Effect of Compound Exposure to Bisphenol A and Nonylphenol on the Development and Fertility of Fetal Mice

Takashi Kimura[1], Naoko Kimura[1] and Kiyoshi Totsukawa[1]*

[1]Laboratory of Animal Reproduction, Graduate School of Agricultural Science, Yamagata University, 1-23 Wakaba-machi, Tsuruoka 997-8555, Japan

Abstract: *Bisphenol A (BPA) and nonylphenol (NP) are endocrine disrupting chemicals (EDCs) which induce reproductive abnormalities. While single exposure studies have been reported, few investigations of the simultaneous administration of several chemicals to animals have been conducted. The purpose of this study was to estimate the effect of gestational exposure to BPA and NP simultaneously on reproductive function. Pregnant female ICR mice were given BPA, NP or BPA plus NP by subcutaneous injection from gestational day (GD) 5 to delivery. The daily doses of BPA and NP were 1/1,000 or 1/100 the median lethal doses (LD_{50}; BPA: 2,500 mg/kg, NP: 1,231mg/kg). On postnatal day (PND) 1, the pups (F1) were thinned out to 8. On PND 42, the body weights of some F1 were recorded and they were sacrificed; the livers, testes, epididymes, ovaries, and uteri were then weighed. The remaining F1 mice were mated with non-treated heterosexual mice. On GD 17, female mice were dissected to count the total number of fetuses and dead fetuses. All NP-treated mice exhibited decreased body and testis weight on PND 42. The pregnancy rate was 100% in all treated female groups, although it declined in untreated female mice mated with male mice from some treatment groups. The average dead fetus rate changed significantly according to the dosage combination. This study shows that BPA and NP can enhance, suppress or be neutral for the effect of the other in combined exposure to BPA and NP.*

Key words: *Bisphenol A, Nonylphenol, Compound exposure, Fetal exposure*

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*To whom correspondence should be addressed.
e-mail: naonao@tds1.tr.yamagata-u.ac.jp

Introduction

Animal reproductive function is regulated by the endocrine system. Endocrine disrupting chemicals (EDCs) such as xenoestrogens are released into the environment and can interfere with the endocrine system and exert various effects including reproductive function disorder in vertebrates.

Bisphenol A (BPA) is a high-volume production, industrial chemical used as a monomer in the manufacture of polycarbonate and epoxy resin products including formula bottles, food-can linings and dental sealants. Trace amounts of BPA have been reported to leach from these consumer products [1, 2].

Previous reports suggest that BPA has weak estrogenic activity in yeast-based receptor assays [3]. In uterotrophic assays, BPA also exhibits weak estrogen-like activity in mature ovariectomized [4] and immature [5,6] mice. Furthermore, BPA alters the postnatal growth rate of female mice after *in utero* exposure and induces early puberty [7], and fetal exposure to BPA altered the expression of the estrogen receptor after birth [8].

Nonylphenol polyethoxylates (NPEs) are non-ionic surfactants used in detergents, plastic additives, paints, herbicides, insecticides and many other synthetic products. Nonylphenol (NP), a major biodegradation product of NPEs [9], is found in water and river deposits. NP is also reported to leach from plastics used in food processing [10].

NP mimics a weak estrogen-like action, and it was reported that NP stimulated the proliferation of MCF-7 cells (from a human estrogen-sensitive breast cancer cell line) [11]. *In vivo* studies have shown that NP accelerated vaginal opening and increased uterine weight in female rats [12], and reduced sperm

production and decreased testis weight in male rats [13].

The purpose of this study was to estimate the effect on the reproductive system of the offspring of ICR mice exposed to BPA and NP during pregnancy. While single exposure studies have been reported, few investigations of the simultaneous administration of several chemicals to mammals have been conducted. BPA and NP have been detected in river water [14], which shows that humans may be exposed to them through the food chain. Furthermore, these chemicals have been detected in human placenta and/or cord blood [15], further proof that humans really are exposed to various chemical substances. Therefore, it is significant to study the effects of compound exposures.

