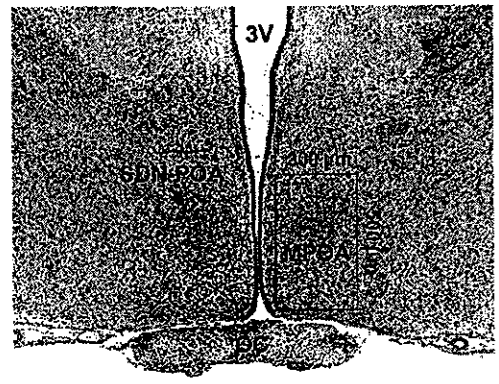


Fig. 4
Schematic view of male hypothalamic MPOA at PND 2. The enclosed area was microdissected from sections of methacarn-fixed paraffin-embedded brain slices for gene expression analysis. Note the intensely stained SDN region. Abbreviations: OC: optic chiasm; 3V: 3rd ventricle; MPOA: medial preoptic area; SDN-POA: sexually dimorphic nucleus of preoptic area.

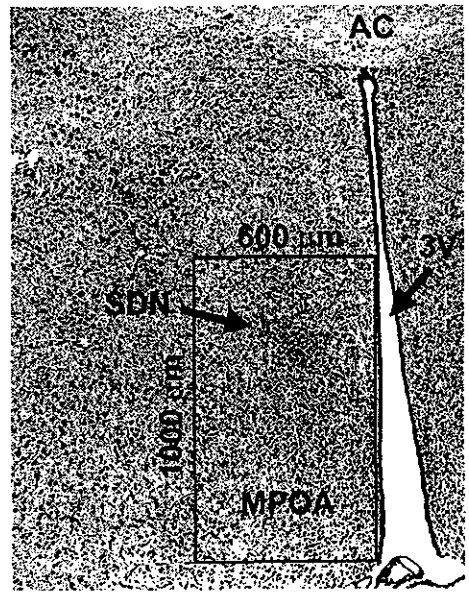
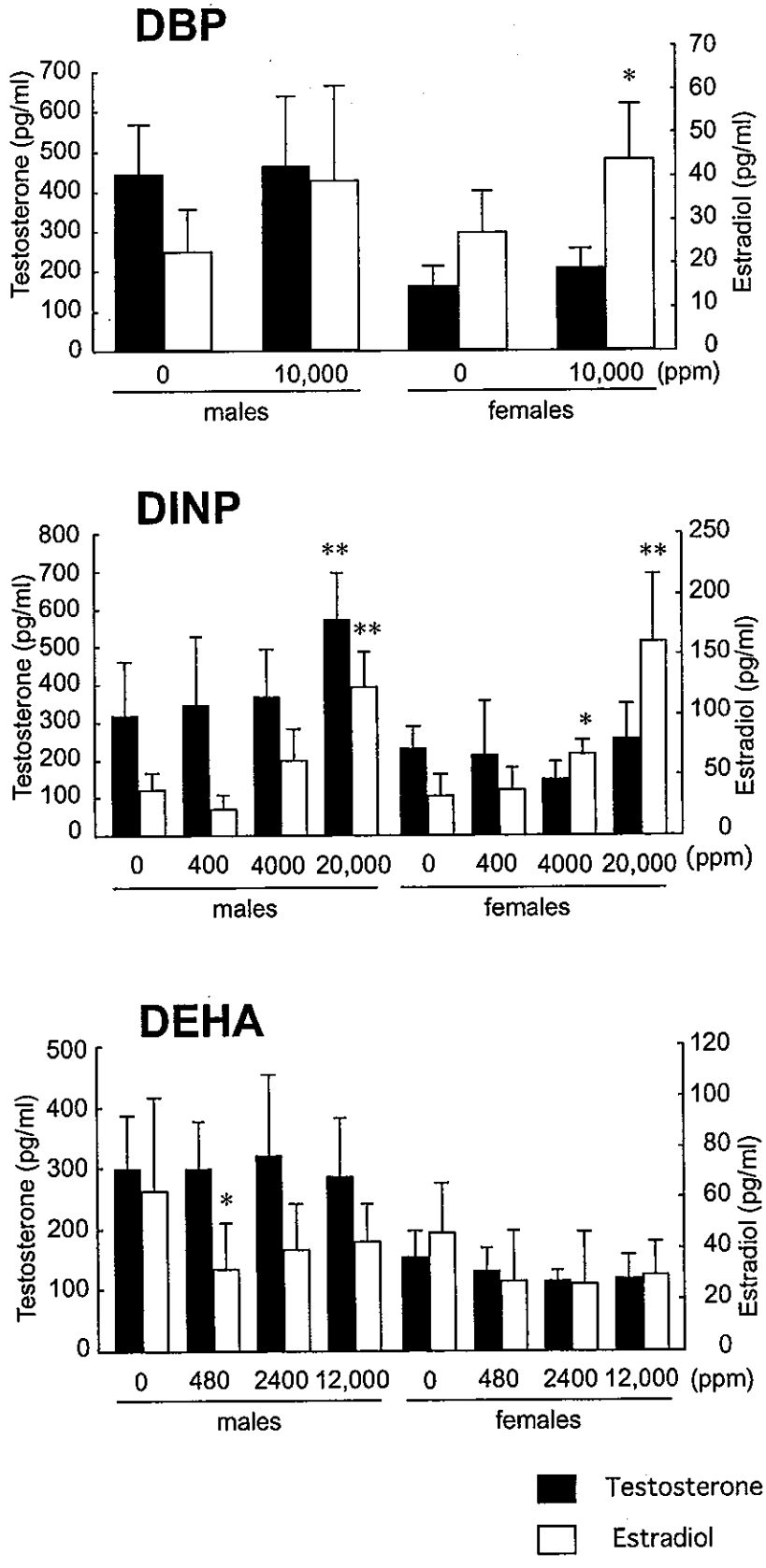


