Effects of Prenatal Exposure to Styrene Trimers on Genital Organs and Hormones in Male Rats

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Styrene trimers migrate from polystyrene food container into foods. We evaluated the estrogenic activity of styrene trimers such as 2,4,6-triphenyl-1-hexene (ST-1), 1a-phenyl-4a-(1′-phenylethyl)tetralin (ST-2), 1a-phenyl-4e-(1′-phenylethyl)tetralin(ST-3), 1e-phenyl-4a-(1′-phenylethyl)tetralin (ST-4), and 1e-phenyl-4e-(1′-phenylethyl)tetralin (ST-5) using the reporter-gene assay with MVLN cells stably expressing the estrogen-stimulated reporter gene, and it was confirmed that ST-1, ST-3, and ST-4 had estrogen-like activity. On the other hand, ST-2 and ST-5 had anti-estrogen-like activity. We examined the estrogenic activity *in vivo* of ST-1, ST-3, and ST-4. The styrene trimers were administered to pregnant rats, and the effects on the offspring were examined. ST-1, ST-3, or ST-4 (0, 10, 100, 1000 μg/kg body wt/day) were subcutaneously injected into pregnant rats from gestational Day 11 through 17, and the male offspring were sacrificed on postnatal days (PND) 101–103. In the ST-4 treatment groups, the relative anogenital distance on PND 3 was significantly shortened. The relative testis weight was remarkably decreased in all styrene trimer treatment groups. Relative weights of the prostate and epididymides significantly decreased in the ST-4 treatment groups. The relative brain weight was markedly reduced in the ST-3 and ST-4 treatment groups. A significant decrease of the Sertoli cell count was observed in the ST-1 and ST-4 treatment groups. The serum follicle stimulating hormone level was remarkably reduced in all styrene trimer treatment groups. The luteinizing hormone level was significantly decreased and the testosterone level increased in the ST-1 and ST-4 groups. These results suggest that prenatal exposure to estrogenic styrene trimers at low levels obstructed genital organ development, and disrupted the endocrine systems of male rat offspring. Exp Biol Med 232:301–308, 2007

Key words: styrene trimer; estrogenic activity; male rats; organ weight; Sertoli cell count; endocrine disruption

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Polystyrene resin used for food containers, such as take-out food containers, coffee cups, meat trays, soup bowls, and salad boxes, contains high levels of styrene trimers (650–20,770 μg/g, Ref. 1; 150-7310 μg/g, Ref. 2) as an impurity. Styrene trimers 2,4,6-triphenyl-1-hexene (ST-1), 1a-phenyl-4a-(1′-phenylethyl)tetralin (ST-2), 1a-phenyl-4e-(1′-phenylethyl)tetralin (ST-3), 1e-phenyl-4a-(1′-phenylethyl)tetralin (ST-4), and 1e-phenyl-4e-(1′-phenylethyl)tetralin (ST-5) migrate from polystyrene lunch boxes (88.1–1290 ng/cm^2, Ref. 3) into vegetable oil by heating in a microwave oven or incubation for 24 hrs at 20°C, and from instant noodle cups (0–33.8 μg/cup, Ref. 4) into foods by cooking. In the previous study, we showed that the styrene trimers ST-1, ST-2, ST-3, ST-4, and ST-5 had binding affinity for estrogen receptor-α (5). It is supposed that the styrene trimers have estrogen-like activities and affect the endocrine system. In this study, to evaluate the estrogenic activity of these styrene trimers, we carried out the reporter-gene assay with MVLN cells stably expressing the estrogen stimulated-luciferase reporter gene. It was revealed that ST-1, ST-3, and ST-4 had estrogen-like activity. The effects of prenatal exposure to ST-1, ST-3, or ST-4 on male Sprague-Dawley rat offspring were examined.

Materials and Methods

Chemicals. Styrene trimers were purchased from Hayashi Pure Chemical Industry (Osaka, Japan). 17β-estradiol (E_2) and bisphenol A were obtained from Calbiochem (Richmond, CA) and Wako Pure Chemical Industries (Osaka, Japan), respectively. Test chemicals were dissolved in ethanol. The chemical structures of these compounds are shown in Figure 1.

***In Vitro* Study.** *Reporter-Gene Assay.* The assay to evaluate luciferase production of MVLN cells obtained from M. Pons (INSERM, Montpellier, France) was performed according to a technique described by Pons *et al*. (6). The solution of chemicals in ethanol was diluted with phenol red–free Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) with 1% FBS treated by charcoal-

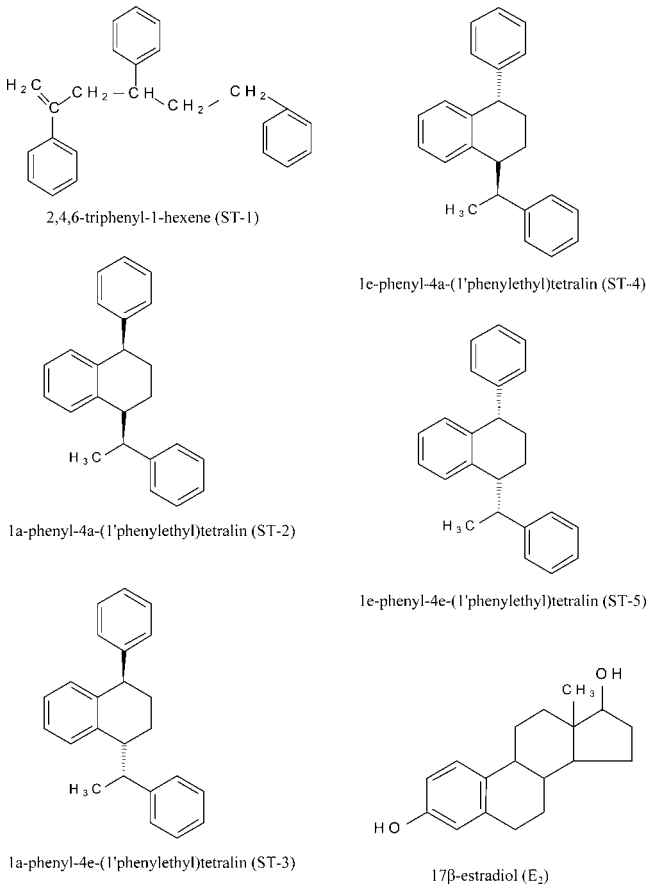

Figure 1. Chemical structure of styrene trimers.