Materials and Methods

Animals and treatment

ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan) and bred in our laboratory. Mice were housed in polycarbonate cages with autoclaved chips as bedding and placed in an animal room with a 12h light / dark cycle (light: 06:00–18:00) under controlled temperature (24±2°C). Food (F1; Funabashi, Chiba, Japan) and tap water were provided *ad libitum.* Eight-week-old female mice were mated with male mice. Vaginal plug detection was considered day 0 of pregnancy. The pregnant female mice were separated from the males and divided into 9 treatment groups (*n*=6): (i) control; (ii) low-dose BPA (2.500 mg/kg; LB); (iii) high-dose BPA (25.00 mg/kg; HB); (iv) low-dose NP (1.231 mg/kg; LN); (v) high-dose NP (12.31 mg/kg; HN); (vi) mixture of LB and LN (LBLN); (vii) mixture of HB and HN (HBHN); (viii) mixture of LB and HN (LBHN); (ix) mixture of HB and LN (HBLN). The BPA and NP doses were set at 1/1,000 or 1/100 of the LD_{50} (BPA: 2,500 mg/kg, NP: 1,231 mg/kg). We considered LD_{50}/1,000 and LD_{50}/100 to be low-dose and high-dose, respectively. Gestational mice were housed individually. Control animals received only corn oil (Ajinomoto Co., Inc., Tokyo, Japan). Corn oil was also used as the vehicle for BPA and NP. The dams were treated daily by subcutaneous injection from gestational day (GD) 5 to delivery (postnatal day (PND) 0). The amount of administration was calculated based on the body weight of the animal on the day of treatment. BPA and/or NP was dissolved in 0.05 ml corn oil and injected into the dorsal region of the neck. On PND 1, pups (F1) were thinned out to 8 per dam. The F1 mice were weaned on PDN 21 and housed with same-sex littermates.

Litter data

On PND 42, 6 F1 males and females per group were selected at random and mated with non-treated heterosexual mice (male: female = 1: 3). On GD 17, the female mice were dissected and their uteri observed. The total number of fetuses and dead fetuses (F2) were counted. The body weights of the remaining F1 were recorded, then they were sacrificed and their livers, testes, epididymes, ovaries, and uteri were weighed.

Statistical analysis

The data are expressed as the mean ± SD. The pregnancy rate was analyzed used the chi-squared test. All other data were analyzed by one-way ANOVA and Fisher’s PLSD. In analyses of the mean number of dead fetuses and the average dead fetus rate, the HN group (0.00) was excluded. A P value of less than 0.05 was considered statistically significant.

Results

F1 body weight and organ weight

The F1 male and female body and organ weights are shown in Tables 1 and 2, respectively.

There were significant differences in body weight on PND 42 among the groups. The body weight of male (Table 1) and female (Table 2) F1, treated *in utero*, decreased in all NP-treated groups (LN, HN, LBLN, HBLN, LBHN and HBHN) compared to the control.

The absolute liver weights of the LB, LN, HN, LBHN and HBLN groups in males decreased, although the relative liver weights of all groups showed no significant difference compared to the control (Table 1). The absolute liver weight of LBLN was increased and HBLN was decreased compared with LN and HB respectively. The relative liver weights of HBHN, LBHN and HBLN were increased compared to HN, LB and LN. In females, LN and HBLN absolute liver weights were decreased compared to the control (Table 2). The relative liver weight of the HBHN group, however, was significantly increased in females. The absolute liver weight of HBLN in females was decreased compared to HB. The relative liver weight of HBHN was increased compared to HB.

The absolute testis weight was decreased in all NP-treated groups compared to the control, although there was no significant difference among groups in the relative testis weights (Table 1). The absolute testis weights of the compound exposure groups (LBLN, HBHN, LBHN and HBLN) were significantly decreased compared to the BPA single exposure groups (LB and

