

Table 3-1: Percentage of MEHP-induced apoptotic spermatogenic cells. Apoptotic spermatogenic cells are detected by PENEL staining. Arrows indicate spermatogenic cells undergoing apoptosis.

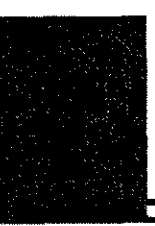
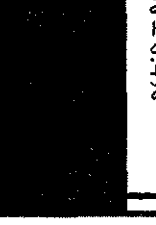
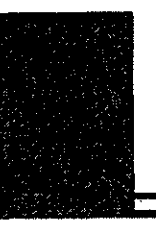
TIME	Concentration of MEHP (nmol/ml)				
	Control	1x10 ⁻⁶	1x10 ⁻³	1	100
1 hr	0.5% ± 0.4% 	0.9% ± 0.5% 	1.1% ± 0.4% 	1.5% ± 0.4% 	1.7% ± 0.4% 
3 hr	1% ± 0.4% 	1.4% ± 0.4% 	1.6% ± 0.4 	2% ± 0.3% 	2.5% ± 0.3% 
6 hr	1.2% ± 0.4% 	2.3% ± 0.6% 	2.5% ± 0.7% 	3% ± 0.6% 	6.8% ± 1.1% 
9 hr	2.8% ± 0.4% 	6.1% ± 1.1% 	7.4% ± 0.7% 	9.8% ± 2.5% 	14% ± 5.2% 

Table 3-2 MEHP-induced apoptotic spermatogenic cells per 10 round seminiferous tubules

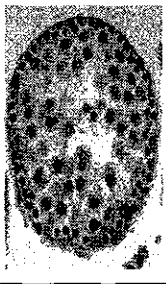
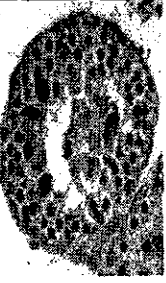
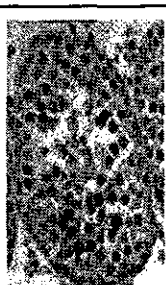
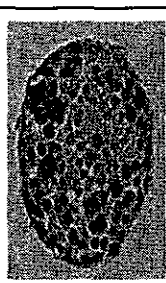
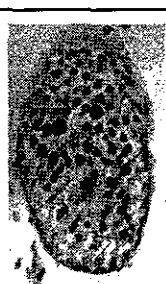
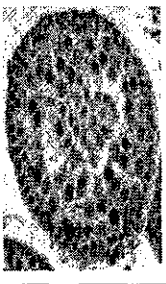
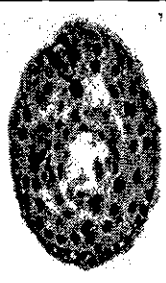
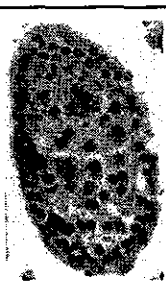
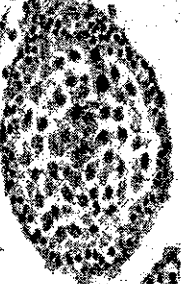
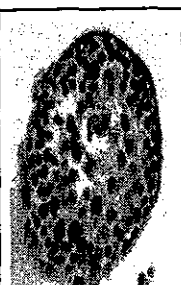
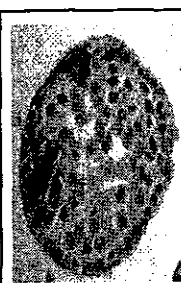
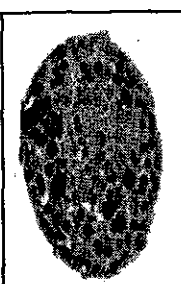
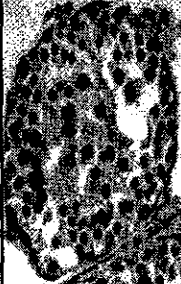
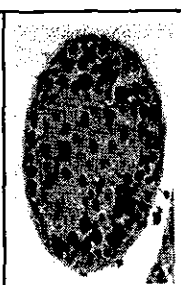
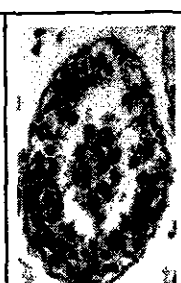
	Control	1x10 ⁻⁶ (nmol/ml)	1x10 ⁻³ (nmol/ml)	1 (nmol/ml)	100 (nmol/ml)
1 hr	 6.3 ± 1.7	 9 ± 3.1	 12.3 ± 2.3	 15.7 ± 1.9	 19.7 ± 0.3
3 hr	 6.7 ± 0.9	 9.3 ± 1.2	 14.3 ± 0.9	 17 ± 0.6	 20.7 ± 1.9
6 hr	 11 ± 2.1	 11.3 ± 2.0	 15.3 ± 2.7	 20.3 ± 1.2	 22.7 ± 3.5
9 hr	 15 ± 3.2	 24 ± 1.5	 28 ± 0.6	 28.7 ± 3.5	 31.3 ± 0.3