dextran stripping to various concentrations. A lysing and luminescent compound, PicaGene LT2.0HS (Toyo Ink, Tokyo, Japan), was used to generate a luminescent response, the intensity was measured using a microplate luminometer, LB96V (Berthold, Wildbad, Germany), and the luciferase activity was subsequently determined. To evaluate cell toxicity, we calculated the number of cells by measuring the amount of protein stained with sulforhodamine B (Wako) as described by Brotons *et al*. (7).

***In Vivo* Study.** *Animals.* Pregnant specific pathogen-free Sprague-Dawley rats (9 weeks old) purchased from Charles River Japan (Kanagawa, Japan) were received on Day 5 of gestation, and individually housed in polycarbonate plastic cages with wood chip bedding (Clean-chip, CLEA Japan Inc., Tokyo, Japan). The animal room was kept at 23 ± 1°C and 50 ± 5% relative humidity. The room's incoming air was passed through a HEPA filter with 99.9% efficiency. Food (CE-2 diet, CLEA) and water were provided *ad libitum*. All procedures involving animals were approved by the institutional animal care and use committee and conformed to the guidelines in The UFAW Handbook on the Care and Management of Laboratory Animals.

Exposure Study Design. Three styrene trimers, ST-1, ST-3, and ST-4, were individually investigated using 24, 32, and 19 pregnant rats, respectively. The various concentrations of chemical solutions in ethanol were diluted with physiological saline (1% ethanol solution). Various doses of the chemicals (0, 10, 100, or 1000 μg/kg body wt/day in saline solution at a volume of 1.0 ml/100g body wt) were subcutaneously administered to the pregnant animals once a day for 7 days from gestational Days 11 through 17. All male and female offspring were kept with the litters until weaning. No effect on male/female ratio was related to chemicals and/or dose. The treatments induced no maternal toxicity, and there was no reduction of litter sizes. Male offspring were provided for this study and individually housed in stainless steel cages from postnatal day (PND) 24. The body weight of male offspring was recorded at frequent intervals until a terminal day. The anogenital distance (AGD) and crown rump length (CRL) were recorded on PND 3. Animals were sacrificed on PND 101–103 under ether anesthesia, and blood was collected. Ventral prostates, testes, epididymides, and brains were removed and weighed.

Histological Preparation. The left and right testes were fixed by phosphate-buffered 10% formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for microscopy. For examination of seminiferous tubules, quantitative evaluation of Sertoli cells was performed. A total of four seminiferous tubules per animal (two tubules per testis) exhibiting a round shape were randomly selected, and the numbers of Sertoli cells were counted.

Serum Hormonal Assay. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone in serum were determined by enzyme immunoassay (EIA) systems. FSH and LH EIA kits for rats were purchased from Amersham Biosciences (Piscataway, NJ). The testosterone EIA kit was obtained from Cayman Chemical (Ann Arbor, MI).

Statistical Analysis. The AGD data on PND 3 being an index of prenatal organ development were analyzed using litter mean. The other data on PND 101–103, being indexes of postnatal organ development, were analyzed using offspring mean values. Quantitative continuous variables such as body weights, organ weights, and hormonal levels were analyzed first using Bartlett's test for equal variances. Based on the outcome of Bartlett's test, either parametric or nonparametric ANOVA was performed. Once significance was established, the significance of differences between the control and styrene trimer treatment groups was assessed using Student's *t* test. *P* values less than 0.05 were considered significant.

Results

***In Vitro* Study.** With luciferase activity induced by 3.0×10^{-10} *M* E_2 being expressed as 100%, the luciferase activity in the presence of various concentrations of styrene trimers is shown in Figure 2. The EC_{50} values are the concentrations of test compounds required for 50% of the luciferase activity at 3.0×10^{-10} *M* E_2 (Table 1). ST-1, ST-3, and ST-4 increased luciferase activity concentration-