Table 1. F_1 Male body weight, liver, testis and epididymis weight on PND 42

Treatment Group			*n*	Body weight (g)	Liver		Testis		Epididymis	
	BPA (mg/kg/day)	NP (mg/kg/day)			Absolute (g)	Relative (%)	Absolute (mg)	Relative (%)	Absolute (mg)	Relative (%)
Control	0	0	18	35.0 ± 2.1^{a}	1.513 ± 0.20^{a}	4.323 ± 0.47$^{a, b, c}$	227.9 ± 33.2^{a}	0.652 ± 0.090	61.7 ± 9.07$^{a, b}$	0.177 ± 0.024^{a}
LB	2.500	0	21	34.3 ± 1.4^{a}	1.406 ± 0.17$^{b, c, d}$	4.104 ± 0.50^{a}	223.8 ± 27.4$^{a, b}$	0.652 ± 0.073	60.1 ± 5.10$^{a, b, c}$	0.175 ± 0.014^{a}
HB	25.00	0	18	35.2 ± 1.7^{a}	1.486 ± 0.14$^{a, b}$	4.227 ± 0.34$^{a, b, c}$	223.1 ± 23.7$^{a, b}$	0.635 ± 0.065	61.1 ± 5.76$^{a, b, c}$	0.174 ± 0.014^{a}
LN	0	1.231	14	31.7 ± 1.4$^{b, c, d}$	1.303 ± 0.16^{c}	4.106 ± 0.44^{a}	198.9 ± 23.0^{c}	0.628 ± 0.074	57.3 ± 4.00$^{c, d}$	0.181 ± 0.011$^{a, b}$
HN	0	12.31	15	32.0 ± 2.5$^{b, c}$	1.341 ± 0.19$^{c, d}$	4.184 ± 0.38$^{a, b}$	207.0 ± 34.8$^{b, c}$	0.646 ± 0.089	63.0 ± 7.21^{a}	0.197 ± 0.016^{c}
LBLN	2.500	1.231	18	32.9 ± 1.7^{b}	1.417 ± 0.09$^{a, b, d}$	4.310 ± 0.15$^{a, b, c}$	201.5 ± 14.4^{c}	0.615 ± 0.057	59.8 ± 4.42$^{a, b, c, d}$	0.183 ± 0.018$^{a, b}$
HBHN	25.00	12.31	16	32.4 ± 2.3$^{b, c}$	1.443 ± 0.16$^{a, b, d}$	4.450 ± 0.30^{c}	197.2 ± 24.2^{c}	0.608 ± 0.062	57.7 ± 5.96$^{b, c, d}$	0.178 ± 0.016$^{a, b}$
LBHN	2.500	12.31	19	31.4 ± 2.0$^{c, d}$	1.375 ± 0.10$^{c, d}$	4.386 ± 0.16$^{b, c}$	193.9 ± 20.4^{c}	0.620 ± 0.071	56.0 ± 4.72^{d}	0.179 ± 0.013$^{a, b}$
HBLN	25.00	1.231	20	30.6 ± 2.1^{d}	1.342 ± 0.14$^{c, d}$	4.384 ± 0.29$^{b, c}$	197.7 ± 21.6^{c}	0.650 ± 0.088	58.0 ± 6.56$^{b, c, d}$	0.190 ± 0.023$^{b, c}$

Mean ± SD. $^{a–d}$Different superscripts indicate statistically significant differences (P<0.05). There was no significant difference in the relative testis weights among groups.