Table 3-3 Percentage of MEHP-induced apoptotic spermatogenic cells per total cells in adult monkey testis (*in vitro*). Arrows indicate apoptotic spermatogenic cell (TUNEL staining).

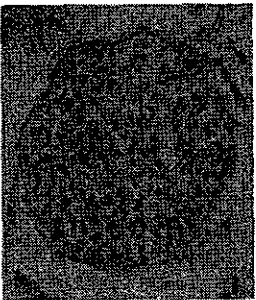
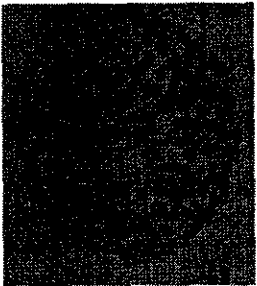
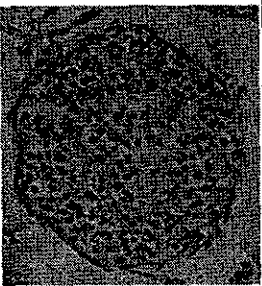
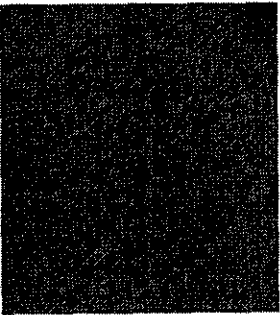
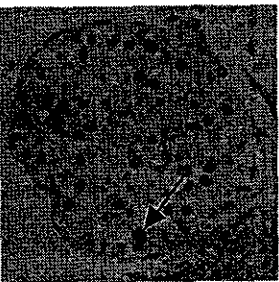
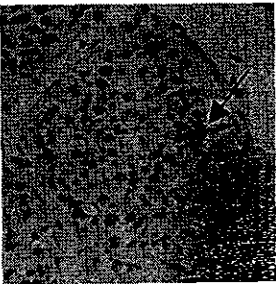
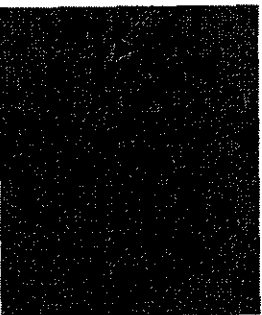
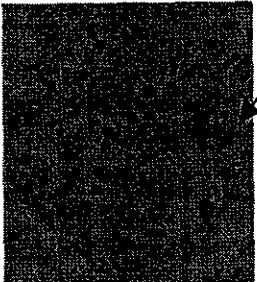
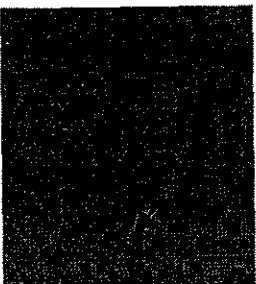
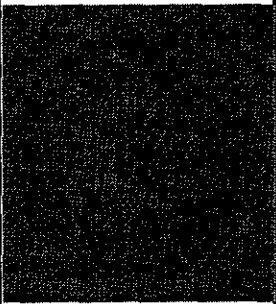
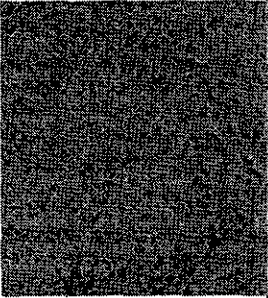
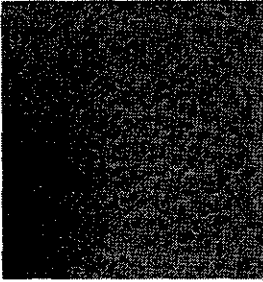
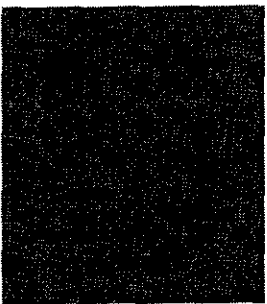
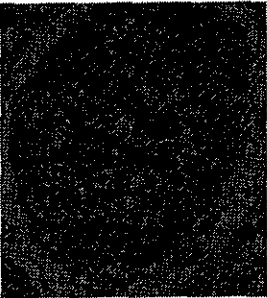
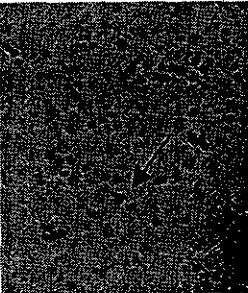
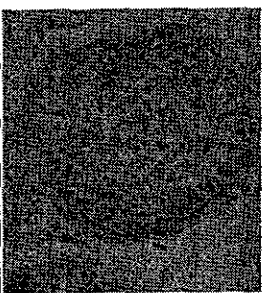
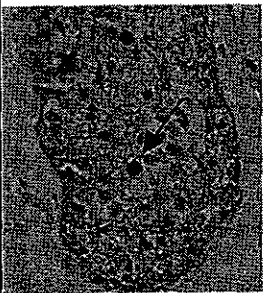
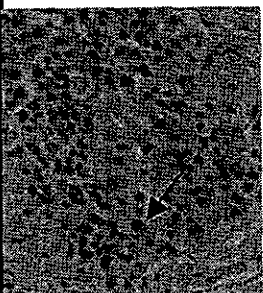
TIME	Concentration of MEHP (nmol/ml)		
	Control	1 nmol/ml	100 nmol/ml
3 hr	 0% ± 0%	 0% ± 0%	 0% ± 0%
6 hr	 0.05% ± 0.05%	 0.06% ± 0.02%	 0.11% ± 0.06%
9 hr	 0.07% ± 0.03%	 0.19% ± 0.08%	 0.36% ± 0.13%