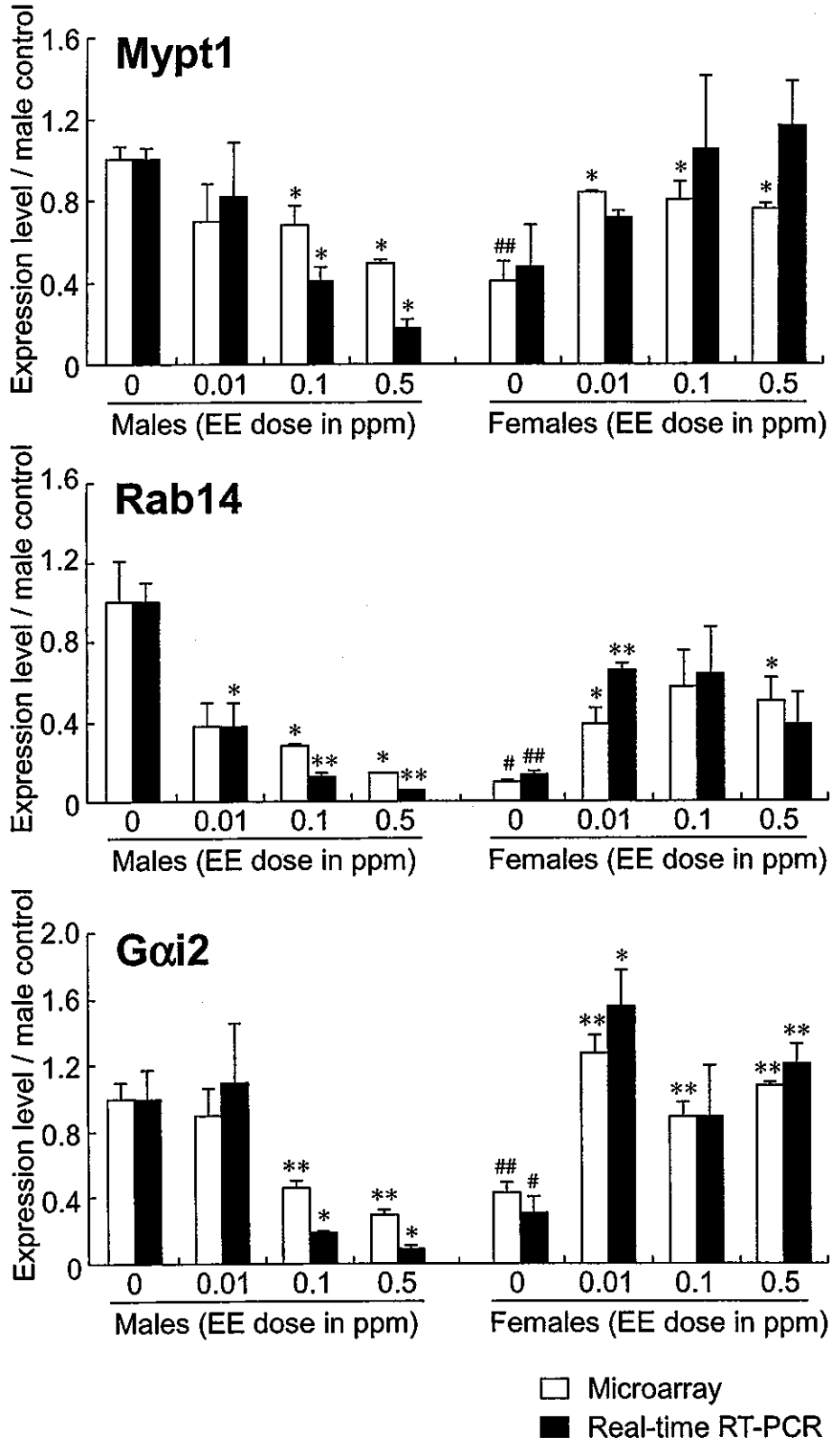
Fig. 5
Schematic view of male hypothalamic MPOA at PND 10. The enclosed area was microdissected from sections of methacarn-fixed paraffin-embedded brain slices for gene expression analysis. Note the intensely stained SDN region. Abbreviations: AC: anterior commissure; 3V: 3rd ventricle; MPOA: medial preoptic area; SDN: sexually dimorphic nucleus.