Table 2. F_1 Female body weight, liver, ovary and uterine weight on PND 42

Treatment Group			*n*	Body weight (g)	Liver		Ovary		Uterus	
	BPA (mg/kg/day)	NP (mg/kg/day)			Absolute (g)	Relative (%)	Absolute (mg)	Relative (%)	Absolute (mg)	Relative (%)
Control	0	0	18	27.6 ± 1.7$^{a, b}$	1.095 ± 0.14$^{a, b}$	3.967 ± 0.42$^{a, b}$	10.4 ± 2.3$^{a, b}$	0.038 ± 0.008	135.4 ± 47.9^{a}	0.494 ± 0.180$^{a, b, c}$
LB	2.500	0	15	26.3 ± 2.2$^{a, c}$	1.037 ± 0.16$^{a, b, c}$	3.925 ± 0.41$^{a, b}$	9.6 ± 2.6$^{a, b}$	0.036 ± 0.009	95.3 ± 23.7^{b}	0.364 ± 0.089^{a}
HB	25.00	0	18	28.5 ± 2.2^{b}	1.117 ± 0.21$^{a, b}$	3.904 ± 0.63$^{a, b}$	10.6 ± 2.2^{a}	0.037 ± 0.008	134.3 ± 55.0^{a}	0.470 ± 0.185$^{a, b, c}$
LN	0	1.231	21	25.2 ± 1.7$^{c, d}$	0.971 ± 0.10^{c}	3.859 ± 0.25^{a}	9.2 ± 2.0$^{a, b}$	0.037 ± 0.007	125.0 ± 52.7$^{a, b}$	0.490 ± 0.186$^{a, b, c}$
HN	0	12.31	20	25.2 ± 2.2$^{c, d}$	1.052 ± 0.20$^{a, b, c}$	4.165 ± 0.54$^{b, c}$	9.1 ± 2.7^{b}	0.036 ± 0.011	109.1 ± 43.4$^{a, b}$	0.434 ± 0.156$^{a, b}$
LBLN	2.500	1.231	18	25.5 ± 2.3^{c}	1.024 ± 0.13$^{a, c}$	4.012 ± 0.22$^{a, b}$	9.8 ± 1.5$^{a, b}$	0.039 ± 0.004	127.6 ± 60.0$^{a, b}$	0.497 ± 0.218$^{b, c}$
HBHN	25.00	12.31	18	26.1 ± 2.1^{c}	1.133 ± 0.12^{b}	4.332 ± 0.24^{c}	9.9 ± 1.8$^{a, b}$	0.038 ± 0.007	97.8 ± 29.8^{b}	0.377 ± 0.119$^{a, b}$
LBHN	2.500	12.31	17	25.1 ± 2.5$^{c, d}$	1.029 ± 0.17$^{a, b, c}$	4.090 ± 0.41$^{a, b, c}$	9.9 ± 1.8$^{a, b}$	0.040 ± 0.006	110.0 ± 52.9$^{a, b}$	0.433 ± 0.180$^{a, b}$
HBLN	25.00	1.231	16	23.9 ± 1.8^{d}	0.984 ± 0.10^{c}	4.115 ± 0.18$^{a, b, c}$	9.9 ± 1.7$^{a, b}$	0.042 ± 0.007	140.6 ± 65.2^{a}	0.599 ± 0.302^{c}

Mean ± SD. $^{a–d}$Different superscripts indicate statistically significant differences (P<0.05). There was no significant difference in the relative ovary weights among groups.

HB). While the absolute epididymis weights were decreased in the LN and LBHN groups, its relative weight in the HN and HBLN groups was significantly increased compared to the control (Table 1). The absolute and relative epididymis weights in the HBHN and LBHN groups were decreased compared to HN.

There was no significant difference in the relative ovary weights among the groups and in the absolute ovary weights of all treatment groups compared to the control (Table 2). The absolute uterine weights of the LB and HBHN groups were significantly decreased, whereas the relative uterine weight showed no significant difference compared to the control (Table 2). The absolute uterus weight of HBHN was decreased compared to HB. In comparison with LB, the relative uterus weight of LBLN was increased.

F2 fetus survivability

On PDN 42, 6 F1 males and females per group were mated with non-treated heterosexual mice. On GD 17, female mice were dissected and their uteri observed to count the total number of fetuses and dead fetuses. Table 3 shows the results of untreated females mated with treated males. Table 4 shows the results of treated females mated with untreated males.

The pregnancy rate was 100% in all treated female groups (Table 4). Although there was no significant difference, the pregnancy rates of untreated females tended to decline in mating with male mice from the HN, LBLN and HBHN groups (Table 3). The number of fetus per untreated dam decreased significantly after mating with male mice from the LN, HN, LBLN and HBHN groups compared with the control (Table 3). Also, the number of fetus in untreated females mated with male mice from the HBHN decreased compared to HB, and in untreated females mated with the male mice from the HBLN increased compared to LN. There was no significant difference in the number of fetuses per dam between any of the treated female groups and the control, while the LBLN group showed a decrease compared to LB and HBHN group showed an increase compared to HN (Table 4). The average dead fetus rate

