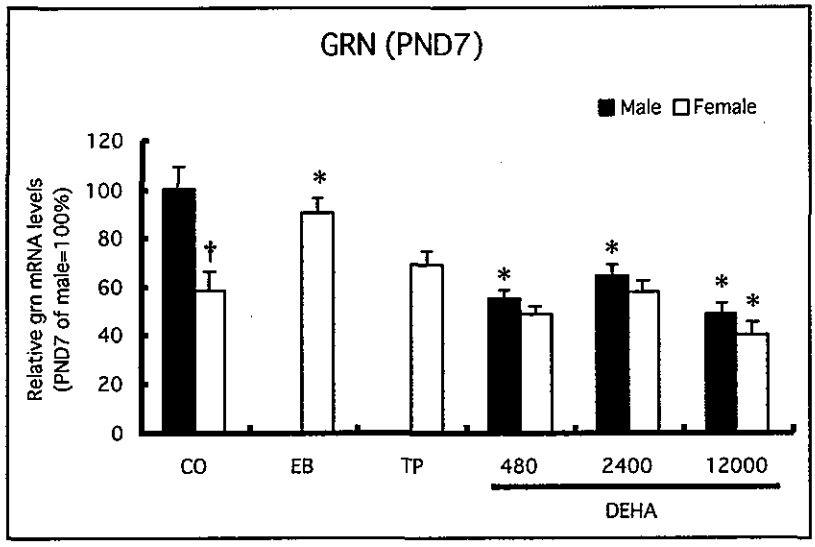

Fig. 1-45
7日齢ラットの視床下部 granulin (grn) 遺伝子発現に対する性ステロイドおよびDEHAの影響.
† : p<0.05 male vs. female, * : p<0.05 vs. CO

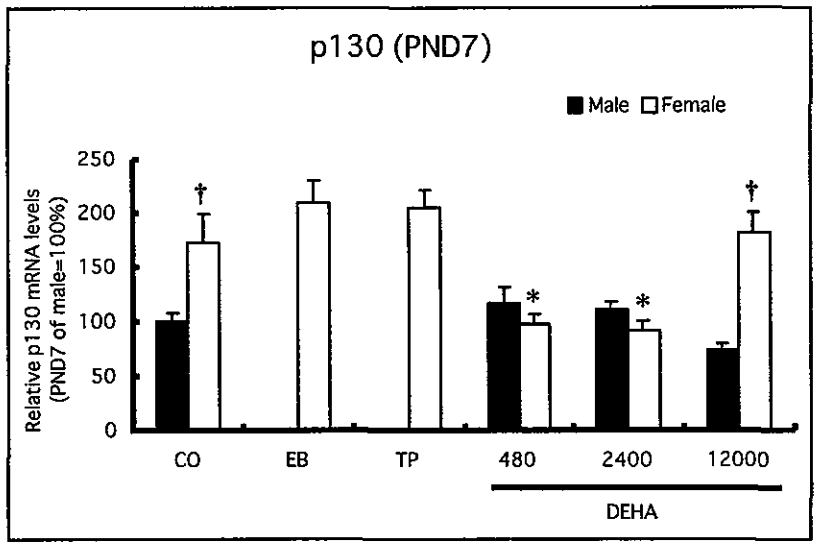

Fig. 1-46
7日齢ラットの視床下部 p130 遺伝子発現に対する性ステ ロイドおよびDEHAの影響.
† : p<0.05 male vs. female, * : p<0.05 vs. CO

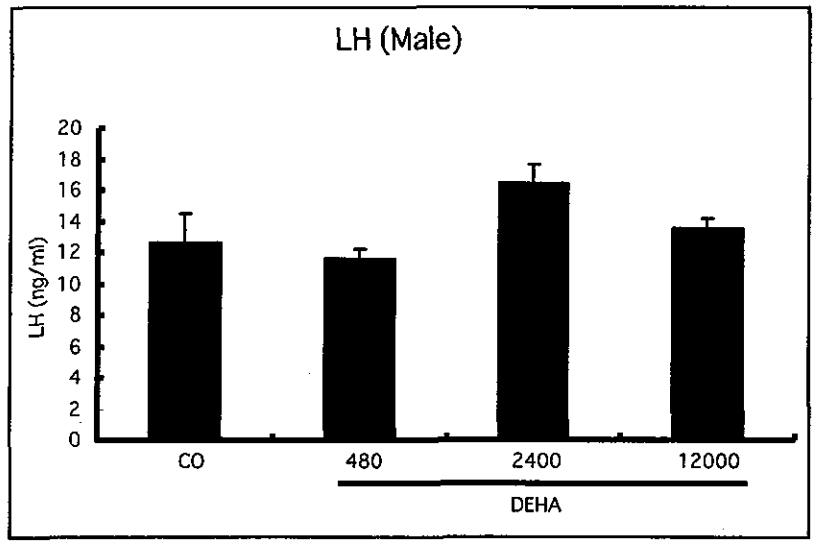

Fig. 1-47
21週齢雄ラットの血清中LH濃度に対するDEHAの影響.