*p<0.05, vs. corresponding control

**p<0.01, vs. corresponding control

Fig. 6
Serum sex steroid levels in offspring exposed gestationally and lactationally to DBP, DINP, or DEHA.



*, **: Significantly different from the corresponding controls (*p<0.05, **p<0.01).
#, ##: Significantly different from male values (# p<0.05, ##p<0.01).

Fig. 7
Confirmation of microarray data by real-time RT-PCR in the EE study.

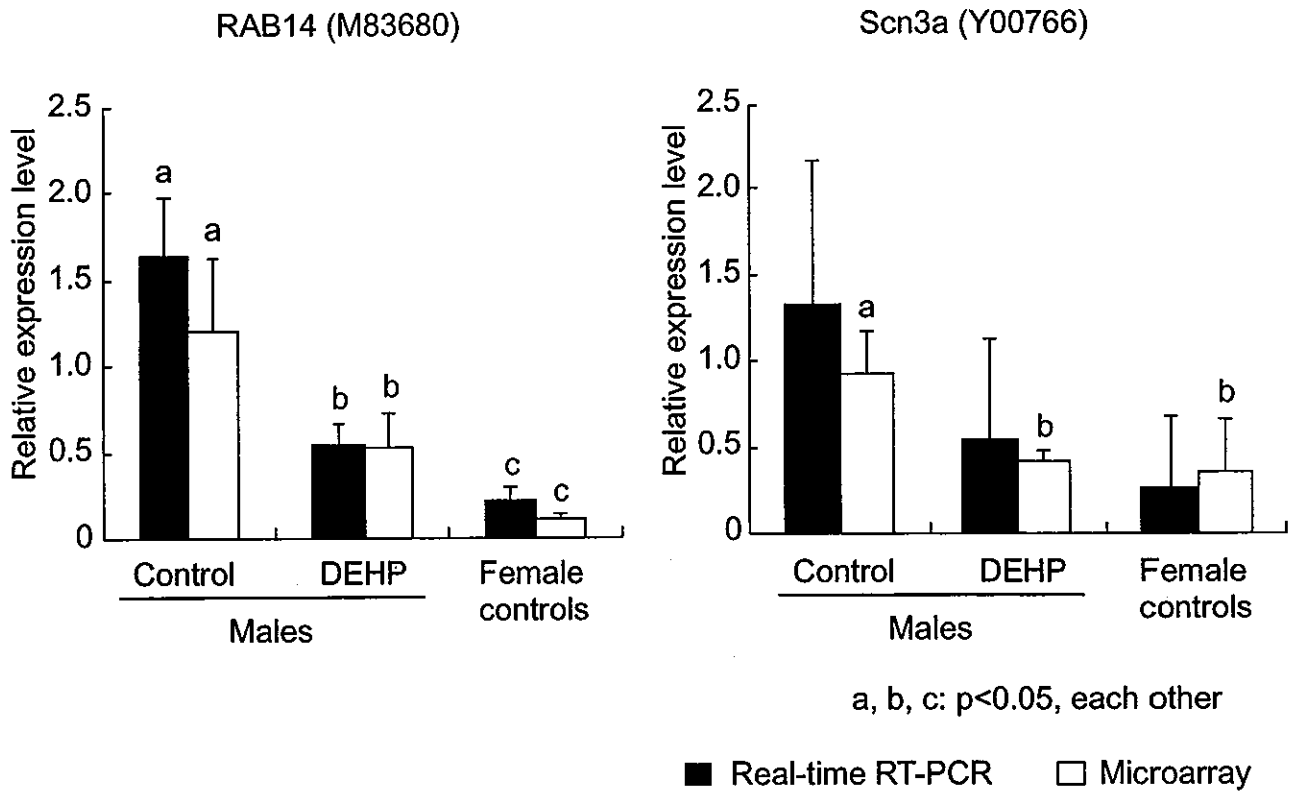


Fig. 8
Confirmation of microarray data by real-time RT-PCR in the DEHP study.

