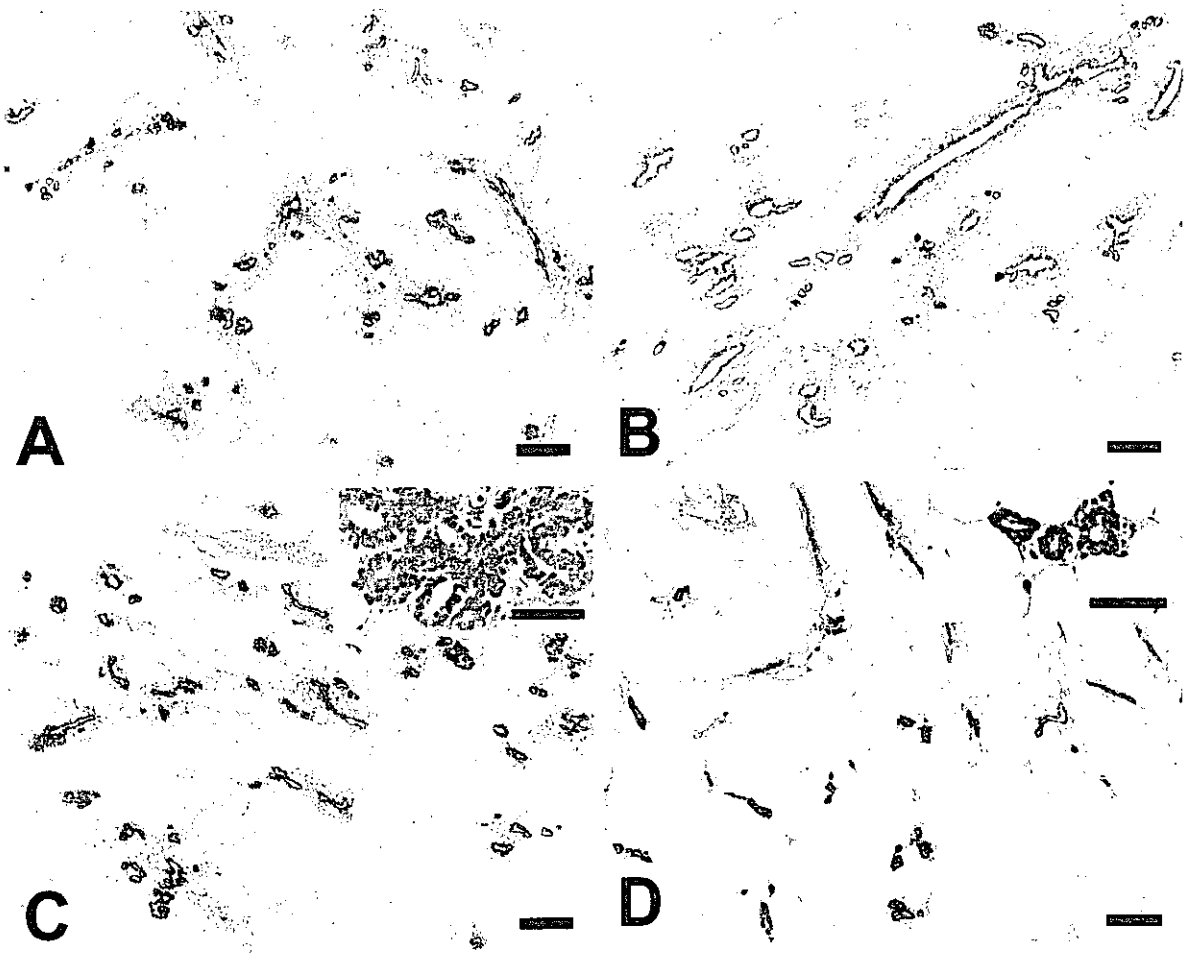

Fig. 6. Histopathological changes of the mammary gland in offspring at PND 21 following exposure to DBP during the late gestation and lactation periods (A–D). (A). Mammary gland of a control male. (B). Dilatation of alveolar buds and ducts in a male exposed to DBP at 20 ppm. (C). Mammary gland of a control female. Inset shows normal branching of alveolar buds from a terminal ductule. (D). Hypoplasia of alveolar buds of the mammary gland in a female exposed to DBP at 20 ppm. Inset illustrates poor branching of alveolar buds. Bar = 50 μm (insets in C and D); 200 μm (A–D).