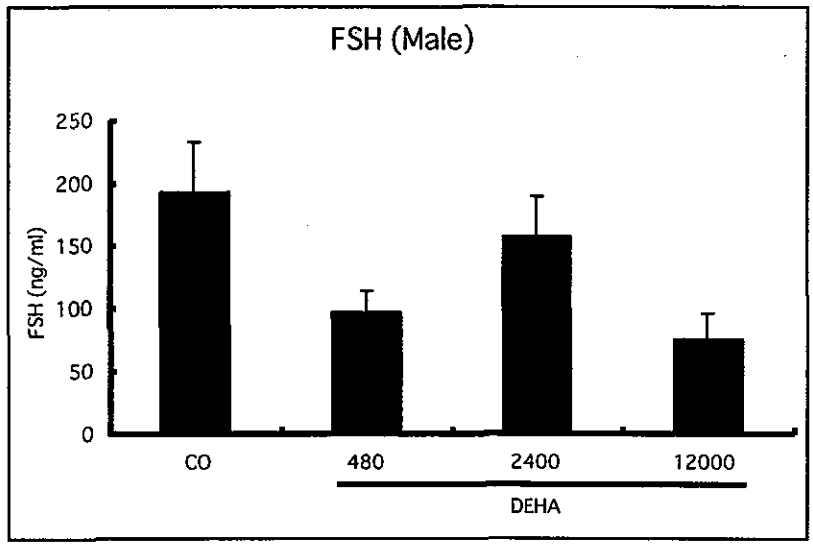

Fig. 1-48
21週齢雄ラットの血清中FSH濃度に対するDEHAの影響.

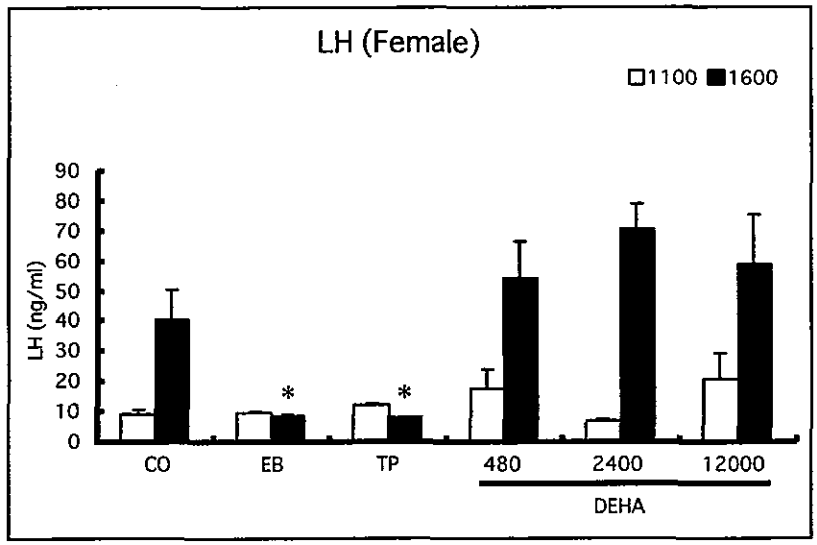

Fig. 1-49
21週齢雌ラットの血清中LH濃度に対するDEHAの影響.
＊：$p<0.05$ vs. CO

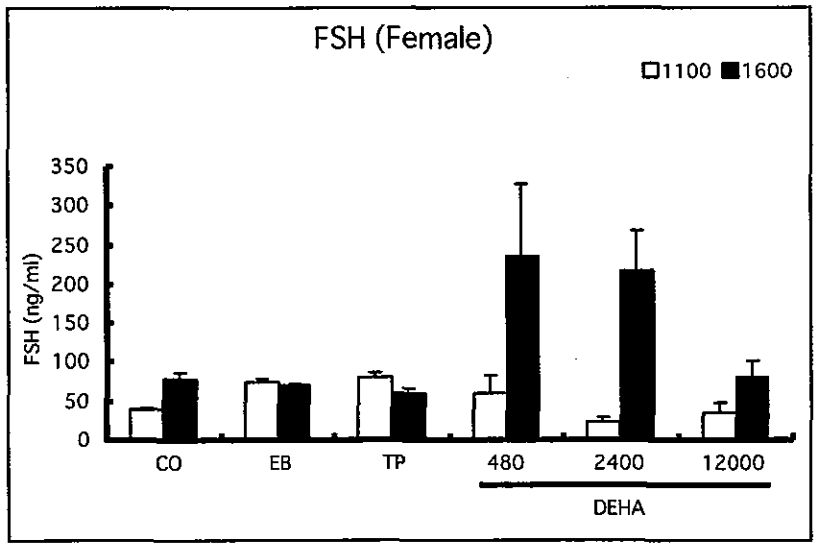

Fig. 1-50
21週齢雌ラットの血清中FSH濃度に対するDEHAの影響.