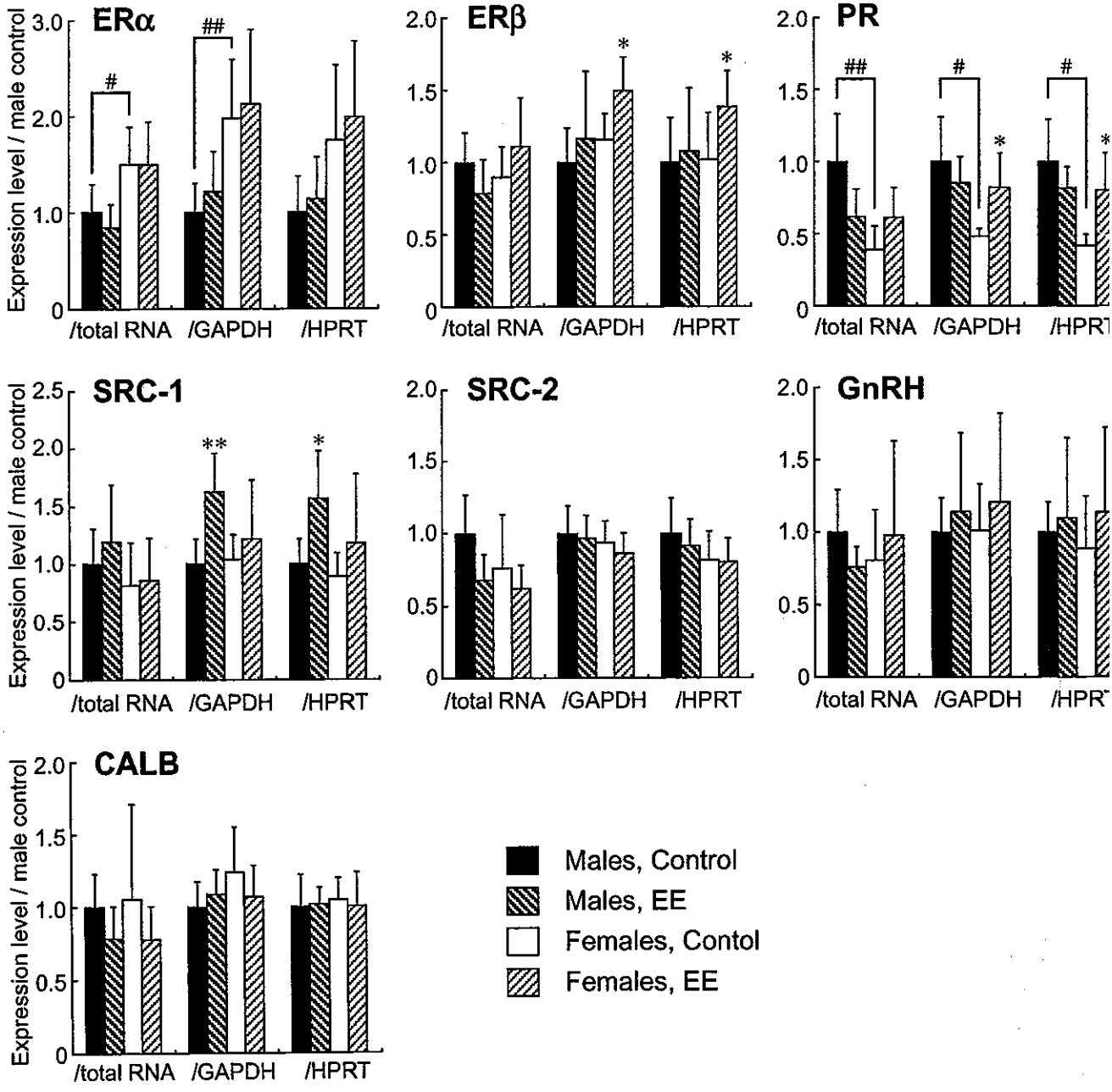


Fig. 9

Gene expression data for the MPOA at PND 10 of pups exposed maternally to EE. Data are ratios to male control values, expressed as mean ± SD, and normalized for the input amount of total RNA, or expression of GAPDH or HPRT
#, ##: Significant differences between males and females (#$p < 0.05$, ##$p < 0.01$ by Student's *t*-test). *, **: Significant differences from the controls of the correspond ing sex (*$p < 0.05$, **$p < 0.01$ by Student's *t*-test).