Table 3-4 Percentage of MEHP-induced apoptotic spermatogenic cells in adult Shiba goat testis (*in vitro*). Arrows indicate apoptotic spermatogenic cells (TUNEL staining).

TIME	Concentration of MEHP (nmol/ml)		
	Control	1 nmol/ml	100 nmol/ml
3 h	 0% ± 0%	 0% ± 0%	 0% ± 0%
6 h	 0% ± 0%	 0%± 0%	 0.01% ± 0.02%
9 h	 0% ± 0%	 0.02% ± 0.02%	 0.04% ± 0.04%

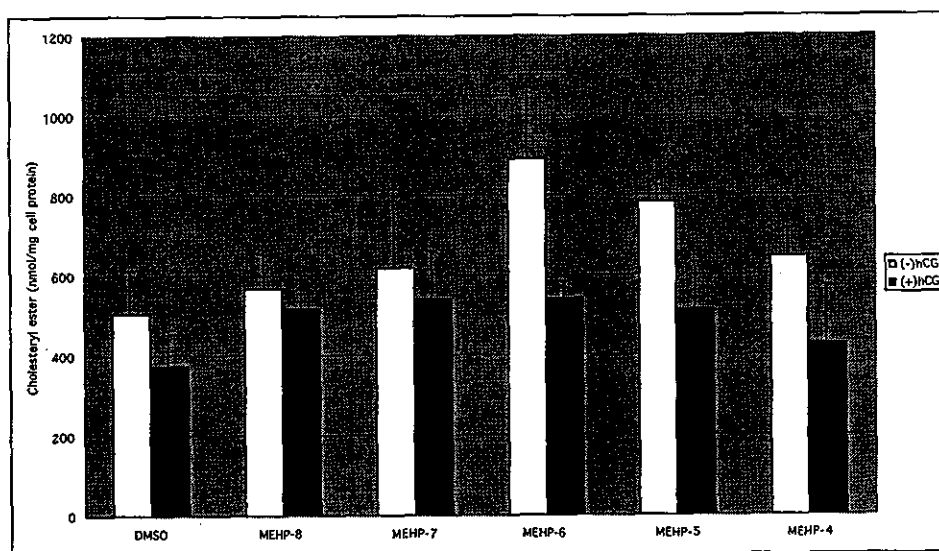


Fig. 4-1

MEHPによる細胞内コレステロールエステルの蓄積

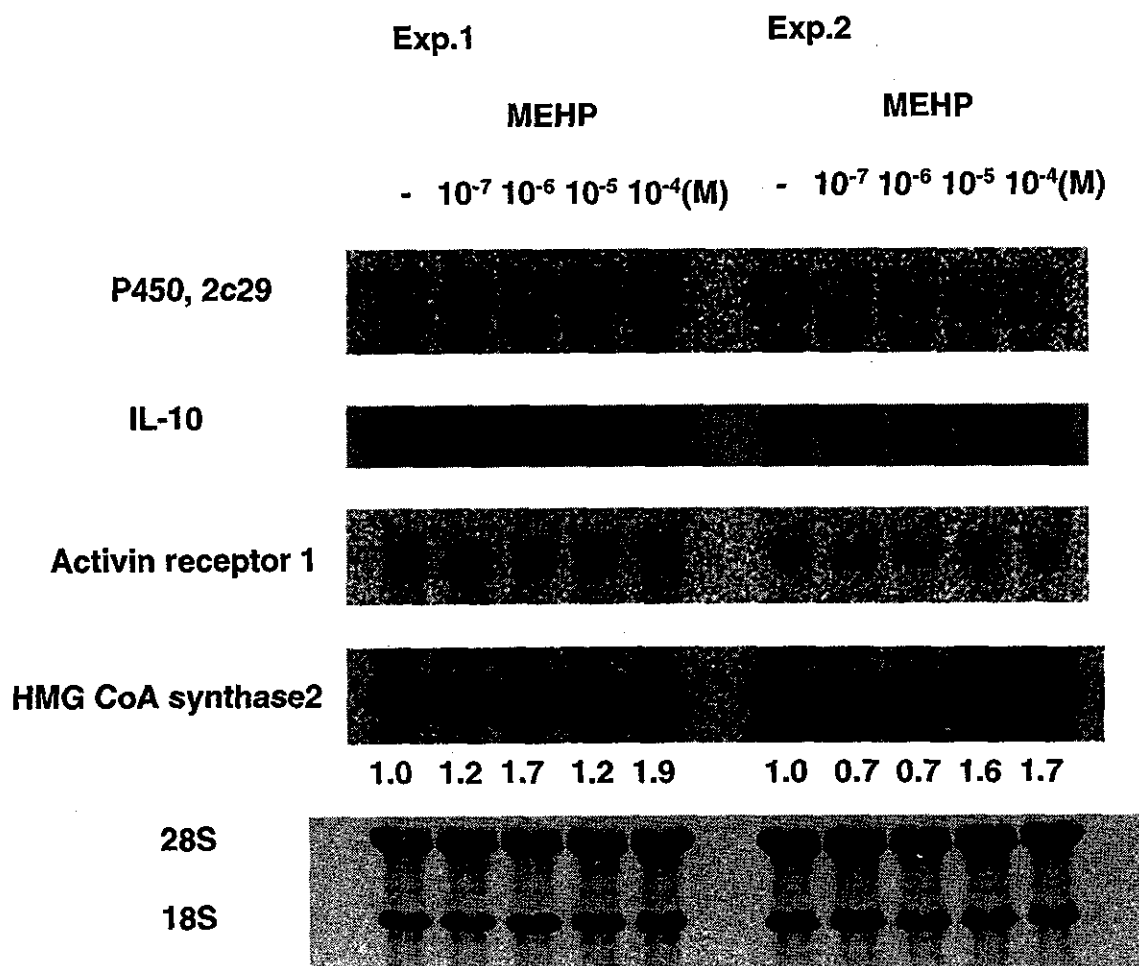


Fig. 4-2 Northern blotによる遺伝子発現変化の確認