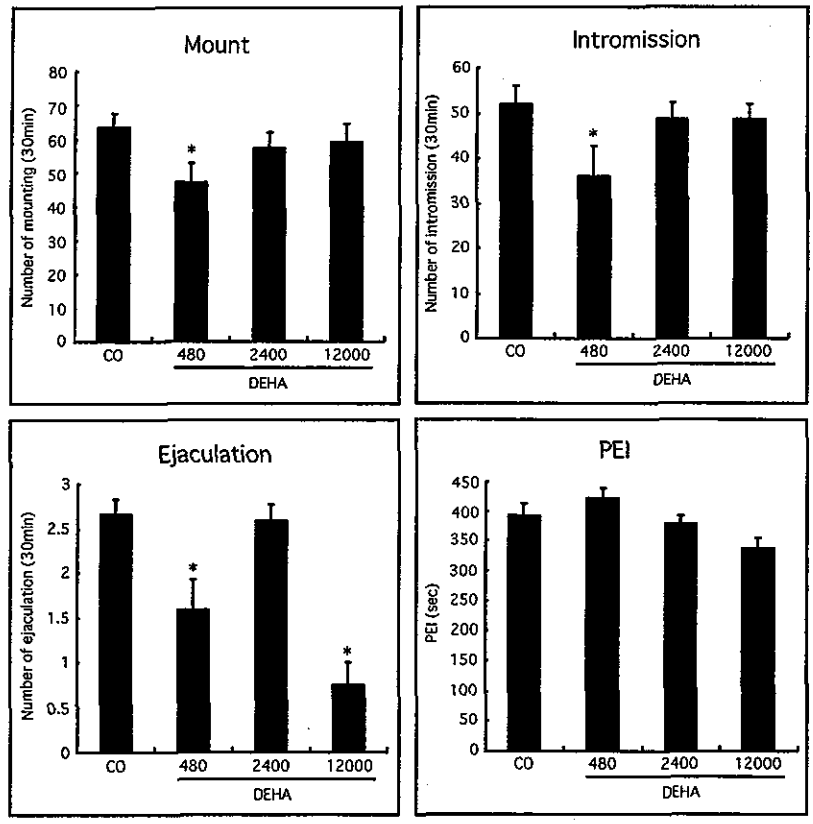

Fig. 1-51
11週齢雄ラットの性行動に対するDEHAの影響.
＊：p<0.05 vs. CO

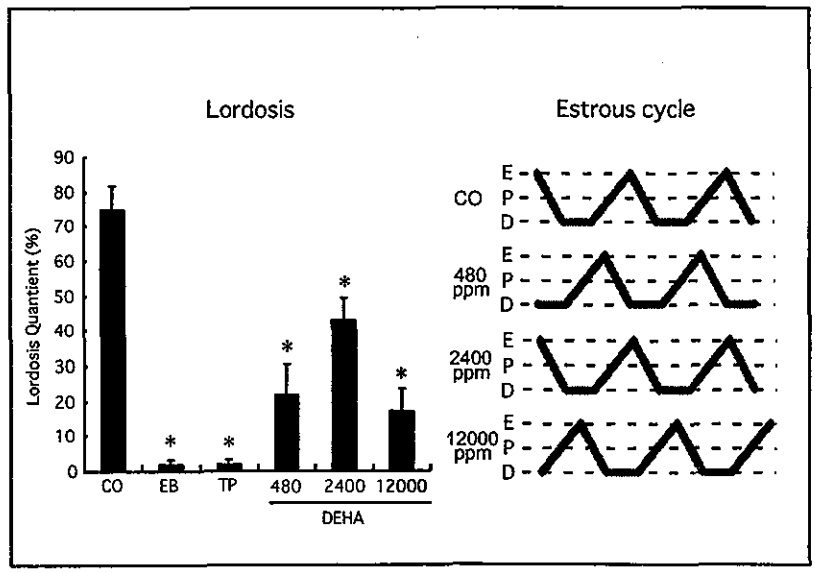

Fig. 1-52
11週齢雌ラットのロードーシス反射の出現及び性周期に 対する
DEHAの影響　　＊：p<0.05 vs. CO

Table 1-1

Summary of the previously assessed endocrine effects of EACs in offspring after perinatal exposure.