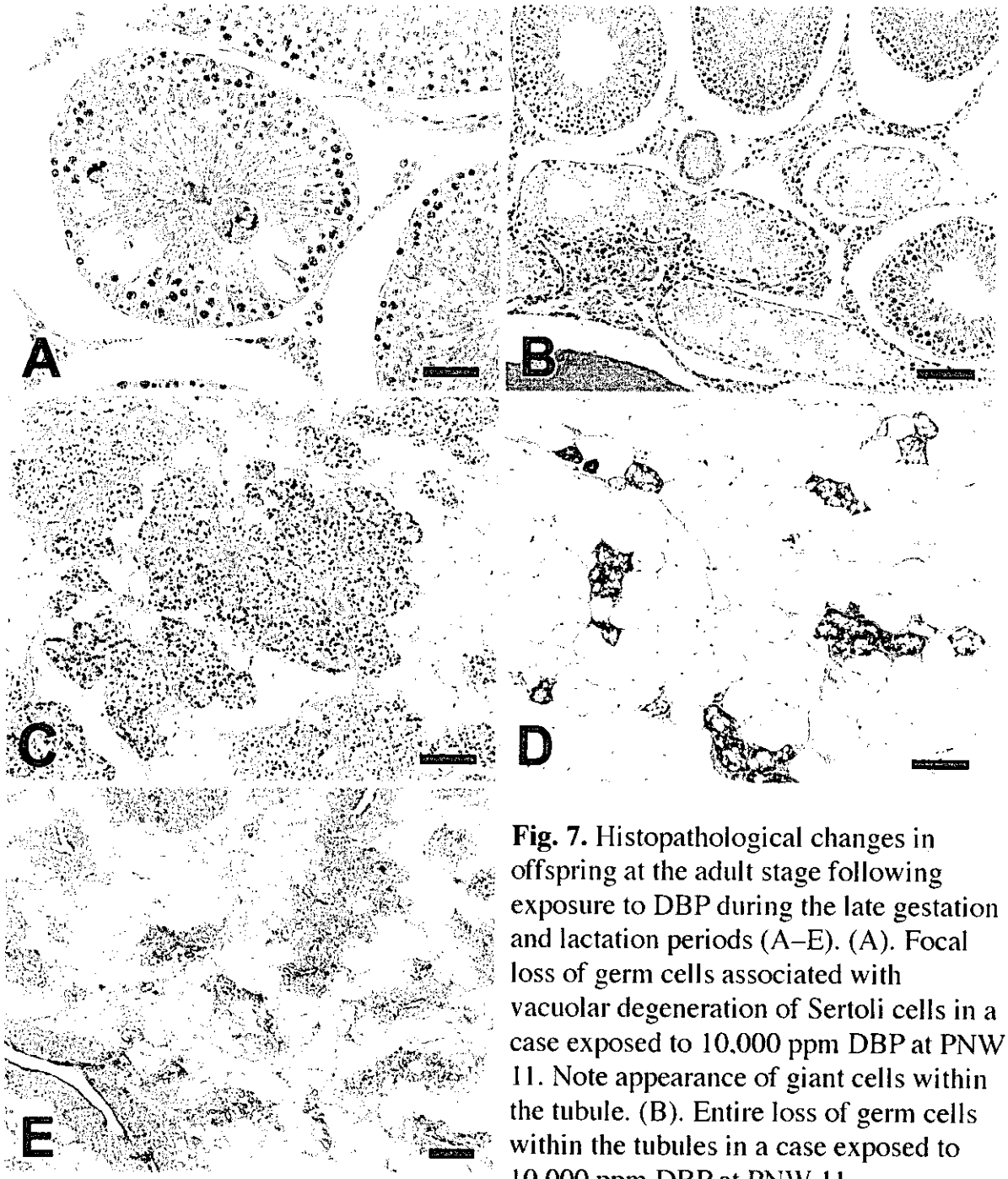

Fig. 7. Histopathological changes in offspring at the adult stage following exposure to DBP during the late gestation and lactation periods (A–E). (A). Focal loss of germ cells associated with vacuolar degeneration of Sertoli cells in a case exposed to 10,000 ppm DBP at PNW 11. Note appearance of giant cells within the tubule. (B). Entire loss of germ cells within the tubules in a case exposed to 10,000 ppm DBP at PNW 11. Affected tubular sections are composed solely of Sertoli cells, the feature being so-called 'Sertoli-cell only appearance'. Note Leydig cell hyperplasia around the tubular lesions. (C). Mammary gland of a control male at PNW 11. (D). Vacuolar degeneration of alveolar cells in the mammary gland of a male exposed to 10,000 ppm DBP. Alveolar atrophy is also evident in this case. (E). Alveolar atrophy associated with fibrosis of surrounding connective tissue in the mammary gland of a male exposed to 20 ppm DBP. Bar = 50 μm (A); 100 μm (B–D); 200 μm (E).

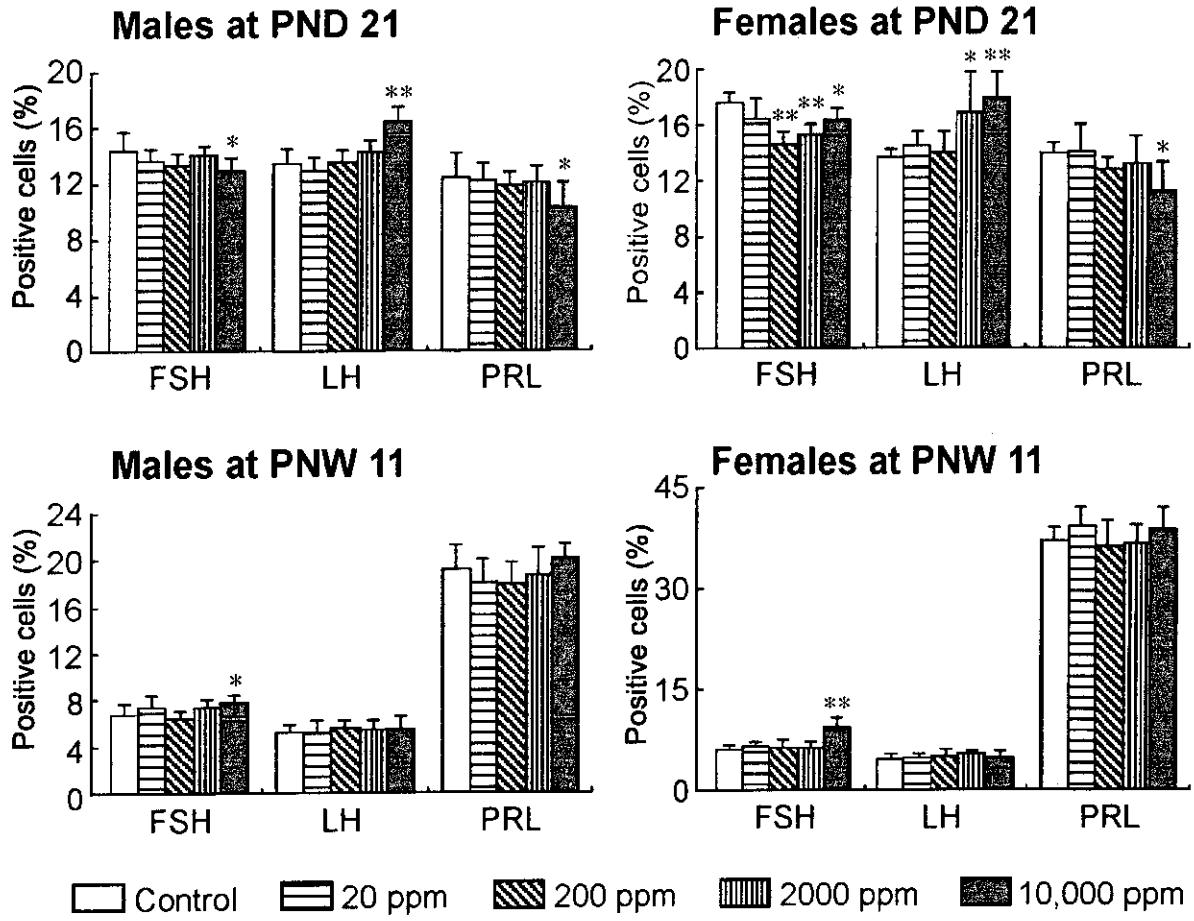

Fig. 8.
Percentages of FSH, LH, and PRL-positive cells in the anterior pituitary of offspring at PND 21 and PNW 11 following exposure to DBP during the late gestation and lactation. Asterisks indicate statistically significant differences between controls and DBP-exposed animals (*, $p < 0.05$, **, $p < 0.01$ by Dunnett's test or Dunnett-type rank-sum test).

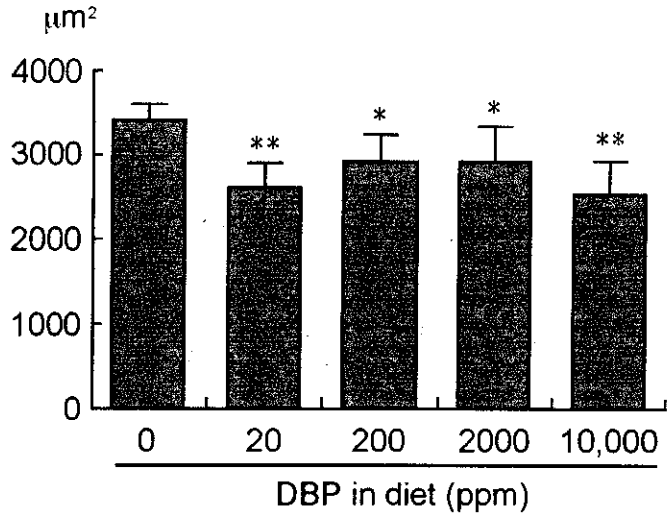

Fig. 9.
Average size of mammary alveolar buds in male offspring at PNW 11 following exposure to DBP during the late gestation and lactation periods. Asterisk sindicate statistically significant differences between controls and DBP-exposed animals (*, $p < 0.05$, **, $p < 0.01$ by Dunnett's test or Dunnett-type rank-sum test).