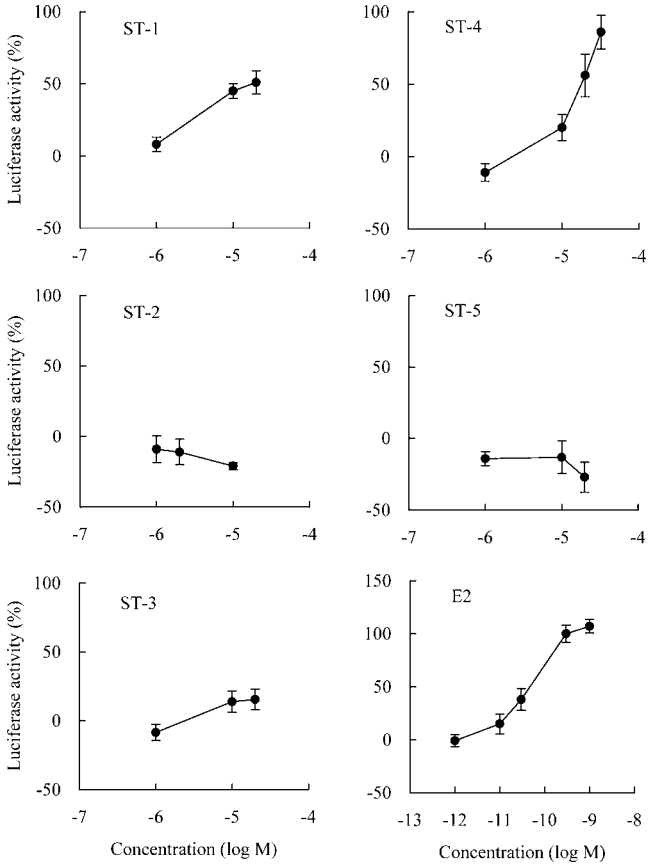

Figure 2. Effects of styrene trimers on luciferase activity in MVLN cells. The luciferase activity obtained at different concentrations of test chemicals was compared with that obtained at 3×10^{-10} *M* E_2. The 0% value was obtained as the solvent control. Each point is the mean ± SD.

dependently. The EC_{50} values of ST-1 and ST-4 were almost the same (1.8×10^{-5} *M*). Maximal activity of ST-4 was 86% at 3.2×10^{-5} *M*, which was the highest among the styrene trimers tested; that of ST-1 was 51% at 2.0×10^{-5} *M*; and that of ST-3 was 16% at 2.0×10^{-5} *M*. Other styrene trimers did not express luciferase activity.

Table 1. Effects of Styrene Trimers on Luciferase Activity in MVLN Cells[a]

Compound	Agonist activity EC_{50} (M)[b]	Antagonist activity IC_{50} (M)[c]
E_2	4.7×10^{-11}	—
Bisphenol A	5.5×10^{-6}	—
ST-1	1.8×10^{-5}	—
ST-2	NE	1.2×10^{-5}
ST-3	ND	—
ST-4	1.8×10^{-5}	—
ST-5	NE	1.3×10^{-5}

[a] E_2, 17β-estradiol; ST-1, 2,4,6-triphenyl-1-hexene; ST-2, 1a-phenyl-4a-(1′-phenylethyl)tetralin; ST-3, 1a-phenyl-4e-(1′-phenylethyl)tetralin; ST-4, 1e-phenyl-4a-(1′-phenylethyl)tetralin; ST-5, 1e-phenyl-4e-(1′-phenylethyl)tetralin; NE, no effect; ND, value could not be estimated from the response curve.
[b] EC_{50}, concentration of the test compound producing 50% of the luciferase activity at 3×10^{-10} *M* E_2.
[c] IC_{50}, concentration of the test compound required for a 50% reduction in luciferase activity at 3.0×10^{-10} *M* E_2.

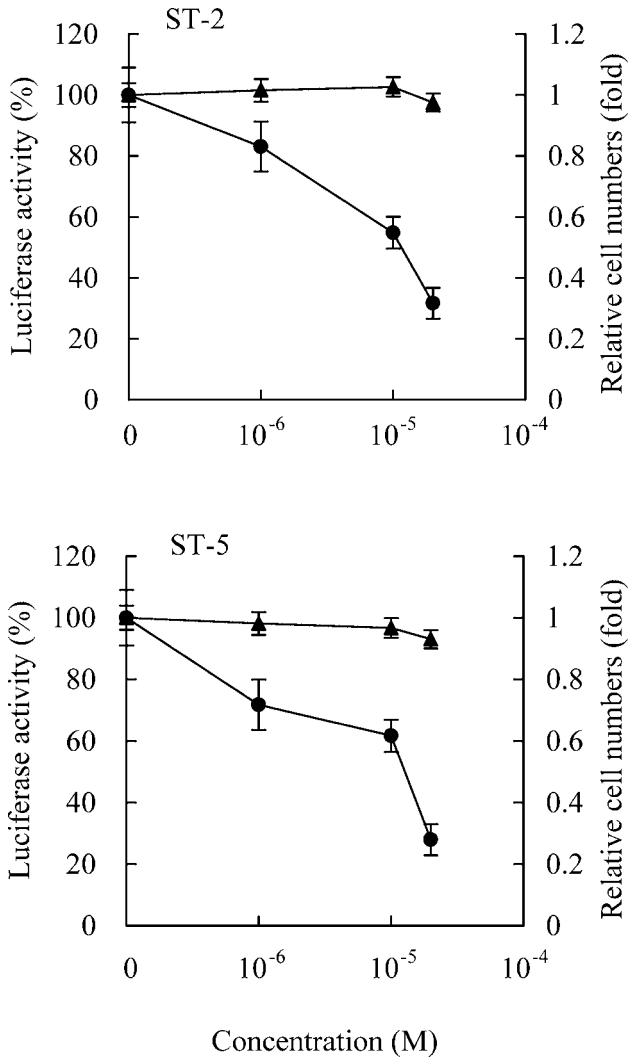

Figure 3. Inhibition of 17β-estradiol-induced luciferase activity by styrene trimers in MVLN cells. MVLN cells were treated with various concentrations of ST-2 or ST-5 in the presence of 3×10^{-10} *M* 17β-estradiol. The luciferase activity (•) in the presence of E_2 alone is shown as 100%, and the relative cell numbers (▲) in the solvent control are shown as 1.0-fold. Each point is the mean ± SD.

We treated MVLN cells with various concentrations of ST-2 or ST-5 in the presence of 3.0×10^{-10} *M* E_2. ST-2 and ST-5 inhibited the luciferase activity induced by E_2 (Fig. 3); the concentrations for 50% inhibition (IC_{50}) of ST-2 and ST-5 were 1.2×10^{-5} and 1.3×10^{-5} *M*, respectively (Table 1).