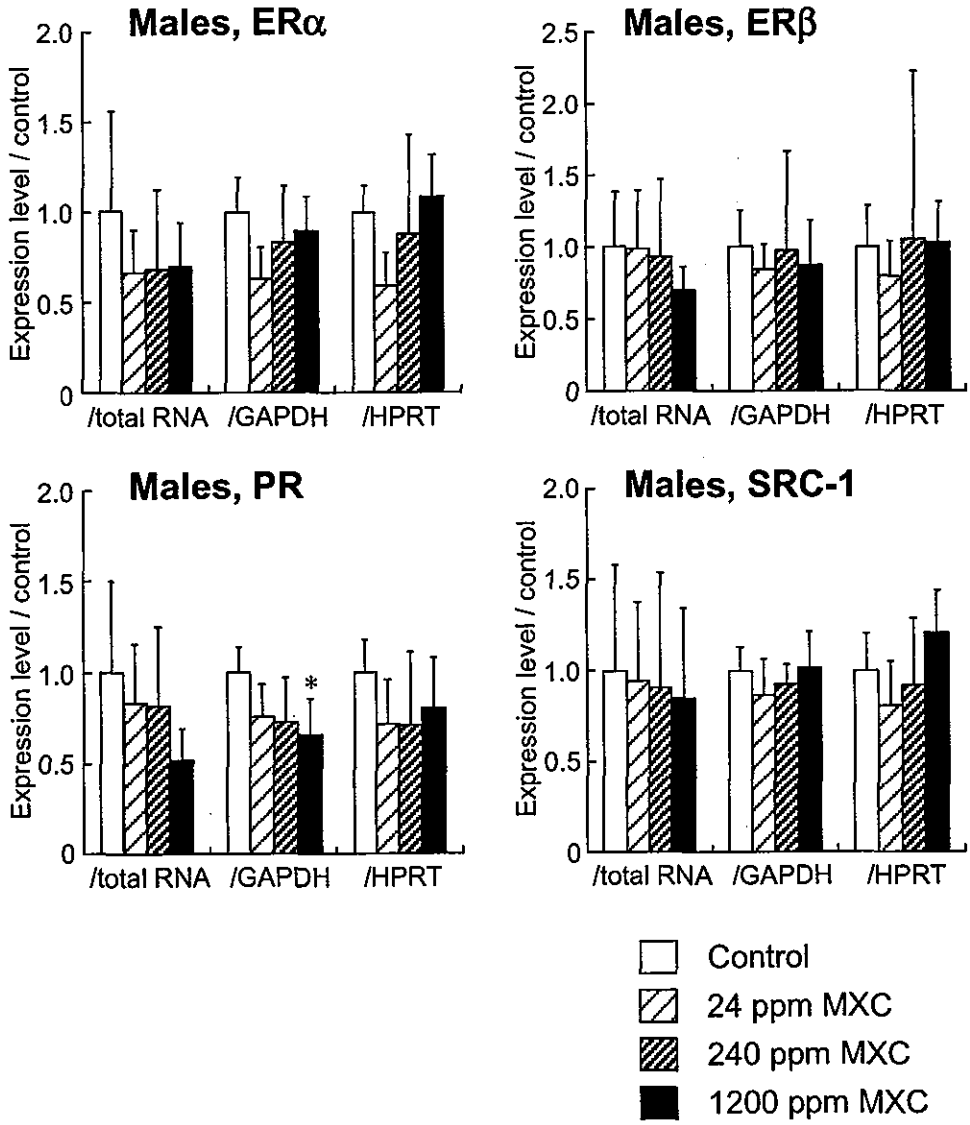


Fig. 10
Gene expression data for the MPOA at PND 10 of male pups exposed maternally to MXC. Data are ratios to control values, expressed as mean ± SD, and normalized for the input amount of total RNA, or GAPDH- or HPRT-expression. *: Significantly different from the controls (*$p < 0.05$ by Dunnett's test).

