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Effects of bisphenol A given neonatally on reproductive functions of male rats

Hideo Kato[a,b,c], Tadakazu Furuhashi[a], Masami Tanaka[d], Yoshinao Katsu[c], Hajime Watanabe[c], Yasuhiko Ohta[e], Taisen Iguchi[c,*]

[a] *Nihon Bioresearch Inc., 6-104 Majima, Fukuju-cho, Hashima 501-6251, Japan*
[b] *The United Graduate School of Veterinary Science, Yamaguchi University, 1677-1, Yoshida 753-8515, Japan*
[c] *Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, National Institutes of Natural Sciences, 5-1, Higashiyama, Myodaiji, Okazaki, Aichi 444-8787, Japan*
[d] *Department of Pharmacology, St. Marianna University School of Medicine, 2-16-1, Sugao, Miyamae, Kawasaki 216-8511, Japan*
[e] *Laboratory of Experimental Animal Science, Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Minami, Koyama, Tottori 680-8553, Japan*

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Abstract

Male Sprague–Dawley rats (Crj:CD (IGS) were treated neonatally with bisphenol A (BPA) to evaluate effects on reproductive parameters. Animals were given BPA subcutaneously in corn oil to dosages of 0.002–97 mg/kg body weight, or 0.9 mg/kg 17β-estradiol (E2) once a day from postnatal day (PND) 0 to PND 9. Preputial separation, copulatory rate, fertility rate, sperm analysis, serum testosterone levels, and gene expression in the testis were assessed. Males in the E2 group showed a decrease in testis weight and alterations of estrogen-mediated gene expression in the testis on PND 10, and by PND 150 incomplete preputial separation, decreases in the copulatory rate, testicular and accessory organ weights and number of sperm. In contrast, males in all BPA groups showed normal reproductive parameters. These results indicate that in male rats, BPA given during the neonatal period neither affected reproductive function nor evoked estrogen-mediated gene responses in the testis.

Keywords: Bisphenol A; Low dose; Reproductive function; Neonatal period; Male rat; Gene expression; 17β-estradiol

1. Introduction

The relationship between chemical pollution and reproductive health is of major public health concern. Some etiological studies have disclosed regional declines in sperm count [1,2] and increases in hypospadias [3]; however, some studies have not reported similar findings [4–9]. The relation between chemical pollution and reproductive abnormalities has also been described in wildlife [10]. Such abnormalities have been attributed partly to estrogenic activity of the contaminants [3]. A potent synthetic estrogen, diethylstilestrol (DES) administered as a pharmaceutical agent to pregnant women between the late 1940s and early 1970s in the USA and Europe resulted in an increase in the risk of urogenital tract abnormalities, the so-called DES syndrome, in daughters and sons of DES treated women [11–13].

Bisphenol A (BPA) is used in the manufacture of polystyrene and epoxy resins. It is reported to have weak binding affinity to the estrogen receptors (ER)α and ERβ [14] and scant estrogenic activity when measured by the yeast two-hybrid assay [15]. Nevertheless, BPA has been detected in human urine [16], maternal blood samples [17], fetal placental units [17–20], and ovarian follicular fluids [20].

Oral administration of BPA at very low-dose levels (2 and 20 μg/kg) has been reported to cause reduced sperm production [21] and increased prostate weight [22]. These effects were reported in CF-1 mice exposed orally during prenatal development from gestation day (GD) 11 to GD 17, but comparable to the dose to which humans are usually exposed to in their daily lives. Results with BPA at low-dose levels given to mothers have been inconsistent. Some studies reported reproductive changes in male offspring including a reduction of sperm production

* Corresponding author. Tel.: +81 564 59 5235; fax: +81 564 59 5236.
E-mail address: taisen@nibb.ac.jp (T. Iguchi).