***In Vivo* Study.** The order of luciferase activity *in vitro* was as follows: ST-4 > ST-1 > ST-3; therefore, we carried out the experiments in this order.

Exposure to ST-4. The AGD in ST-4 treatment groups were shorter than that of the control, and the relative AGD (AGD/CRL) significantly decreased (0, 9.87; 10, 9.32; 100, 8.99; 1000 μg/kg, 9.13%; Table 2). The body weight in the ST-4 treatment groups was heavier than that in the control, and the body weight significantly increased at 1000

Table 2. Effects of Prenatal Exposure to Styrene Trimers on Anogenital Distance in Male Rats[a]

	No. of litters	AGD (mm)	CRL (mm)	AGD/CRL (%)
Dose of ST-4 (μg/kg body wt)				
0	5	4.38 ± 0.25	44.41 ± 1.13	9.87 ± 0.38
10	5	4.06 ± 0.15*	43.54 ± 0.75	9.32 ± 0.37*
100	4	4.04 ± 0.09*	45.02 ± 1.70	8.99 ± 0.23**
1000	5	4.09 ± 0.16	44.82 ± 1.08	9.13 ± 0.33*
Dose of ST-1 (μg/kg body wt)				
0	6	4.54 ± 0.50	43.36 ± 0.96	10.49 ± 1.16
10	6	4.30 ± 0.41	44.63 ± 1.43	9.63 ± 0.66
100	6	4.24 ± 0.27	44.42 ± 1.43	9.54 ± 0.50
1000	6	4.26 ± 0.23	41.45 ± 1.06**	10.29± 0.50
Dose of ST-3 (μg/kg body wt)				
0	8	3.26 ± 0.27	40.98 ± 1.13	7.96 ± 0.52
10	8	3.22 ± 0.22	42.18 ± 1.26**	7.65 ± 0.64
100	8	3.21 ± 0.18	42.82 ± 0.78**	7.51 ± 0.42
1000	8	3.53 ± 0.12*	43.36 ± 1.16**	8.15 ± 0.24

[a] ST-4, 1e-phenyl-4a-(1′-phenylethyl)tetralin; ST-1, 2,4,6-triphenyl-1-hexene; ST-3, 1a-phenyl-4e-(1′-phenylethyl)tetralin; AGD, anogenital distance; CRL, crown rump length. Each value is the mean ± SD. Styrene trimers were injected into pregnant rats from gestational Days 11 through 17. These values were measured on PND 3. Significantly different from control: $*P < 0.05$, $**P < 0.01$.

μg/kg on and after PND 38 (Fig. 4). The organ weights are shown in Table 3. Prostate weight decreased in the ST-4 treatment groups (0, 571; 10, 514; 100, 536; 1000 μg/kg, 508 mg), and the reduction was significant at 10 and 1000 μg/kg. There was no difference in the weights of the epididymides and testes between the ST-4 treatment groups and the control. The relative weight of epididymides, however, significantly decreased at 10 μg/kg, and that of the testes also exhibited a remarkable decrease at 10 and 1000 μg/kg. A significant decrease in the brain weight was observed in ST-4-treated rats (0, 2147; 10, 2085; 100, 2054; 1000 μg/kg, 2090 mg), and the relative weight also reduced in the ST-4 treatment groups. The number of Sertoli cells in the ST-4-treated rats was significantly smaller than that in the control (0, 26.5; 10, 23.8; 100, 21.5; 1000 μg/kg, 21.8 cells/seminiferous tubule; Table 4). No other testicular histological alterations were observed. Serum reproductive hormone levels are shown in Figure 5. FSH levels significantly decreased in all ST-4 treatment groups (0, 58.9; 10, 34.9; 100, 36.9; 1000 μg/kg, 23.7 ng/ml). LH levels also significantly decreased in all ST-4 treatment groups (0, 5.72; 10, 4.28; 100, 4.30; 1000 μg/kg, 2.75 ng/ml). Testosterone levels at 0, 10, 100, and 1000 μg/kg were 5.04, 9.09, 8.30, and 10.15 ng/ml, respectively. The increase in the testosterone level at 1000 μg/kg was significant.

Exposure to ST-1. The relative AGD in the ST-1 treatment groups tended to be shorter than that in the control, but the difference was not significant (Table 2). The body weight of rats treated at 10 and 100 μg/kg was significantly heavier than that of the control on and after PND 62 (Fig. 4). Although there was no difference in the weight of the prostate, epididymides, testes, and brain between the ST-1 treatment groups and the control, the relative weights of the testes and brain significantly