Table 1

Effects on dams and offspring until prepubertal necropsy of exposure to di-*n*-butyl phthalate (DBP) during late gestation and lactation

	DBP in diet (ppm)				
	0	20	200	2000	10,000
No. of dams examined	7	7	6	8	6
Maternal parameter					
Body weight gain (g/day)					
GD 15–GD 20	14.1 ± 1.4 [a]	11.5 ± 1.7*	13.8 ± 2.5	13.1 ± 1.5	11.2 ± 2.3*
PND 2–PND 10	4.2 ± 0.6	4.3 ± 1.9	4.5 ± 2.2	4.1 ± 1.5	4.3 ± 1.6
PND 10–PND 21	-1.0 ± 1.6	-1.5 ± 1.6	-1.2 ± 1.4	-0.7 ± 1.0	-1.4 ± 1.0
Food consumption (g/day)					
GD 15–GD 20	27.4 ± 2.2	26.4 ± 2.6	26.0 ± 2.5	24.6 ± 6.0	25.5 ± 3.8
PND 2–PND 10	35.7 ± 1.9	37.6 ± 5.2	36.2 ± 2.7	35.6 ± 3.6	35.1 ± 4.4
PND 10–PND 21	43.3 ± 11.6	49.6 ± 5.5	47.0 ± 6.5	47.6 ± 4.7	44.9 ± 13.0
DBP intake (mg/kg/day)					
GD 15–GD 20	0	1.5 ± 0.1	14.4 ± 0.8	148.2 ± 15.3	712.3 ± 128.9
PND 2–PND 10	0	2.4 ± 0.4	22.7 ± 1.4	223.6 ± 17.5	1108.5 ± 162.7
PND 10–PND 21	0	3.0 ± 0.4	28.5 ± 4.9	290.9 ± 36.4	1371.8 ± 376.9
Duration of pregnancy (days)	21.2 ± 0.5	21.5 ± 0.5	21.6 ± 0.5	21.3 ± 0.5	21.3 ± 0.5
Offspring parameter					
No. of live offspring	13.3 ± 2.2	11.0 ± 2.6	13.7 ± 1.9	12.4 ± 1.7	12.8 ± 1.9
Male ratio (%)	65.6 ± 14.2	51.0 ± 17.8	47.4 ± 13.5	43.9 ± 15.7*	24.7 ± 4.5**
BW, PND 2 (g)					
Males	6.7 ± 0.5	7.6 ± 0.6*	7.1 ± 0.8	7.0 ± 0.7	6.5 ± 0.5
Females	6.4 ± 0.5	7.3 ± 0.7*	6.8 ± 0.8	6.5 ± 0.6	6.0 ± 0.4
AGD, PND 2 (mm)					
Males	3.7 ± 0.2	3.9 ± 0.2	3.8 ± 0.3	3.8 ± 0.2	3.0 ± 0.1**
Females	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Nipples/areolae in males at PND 14					
No. of identified animals (%)	0	4	13	15	100##
Relative organ weights, PND 21					
No. of offspring examined	8	8	8	8	8
Males					
BW (g)	52.9 ± 7.0	49.8 ± 3.9	52.8 ± 3.3	55.5 ± 4.8	46.7 ± 3.0
Liver (g/100g BW)	3.66 ± 0.20	3.47 ± 0.29	3.67 ± 0.08	3.81 ± 0.17	4.72 ± 0.25**
Kidneys (g/100g BW)	1.07 ± 0.06	1.04 ± 0.06	1.09 ± 0.07	1.11 ± 0.07	1.10 ± 0.04
Brain (g/100g BW)	2.80 ± 0.35	3.00 ± 0.11	2.80 ± 0.24	2.72 ± 0.22	3.13 ± 0.22*
Adrenals (mg/100g BW)	28.87 ± 5.38	25.23 ± 3.80	23.57 ± 7.84	24.11 ± 7.35	30.88 ± 4.54
Testes (g/100g BW)	0.43 ± 0.03	0.41 ± 0.04	0.40 ± 0.03	0.40 ± 0.04	0.35 ± 0.03**
Epididymides (g/100g BW)	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01
Females					
BW (g)	50.2 ± 7.2	50.3 ± 6.4	50.9 ± 4.8	49.2 ± 10.0	44.0 ± 6.1
Liver (g/100g BW)	3.81 ± 0.23	3.56 ± 0.20	3.86 ± 0.15	3.73 ± 0.15	4.82 ± 0.26**
Kidneys (g/100g BW)	1.11 ± 0.09	1.08 ± 0.05	1.17 ± 0.05	1.23 ± 0.16	1.13 ± 0.05
Brain (g/100g BW)	2.88 ± 0.33	2.87 ± 0.28	2.87 ± 0.26	3.03 ± 0.76	3.26 ± 0.55
Adrenals (mg/100g BW)	29.39 ± 5.76	27.43 ± 6.79	29.04 ± 6.02	23.95 ± 4.53	28.80 ± 6.02
Ovaries (mg/100g BW)	35.39 ± 8.38	33.14 ± 7.12	35.71 ± 4.71	30.79 ± 6.62	32.71 ± 6.42
Uterus (g/100g BW)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01

[a] Mean ± SD.

Abbreviations: DBP, di-*n*-butyl phthalate; GD, gestational day; PND, postnatal day; BW, body weight; AGD, anogenital distance.

* ,** Significantly different from the controls by Dunnett's test or Dunnett-type rank-sum test (* $p<0.05$, ** $p<0.01$).

Significantly different from the controls by Fisher's exact probability test ($p<0.01$).

Table 2

Onset of puberty and estrous cyclicity in the offspring exposed to di-*n*-butyl phthalate (DBP) during the late gestation and lactation periods.

	DBP in diet (ppm)				
	0	20	200	2000	10,000
Onset of puberty					
Males No. of animals examined	20	20	16	24	11
Age by day	42.1 ± 1.3 [a]	41.2 ± 1.5	40.8 ± 1.2*	41.7 ± 1.5	42.5 ± 1.9
Females No. of animals examined	20	20	16	24	18
Age by day	34.1 ± 1.8	33.5 ± 1.8	34.3 ± 2.1	34.8 ± 2.1	35.6 ± 2.0
Estrous cyclicity (Normal/ED/EE)					
PNW 8–11	(7/1/0)	(7/1/0)	(7/1/0)	(6/2/0)	(4/4/0)
PNW 17–20	(9/1/0)	(9/1/0)	(7/0/1)	(7/3/0)	(9/1/0)

[a] Mean ± SD

Abbreviations: DBP, di-*n*-butyl phthalate; PNW, postnatal week; ED, extended diestrus ; EE, extended estrus.

* Significantly different from the controls by Dunnett's test or Dunnett-type rank-sum test ($p<0.05$).

Table 3

Organ weights at necropsy in PNWs 11 and 20 for offspring exposed to di-*n*-butyl phthalate (DBP) during the late gestation and lactation periods.