Chemical	Sex	Dose (ppm in diet)	Results
EE [a,b,c]	M	0.5	Reduced neonatal body weights and body weight gain during PNDs 2-10 Delayed onset of puberty Decrease of SDN-POA volume
	F	0.5	Reduced neonatal body weights and body weight gain during PNDs 2-10 Early onset of puberty Irregularity in estrous cyclicity Increased relative weights of adrenals and pituitaries at PNW 11 Histopathological alterations in the endocrine organs at PNW 11 - Increase of follicles/decrease of corpora lutea in the ovaries - Hypertrophy of the endometrial epithelium - Diffuse hyperplasia of the anterior pituitaries - Lobular hyperplasia in the mammary glands Population changes in the pituitary hormone-immunoreactive cells - Increase of the PRL-immunoreactive cell ratio at PNW 3
MXC [b,d]	M	24, 240	No alterations in the endocrine/reproductive system
		1200	Reduced body weight gain during PNDs 2-10 Reduced absolute and relative weights of the testes at PNW 3 Delayed onset of puberty Population changes in the pituitary hormone-immunoreactive cells - Decrease of LH-, FSH-, and PRL-immunoreactive cell ratios at PNW 3
	F	24	No alterations in the endocrine/reproductive system
		240	Pituitary hormone-immunoreactive cell population - Increase of PRL at PNW 11
		1200	Reduced body weight gain during PNDs 2-10 Early onset of puberty Irregular estrous cyclicity Decreased absolute and relative weights of the ovaries at PNW 11 Histopathological alterations in the endocrine organs at PNW 11 - Increase of follicles/ decrease of corpora lutea in the ovaries - Hypertrophy of the endometrial epithelium - Diffuse hyperplasia of the anterior pituitaries - Vaginal mucosal hyperplasia - Lobular hyperplasia of the mammary glands Population changes in the pituitary hormone-immunoreactive cells - Decrease of LH-immunoreactive cell ratio at PNW 3 - Increase of FSH- and PRL-immunoreactive cell ratios at PNW 11
DINP [b,d]	M/F	400, 4000	No alterations in the endocrine/reproductive system
	M	20,000	Reduced body weight gain during PNDs 2-10 and PNDs 21-42 Reduced absolute and relative weights of the testes at PNW 3 Histopathological alterations in the gonadal organs at PNW 11 - Degeneration of meiotic spermatocytes at stage XIV in the testes - Vacuolar degeneration of Sertoli cells in the testes
	F	20,000	Reduced body weight gain during PNDs 2-10 Histopathological alterations in the gonadal organs at PNW 11 - Decrease of corpora lutea in the ovaries
GEN [b,d]	M	20, 200, 1000	Reduced body weights of males at PNW 11
	F	20, 200, 1000	No alterations in the endocrine/reproductive system

Data are summarized from [a] Masutomi et al. (2004a), [b] Masutomi et al. (2004b), [c] Shibutani et al. (2005), and [d] Masutomi et al. (2003), respectively.

Abbreviations: EE, ethinylestradiol; MXC, methoxychlor; DINP, diisononyl phthalate; GEN, genistein; M, males; F, females; PND, postnatal day; SDN-POA, sexually dimorphic nucleus of the preoptic area; PNW, postnatal week; PRL, prolactin, LH, luteinizing hormone; FSH, follicle-stimulating hormone.

Table 1-2

Sequences of primers and probes used for real-time RT-PCR[a]

Gene	Accession No.		Sequence	Product size
ERα	Y00102	sense	5'-GGGCTTCCCCAACACCAT-3'	65 bp
		antisense	5'-CGTTTCAGGGATTCGCAGAA-3'	
		probe[b]	5'-TGAGAACTCCCAGGCTCCCCACAA-3'	
ERβ	U57439	sense	5'-TGCTGGATGGAGGTGCTAATG-3'	82 bp
		antisense	5'-CGAGGTCGGGAGCGAAA-3'	
PR	NM_022847	sense	5'-GGGCACTGGCTGTGGAATT-3'	77 bp
		antisense	5'-CATGCCCGCCAGGATCT-3'	
SRC-1	XM_233944	sense	5'-CCAGGCCCAGCAGAAGAG-3'	65 bp
		antisense	5'-CACATTCCTTTAAAAGTGGTTATTCAGT-3'	
		probe[c]	5'-CTCCTTCAGCAGCTAC-3'	
SRC-2	NM_031822	sense	5'-CCGCCAGCCGCTAATG-3'	64 bp
		antisense	5'-GCCAGGCCTCAAAGTCAAGTT-3'	
		probe[b]	5'-CCAGATCAGCGGCGTTTCCAATG -3'	
GnRH	M31670	sense	5'-CCCCAGAACTTCGAATGCA -3'	70 bp
		antisense	5'-GTTCCAGAGCTCTCTGCAGAT-3'	
		probe[b]	5'-CGAGGTCGGGAGCGAAA-3'	
CALB	M27839	sense	5'-AGAATTTCCTGCTGCTCTTTCG-3'	77 bp
		antisense	5'-GTCATACTTTCTCCAAGTCTTCATGAA-3'	
		probe[b]	5'-TGCCAGCAACTGAAGTCCTGCGA-3'	
HPRT	X62085	sense	5'-GCCGACCGGTTCTGTCAT-3'	71 bp
		antisense	5'-GGTCATAACCTGGTTCATCATCAC-3'	
		probe[c]	5'-CAGTCCCAGCGTCGTG-3'	

[a]For GAPDH, commercially available TaqMan® Rodent GAPDH Control Reagents (Applied Biosystems) were used (Sequence information not available).

[b] TaqMan® probe.

[c] TaqMan® MGB probe.

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; SRC, steroid receptor coactivator; GnRH, gonadotrophin releasing hormone; CALB, calbindin-D; HPRT, hypoxanthine-guanine phosphoribosyl transferase.

Table 1-3

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 1-4

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 1-5

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 1-6

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 1-7

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 1-8

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 1-9

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 1-10
Histopathological changes in offspring exposed gestationally and lactationally to DINP.