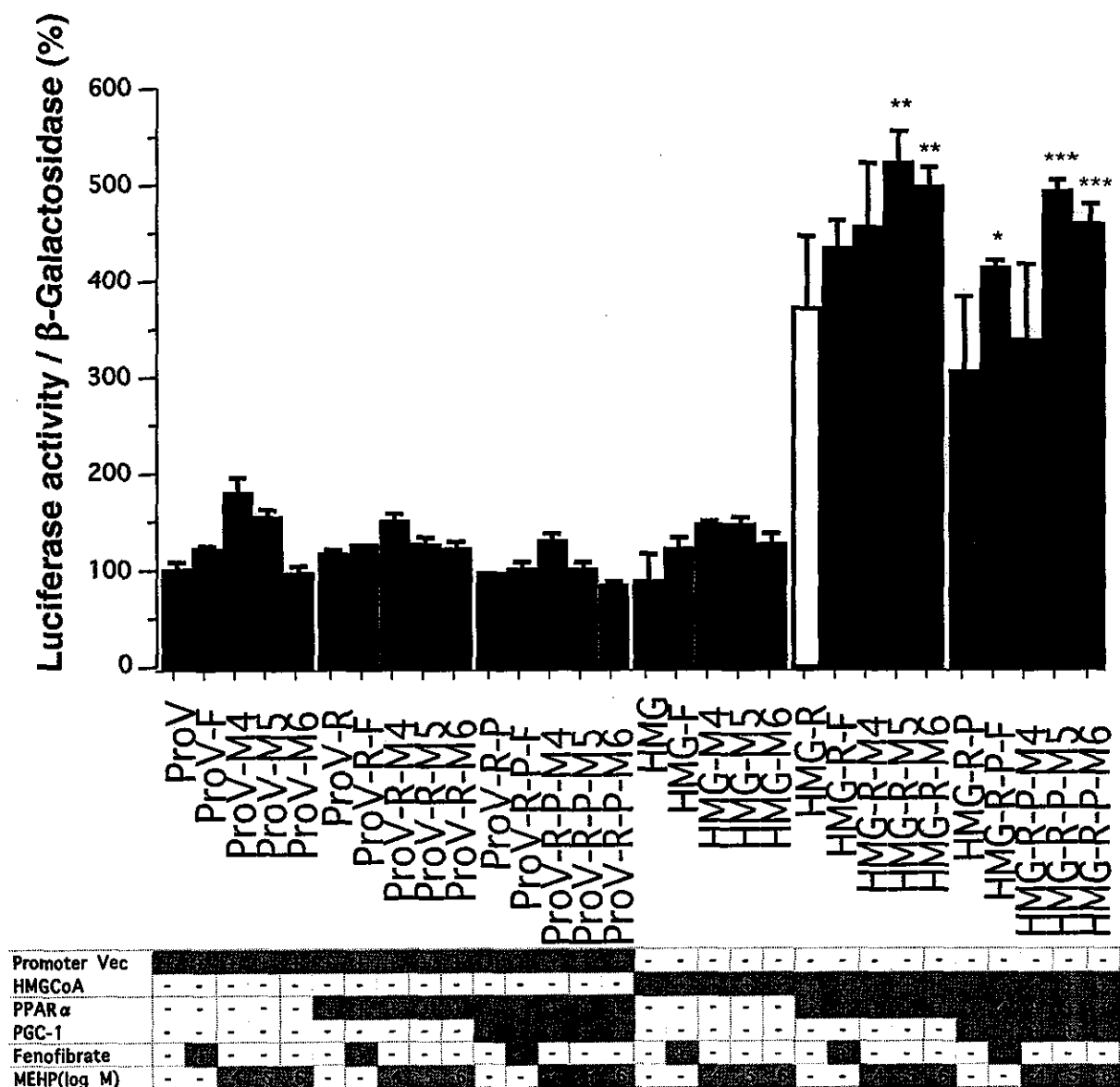


Fig. 4-4 MEHPによるHMG CoA synthase 2遺伝子のプロモーターの活性化

**p<0.01 versus HMG-R

***p<0.001, *p<0.05 versus HMG-R-P

-54 -35 Motif : PPARalpha/RXR-alpha
agaccttttggcccagttttt

aaaaactgggccaaggtct
CWRWCTAGGNCAAAGGTCA (コンセンサス配列)