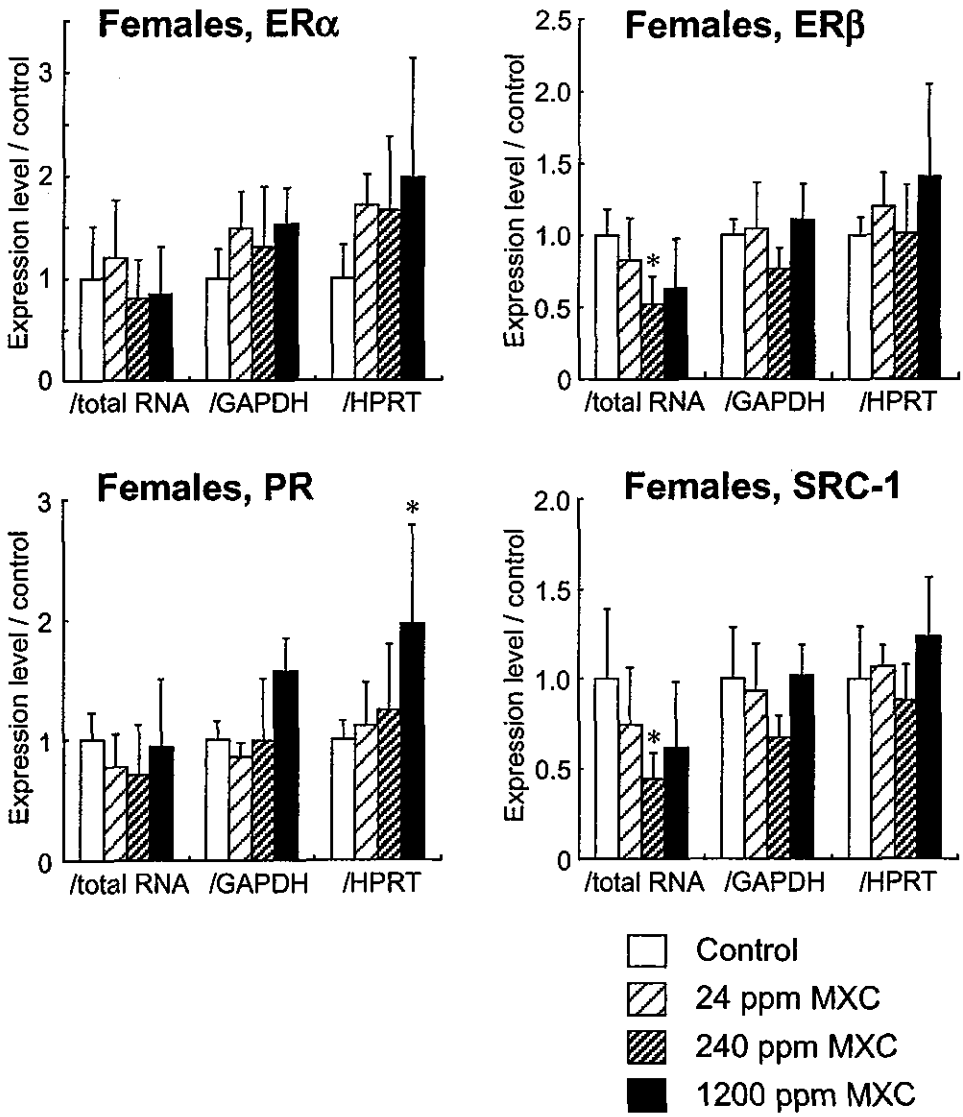


Fig. 11
Gene expression data for the MPOA at PND 10 of female pups exposed maternally to MXC. Data are ratios to control values, expressed as mean ± SD, and normalized for the input amount of total RNA, or GAPDH- or HPRT-expression. *: Significantly different from the controls (*$p < 0.05$ by Dunnett's test).