	DBP in diet (ppm)				
	0	20	200	2000	10,000
PNW 11					
No. of animals examined	8	8	8	8	10 (8)[b]
Males					
BW (g)	398.0 ± 15.8[a]	398.1 ± 24.7	429.8 ± 29.8	410.3 ± 51.9	424.8 ± 32.4
Liver (g/100g BW)	3.41 ± 0.20	3.36 ± 0.21	3.38 ± 0.27	3.40 ± 0.30	3.38 ± 0.21
Kidneys (g/100g BW)	0.69 ± 0.04	0.66 ± 0.03	0.68 ± 0.05	0.67 ± 0.04	0.61 ± 0.04**
Brain (g/100g BW)	0.50 ± 0.03	0.51 ± 0.03	0.47 ± 0.04	0.50 ± 0.06	0.49 ± 0.03
Pituitary (mg/100g BW)	2.50 ± 0.29	2.90 ± 0.20*	2.98 ± 0.25*	3.04 ± 0.29**	2.77 ± 0.41
Adrenals (mg/100g BW)	13.60 ± 1.71	12.30 ± 1.19	13.58 ± 1.40	12.15 ± 1.64	11.88 ± 2.34
Testes (g/100 g BW)	0.79 ± 0.03	0.80 ± 0.06	0.77 ± 0.09	0.84 ± 0.12	0.73 ± 0.14
Epididymides (g/100g BW)	0.24 ± 0.02	0.24 ± 0.02	0.22 ± 0.03	0.23 ± 0.03	0.19 ± 0.06
Prostate, ventral (g/100g BW)	0.12 ± 0.03	0.16 ± 0.02	0.17 ± 0.03**	0.15 ± 0.05	0.13 ± 0.03
Seminal vesicles(g/100g BW)	0.30 ± 0.05	0.29 ± 0.02	0.32 ± 0.04	0.25 ± 0.11	0.26 ± 0.05
Females					
BW (g)	267.6 ± 16.7	267.2 ± 21.5	286.6 ± 33.7	270.5 ± 39.9	270.9 ± 18.2
Liver (g/100g BW)	3.41 ± 0.17	3.39 ± 0.23	3.72 ± 0.20	3.52 ± 0.51	3.28 ± 0.24
Kidneys (g/100g BW)	0.64 ± 0.08	0.67 ± 0.06	0.66 ± 0.04	0.67 ± 0.06	0.62 ± 0.05
Brain (g/100g BW)	0.69 ± 0.05	0.73 ± 0.05	0.67 ± 0.09	0.71 ± 0.08	0.69 ± 0.03
Pituitary (mg/100g BW)	5.42 ± 0.68	5.24 ± 0.59	5.04 ± 0.54	4.94 ± 0.52	3.48 ± 0.54**
Adrenals (mg/100g BW)	23.14 ± 2.78	21.57 ± 2.53	23.26 ± 2.75	21.39 ± 2.55	21.55 ± 2.12
Ovaries (mg/100g BW)	33.99 ± 3.03	38.32 ± 7.17	33.63 ± 3.75	37.15 ± 5.43	34.83 ± 5.09
Uterus (g/100g BW)	0.16 ± 0.02	0.16 ± 0.03	0.15 ± 0.03	0.18 ± 0.03	0.18 ± 0.03
PNW 20					
No. of animals examined	10	10	8	10	0 (10)[b]
Males					
BW (g)	541.1 ± 66.3	582.5 ± 55.0	612.3 ± 79.7	567.3 ± 61.7	n.a.
Liver (g/100g BW)	2.82 ± 0.15	2.92 ± 0.29	3.12 ± 0.23	2.94 ± 0.37	n.a.
Kidneys (g/100g BW)	0.59 ± 0.03	0.59 ± 0.05	0.60 ± 0.05	0.57 ± 0.05	n.a.
Brain (g/100g BW)	0.39 ± 0.05	0.37 ± 0.04	0.35 ± 0.05	0.38 ± 0.04	n.a.
Pituitary (mg/100g BW)	2.69 ± 0.32	2.70 ± 0.45	2.73 ± 0.42	2.76 ± 0.24	n.a.
Adrenals (mg/100g BW)	10.59 ± 1.69	9.17 ± 1.62	9.85 ± 1.51	10.35 ± 2.91	n.a.
Testes (g/100g BW)	0.67 ± 0.08	0.62 ± 0.09	0.58 ± 0.07	0.67 ± 0.09	n.a.
Epididymides (g/100g BW)	0.26 ± 0.04	0.24 ± 0.03	0.23 ± 0.03	0.26 ± 0.03	n.a.
Prostate, ventral (g/100g BW)	0.15 ± 0.04	0.12 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	n.a.
Seminal vesicles (g/100g BW)	0.30 ± 0.05	0.27 ± 0.03	0.26 ± 0.04	0.27 ± 0.05	n.a.
Females					
BW (g)	339.5 ± 34.5	346.5 ± 24.0	385.0 ± 45.5	378.7 ± 50.3	339.2 ± 49.1
Liver (g/100g BW)	2.76 ± 0.11	2.87 ± 0.13	2.75 ± 0.29	2.79 ± 0.17	2.79 ± 0.34
Kidneys (g/100g BW)	0.52 ± 0.05	0.56 ± 0.05	0.54 ± 0.03	0.52 ± 0.04	0.51 ± 0.05
Brain (g/100g BW)	0.59 ± 0.07	0.58 ± 0.05	0.54 ± 0.07	0.53 ± 0.07	0.59 ± 0.07
Pituitary (mg/100g BW)	6.03 ± 0.87	5.71 ± 0.83	5.05 ± 0.72*	5.04 ± 0.68*	4.64 ± 0.80**
Adrenals (mg/100g BW)	18.52 ± 4.16	19.63 ± 2.27	19.66 ± 3.24	17.54 ± 4.33	17.09 ± 3.01
Ovaries (mg/100g BW)	28.01 ± 4.77	28.05 ± 3.82	23.34 ± 4.41	26.54 ± 4.62	24.53 ± 4.84
Uterus (g/100g BW)	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.03	0.13 ± 0.03	0.16 ± 0.03

[a] Mean ± SD.

[b] No. of females examined.

Abbreviations: DBP, di-*n*-butyl phthalate; BW, body weight; n.a., not available.

* ,** Significantly different from the controls by Dunnett's test or Dunnett-type rank-sum test (* $p<0.05$, ** $p<0.01$).

Table 4

Histopathological findings for offspring exposed to di-*n*-butyl phthalate (DBP) during the late gestation and lactation periods.

		DBP in diet (ppm)				
PND 21		0	20	200	2000	10,000
Males	No. of animals examined	8	8	8	8	8
Testis						
Reduction of spermatocyte development (±/+/++) [a]		0	4[b] (2/2/0) [c]*	4(4/0/0) *	8(1/6/1)**,##	8(0/0/8)**,##
Aggregated foci of Leydig cells		0	0	1	8**	8**
Epididymis						
Decreased ductular cross sections, epididymal duct (+/++)		0	0	0	5(5/0)**,#	7(5/2)**,##
Mammary gland						
Dilatation of alveolar bud (±/+/++)		0	2(0/1/1)	2(0/2/0)	2(2/0/0)	1(0/1/0)
Dilatation of duct (±/+/++)		0	2(0/0/2)	3(0/3/0)	1(1/0/0)	3(0/3/0)
Liver						
Liver cell hypertrophy (+/++/+++)		0	0	0	0	8(2/3/3)**,##
Females	No. of animals examined	8	8	8	8	8
Mammary gland						
Hypoplasia of alveolar bud (±/+)		0	4(2/2) *	3(1/2)	4(3/1) *	4(0/4) *
Liver						
Liver cell hypertrophy (++/+++)		0	0	0	0	8(2/6)**,##
PNW 11						
Males	No. of animals examined	8	8	8	8	10
Testis [d]						
Loss of germ cell development (±/+/++/+++)		0	0	1(1/0/0/0)	4(4/0/0/0) *	9(7/0/0/2)**,##
Leydig cell hyperplasia (+/++/+++)		0	0	0	0	2(1/0/1)
Epididymis [d]						
Intraductular cellular debris (±)		1(1)	0	0	0	4(4)
Hypoplasia		0	0	0	0	2
Prostate, ventral lobe						
Flattening of surface epithelia (±/+/++/+++)		2(0/2/0/0)	6(3/2/1/0) *	3(0/2/0/1)	3(0/0/3/0)	9(1/6/2/0)**,#
Mammary gland						
Vacuolar degeneration, alveolar cells (±/+/++/+++)		1(0/1/0/0)	8(0/2/6/0)**,##	6(0/3/3/0)**,#	8(0/3/5/0)**,##	9(0/1/5/3)**,##
Alveolar atrophy (±/+/++/+++)		0	6(2/3/0/1)**,#	2(1/1/0/0)	6(0/4/1/1)**,#	5(0/2/3/0)*,#
Females	No. of animals examined	8	8	8	8	8
Pituitary						
Small in size		0	0	0	0	6**
PNW 20						
Males	No. of animals examined	10	10	8	10	n.a.
Testis [d]						
Loss of germ cell development (±/+/++/+++)		1(0/1/0/0)	2(0/1/0/1)	2(0/2/0/0)	5(5/0/0/0)	n.a.
Leydig cell hyperplasia (±/+/++)		1(1/0/0)	1(0/0/1)	1(0/0/1)	0	n.a.
Prostate, ventral lobe						
Flattening of surface epithelia (±/+/++)		3(1/1/1)	2(0/1/1)	4(1/0/3)	7(0/4/3)	n.a.
Mammary gland						
Vacuolar degeneration, alveolar cells (±/+/++)		2(2/0/0)	5(2/2/1)	6(3/3/0)*,#	6(0/4/2)	n.a.
Alveolar atrophy (±/+/++/+++)		1(0/1/0/0)	5(0/3/2/0)	8(0/8/0/0)**,##	8(0/6/2/0)**,##	n.a.
Females	No. of animals examined	10	10	8	10	10
No abnormalities		10	10	8	10	10