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[23–25], whereas others failed to demonstrate these changes [26–28]. The National Toxicology Program has evaluated the discrepancies in the scientific evidence on low-dose effects [29]. The preponderance of evidence favored the conclusion that there are no low-dose effects of BPA; however, the possibility of a low-dose effect could not be discounted because the effects being measured were subtle and can be influenced by large number of variables that are difficult to control.

Most studies were conducted to expose mothers, but not neonates, to low-dose levels of BPA. In male rats and mice, neonatal treatment of estrogen for 10–30 days results in a long-lasting suppression of spermatogenesis [30,31]; however, if the commencement of estrogen injection is delayed until the 10th postnatal day, then a permanent arrest of spermatogenesis takes place in rats and mice [32,33].

The present study has evaluated the effect of neonatal exposure of low dose of BPA in male rats. BPA treatment was by subcutaneous administration as the dosing route rather than oral administration to replicate exposure conditions of a former experiment that studied BPA in neonatal female rats [34]. Low-dose effects on male reproductive functions were analyzed using end points for general reproductive toxicity studies. Under the hypothesis that male reproductive parameters would be adversely affected by BPA exposure, we analyzed gene expression for the steroidogenic enzymes in the testis. Quantification of gene expression, which was identified by the subpanel of the National Toxicology Program as an additional research to clarify uncertainties [29], is sensitive and easily measured molecular end point.

2. Materials and methods

2.1. Animal rearing and treatment

Pregnant Sprague–Dawley rats (Crj:CD, IGS) were used in this study. The animals were housed individually in cages placed in an animal room on a 12-h light/12-h dark cycle (lighting: 06:00–18:00 h) with controlled temperature (21–25 C), relative humidity (45–65%), and filter-sterilized fresh air changes. The animals were given free access to CRF-1 diet manufactured from natural raw materials (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum. Estrogenic activity of CRF-1 diet is relatively low compared to the other commercially available certified rodent diets in Japan [35]. Twelve dams which spontaneously delivered offspring on the same day were used for each of three tests, in which the neonates were necropsied on PND 10, PND 35, or PND 150. The day of delivery was defined as PND 0. A total of 56 male neonates whose body weights were similar were selected from all male neonates delivered, and eight foster mothers nursed seven male neonates each. Seven males from each litter were allotted to seven groups. The remaining males and all female neonates were not used in this study. The male neonates were given the vehicle (ethanol) only, which served as the control, 24 ng, 120 ng, 600 ng, 3 μg, or 1 mg of bisphenol A [BPA; 2,2-bis (4-hydroxyphenol) propane, Kanto Chemical Co., Inc., Tokyo, Japan], or 10 μg of 17β-estradiol (E2, Sigma Chemical Co., St. Louis, MO) once a day from PNDs 0 to 9. BPA was dissolved in ethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to 500 mg/ml. The solution thus prepared was diluted with ethanol to BPA concentrations of 12, 60, 300, and 1500 μg/ml. Each dilution prepared was mixed with corn oil (Wako) to a concentration of 1/10. The control group was treated with a mixture of ethanol and alcohol at a volumetric ratio of 1:9. E2 was likewise dissolved at a dose of 500 μg/ml in the ethanol–oil solution. The animals were given a subcutaneous injection of 0.02 ml solution dorsally. To test effects of BPA on three different progressive stages, necropsy was performed on PNDs 10, 35, and 150. At all stages, eight male neonates of each group were given the vehicle, BPA, or E2.

Average doses of BPA in the present study were calculated on the basis of the body weight from PNDs 0 to 9 as reported previously [34]: the doses of BPA per kg body weight were 2 μg (1–3.5 μg) in the 24 ng group, 11 μg (4.8–18 μg) in the 120 ng group, 56 μg (24–87 μg) in the 600 ng group, 277 μg (124–429 μg) in the 3 μg group and 97 mg (43–152 mg/kg) in the 1 mg group. The dose of E2 per kg body weight was 0.9 mg (0.4–1.4 mg) in the 10 μg group.