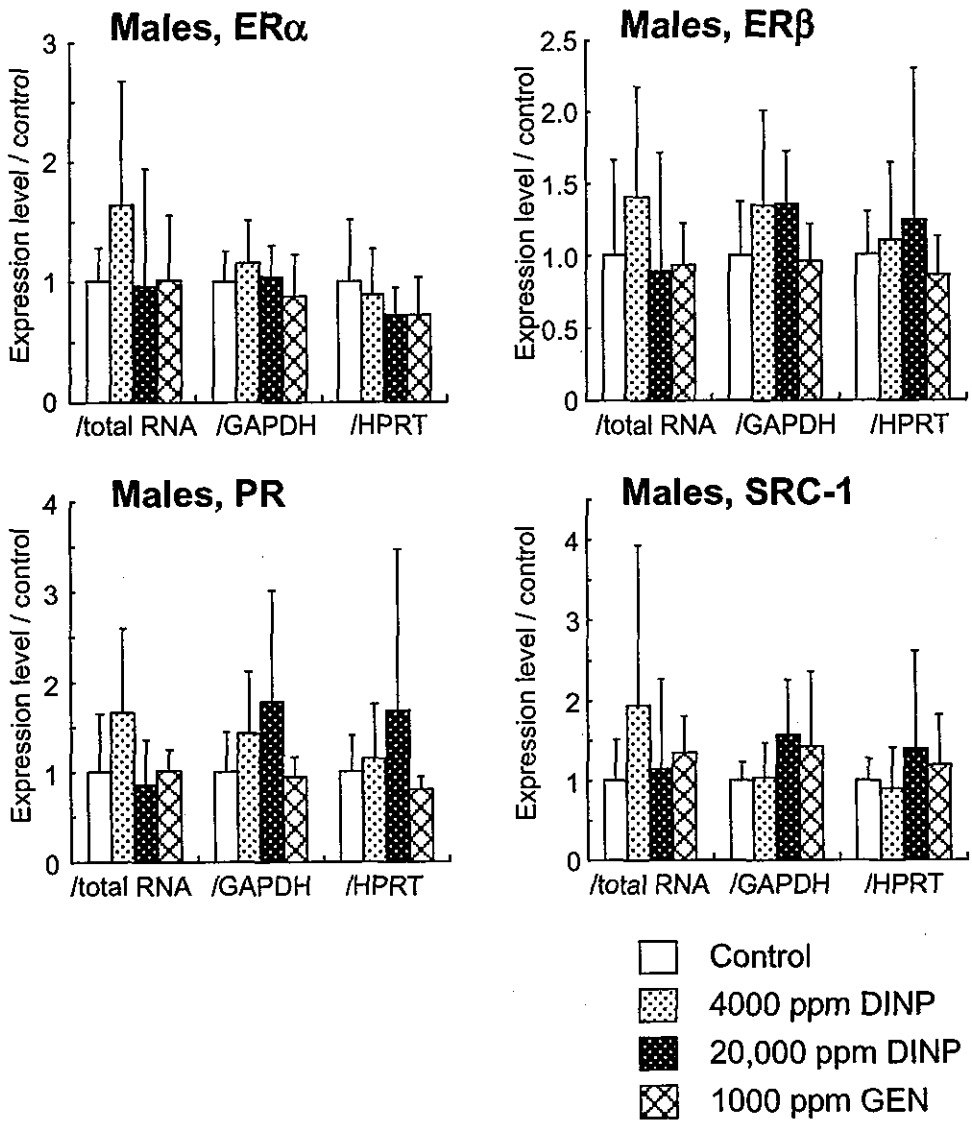


Fig. 12
Gene expression data for the MPOA at PND 10 of male pups exposed maternally to DINP or GEN. Data are ratios to control values, expressed as mean ± SD, and normalized for the input amount of total RNA, or GAPDH- or HPRT-expression.