[a] Grade of change; ±, minimal; +, slight; ++, moderate; and +++, severe.

[b] Total No. of animals with each finding.

[c] No. of animals with each grade.

[d] Lesions in these organs appeared either unilaterally or bilaterally, and the higher score was selected when lesions appeared bilaterally.

Abbreviations: DBP, di-*n*-butyl phthalate; n.a., not available.

*,** Significantly different from the controls by Fisher's exact probability test (*$p < 0.05$, **$p < 0.01$).

#,## Significantly different from the controls by Mann-Whitney's *U*-test (#$p < 0.05$, ##$p < 0.01$).

Table 5

Effects on dams and offspring until prepubertal necropsy of exposure to diisononyl phthalate (DINP) during late gestation and lactation.

	DINP in diet (ppm)			
	Control	400	4000	20000
No. of dams examined	8	10	8	10
Maternal parameter				
BW change (g/day)				
GD15-GD20	13.2 ± 3.4[a]	13.8 ± 1.7	14.7 ± 2.1	6.1 ± 1.6**
PND2-PND10	4.7 ± 1.9	4.5 ± 2.2	4.8 ± 1.4	-0.9 ± 2.0**
PND10-PND21	-1.6 ± 1.2	-1.2 ± 1.3	-0.5 ± 0.8	-2.4 ± 1.5
Food intake (g/day)				
GD15-GD20	27.0 ± 2.7	27.4 ± 2.8	26.4 ± 2.9	15.4 ± 2.9**
PND2-PND10	41.6 ± 5.7	42.1 ± 3.5	42.0 ± 3.6	29.2 ± 4.5**
PND10-PND21	53.5 ± 12.9	55.8 ± 7.0	53.1 ± 11.6	38.0 ± 5.7**
DINP intake (mg/kg/day)				
GD15-GD20	0	28.4 ± 2.5	269.9 ± 18.4	825.8 ± 146.4
PND2-PND10	0	51.0 ± 6.4	506.4 ± 46.7	2142.1 ± 241.9
PND10-PND21	0	62.8 ± 10.8	593.4 ± 140.3	2823.9 ± 296.3
Duration of pregnancy (days)	21.4 ± 0.5	21.8 ± 0.4	21.6 ± 0.5	21.4 ± 0.5
Offspring parameter				
No. of live offspring	12.3 ± 3.5	13.3 ± 3.3	14.3 ± 1.8	13.5 ± 1.4
Male ratio (%)	47.5 ± 16.9	45.2 ± 14.1	50.0 ± 12.1	49.5 ± 8.9
Body weight at PND2 (g)				
Males	7.5 ± 1.0	7.4 ± 0.8	7.1 ± 0.6	5.8 ± 0.8**
Females	7.0 ± 0.9	7.0 ± 0.9	6.7 ± 0.6	5.6 ± 0.8**
AGD at PND2 (mm)				
Males	4.0 ± 0.2	4.2 ± 0.2	4.1 ± 0.2	3.8 ± 0.3
Females	2.1 ± 0.1	2.2 ± 0.2*	2.2 ± 0.1*	2.1 ± 0.1
Nipples/areolae in males at PND 14				
No. of identified animals (%)	0%	19.4%*	34.6%**	30.8%**
Relative organ weights at PND 21				
No. of animals examined	10	10	10	10
Males				
BW (g)	62.0 ± 4.5[a]	55.4 ± 5.0**	54.0 ± 4.4**	26.0 ± 3.8**
Liver (g/100g BW)	3.85 ± 0.12	3.68 ± 0.27	3.97 ± 0.31	3.85 ± 0.34
Kidneys (g/100g BW)	1.09 ± 0.06	1.10 ± 0.08	1.24 ± 0.07**	1.29 ± 0.12**
Brain (g/100g BW)	2.48 ± 0.13	2.73 ± 0.21	2.84 ± 0.23*	5.18 ± 0.56**
Adrenals (mg/100g BW)	27.9 ± 4.3	23.4 ± 5.2	25.6 ± 7.0	19.4 ± 11.8
Testes (g/100g BW)	0.38 ± 0.08	0.42 ± 0.03	0.44 ± 0.03	0.47 ± 0.04*
Epididymides (g/100g BW)	0.06 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.02**
Females				
BW (g)	60.3 ± 5.2	54.2 ± 4.5**	51.6 ± 2.2**	25.1 ± 3.1**
Liver (g/100g BW)	4.07 ± 0.22	3.83 ± 0.21	4.04 ± 0.32	4.13 ± 0.24
Kidneys (g/100g BW)	1.19 ± 0.07	1.17 ± 0.07	1.26 ± 0.09	1.30 ± 0.06**
Brain (g/100g BW)	2.50 ± 0.17	2.73 ± 0.17	2.85 ± 0.12*	5.24 ± 0.52**
Adrenals (mg/100g BW)	24.9 ± 5.9	25.5 ± 4.6	25.7 ± 7.5	23.4 ± 13.6
Ovarys (mg/100g BW)	27.8 ± 11.8	29.6 ± 7.5	26.6 ± 3.5	23.7 ± 10.6
Uterus (g/100g BW)	0.06 ± 0.01	0.07 ± 0.01*	0.08 ± 0.01*	0.11 ± 0.02**

[a] Mean ± SD.

Abbreviations: GD, gestational day; PND, postnatal day; PNW. Postnatal weeks; BW, body weight; AGD, anogenital distance.

*,**: Significantly different from the controls (* $p<0.05$, ** $p<0.01$).

Table 6

Onset of puberty and estrous cyclicity in the offspring exposed to diisononyl phthalate (DINP) during the late gestation and lactation periods.

	DINP in diet (ppm)			
	Control	400	4000	20000
Onset of purberty				
Male				
Age by day	41.8 ± 2.1[a]	41.9 ± 1.7	40.8 ± 1.4	45.9 ± 2.8**
BW at onset (g)	204.0 ± 19.1	198.9 ± 19.5	187.7 ± 11.9**	165.2 ± 14.2**
Female				
Age by day	34.8 ± 1.8	34.4 ± 1.7	34.1 ± 1.5	38.3 ± 3.1**
BW at onset (g)	125.8 ± 11.4	120.3 ± 14.6	117.7 ± 15.0	98.9 ± 11.6**
Estrous cyclicity				
PNW 8-11 (ED)	10 (0)	10 (1)	10 (2)	10 (1)
PNW 17-20 (ED)	10 (2)	10 (2)	10 (1)	10 (1)

[a] Mean ± SD.