Table 3. F_2 Fetus data (male treated), pregnancy rate, number of fetuses per dam, no. of dead fetuses and average dead fetus rate

Treatment groups	BPA (mg/kg/day)	NP (mg/kg/day)	*n*	Pregnancy rate (%)	No. of fetus/dam	No. of dead fetus/dam	Average dead fetus rate (%)
Control	0	0	18	100.0 (18/18)	15.6 ± 1.34[a, b]	0.50 ± 0.69[a]	3.08 ± 4.11[a]
LB	2.500	0	18	100.0 (18/18)	14.8 ± 1.90[a, b, c]	0.39 ± 0.59[a]	2.61 ± 3.99[a]
HB	25.00	0	18	100.0 (18/18)	16.5 ± 1.74[a]	1.11 ± 0.99[a, b, c]	6.51 ± 5.64[a, b]
LN	0	1.231	18	100.0 (18/18)	13.3 ± 3.21[c]	1.56 ± 2.17[b, c]	11.11 ± 14.72[b]
HN	0	12.31	18	83.3 (15/18)	12.9 ± 3.45[c]	0.60 ± 0.80[a, b]	4.00 ± 5.28[a]
LBLN	2.500	1.231	18	88.9 (16/18)	13.6 ± 2.74[c]	1.69 ± 1.99[c]	11.41 ± 13.05[b]
HBHN	25.00	12.31	18	88.9 (16/18)	13.1 ± 3.15[c]	1.50 ± 1.41[b, c]	10.92 ± 10.27[b]
LBHN	2.500	12.31	18	100.0 (18/18)	14.2 ± 2.74[b, c]	0.61 ± 0.83[a, b]	4.59 ± 6.08[a]
HBLN	25.00	1.231	18	100.0 (18/18)	15.6 ± 3.11[a, b]	1.22 ± 1.90[a, b, c]	6.84 ± 10.46[a, b]

[a–c]Different superscripts indicate statistically significant differences (P<0.05). There was no significant difference in the pregnancy rates among groups.

Table 4. F_2 Fetus data (female treated), pregnancy rate, number of fetuses per dam, no. of dead fetuses and average dead fetus rate

Treatment groups	BPA (mg/kg/day)	NP (mg/kg/day)	*n*	Pregnancy rate (%)	No. of fetus/dam	No. of dead fetus/dam	Average dead fetus rate (%)
Control	0	0	6	100.0 (6/6)	14.8 ± 2.03[a, b, c, d]	0.50 ± 0.50[a, b]	3.37 ± 3.69
LB	2.500	0	6	100.0 (6/6)	16.7 ± 0.94[a]	1.50 ± 1.50[a]	9.00 ± 8.26
HB	25.00	0	6	100.0 (6/6)	15.5 ± 1.80[a, b, c]	0.83 ± 0.69[a, b]	5.38 ± 4.62
LN	0	1.231	6	100.0 (6/6)	14.3 ± 1.60[b, c, d]	0.67 ± 0.75[a, b]	4.65 ± 4.96
HN	0	12.31	6	100.0 (6/6)	13.3 ± 1.49[b, d]	0.00 ± 0.00*	0.00 ± 0.00*
LBLN	2.500	1.231	6	100.0 (6/6)	13.0 ± 2.89[d]	0.67 ± 0.75[a, b]	5.13 ± 5.41
HBHN	25.00	12.31	6	100.0 (6/6)	15.8 ± 1.34[a, c]	0.17 ± 0.37[b]	1.05 ± 2.48
LBHN	2.500	12.31	6	100.0 (6/6)	14.5 ± 1.26[a, b, c, d]	0.67 ± 1.11[a, b]	4.60 ± 8.45
HBLN	25.00	1.231	6	100.0 (6/6)	15.5 ± 1.50[a, b, c]	1.00 ± 1.15[a, b]	6.45 ± 7.50

[a–d]Different superscripts indicate statistically significant differences (P<0.05). *HN was excluded from statistical analysis. There was no significant difference in the pregnancy rates among groups. There was no significant difference in the average dead fetus rates among groups.

in untreated females mated with males from LN, LBLN and HBHN group mice was significantly increased, while in untreated females mated with males from the HB and HBLN tended to increase (Table 3). The average dead fetus rate in untreated females mated with males from the LBLN or HBHN was significantly increased compared to LB or HN, respectively. There was no significant difference in the average dead fetus rate between any of the treated female groups (Table 4).