		DINP in maternal diet (ppm)			
		0	400	4000	20,000
Males, Week 3	No. of animals examined	10	10	10	10
Testis					
Reduction of tubular size (±/+/++) [a]		1[b](0/1/0)[c]	0	2(2/0/0)	10(0/0/10)"
Reduction of spermatocyte development (±/+/++)		3(2/0/1)	2(2/0/0)	5(1/3/1)	10(0/1/9)"
Tubules with only Sertoli cells (present)		0	0	1	0
Aggregated foci of Leydig cel Incidence [d]	>20 cells	0	0	0.6±1.1*	4.0±2.3**
	≤20 cells	3.9±2.0	8.6±6.1	9.3±4.4*	10.7±3.0**
Epididymis					
Decreased ductular cross section, epididymal duct (±/+)		0	2(1/1)	4(2/2)'	6(2/4)"
Small ductular size, epididymal duct (±/+/++)		0	2(1/1/0)	1(1/0/0)	10(0/4/6)"
Mammary glands					
Alveolar bud hypoplasia (+/++)		0	0	0	10(4/6)"
Adrenals					
Cortical atrophy (±/+/++)		0	2(1/1/0)	2(0/2/0)	10(0/3/7)"
Pituitary					
Diffuse atrophy, anterior lobe (++)		0	0	1	10"
Liver					
Decrease of cytoplasmic glycogen granules (±/+/++/+++)		5(4/1/0/0)	8(5/2/1/0)	10(0/10/0/0)'	10(0/0/2/8)"
Increase of cytoplasmic eosinophilia (±/+/++)		0	3(3/0/0)	10(0/10/0)"	10(0/0/10)"
Kidneys					
Dilatation, collecting tubules (±/+/++)		0	0	0	6(1/2/3)"
Mineralization (+/++)		0	0	0	3(2/1)
Females, Week 3	No. of animals examined	9	10	10	10
Ovary					
Size reduction of follicles/interstitial cell layer (±/+/++)		0	5(1/4/0)'	6(2/4/0)"	10(0/0/10)"
Mammary glands					
Bud hypoplasia (±/+/++)		0	0	3(2/1/0)	10(1/3/6)"
Adrenals					
Cortical atrophy (+/++)		0	1(1/0)	1(1/0)	10(2/8)"
Pituitary					
Diffuse atrophy, anterior lobe (++/+++)		0	0	0	10(7/3)"
Liver					
Decrease of cytoplasmic glycogen granules (±/+/++/+++)		4(3/1/0/0)	4(4/0/0/0)	9(1/4/4/0)"	10(0/0/4/6)"
Increase of cytoplasmic eosinophilia (±/+/++)		0	0	8(2/6/0)"	10(0/0/10)"
Kidneys					
Dilatation, collecting tubules (±/+)		0	0	1(1/0)	9(4/5)"
Mineralization, medulla (±/+)		0	0	0	4(3/1)'
Males, Week 11	No. of animals examined	10	10	10	10
Testis					
Focal tubular atrophy (±/+)		2(2/0)	0	3(2/1)	5(5/0)
Sertoli cell vacuolation (±/+)		0	0	4(2/2)'	5(5/0)'
Focal Leydig cell hyperplasia (+)		0	0	1	0
Epididymis					
Intraductular cellular debris (±)		0	0	6"	4'
Adrenals					
Cortical atrophy (±/+)		0	0	1(0/1)	7(5/2)"
Females, Week 11	No. of animals examined	10	10	10	10
Ovary					
Increase of follicles (+)		1	3	6'	8"
Males, Week 20	No. of animals examined	10	10	10	10
Testis					
Focal tubular atrophy (±)		2	1	2	5
Epididymis					
Intraductular cellular debris (±)		1	1	2	0
Females, Week 20	No. of animals examined	10	10	10	10
Ovary					
Increase of follicles (+)		1	3	1	2

[a] Grade of change; ±, minimal; +, slight; ++, moderate; +++, severe.
[b] Total No. of animals with each change.
[c] No. of animals for each grade.
[d] Expressed as mean±SD.
*,** Significantly different from the controls using parametric or non-parametric statistic method ($*p < 0.05$, $**p < 0.01$).
',"Significantly different from the controls using Fisher's exact probability test ($'p < 0.05$, $"p < 0.01$).
',"Significantly different from the controls using Mann-whitney's U-test ($'p < 0.05$, $"p < 0.01$).

Table 1-11

Effects on dams and offspring until prepubertal necropsy by exposure to di-ethylhexyl adipate (DEHA) during lat and lactation.