Abbreviations: BW, body weight; ED, extended diestrus.

*,**: Significantly different from the controls (* $p<0.05$, ** $p<0.01$).

Table 7

Organ weights at necropsy in PNWs 11 and 20 for offspring exposed to diisononyl phthalate (DINP) during the late gestation and lactation periods.

	DINP in diet (ppm)			
	0	400	4000	20000
PNW 11				
No. of animals examined	10	10	10	10
Males				
BW (g)	437.2± 32.9[a]	422.5± 30.6	401.4± 29.5*	327.3± 29.3**
Liver (g/100g BW)	3.39± 0.21	3.35± 0.17	3.25± 0.27	3.24± 0.15
Kidneys (g/100g BW)	0.65± 0.04	0.65± 0.05	0.65± 0.04	0.66± 0.06
Brain (g/100g BW)	0.48± 0.03	0.47± 0.03	0.50± 0.03	0.57± 0.05**
Pituitary (mg/100g BW)	2.69± 0.20	2.60± 0.34	2.69± 0.26	2.94± 0.29
Adrenals (mg/100g BW)	13.4± 1.1	13.0± 1.3	14.1± 2.1	13.0± 2.0
Testes (g/100g BW)	0.80± 0.04	0.77± 0.05	0.88± 0.07*	0.88± 0.08*
Epididymides (g/100g BW)	0.24± 0.02	0.23± 0.02	0.25± 0.02	0.25± 0.02
Prostate ventral (g/100g BW)	0.09± 0.01	0.09± 0.02	0.09± 0.02	0.09± 0.01
Prostate dorsolateral (g/100g BW)	0.34± 0.03	0.32± 0.06	0.35± 0.05	0.36± 0.04
Seminal vesicle (g/100g BW)	0.23± 0.03	0.21± 0.04	0.24± 0.04	0.23± 0.09
Females				
BW (g)	281.9± 12.0	271.0± 22.0	287.3± 29.9	233.9± 31.2*
Liver (g/100g BW)	3.48± 0.29	3.43± 0.14	3.50± 0.38	3.39± 0.32**
Kidneys (g/100g BW)	0.66± 0.03	0.63± 0.03	0.63± 0.04	0.61± 0.04
Brain (g/100g BW)	0.69± 0.03	0.70± 0.04	0.67± 0.07	0.78± 0.08*
Pituitary (mg/100g BW)	4.59± 0.36	4.17± 0.39	4.35± 0.67	5.44± 0.78**
Adrenals (mg/100g BW)	23.5± 3.7	22.0± 3.1	22.2± 1.9	22.2± 3.9
Ovaries (mg/100g BW)	31.5± 3.0	32.9± 5.4	35.8± 6.2	33.4± 5.2
Uterus (g/100g BW)	0.16± 0.03	0.17± 0.04	0.16± 0.03	0.19± 0.03
PNW 20				
No. of animals examined	10	10	10	10
Males				
BW (g)	624.9 ± 61.4[a]	589.5 ± 60.0	583.8 ± 69.4	452.2 ± 50.5**
Liver (g/100g BW)	2.98 ± 0.16	2.99 ± 0.15	3.21 ± 0.26	2.82 ± 0.27
Kidneys (g/100g BW)	0.57 ± 0.07	0.56 ± 0.05	0.56 ± 0.04	0.58 ± 0.04
Brain (g/100g BW)	0.36 ± 0.03	0.37 ± 0.03	0.37 ± 0.05	0.45 ± 0.05**
Pituitary (mg/100g BW)	1.67 ± 0.53	1.85 ± 0.42	1.76 ± 0.26	1.99 ± 0.26
Adrenals (mg/100g BW)	9.4 ± 1.2	9.2 ± 1.7	9.1 ± 1.6	9.8 ± 1.4
Testes (g/100g BW)	0.63 ± 0.09	0.62 ± 0.06	0.68 ± 0.09	0.72 ± 0.08*
Epididymides (g/100g BW)	0.23 ± 0.02	0.22 ± 0.02	0.24 ± 0.03	0.30 ± 0.08*
Prostate ventral (g/100g BW)	0.10 ± 0.03	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.04
Prostate dorsolateral (g/100g BW)	0.38 ± 0.12	0.32 ± 0.06	0.35 ± 0.06	0.41 ± 0.04
Seminal vesicle (g/100g BW)	0.25 ± 0.08	0.21 ± 0.04	0.23 ± 0.05	0.28 ± 0.04
Females				
BW (g)	370.2 ± 25.3	357.7 ± 26.7	366.5 ± 21.2	295.3 ± 32.6**
Liver (g/100g BW)	2.96 ± 0.28	2.94 ± 0.24	3.06 ± 0.28	3.03 ± 0.20
Kidneys (g/100g BW)	0.62 ± 0.25	0.53 ± 0.03	0.54 ± 0.04	0.53 ± 0.05
Brain (g/100g BW)	0.56 ± 0.03	0.56 ± 0.06	0.55 ± 0.04	0.65 ± 0.07*
Pituitary (mg/100g BW)	4.34 ± 0.66	4.38 ± 0.60	4.23 ± 0.72	5.08 ± 1.20
Adrenals (mg/100g BW)	17.3 ± 2.4	16.5 ± 2.5	17.5 ± 2.2	18.9 ± 3.6
Ovaries (mg/100g BW)	22.4 ± 2.8	23.0 ± 3.0	22.6 ± 3.0	24.9 ± 4.9
Uterus (g/100g BW)	0.15 ± 0.03	0.14 ± 0.02	0.15 ± 0.03	0.19 ± 0.05*

[a] Mean ± SD.

Abbreviations: BW, body weight.

*, **: Significantly different from the controls (* $p<0.05$, ** $p<0.01$).

Table 8

Histopathological changes in animals exposed perinatally to diisononyl phthalate (DINP).

	DINP in diet (ppm)			
	0	400	4000	20000
PND 21 No. of animals examined	10	10	10	10
Testis				
Reduction of spermatocyte development (±/+/++)[a]	0	2[b](2/0/0)[c]	6(0/5/1)	10(0/4/6)
Aggregated foci of Leydig cells	0	9	10	10
Mammary gland				
Hypoplasia of alveolar bud (+/++)	0	0	0	9(1/8)
PNW 11 No. of animals examined	10	10	10	10
Testis				
Loss of germ cell development (±/+)	0	0	3(2/1)	2(2/0)
Sertoli cell vacuolation (±/+)	0	0	4(3/1)	7(7/0)
Leydig cell hyperplasia, focal	0	0	1	0
Mammary gland				
Alveolar atrophy (±/+/++)	6(3/2/1)	7(6/1/0)	5(3/2/0)	5(2/1/2)

[a] Grade of change; ±, minimal; +, slight; ++, moderate; and +++, severe.

[b] Total No. of animals with each finding.

[c] No. of animals with each grade.

Abbreviations: DINP, diisononyl phthalate; PND, postnatal day; PNW, postnatal week..

*,** Significantly different from the controls by Fisher's exact probability test ($*p < 0.05$, $**p < 0.01$).

#,## Significantly different from the controls by Mann-Whitney's *U*-test ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$).

Table 9

Number of genes showing sexual dimorphic expression as well as altered expression by ethinylestradiol (EE) at 0.5 ppm in the microdissected medial preoptic area (>2-fold, $p<0.05$).

	Males	Females
Untreated controls		
Predominantly expressed	57	14
EE at 0.5 ppm		
Up-regulated	20	55
Down-regulated	183 (22)[a]	2

[a] Number in parenthesis represents genes showing up-regulation by 0.5 ppm EE in females.