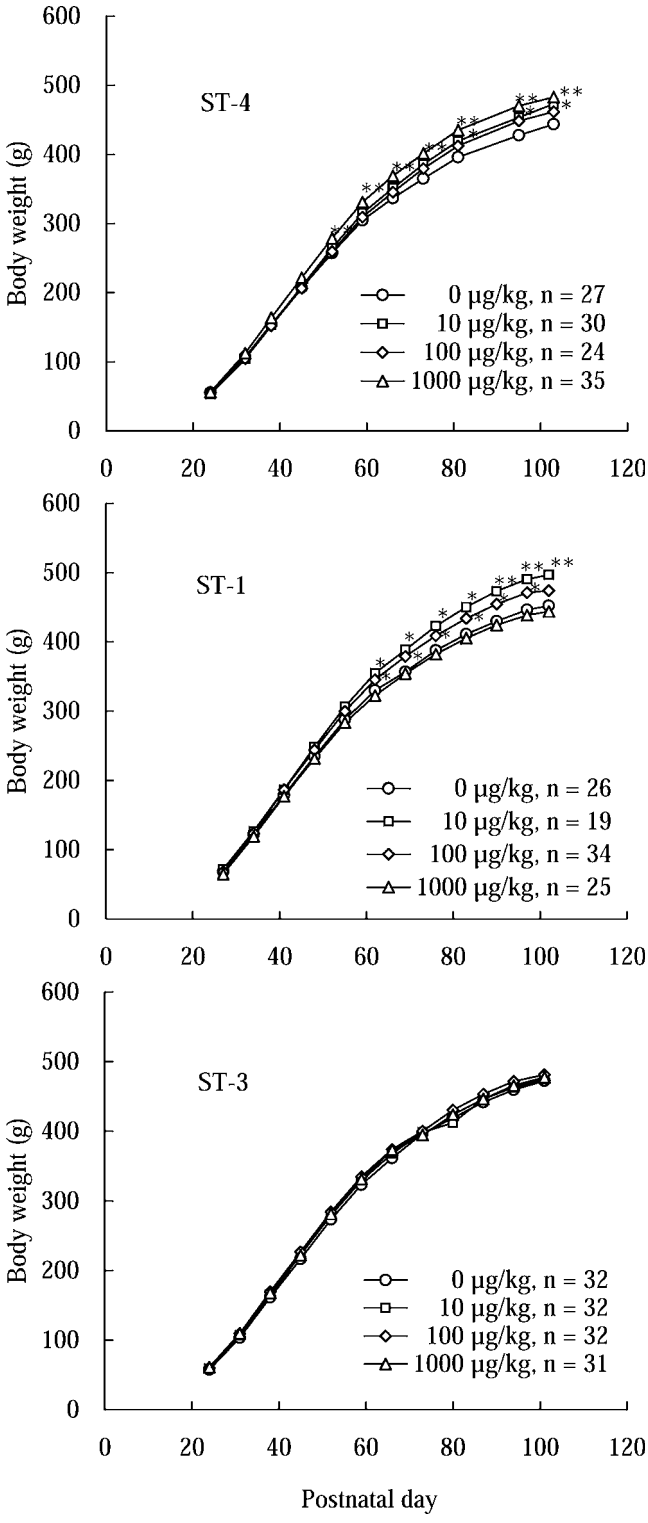

Figure 4. Body weight of male rat offspring prenatally exposed to styrene trimers. Each point is the mean of male offspring. Significantly different from control: $*P < 0.05$, $**P < 0.01$.

Table 3. Effects of Prenatal Exposure to Styrene Trimers on Organ Weight of Male Rats[a]

	No. of animals	Body weight (g)	Organ weight (mg)				Relative organ weight (mg/100g bw)			
			Prostate	Epididymides	Testes	Brain	Prostate	Epididymides	Testes	Brain
Dose of ST-4 (μg/kg body wt)										
0	27	444 ± 49	571 ± 116	1030 ± 83	3135 ± 252	2147 ± 70	129 ± 26	233 ± 18	711 ± 60	489 ± 52
10	30	473 ± 36*	514 ± 101*	1027 ± 77	3049 ± 177	2085 ± 66**	109 ± 24**	218 ± 23**	648 ± 57**	443 ± 35**
100	24	462 ± 55	536 ± 114	1045 ± 61	3158 ± 194	2054 ± 81**	116 ± 21	228 ± 21	690 ± 66	450 ± 52*
1000	35	483 ± 46**	508 ± 125*	1066 ± 62	3158 ± 179	2090 ± 62**	105 ± 25**	223 ± 23	659 ± 69**	436 ± 38**
Dose of ST-1 (μg/kg body wt)										
0	26	452 ± 49	562 ± 115	1034 ± 70	3249 ± 322	2117 ± 64	127 ± 34	231 ± 23	724 ± 84	473 ± 51
10	19	497 ± 59**	589 ± 104	1077 ± 100	3239 ± 219	2136 ± 71	120 ± 23	219 ± 23	658 ± 67**	435 ± 43*
100	34	474 ± 42	619 ± 140	1067 ± 65	3161 ± 211	2111 ± 82	132 ± 35	226 ± 20	670 ± 59**	448 ± 39*
1000	25	444 ± 32	599 ± 112	1040 ± 48	3381 ± 405	2134 ± 66	135 ± 23	235 ± 18	766 ± 105	483 ± 30
Dose of ST-3 (μg/kg body wt)										
0	32	472 ± 48	564 ± 112	1049 ± 86	3151 ± 332	2168 ± 78	121 ± 26	224 ± 26	671 ± 72	463 ± 41
10	32	474 ± 35	571 ± 96	1012 ± 127	2958 ± 470	2147 ± 70	121 ± 22	214 ± 28	626 ± 101*	455 ± 31
100	32	481 ± 40	575 ± 136	1054 ± 127	3168 ± 483	2143 ± 66	119 ± 26	220 ± 27	660 ± 98	448 ± 37
1000	31	477 ± 43	540 ± 87	1062 ± 116	3139 ± 283	2143 ± 63	114 ± 21	223 ± 25	660 ± 58	452 ± 39

[a] ST-4, 1e-phenyl-4a-(1′-phenylethyl)tetralin; ST-1, 2,4,6-triphenyl-1-hexene; ST-3, 1a-phenyl-4e-(1′-phenylethyl)tetralin. Each value is the mean ± SD. Various doses of styrene trimers were subcutaneously injected into pregnant rats from gestational Days 11 through 17. Significantly different from control: $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 4. Number of Sertoli Cells in Rats Prenatally Exposed to Styrene Trimers[a]