Fig. 4-5
HMG CoA synthase 2遺伝子のプロモーター領域におけるPPAR α 結合部位

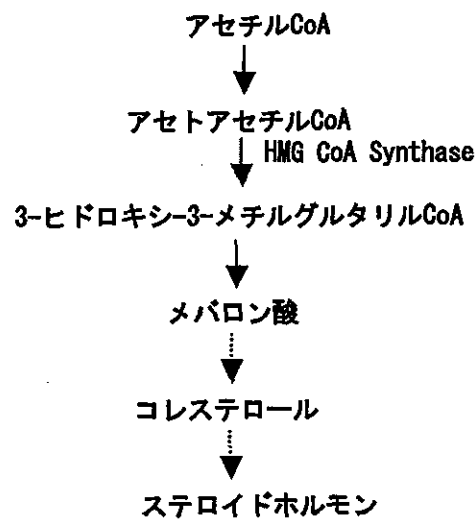


Fig. 4-6
HMG CoA synthase 2のLeydig細胞における働き

別紙 4

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Shibutani, M., Uneyama, C.	Methacarn a fixation tool for multipurpose genetic analysis from paraffin-embedded tissues.	Conn, M.	Methods Enzymol, Vol. 356	Academic Press	New York	2002	114-125
Shibutani, M., Uneyama, C., Masutomi, N., et al.	Application of methacarn fixation for genetic analysis in microdissected paraffin-embedded tissue specimens.	Inoue, T., Pennie, W.D.	Toxicogenomics	Springer	Berlin	2002	100-106
Shibutani, M., Uneyama, C.	Methacarn fixation for genomic DNA analysis in microdissected cells.	Murray, G.I., Curran, S.	Methods in Molecular Biology	Humana Press	Totowa	2004	11-25

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Uneyama, C. Shibutani, M., et al.	Methacarn fixation for genomic DNA analysis in microdissected paraffin-embedded tissue specimens.	J. Histochem. Cytochem.	50	1237-1245	2002
Masutomi, N., Shibutani, M., et al.	Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life.	Toxicology	192	149-170	2003
Masutomi, N., Shibutani, M., et al.	Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals.	Arch. Toxicol.	78	232-240	2004
Takagi, H., Shibutani, M., et al.	Microdissected region-specific gene expression analysis with methacarn-fixed paraffin-embedded tissues by real-time RT-PCR.	J. Histochem. Cytochem.	52	903-913	2004
Lee, K-Y., Shibutani, M., et al.	Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation.	Toxicology	203	221-238	2004
Shibutani, M., Masutomi, N., Uneyama, C., et al.	Down-regulation of GAT-1 mRNA expression in the microdissected hypothalamic medial preoptic area of rat offspring exposed maternally to ethinylestradiol.	Toxicology	208	35-48	2005
Takagi, H., Shibutani, M., et al.	Impact of maternal dietary exposure to endocrine-acting chemicals on progesterone receptor expression in microdissected hypothalamic medial preoptic areas of rat offspring.	Toxicol. Appl. Pharmacol.			in press
Yonehara, K., Suzuki, M., Nishihara, M., et al.	Sex-related differences in gene expression in neonatal rat hypothalamus assessed by cDNA microarray analysis.	Endocrine J.	49	131-137	2002
Yonehara, K., Suzuki, M., Nishihara, M., et al.	Androgen induces p130 mRNA expression in the neonatal rat hypothalamus.	Neurosci. Lett.	334	107-110	2002
Yonehara, K., Suzuki, M., Nishihara, M., et al.	Expression analysis of estrogen and androgen target genes in neonatal rat hypothalamus.	J. Reprod. Dev.	49	547-552	2003