Discussion

We investigated the effects of BPA, NP, and a mixture of BPA and NP exposure *in utero*. In this study, the body weight of the NP-treated group decreased significantly. Cunny *et al.* [16] reported that 2,000 ppm NP dietary administration for 90 days (approximate dietary intake of 150 mg/kg/day) induced a small decrease in body weight gain in rats. In addition, Tyl *et al.* [17] showed that 750 and 7,500 ppm BPA dietary exposure (approximate dietary intake of 50, 500 mg/kg/day) decreased the liver weight in CD Sprague-Dawley rats. Benjamin *et al.* [18] reported that 100 mg/kg BPA plus 100 mg /kg NP oral administration did not induce change, but 100 mg/kg BPA administration decreased liver weight significantly compared with control rats. On the other hand, the administration of 500, 750, 1,000, or 1,250 mg/kg/day BPA by gastric intubation in mice increased the relative liver weight [19]. Increases in relative liver weight with histopathological changes were observed in a study carried out by Nagao *et al.* [20], who administered 50 mg/kg/day NP. In the present study, the absolute liver weights of male LB, LN, HN, LBHN and HBLN group mice and female LN and HBLN

group mice were significantly decreased, while the relative liver weight of HBHN females was significantly increased compared with the control. This suggests that the treatment effect on the liver varied according to the administration dose or interaction with other chemicals.

Kabuto *et al.* [21] reported that feeding pregnant/ lactating mothers BPA at 5 or 10 μg/ml of drinking water reduced the testis weights of their pups. Moreover, exposure to 100, 250 and 400 mg/kg/day NP reduced the testis and epididymis weights [22]. In our results, the absolute testis weight of all NP-treated groups decreased, although that of groups treated with BPA alone did not decrease compared to the control. The relative epididymis weights of all treatment groups were not significantly different from the control. The absolute epididymis weights were decreased in the LN and LBHN groups compared with the control. The relative epididymis weights of the HN and HBLN groups, however, were significantly increased when compared with the control group. The results of this study contradict those of previous studies, and the reason for this may be the different route of administration.

The results of fetus survivability indicate that males were affected by treatment more than females. For instance, in the average dead fetus rate, there was a significant difference among untreated females mated with males from the treated groups, although treated females showed no significant differences. This may be explained by differences in hormone sensitivity. In the present study, the pregnancy rate of untreated females mated with males from the HN, LBLN and HBHN groups tended to decrease. Additionally, the average dead fetus rate was increased in untreated females mated with males from the LN, LBLN and HBHN groups by more than 10%. Kabuto *et al.* [21] reported that BPA administration increased dose-dependently the thiobarbituric acid-reactive substances (TBARS) levels in testis and showed that exposure to 10 μg/ml BPA in drinking water significantly increased TBARS, although 5 μg/ml exposure did not lead to a significant increase. They concluded that 10 μg/ml BPA administration was too severe to up-regulate catalase activity in the testis, and a decrease in its activity caused an increase in the TBARS level. In the present study, the dead fetus rate in untreated females mated with HB group males, but not LB, tended to increase probably because testis cells were damaged by hyperoxidation. NP also induces hyperoxidation. Chitra's study [23] showed that lipid peroxidation increased significantly in the epididymal sperm of male rats. Han *et al.* [24] observed that NP treatment induced the apoptosis of testis cells. Testis dysfunction and/or reduced motility of sperm, caused by hyperoxidation, might have induced a high fetal death rate, such as those shown by untreated females mated with LN, LBLN and HBHN group mice in the present study.

In the study of EDCs, there are many cases in which low-dose exposure shows a greater effect than high-dose exposure. This differs from general toxicity which acts dose dependently. EDCs are well-known to an effect of inverted-U shape. This phenomenon might explain the increase in the average dead fetus rate seen in untreated females mated with male LN mice compared to male HN mice.