	DEHA in diet (ppm)			
	0	480	2400	12000
No. of dams examined	10	10	10	10
Maternal parameter				
BW change (g/day)				
GD15-GD20	13.9 ± 3.06[a]	14.6 ± 1.4	14.6 ± 2.4	12.3 ± 1.9
PND2-PND10	4.3 ± 1.5	5.6 ± 2.2	5.7 ± 1.3	4.4 ± 2.5
PND10-PND21	-1.0 ± 1.0	-0.7 ± 0.8	-0.8 ± 0.8	-0.6 ± 1.2
Food intake (g/day)				
GD15-GD20	28.1 ± 5.6	23.6 ± 3.0	23.6 ± 2.3	20.8 ± 2.7**
PND2-PND10	44.5 ± 5.5	44.2 ± 6.7	44.7 ± 6.2	38.6 ± 3.5
PND10-PND21	61.3 ± 7.6	64.2 ± 7.3	64.9 ± 8.2	56.5 ± 4.8
DEHA intake (mg/kg/day)				
GD15-GD20	0	32.9 ± 3.3	166.0 ± 8.7	751.4 ± 83.4
PND2-PND10	0	71.3 ± 9.3	350.5 ± 34.8	1677.4 ± 198.0
PND10-PND21	0	97.6 ± 10.0	481.0 ± 58.2	2333.3 ± 230.0
Duration of pregnancy (days)	21.7 ± 0.5	21.8 ± 0.4	21.5 ± 0.5	21.5 ± 0.5
Offspring parameter				
No. of live offspring	11.6 ± 4.2	12.8 ± 1.8	11.7 ± 2.9	13.2 ± 1.5
Male ratio (%)	51.6 ± 22.5	44.1 ± 12.1	51.3 ± 14.6	51.1 ± 12.1
Body weight at PND2 (g)				
Males	7.3 ± 1.4	7.1 ± 0.9	7.3 ± 1.0	6.6 ± 0.7**
Females	7.0 ± 1.0	6.7 ± 0.8	6.6 ± 1.2	6.2 ± 0.7**
AGD at PND2 (mm)				
Males	3.9 ± 0.5	4.0 ± 0.3	3.9 ± 0.5	3.9 ± 0.3
Females	2.0 ± 0.2	2.0 ± 0.2	1.9 ± 0.2**	2.0 ± 0.2
Nipples/areolae in males at PND14				
No. of identified animales (%)	4.4%	27.7%**	24.5%**	34.0%**
Relative organ weights at PND21				
No. of animales examined	10	10	10	10
Males				
BW (g)	48.3 ± 9.1	48.2 ± 4.9	46.9 ± 6.6	42.4 ± 6.1
Liver (g/100gBW)	3.58 ± 0.28	3.76 ± 0.28	3.65 ± 0.29	3.63 ± 0.25
Kidneys (g/100gBW)	1.10 ± 0.10	1.10 ± 0.06	1.12 ± 0.12	1.09 ± 0.05
Brain (g/100gBW)	3.15 ± 0.44	3.06 ± 0.24	3.16 ± 0.37	3.47 ± 0.39
Adrenals (mg/100gBW)	27.4 ± 4.0	28.8 ± 5.7	28.3 ± 3.2	27.2 ± 2.7
Testes (g/100gBW)	0.42 ± 0.03	0.40 ± 0.04	0.42 ± 0.04	0.41 ± 0.04
Epididymides (g/100gBW)	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
Females				
BW (g)	48.3 ± 6.4	48.0 ± 5.4	46.2 ± 5.4	40.8 ± 3.4**
Liver (g/100gBW)	3.75 ± 0.20	3.85 ± 0.35	3.70 ± 0.18	3.75 ± 0.31
Kidneys (g/100gBW)	1.13 ± 0.05	1.18 ± 0.05	1.16 ± 0.07	1.17 ± 0.08
Brain (g/100gBW)	3.08 ± 0.35	2.99 ± 0.27	3.11 ± 0.30	3.47 ± 0.31**
Adrenals (mg/100gBW)	27.6 ± 5.3	29.1 ± 5.2	32.1 ± 6.9	31.7 ± 5.6
Ovaries (mg/100gBW)	31.5 ± 7.9	36.8 ± 7.5	27.6 ± 4.3	32.5 ± 8.5
Uterus (g/100gBW)	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.01

[a] Mean±SD.

Abbreviations: GD,gestational day; PND,postnatal day; PNW,Postnatal weeks; BW, Body weight; AGD,anogenit

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

Table 1-12

Onset of puberty and estrous cyclicity in the offspring exposed to di-ethylhexyl adipate (DEH during the late gestation and lactation periods.

	DEHA in diet (ppm)			
	0	480	2400	12000
Onset of puberty				
Males				
Age by day	41.1 ± 2.3[a]	41.3 ± 1.6	41.0 ± 1.6	42.4 ± 1.9 *
BW at onset (g)	193.2 ± 17.1	186.7 ± 13.9	187.6 ± 14.8	186.3 ± 20.6
Females				
Age by day	36.3 ± 2.3	36.8 ± 2.1	35.6 ± 2.3	37.3 ± 3.2
BW at onset (g)	128.7 ± 17.7	125.1 ± 12.1	121.3 ± 12.7	118.5 ± 15.1
Estrous cyclicity				
PNW 8-11 (ED)	10(2)	10(1)	10(1)	10(2)
PNW 17-20 (ED)	10(1)	10(0)	10(0)	10(0)

[a]Mean±SD.