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Suzuki, M., Lee, H.C., Nishihara, M., et al.	Effects of methoxychlor exposure during perinatal period on reproductive function after maturation in rats.	J. Reprod. Dev.	50	455-461	2004
鈴木正寿, 西原真杉	脳の性分化における分子機構—グラニューリンの関わり—	性差と医療	2	157-160	2005
Mitsuhashi, M., Morimura, K., Fukushima, S., et al.	Di-n-butyl phthalate is toxic to the male reproductive system and its toxicity is enhanced by thioacetamide induced liver injury.	J. Toxicol. Pathol.	17	177-185	2004
Shirai, T., Asamoto, M.	Application of toxicogenomics to the endocrine disruption issue.	Pure Appl. Chem.	75	2419-2422	2003
Tsutsumi, T., Ichihara, T., Shirai, T., et al.	Renal toxicity induced by folic acid is associated with the enhancement of male reproductive toxicity of di(n-butyl) phthalate in rats.	Reprod. Toxicol.	18	35-42	2004
Andriana, B. B., Kurohmaru, M., et al.	Effects of mono (2-ethylhexyl)phthalate (MEHP) on testes in rats in vitro.	Okajimas Folia Anat. Jpn.	80	127-136	2004
Andriana, B.B., Tay, T.W., Kurohmaru, M., et al.	An ultrastructural study on cytotoxic effects of mono(2-ethylhexyl) phthalate (MEHP) on testes in Shiba goats in vitro.	J. Vet. Sci.	5	235-240	2004
Awal, M.A., Kurohmaru, M., et al.	Mono-(2-ethylhexyl)phthalate (MEHP) induces spermatogenic cell apoptosis in guinea pig testes at prepubertal stage in vitro.	Int. J. Toxicol.	23	349-355	2004
Awal, M.A., Kurohmaru, M., et al.	Mono(2-ethylhexyl) phthalate (MEHP) induces testicular alterations in guinea pigs at prepubertal stage.	Tissue & Cell			in press
Takahashi, M., Tsuboyama-Kasaoka, N., Ezaki, O., et al.	Fish oil feeding alters liver gene expressions to defend against PPAR α activation and ROS production.	Am. J. Physiol. Gastrointest. Liver Physiol.	282	G338-G348	2002
Nakatani, T., Tsuboyama-Kasaoka, N., Ezaki, O., et al.	Mechanism for peroxisome proliferator-activated receptor- α activator-induced up-regulation of UCP2 mRNA in rodent hepatocytes.	J. Biol. Chem.	277	9562-9569	2002
Yajima, H., Ikeshima, E., Ezaki, O., et al.	Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor (PPAR) and and reduce insulin resistance.	J. Biol. Chem.	279	33456-33462	2004
小泉睦子, 江馬 眞ら	DINP の無毒性量と耐容 1 日摂取量の算定.	日本食品化学学会誌	9	39-45	2002
Ema, M., Miyawaki, E., Hirose, A., et al.	Decreased anogenital distance and increased incidence of undescended testes in fetuses of rats given monobutyl phthalate, a major metabolite of butyl benzyl phthalate.	Reprod. Toxicol.	17	407-412	2003
Hirose, A., Hasegawa, R., Ema, M., et al.	Revision and establishment of Japanese drinking water quality guidelines for di(2-ethylhexyl) phthalate, toluene and vinyl chloride-differences from the latest WHO guideline drafts.	J. Toxicol. Sci.	29	535-539	2004
Fukui, Y., Ema, M., et al.	Comments from the Behavioral Teratology Committee of the Japanese Teratology Society on OECD Guideline for the Testing of Chemicals, Proposal for a New Guideline 426, Developmental Neurotoxicity Study, Draft Document (September 2003).	Cong. Anom.	44	172-177	2004

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
高橋美加, 平田睦子 江馬 眞 ら	OECD化学物質対策の動向(第6報)、第14回 OECD高生産量化学物質初期評価会議(2002年 パリ)	化学生物総合 管理学会誌	1	46-55	2004
高橋美加, 平田睦子 江馬 眞 ら	OECD化学物質対策の動向(第5報).	国立医薬品食 品衛生研究所 報告	122	37-42	2004

研究成果の刊行物・別刷