The effect of postnatal exposure to estrogen on the development of male reproductive organs is to decrease spermatogonia and spermatocytes causing morphological abnormality of seminiferous tubules without tubular lumen [25]. The estrogen-like action of BPA and/or NP might be reason for the decreases in absolute testis or epididymis weights and mean number of fetuses in untreated females mated with treated males and their increase in dead fetus rate.

The absolute testis weights of compound exposure groups (LBLN, HBHN, LBHN and HBLN) were significantly decreased in comparison with BPA single exposure groups (LB and HB), though compound exposure groups showed no significant differences from LN and HN. These results indicate that the effect of BPA is neutral, or that BPA is neither an enhancer nor a suppressor. On the other hand, the decline in the absolute liver weight of male HBLN mice compared to HB indicates that high-dose BPA suppresses the effect of low-dose NP. The absolute uterus weight of female HBHN mice was decreased compared to HB. This suggests that high-dose BPA enhanced the effect of HN tending to decrease the uterus weight compared to the control. The absolute epididymis weights in HBHN and LBHN mice were significantly decreased compared to HN, and tended to be decreased compared to LB and HB. This indicates that a mixture of BPA and NP can induce effects which do not show in individual exposures. The average dead fetus rate of untreated female mice mated with treated male HBHN (10.92%) mice was approximately equal to the sum of that attributable to male HB (6.51%) and HN (4.00%) mice, whereas the average dead fetus rate attributable to male HBLN (6.84%) mice was lower than that observed following independent exposure to NP (LN; 11.11%). The average dead fetus rates attributable to male LBLN (11.41%) and LBHN (4.59%) mice were similar those

attributable to male LN (11.11%) and HN (4.00%) mice, respectively. These suggest that BPA and NP act as enhancers, suppressers or are neutral when the combination quantity changes.

The effects of various EDCs have been investigated. While studies of single chemical exposure have been conducted, few investigations, such as the administration of several chemicals to animals, have been conducted. We investigated the effects of BPA, NP, and a mixture of BPA and NP on mice. This study shows that BPA and NP can enhance, suppress or be neutral for the effect of the other, and the effect following compound administration can be a summation of individual exposure effects of BPA and NP. Our study indicates that the effect of EDCs significantly varies with their interaction with other compounds, including other EDCs. If future studies elucidate the mechanism of individual EDCs, it may become possible to predict the effects of compound exposures.