Abbreviation: EE, ethinylesradiol.

Table 10

List of genes showing dose-dependent down-regulation by ethinylestradiol (EE) in the microdissected medial preoptic area in males (>2-fold, $p<0.05$).

Accession No.	EE (ppm) 0.01	0.1	0.5	Gene Name	Description
From 0.01 ppm					
M83680	0.4[a]	0.3	0.1	RAB14	GTPase Rab14
AA893065	0.4	0.3	0.2	Pcmt1	protein-L-isoaspartate (D-aspartate) O-methyltransferase
U66478	0.4	0.5	0.3	Mad1	mothers against dpp 1 homolog
AA859832	0.5	0.4	0.2		EST
X17682	0.5	0.5	0.3	Map2	microtubule-associated protein 2
U62897	0.5	0.5	0.2	Cpd	carboxypeptidase D precursor
AI113289	0.5	0.4	0.1	Ptpn1	protein tyrosine phosphatase, non-receptor type 1
AA957917	0.4	0.2	0.1	Slc7a1	solute carrier family 7 member A1
From 0.1 ppm					
D10706	1.3	0.2	0.0	Oaz1	ornithine decarboxylase antizyme
AI175486	1.0	0.2	0.1	Rps7	ribosomal protein S7
AI008131	0.5	0.3	0.1	Amd1a	S-Adenosylmethionine decarboxylase 1A
AI104389	0.7	0.4	0.1	Th	tyrosine hydroxylase
Y00766	0.7	0.2	0.1	Scn3a	sodium channel, voltage-gated, type III, alpha polypeptide
D17711	0.7	0.3	0.1	CSBP	heterogeneous nuclear ribonucleoprotein K
D13127	0.7	0.4	0.1	Atp5o	ATP synthase, H+ transporting
AA799732	0.5	0.2	0.1		EST
X15013	0.9	0.3	0.2		ribosomal protein L7a
AF090135	0.5	0.3	0.2	Veli1	lin-7-Ba
AA859496	0.8	0.4	0.2	Gch	GTP cyclohydrolase 1
L13619	1.1	0.4	0.2	CL-6	growth response protein
X53428	0.7	0.3	0.2	Gsk3b	glycogen synthase kinase 3 beta
M16112	0.8	0.4	0.2	Camk2b	calcium/calmodulin-dependent protein kinase
L04739	1.0	0.4	0.2		plasma membrane calcium ATPase isoform 1
D26564	1.0	0.4	0.2	Cdc37	brain specific protein
AA892006	0.8	0.4	0.2		EST
AA891812	1.0	0.4	0.2		EST
X73653	0.6	0.4	0.2	Gsk3b	glycogen synthase kinase 3 beta
D00688	0.8	0.4	0.2	Maoa	monoamine oxidase, partial cds.
AI638989	0.5	0.4	0.2		EST
AA848218	0.8	0.4	0.2		EST
Z38067	1.1	0.4	0.3		c-myc
J00797	1.0	0.4	0.3		alpha-tubulin gene
AF055286	0.7	0.5	0.3	Slc22a3	solute carrier family 22
AA799537	1.0	0.4	0.3		EST
AI230614	0.6	0.4	0.3	Atp1b1	ATPase Na+/K+ transporting beta 1 polypeptide
AF084186	1.3	0.4	0.3	A2A	alpha-fodrin
D10699	1.2	0.5	0.3	Uchl1	ubiquitin carboxy-terminal hydrolase L1
AI639410	0.8	0.5	0.3		EST
M12672	0.9	0.5	0.3	Gnai2	GTP-binding protein alpha-i2
S82383	0.8	0.4	0.3		slow-twitch alpha TM/hTMnm homolog
M17526	0.5	0.5	0.3	Gnao	guanine nucleotide binding protein, alpha o
M89945	0.8	0.5	0.3	Fdps	farnesyl diphosphate synthase gene
X84047	1.1	0.4	0.3	Gas	G protein; XLas protein
AA817892	1.1	0.5	0.3	Gnb2	guanine nucleotide binding protein beta 2

[a] Relative expression ratio as compared to the level in untreated controls.

Abbreviation: EE, ethinylestradiol; EST, expressed sequence tag.

Table 11

List of genes showing dose-dependent up-regulation by ethinylestradiol (EE) in the microdissected medial preoptic area of females (2-fold, P<0.05).

Accession No.	EE (ppm) 0.01	0.1	0.5	Gene name	Description
From 0.01 ppm					
AA799732	5.0[a]	6.7	4.7		EST, moderately similar to mouse DGCR6 protein
M83680	3.9	5.5	5.0	RAB14	GTPase Rab14
AA892228	3.0	2.3	2.6		EST
M12672	3.0	2.1	2.6	Gnai2	GTP-binding protein alpha-i2
L05435	2.7	2.0	2.3	SV2	synaptic vesicle glycoprotein 2 a
U50185	2.2	2.1	2.0	Mypt1	smooth muscle myosin binding subunit
J02827	2.0	2.6	2.0	Bckdha	branched chain alpha-ketoacid dehydrogenase E1 alpha
From 0.1 ppm					
AA799537	1.8	2.1	2.1		EST
AI137790	1.6	2.1	2.1		leydig cell hypercalcemic tumour H-500
X58200	1.9	3.1	2.2	Rpl23	ribosomal protein L23.
AI639282	1.8	3.0	2.1		EST, Moderately similar to DNA-directed RNA polymerase II
AI103498	2.2	4.5	3.3	Rpl5	ribosomal protein L5
AA859896	2.0	4.2	3.0	Macs	myristoylated alanine-rich protein kinase C substrate
AI638985	3.4	4.9	5.2		
AA965154	1.7	2.2	2.1	Ywhae	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activatioprotein
AI170212	2.4	2.6	2.5	Ap3m2	adaptor-related protein complex 3, mu 2
AI137583	2.5	3.0	3.2	Id2	inhibitor of DNA binding 2
AB000776	3.1	3.2	3.1	Sema6b	sema, transmembrane, and cytoplasmic domain,
AA684963	1.1	2.2	2.0		EST
AI176589	1.7	2.3	2.3	Rpl27	ribosomal protein L27

[a] Relative expression ratio as compared to the level in untreated controls.

Abbreviation: EE, ethinylestradiol; EST, expressed sequence tags.

Table 12

List of genes showing sexually dimorphic expression in the medial preoptic area of males and their expression changes by ethinylestradiol (EE) at 0.5 ppm (>2-fold, $p<0.05$).