Abbreviations: BW, Body weight; ED, extended distrus.

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

Table 1-13

Organ weights at PNWs 11 and 20 of offspring exposed to di-ethylhexyl adipate (DEHA) during the late gestation and lactation periods.

	DEHA in diet (ppm)			
	0	480	2400	12000
PNW 11				
Males				
No. of animals examined	10	10	10	10
BW (g)	402.0 ± 20.3[a]	394.4 ± 31.2	403.2 ± 20.3	370.7 ± 50.2
Liver (g/100gBW)	3.29 ± 0.33	3.42 ± 0.33	3.46 ± 0.22	3.33 ± 0.34
Kidneys (g/100gBW)	0.70 ± 0.08	0.68 ± 0.05	0.71 ± 0.07	0.66 ± 0.04
Brain (g/100gBW)	0.52 ± 0.02	0.52 ± 0.04	0.51 ± 0.03	0.54 ± 0.06
Pituitary (mg/100gBW)	3.65 ± 0.56	3.53 ± 0.21	3.86 ± 0.45	3.86 ± 0.30
Adrenals (mg/100gBW)	14.6 ± 2.5	13.5 ± 1.3	12.8 ± 1.6	13.7 ± 2.1
Testes (g/100gBW)	0.81 ± 0.06	0.77 ± 0.07	0.80 ± 0.07	0.77 ± 0.07
Epididymides (g/100gBW)	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.02
Prostate, ventral (g/100gBW)	0.16 ± 0.02	0.14 ± 0.02	0.16 ± 0.04	0.16 ± 0.03
Prostate, dorso-lateral (g/100gBW)	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.16 ± 0.02
Seminal vesicle (g/100gBW)	0.29 ± 0.05	0.26 ± 0.05	0.26 ± 0.04	0.29 ± 0.04
Females				
No. of animals examined	10	10	11	10
BW (g)	272.5 ± 31.1	251.9 ± 33.7	265.7 ± 24.1	244.8 ± 31.5
Liver (g/100gBW)	3.38 ± 0.38	3.18 ± 0.37	3.11 ± 0.29	3.43 ± 0.46
Kidneys (g/100gBW)	0.64 ± 0.04	0.63 ± 0.04	0.66 ± 0.05	0.66 ± 0.04
Brain (g/100gBW)	0.71 ± 0.06	0.75 ± 0.08	0.70 ± 0.06	0.78 ± 0.11
Pituitary (mg/100gBW)	6.22 ± 0.82	6.06 ± 0.60	5.79 ± 0.91	6.91 ± 1.18
Adrenals (mg/100gBW)	23.4 ± 5.5	35.5 ± 15.7	31.0 ± 5.0	34.3 ± 5.5
Ovaries (mg/100gBW)	30.7 ± 5.5	35.5 ± 15.7	31.0 ± 5.0	34.3 ± 5.5
Uterus (g/100gBW)	0.16 ± 0.03	0.18 ± 0.03	0.19 ± 0.07	0.19 ± 0.05
PNW 20				
Males				
No. of animals examined	10	10	10	10
BW (g)	589.5 ± 66.1	530.1 ± 54.9	559.0 ± 70.3	522.1 ± 31.7
Liver (g/100gBW)	2.92 ± 0.26	2.75 ± 0.33	2.88 ± 0.18	2.93 ± 0.23
Kidneys (g/100gBW)	0.58 ± 0.03	0.59 ± 0.04	0.59 ± 0.05	0.59 ± 0.06
Brain (g/100gBW)	0.38 ± 0.03	0.41 ± 0.04	0.40 ± 0.04	0.42 ± 0.03
Pituitary (mg/100gBW)	2.72 ± 0.33	2.96 ± 0.15	2.87 ± 0.36	3.13 ± 0.20 **
Adrenals (mg/100gBW)	9.4 ± 1.5	9.2 ± 1.2	9.0 ± 1.1	8.6 ± 1.1
Testes (g/100gBW)	0.59 ± 0.02	0.62 ± 0.08	0.64 ± 0.09	0.62 ± 0.05
Epididymides (g/100gBW)	0.23 ± 0.02	0.25 ± 0.03	0.25 ± 0.02	0.25 ± 0.02
Prostate, ventral (g/100gBW)	0.16 ± 0.04	0.15 ± 0.03	0.15 ± 0.05	0.17 ± 0.05
Prostate, dorsolateral (g/100gBW)	0.15 ± 0.02	0.15 ± 0.02	0.17 ± 0.04	0.18 ± 0.03
Seminal vesicle (g/100gBW)	0.31 ± 0.04	0.30 ± 0.05	0.32 ± 0.05	0.33 ± 0.05
Females				
No. of animals examined	10	10	10	10
BW (g)	346.7 ± 46.9	329.8 ± 50.5	321.9 ± 28.4	304.2 ± 26.4
Liver (g/100gBW)	2.87 ± 0.41	3.05 ± 0.28	2.85 ± 0.16	2.85 ± 0.17
Kidneys (g/100gBW)	0.56 ± 0.07	0.57 ± 0.05	0.60 ± 0.07	0.59 ± 0.05
Brain (g/100gBW)	0.59 ± 0.07	0.61 ± 0.08 (9)	0.63 ± 0.05	0.67 ± 0.06
Pituitary (mg/100gBW)	5.90 ± 1.10	5.88 ± 0.86	5.89 ± 0.85	6.26 ± 0.87
Adrenals (mg/100gBW)	17.4 ± 4.0	18.2 ± 1.8	18.7 ± 3.5	19.4 ± 3.1
Ovaries (mg/100gBW)	23.8 ± 2.9	25.3 ± 4.7	25.8 ± 4.2	25.5 ± 3.4
Uterus (mg/100gBW)	0.18 ± 0.05	0.21 ± 0.08	0.20 ± 0.05	0.21 ± 0.05