References

1) Brotons, J.A., Olea-Serrano, M.F., Villalobos, M., Pedraza, V. and Olea, N. (1995): Xenoestrogens released from lacquer coatings in food cans. Environ. Health Perspect., 103, 608–612.
2) Olea, N., Pulgar, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M. and Sonnenshein, C. (1996): Estrogenicity of resin-based composites and sealants used in dentistry. Environ. Health Perspect., 104, 298–305.
3) Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J. and McDonnell, D.P. (1997): Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol. Appl. Pharmacol., 143, 205–212.
4) Papaconstantinou, A.D., Umbreit, T.H., Fisher, B.R., Goering, P.L., Lappas, N.T. and Brown, K.M. (2000): Bisphenol A-induced increase in uterine weight and alterations in uterine morphology in ovariectomized B6C3F1 mice: role of the estrogen receptor. Toxicol. Sci., 56, 332–339.
5) Markey, C.M., Michaelson, C.L., Veson, E.C., Sonnenschein, C. and Soto, A.M. (2001): The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environ. Health Prespect., 109, 55–60.
6) Tinwell, H., Joiner, R., Pate, I., Soames, A., Foster, J. and Ashby, J. (2000): Uterotrophic activity of bisphenol A in the immature mouse. Regul. Toxicol. Pharmacol., 32, 118–126.
7) Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenbergh, J.G. and vom Saal, F.S. (1999): Exposure to bisphenol A advances puberty. Nature, 401, 763–764.
8) Iwasaki, T. and Totsukawa, K. (2003): Change in sexual maturation and estrogen receptor expression in mouse fetuses exposed to bisphenol A. Environ. Sci., 10, 239–246.
9) Giger, W., Brunner, P.H. and Schaffner, C. (1984): 4-Nonylphenol in sewage sludge: accumulation of toxic metabolites from nonionic surfactants. Science, 225, 623–625.
10) Gilbert, M.A., Shepherd, M.K., Startin, J.R. and Wallwork, M.A. (1982): Identification by gas chromatography-mass spectrometry of vinyl chloride oligomers and other low-molecular-weight components in poly(vinyl chloride) resins for food packaging applications. J. Chromatogr., 237, 249–261.
11) White, R., Jobling, S., Hoare, S.A., Sumpter, J.P. and Parker, M.G. (1994): Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinol., 135, 175–182.
12) Chapin, R.E., Delaney, J., Wang, Y., Lanning, L., Davis, B., Collins, B., Mintz, N. and Wolfe, G. (1999): The effects of 4-nonylphenol in rats: a multigeneration reproduction study. Toxicol. Sci., 52, 80–91.
13) Fan, Q., Li, W. and Shen, L. (2001): Adverse effects of exposure to p-nonylphenol on reproductive system of young male rats. Zhonghua Yu Fang Yi Xue Za Zhi, 35, 344–346.
14) Furuichi, T., Kannan, K., Giesy, J.P. and Masunaga, S. (2004): Contribution of known endocrine disrupting substances to the estrogenic activity in Tama River water samples from Japan using instrumental analysis and in vitro reporter gene assay. Water Res., 38, 4491–4501.
15) Sakurai, K. and Mori, C. (2000): Fetal exposure to endocrine disruptors. Nippon Rinsho, 58, 2508–2513.
16) Cunny, H.C., Mayes, B.A., Rosica, K.A., Trutter, J.A. and Van Miller, J.P. (1997): Subchronic toxicity (90-day) study withpara-nonylphenol in rats. Regul. Toxicol. Pharmacol., 26, 172–178.
17) Tyl, R.W., Myers, C.B., Marr, M.C., Thomas, B.F., Keimowitz, A.R., Brine, D.R., Veselica, M.M., Fail, P.A., Chang, T.Y., Seely, J.C., Joiner, R.L., Butala, J.H., Dimond, S.S., Cagen, S.Z., Shiotsuka, R.N., Stropp, G.D. and Waechter, J.M. (2002): Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol. Sci., 68, 121–146.
18) Benjamin, L.L.T., Normadiah, M.K. and Mustafa, A.M. (2003): Assessment of pubertal development in juvenile male rats after sub-acute exposure to bisphenol A and nonylphenol. Toxicol. Lett., 143, 261–270.
19) Morrissey, R.E., George, J.D., Price, C.J., Tyl, R.W., Marr, M.C. and Kimmel, C.A. (1987): The developmental toxicity of bisphenol A in rats and mice. Fundam. Appl. Toxicol., 8, 571–582.
20) Nagao, T., Wada, K., Marumo, H., Yoshimura, S. and Ono, H. (2001): Reproductive effects of nonylphenol in rats after gavage administration: a two-generation study. Reprod. Toxicol., 15, 293–315.
21) Kabuto, H., Amakawa, M. and Shishibori, T. (2004): Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci.,

74, 2931–2940.
22) de Jager, C., Bornman, M.S. and van der Horst, G. (1999): The effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats. Andrologia, 31, 99–106.
23) Chitra, K.C., Latchoumycandane, C. and Mathur, P.P. (2002): Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. Arch. Toxicol., 76, 545–551.
24) Han, X.D., Tu, Z.G., Gong, Y., Shen, S.N., Wang, X.Y., Kang, L.N., Hou, Y.Y. and Chen, J.X. (2004): The toxic effects of nonylphenol on the reproductive system of male rats. Reprod. Toxicol., 19, 215–221.
25) Vigueras-Villasenor, R.M., Moreno-Mendoza, N.A., Reyes-Torres, G., Molina-Ortiz, D., Leon, M.C. and Rojas-Castaneda, J.C. (2006): The effect of estrogen on testicular gonocyte maturation. Reprod. Toxicol., 22, 513–520.