	No. of animals	Number of Sertoli cells
Dose of ST-4 (μg/kg body wt)		
0	27	26.5 ± 2.8
10	30	23.8 ± 2.0**
100	24	21.5 ± 2.8**
1000	35	21.8 ± 2.7**
Dose of ST-1 (μg/kg body wt)		
0	26	25.6 ± 2.2
10	19	25.2 ± 2.1
100	34	25.5 ± 1.9
1000	25	25.1 ± 2.0
Dose of ST-3 (μg/kg body wt)		
0	32	26.0 ± 2.4
10	32	24.8 ± 2.1*
100	32	24.3 ± 1.7**
1000	31	23.9 ± 2.1**

[a] ST-4, 1e-phenyl-4a-(1′-phenylethyl)tetralin; ST-1, 2,4,6-triphenyl-1-hexene; ST-3, 1a-phenyl-4e-(1′-phenylethyl)tetralin. Sertoli cells were counted in cross sections of the seminiferous tubules. Each value is the mean ± SD. Various doses of styrene trimers were subcutaneously injected into pregnant rats from gestational Days 11 through 17. Significantly different from control: $^{*}P < 0.05$, $^{**}P < 0.01$.

decreased at 10 and 100 μg/kg (Table 3). There was no significant difference between the number of Sertoli cells in the ST-1 treatment groups and that in the control (Table 4), and no other testicular histological alterations were observed either. Serum reproductive hormone levels are shown in Figure 6. FSH levels at 0, 10, 100, and 1000 μg/kg were 45, 42, 24, and 27 ng/ml, respectively, and those at 100 and 1000 μg/kg significantly decreased. LH levels were significantly lower in all ST-1 treatment groups (0, 5.65; 10, 4.46; 100, 3.56; 1000 μg/kg, 3.51 ng/ml). Testosterone levels significantly increased in all ST-1 treatment groups (0, 6.5; 10, 14.4; 100, 14.9; 1000 μg/kg, 16.9 ng/ml).

Exposure to ST-3. There was no difference in the relative AGD between the ST-3 treatment groups and the control (Table 2). No difference was observed between body weight in the ST-3 treatment groups and that in the control (Fig. 4). There was no difference in the weight of the prostate, epididymides, testes, and brain between the ST-3 treatment groups and the control. However, the relative weight of the testes significantly decreased at 10 μg/kg (Table 3). The number of Sertoli cells in the ST-3-treated rats was significantly smaller than that in the control (0, 26.0; 10, 24.8; 100, 24.3; 1000 μg/kg, 23.9 cells/seminiferous tubule; Table 4). No other testicular histological alterations were observed. The FSH level reduced at 100 μg/kg, but no changes were observed in the levels of LH and testosterone in the ST-3 treatment groups (Fig. 7).

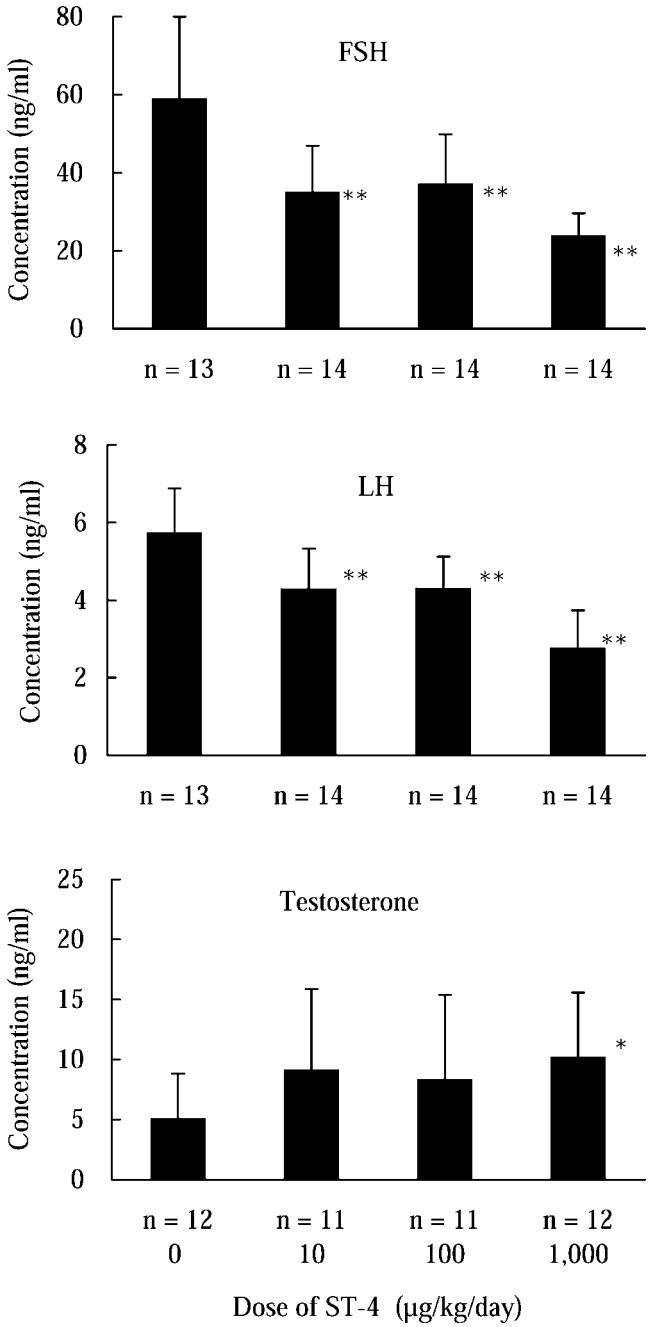

Figure 5. Reproductive hormone levels in the serum of male offspring prenatally exposed to ST-4 on PND 100. Each bar is the mean ± SD. Significantly different from control: $^{*}P < 0.05$, $^{**}P < 0.01$.