Accession No.	Gene name	Description	M>F	Changes at 0.5 ppm EE	
				M	F
Down-regulated in males and up-regulated in females by EE (8)					
M83680	RAB14	GTPase Rab14	10.5[a]	0.1[b]	5.0[c]
M12672	Gai2	GTP-binding protein alpha-i2	2.3	0.3	2.1
U50185	Mypt1	smooth muscle myosin binding subunit	2.5	0.5	2.0
D13309	rDbpB	DNA-binding protein B	2.4	0.4	2.0
AI170212	Ap3m2	adaptor-related protein complex 3, mu 2	3.0	0.3	2.5
AA892394		EST, strong similarity to RNA-binding protein 1	2.5	0.3	2.8
AA799732		EST, similar to DGCR6 protein	3.9	0.1	4.7
AA892228		EST, strong similarity to RNA-binding protein 1	2.0	0.3	2.6
Down-regulated only in males by EE (33)					
AI230614	Atp1b1	ATPase Na+/K+ transporting beta 1 polypeptide	2.7	0.3	-
AI176710	Nr4a3	nuclear receptor subfamily 4, group A, member 3	2.0	0.4	-
AI137331	Rock1	Rho-associated kinase beta	2.8	0.5	-
AI013194	Eif5	eukaryotic initiation factor 5	2.2	0.5	-
AI012183	Nr2f2	nuclear receptor subfamily 2, group F, member 2	3.2	0.4	-
AA925762	Macs	myristoylated alanine-rich protein kinase C	2.5	0.4	-
AA900900	Ralbp1	RalA binding protein 1	2.2	0.3	-
AA894089	Neurodap1	rotein carrying the RING-H2 sequence motif	2.2	0.4	-
AA893065	Pcmt1	protein-L-isoaspartate O-methyltransferase	3.0	0.2	-
AA875659	Inexa	Internexin, alpha	2.6	0.4	-
Y00766	Scn3a	sodium channel, voltage-gated, type III	2.6	0.1	-
X73653	Gsk3b	glycogen synthase kinase 3 beta	2.9	0.2	-
X53428	Gsk3b	glycogen synthase kinase 3 beta	2.3	0.2	-
X17682	Map2	microtubule-associated protein 2	4.3	0.3	-
U66478	Mad1	mothers against decapentaplegic, homolog 1	2.1	0.3	-
U62897	Cpd	carboxypeptidase D precursor	2.2	0.2	-
S81497	LAL	Intracellular hydrolase, lysosomal acid lipase	2.1	0.4	-
M92076	Grm3	Glutamate receptor, metabotropic 3	2.2	0.4	-
M55291	TrkB	neural receptor protein-tyrosine kinase	2.5	0.4	-
M17526	Gnao	guanine nucleotide binding protein, alpha o	2.4	0.3	-
M16112	Camk2b	calcium/calmodulin-dependent protein kinase II	2.0	0.2	-
L13151	GAP	GTPase-activating protein gene	3.6	0.2	-
D10262	CKR	Choline kinase	2.7	0.4	-
AF093268	Ruvbl1	RuvB-like protein 1	2.0	0.4	-
AF090135	Veli1	lin-7-Ba	2.8	0.2	-
AB008538	Alcam	activated leukocyte cell adhesion molecule	3.0	0.3	-
AI145494	Syn2	synapsin II	2.5	0.3	
AA875127		EST	2.4	0.4	-
AA859832		EST	3.5	0.2	-
AA892006		EST	2.5	0.2	-
H31479		EST	2.1	0.5	-
AI639314		EST	2.4	0.3	-
AI638989		EST	2.8	0.2	-
Only up-regulated in females by EE (3)					
AI009191	Fyn	Fyn proto-oncogene	2.5	-	2.1
X58200	Rpl23	ribosomal protein L23	2.1	-	3.1
U38379	Ggh	gamma-glutamyl hydrolase	2.1	-	2.1
Without expression change by EE (13)					
M59980	RK5	potassium voltage gated channel	5.2	-	-
K00994	icabp	intestinal calcium binding protein	4.4	-	-
M83676	RAB12	RAB12, member RAS oncogene family	4.2	-	-
S65355		nonselective-type endothelin receptor	2.9	-	-
AA819643	Prkaa2	AMP-activated protein kinase	2.9	-	-
AA892137		EST	2.8	-	-
X57764	Ednrb	endothelin receptor type B	2.2	-	-
U09793	c-Ki-ras	Kirsten rat sarcoma viral oncogene homologue 2	2.2	-	-
D86041	Ddah1	dimethylarginine dimethylaminohydrolase1	2.1	-	-
AI232078	Ltbp1	latent transforming growth factor beta binding protein 1	2.1	-	-
AI030286	Bdnf	brain derived neurothrophic factor	2.1	-	-
AA894321		EST	2.1	-	-
AA874999		EST	2.0	-	-

EE- ethinylestradiol 0.5 ppm

a. Relative expression ratio in control males as compared to control females

b. Relative expression ratio in EE exposed males as compared to control males

c. Relative expression ratio in EE exposed females as compared to control females

Abbreviations: EE, ethinylestradiol; M, Males; F, females; EST, expressed sequence tag.

Table 13

List of genes encoding G proteins and their related molecules among genes to show male-predominant expression in the medial preoptic area, and their expression changes by 0.5 ppm ethinylestradiol (EE).

Accession No.	Gene name	Description	M>F	Changes at 0.5 ppm EE		Example of reported function	Reference
				M	F		
M83680	RAB14	GTPase Rab14	10.5[a]	0.1[b]	5.0[c]	Low molecular-weight GTPase subfamily	
M12672	Gai2	GTP-binding protein (Gαi2)	2.3	0.3	2.1	GTP binding protein	
U50185	Mypt1	myosine phosphatase	2.5	0.5	2.0	Inhibited by GTPase Rho	Kimura et al., 1996
AI137331	Rock1	Rho-associated kinase β	2.8	0.5	-	Rho-associated kinase	
AI013194	Eif5	eukaryotic initiation factor 5	2.2	0.5	-	Functions as a GTPase-activating protein	Paulin et al., 2001
AA925762	Macs	myristoylated alanine-rich protein kinase C	2.5	0.4	-	Phosphorylated by Rho-associated kinase in human neuronal cells	Ikenoya et al., 2002;
AA900900	Ralbp1	RalA binding protein 1	2.2	0.3	-	GTPase	
Y00766	Scn3a	sodium channel, voltage-gated, type III	2.6	0.1	-	Major targets of G protein-coupled receptor	
X73653	Gsk3b	glycogen synthase kinase 3β	2.9	0.2	-	Activated in neuronal cells by Gα12 and Gα13	Sayas et al., 2002
M92076	Grm3	glutamate receptor, metabotropic 3	2.2	0.4	-	Peculiar family of G protein-coupled receptors	
M55291	TrkB	neural receptor protein-tyrosine kinase	2.5	0.4	-	Activated via a G protein-coupled receptor mechanism	Rogalski et al., 2000
M17526	Gnao	guanine nucleotide binding protein α_o	2.4	0.3	-	GTP binding protein	
M16112	Camk2b	calcium/calmodulin-dependent protein kinase II	2.0	0.2	-	Interacted with Rad-related GTPases	Moyers et al., 1997
L13151	GAP	GTPase-activating protein gene	3.6	0.2	-	GTPase-activating protein	
D10262	CKR	choline kinase	2.7	0.4	-	Regulated by Ras proteins involved in GTPases Ral-GDS	
AI009191	Fyn	Fyn proto-oncogene	2.5	-	2.1	Phosphorylate p250GAP in oligodendrocytes	Taniguchi et al., 2003
M59980	RK5	potassium voltage gated channel	5.2	-	-	G protein-gated channel	
M83676	RAB12	RAB12, member RAS oncogene family	4.2	-	-	Small GTP-binding proteins	
S65355		nonselective-type endothelin receptor	2.9	-	-	G protein-coupled receptor	Bremnes et al., 2000
AA819643	Prkaa2	AMP-activated protein kinase	2.9	-	-	Activated by stimulations of G(q)-coupled receptors.	Kishi et al., 2000
X57764	Ednrb	endothelin receptor type B	2.2	-	-	G protein-coupled receptors	Bremnes et al., 2000
AI030286	Bdnf	Brain derived neurothrophic factor	2.1	-	-	Mediated its neurotrophic signaling by Rho GTPases	Ozdinler and Erzurumlu 2001

[a] Relative expression ratio in control males as compared to control females

[b] Relative expression ratio in EE exposed males as compared to control males

[c] Relative expression ratio in EE exposed females as compared to control females