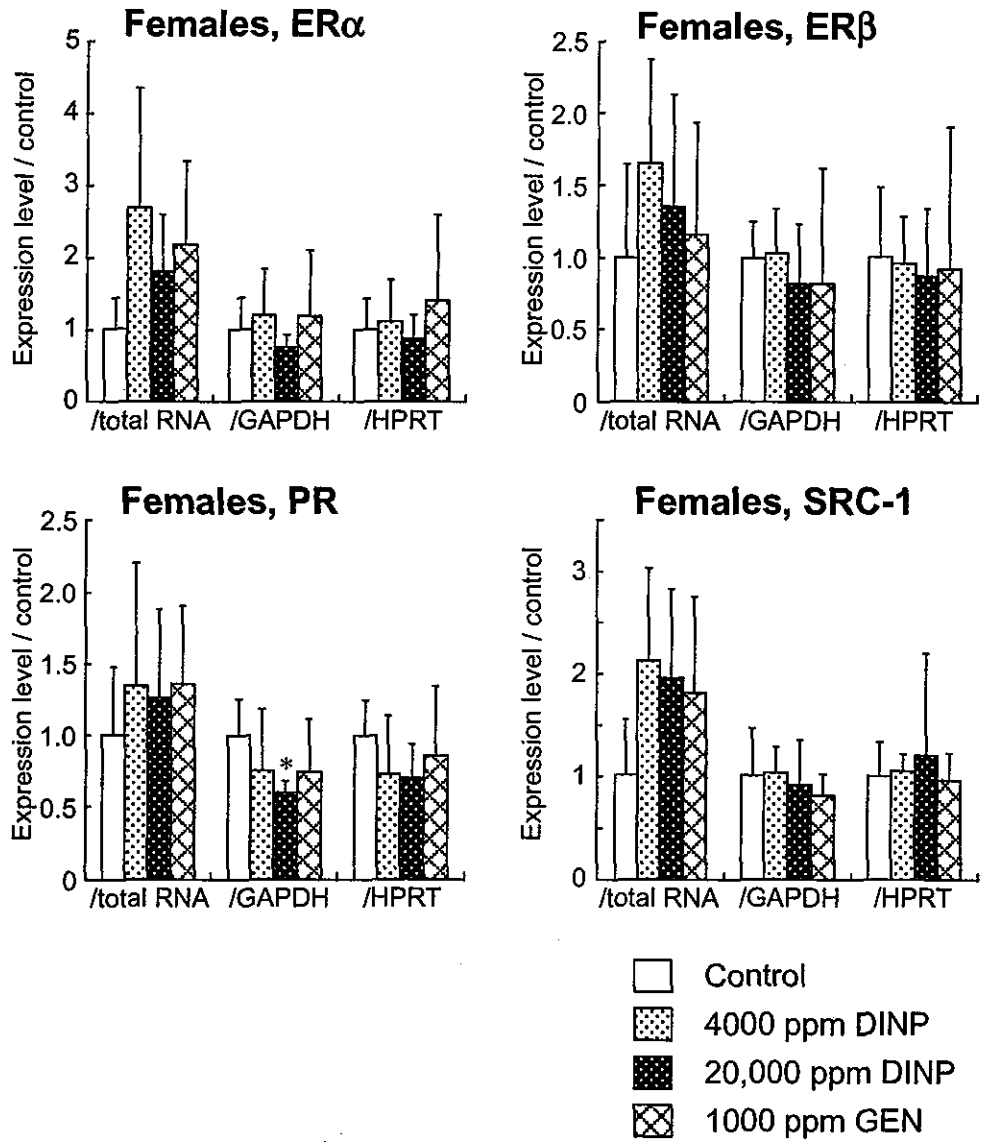


Fig. 13
Gene expression data for the MPOA at PND 10 of female pups exposed maternally to DINP or GEN. Data are ratios to control values, expressed as mean ± SD and normalized for the input amount of total RNA, or GAPDH- or HPRT-expression. *: Significantly different from the controls (*$p < 0.05$ by Dunnett's test).

Table 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 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 3. Effects on dams and offspring until prepubertal necropsy of exposure to diisononyl phthalate (DINP) during late gestation and lactation.

	DINP in diet (ppm)			
	0	400	4000	20,000
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 PNW 3				
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 4. 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)			
	0	400	4000	20,000
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 5. 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	20,000
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 6
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 [a]	>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 ($^{\dagger}p < 0.05$, $^{\#}p < 0.01$).
[†],[#] Significantly different from the controls using Mann-whitney's U-test ($^{\dagger}p < 0.05$, $^{\#}p < 0.01$).

Table 7

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 8

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 9
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 10

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 ($*p < 0.05$, $**p < 0.01$).

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