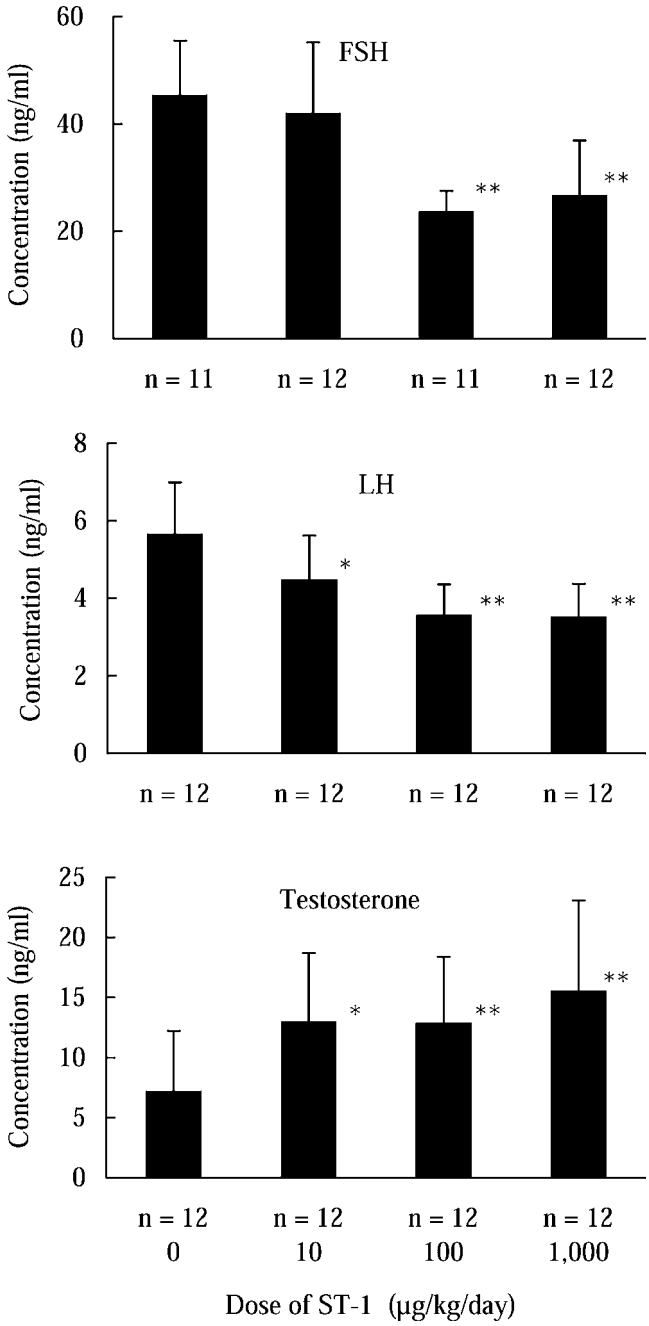

Figure 6. Reproductive hormone levels in the serum of male offspring prenatally exposed to ST-1 on PND 100. Each bar is the mean ± SD. Significant different from control: $^{*}P < 0.05$, $^{**}P < 0.01$.

Discussion

The reporter-gene assay in this study clarified that ST-1, ST-3, and ST-4 were agonists for estrogen, but ST-2 and ST-5 were antagonists. Estrogenic activities of styrene trimers with tetralin structures differed depending on their chemical structures. It is considered that estrogenic activity of ST-4 was the highest among styrene trimers tested because ST-4 had the highest luciferase activity. The values of EC_{50} show that ST-4 and ST-1 had nearly the same estrogenic potency as bisphenol A.

ST-4 affected the AGD, and the relative AGD was significantly reduced at all doses tested. ST-1 or ST-3 did not distinctly reduce the relative AGD. AGD is decreased by estrogenic compounds such as estradiol benzoate, diethylstilbestrol (DES) (8), and phthalate esters (9, 10). It seems that the decrease of the relative AGD was attributed to the estrogenicity of ST-4.

Prenatal exposure to ST-1 or ST-4 increased the body weight of rats. Prenatal exposure to estrogenic compounds such as octylphenol and bisphenol A was reported to

increase the body weight of male rats (11, 12). ST-3 did not induce an increase in body weight. The estrogenicity of ST-3 may have been low in terms of increasing the body weight.

There was a decrease in the relative organ weights of styrene trimer–treated rats. The relative weight of the prostate and epididymides decreased in the ST-4 treatment groups. The relative testes weight reduced at 10 μg/kg in all styrene trimer treatment groups. Estrogenic compounds such as benzyl butyl phthalate, diethylhexyl phthalate (9), DES (11), and E_2 (13) decreased the weight of the prostate and testes. Moreover, these phthalate esters (9), nonylphenol, and DES (14) reduced the weight of epididymides. The decrease of these organ weights was considered to be induced by the estrogenic activity of the styrene trimers. It seems that the significant decrease in the relative testes weight in the 1000 μg/kg ST-4 treatment group was due to the increased body weight. The relative brain weight decreased in ST-1- and ST-4-treated rats. It is possible that rats showing a decrease in the relative weight of the brain received some lesions and impairment in the development and faculty of the brain.

The Sertoli cell is elaborately equipped to support spermatogenesis, and the adult Sertoli cell population is thought to remain relatively stable throughout the life span of the animal (15). It has also been indicated that the spermatid number depends on the number of Sertoli cells (16). The number of Sertoli cells was significantly reduced in the ST-3 and ST-4 treatment groups. It is considered that the decrease in the Sertoli cell count was induced in the fetus, and there is a possibility that spermatogenesis was adversely affected. The estrogenic chemical DES decreased the Sertoli cell number in perinatal rats (17, 18). However, ST-1, having a more potent estrogen-like activity than ST-3, did not induce a decrease in Sertoli cells. FSH is a crucial factor to control Sertoli cell proliferation in the testes of fetal rats (19). Although a reduction in the FSH level was observed in ST-1 treatment groups, ST-1-treated offspring may have undergone this FSH decrease at the stage of Sertoli cell proliferation.