[a]Mean±SD.

Abbreviations: BW, Body weight.

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

Table 1-14

Histopathological changes in offspring exposed gestationally and lactationally to DEHA.

		DEHA in maternal diet (ppm)			
		0	480	2400	12,000
Males, Week 3	No.of animals examined	10	10	10	10
Testis					
Decrease of spermatocyte development (±/+/++) [a]		2[b](1/1/0)[c]	5(1/3/1)	6(3/2/1)	5(1/4/0)
Apoptotic spermatocyte (±/+)		9(9/0)	10(8/2)	10(8/2)	10(5/5)"
Aggregated foci of Leydig cel Incidence[d]	>20 cells	0.1±0.3	0.1±0.3	0.3±0.5	0.7±0.9
	≤20 cells	5.7±2.9	9.9±3.8	9.6±3.5	12.4±5.6*
Epididymis					
Decreased cross section (+/++)		0	0	1(1/0)	3(2/1)
Mammary glands					
Bud hypoplasia (+/++)		0	0	0	6(3/3)"
Females, Week 3	No.of animals examined	10	10	11	10
Ovary					
Size reduction of follicles/interstitial cell layer (±/+/++)		2(1/1/0)	1(0/0/1)	2(1/1/0)	2(0/1/1)
Mammary glands					
Bud hypoplasia		0	0	0	1
Males, Week 11	No.of animals examined	10	10	10	10
Testis					
Tubular atrophy (±/+++)		2(2/0)	0	2(2/0)	2(1/1)
Females, Week 11	No.of animals examined	10	10	11	10
Ovary					
Increase of follicles (±/+)		2(2/0)	2(1/1)	1(1/0)	5(2/3)
Males, Week 20	No.of animals examined	10	10	10	10
No abnormalities detected		10	10	10	10
Females, Week 20	No.of animals examined	10	10	10	10
Ovary					
Increase of follicles (±)		0	1	1	0

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

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

[c] No. of animals for each grade.

[d] Expressed as mean±SD.

*,** Significantly different from the controls using parametric or non-parametric statistic method ($*p < 0.05$, $**p < 0.0$

','' Significantly different from the controls using Mann-whitney's U-test ($'p < 0.05$, $''p < 0.01$).

Table 1-15
Comparison of gene ratios showing presence or absence call and signal ratios in the GAPDH gene between unfixed frozen tissue and methacarn-fixed PETs

Probes	Unfixed frozen, 1x amplified	Unfixed frozen, 2x amplified	MF-PET, 2x amplified
Present	40.3 %	36.9 %	36.4 %
Absent	57.5 %	60.9 %	61.5 %
Marginal	2.2 %	2.2 %	2.1 %
Signal ratio (3'/5') of GAPDH	1.1	12.3	11.3

1x amplified: once amplified by in vitro transcription
2x amplified: twice amplified by in vitro transcription
MF-PET: methacarn-fixed paraffin-embedded tissue

Table 1-16
Correlation analysis of gene expression data between unfixed-frozen tissue and methacarn-fixed PET

	MF-PET, 2x amplified vs. Unfixed, 1x amplified	Unfixed, 2x amplified vs. Unfixed, 1x amplified	MF-PET, 2x amplified vs. Unfixed, 2x amplified
R_1	0.77	0.80	0.98
R_2	0.91	0.63	0.98
$p < 0.05$	22.3 %	27.5 %	0 %

1 x amplified: once amplified by in vitro transcription
2 x amplified: twice amplified by in vitro transcription
MF-PET: methacarn-fixed paraffin-embedded tissue
R1: Pearson's correlation coefficient with raw data
R2: Pearson's correlation coefficient with normalized data

Table 1-17
Mean distance from the poly(A^+) tail to the probe sites of the genes showing presence call only in each preparation

Preparation	No. of genes available [a]	Distance A[b]	Distance B[c]
MF-PET, 2x amplified	6	318	97
Unfixed, 1x amplified	5	847	569

[a] No of genes whose 3' terminal sequence information is available among genes showing presence call only in each preparation.
[b] Distance from the beginning of the poly(A+) tail to the probe located at 5'-most position.
[c] Distance B: distance from the beginning of the poly(A+) tail to the probe located at the 3'-most position

Table 1-18

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.