The styrene trimers disturbed the secretion of serum reproductive hormones. Reduced FSH levels were observed in all styrene trimer treatment groups. It is supposed that styrene trimers induced a disturbance in the hypothalamic-pituitary axis function and FSH secretion was insufficient from the fetal phase. The insufficient FSH secretion induced by ST-3 or ST-4 in the fetus may have resulted in the decreased Sertoli cell count. Moreover, it is supposed that the region of the hypothalamic-pituitary axis inadequately developed and the brain's weight decreased. The decreased LH levels in the ST-1 and ST-4 treatment groups seemed to be due to negative feedback due to the increasing testosterone level.

The effects of prenatal exposure to the styrene trimers are summarized in Table 5, and the intensity of the effects is represented. ST-4 most significantly affected the relative

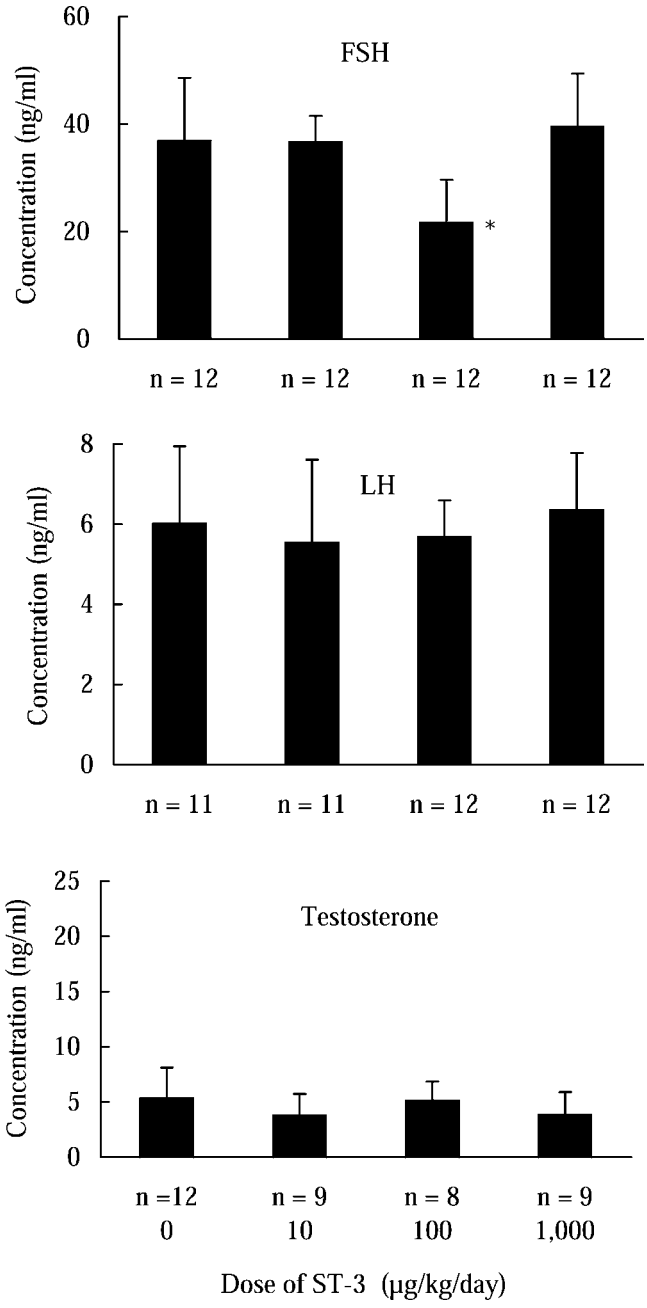

Figure 7. Reproductive hormone levels in the serum of male offspring prenatally exposed to ST-3 on PND 100. Each bar is the mean ± SD. $^*P < 0.01$.

AGD, the weight of the reproductive organs and brain, and the secretion of serum reproductive hormones among the styrene trimers tested. It seems that the estrogenic activity estimated in the reporter-gene assay was reflected in the potency of effects of prenatal exposures except for the decrease in the Sertoli cell count in rats.

When Nagao *et al.* (20) prenatally exposed male rats to polystyrene extract, there were no changes in body and brain weights or AGD. The doses of styrene trimer extract may have been too small to induce any changes.

Table 5. Effects of Prenatal Exposures to Styrene Trimers on Male Rat Offspring[a]

Effects	Compounds		
	ST-4	ST-1	ST-3
Decrease of relative AGD	++	−	−
Increase of body weight	+	+	−
Decrease of relative prostate weight	+	−	−
Decrease of relative epididymides weight	+	−	−
Decrease of relative testes weight	+	+	+
Decrease of relative brain weight	++	+	−
Decrease of Sertoli cell count	++	−	++
Decrease of FSH level	++	+	+
Decrease of LH level	++	++	−
Increase of testosterone level	+	++	−

[a] ST-4, 1e-phenyl-4a-(1′-phenylethyl)tetralin; ST-1, 2,4,6-triphenyl-1-hexene; ST-3, 1a-phenyl-4e-(1′-phenylethyl)tetralin; −, no significant change in the styrene trimer treatment groups; +, a significant change in 1 or 2 treatment groups; ++, a significant change in all treatment groups.

The results of this study suggest that prenatal exposure to estrogenic styrene trimers at low levels obstructs genital organ development and disrupts the endocrine systems of male rat offspring. Styrene trimers migrating from polystyrene containers might affect genital development and the central nervous system of embryos.

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