For animals necropsied on PND 10, the serum testosterone levels and the testicular weight were measured, and histological changes and gene expression in the testis were examined. For animals necropsied on PND 35, in addition to the parameters on PND 10, the seminal vesicle, ventral prostate, and epididymis were weighed. For animals necropsied on PND 150, the males were weaned on PND 21, checked for preputial separation daily from PNDs 35 to 70, and bred to untreated females from PNDs 105 to 130 for confirmation of the fertility. On PND 150, all males were sacrificed to measure some sperm parameters in the left cauda epididymis, gene expression in the testis and serum testosterone levels, and to examine histology of the testis and ventral prostate. At the same time, the organs corresponding to those which were weighed on PND 35 were weighed.

The right testis on PNDs 10, 35, and 150, and ventral prostate on PND 150 were fixed in 10% neutral buffered formalin, cut in paraffin at 4 μm, and stained routinely with hematoxylin and eosin for histological examination. The left testis on PNDs 10, 35, and 150, and left cauda epididymis on PND 150 were frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Mating and cesarean section

The males were paired and mated with untreated females at proestrus on a one-to-one basis from the evening (about 17:00 h) to the next morning (about 09:00 h). Females which had sperm in the vaginal smear the next morning were regarded as having copulated. The day when the sperm was found was defined as Day 0 of gestation. Males which failed to copulate were separated once from the females and mated again with other untreated females at proestrus 3 or 4 days later. Thus, the males were mated with untreated females a maximum of four times until evidence of copulation was observed. Females in which copulation had been confirmed were subjected to Caesarean section on Day 13 of gestation and examined for the number of corpora lutea, embryonic mortality, and implantation sites.

2.3. Measurements of serum testosterone levels

The serum testosterone level was estimated by commercially available radioimmunoassay kit (rat testosterone RIA kit, IMMUNOTECH, Marseille, France). Regarding the serum samples obtained on PND 10, the serum from two to three animals in the same group were stored at −20 °C until use. Testosterone was extracted from the serum with diethyl ether in a volume 10 times as much as the volume of the serum in order to concentrate testosterone because the serum testosterone level on PND 10 was under the lower limit of quantitative analysis (5 ng/ml). The organic and aqueous phases were separated by the dry ice–ethanol method. The ether phase was evaporated by ventilation and reconstituted in a serum diluent provided with the RIA kit. In contrast, the serum samples obtained on PNDs 35 and 150 were subjected to the measurement for each animal without extraction.

2.4. Sperm analysis

To prepare semen samples, the right cauda epididymis was cut into strips in culture fluid for sperm (Medium 199 with 0.5% BSA) at 37 °C and left still for about 5 min. The prepared semen samples were examined for: (1) sperm motility and (2) sperm morphology as described below. Suspensions of sperm were prepared using the left cauda epididymis to examine (3) the number of sperm.

2.4.1. Sperm motility

The diluted semen sample was put into a sample chamber (MICROSLIDES, #HTR1099, VitroCom INC., NJ, USA) to calculate the motile sperm rate (%) and progressive sperm rate (%) using TOX IVOS (Hamilton Thorne Research,

MA, USA). Measurement of sperm motility was performed after the diluted semen sample which was incubated in a CO_2 incubator for 30 min.

2.4.2. Sperm morphology

The semen sample was smeared on a glass slide, fixed in 10% neutral buffered formalin, and stained with 1% eosin solution. Morphology of 300 sperm was observed under a microscope.

2.4.3. Number of sperm

The left cauda epididymis was homogenized (Ultra-Turrax T25 basic, IKA-Labortechnic, Germany) and put into a sample chamber (2X-CEL, Fertility Technologies, Inc., NY, USA). Then the number of sperm was counted using TOX IVOS. The number of sperm per gram was obtained by dividing the number of sperm by weight of the cauda epididymis.

2.5. RNA isolation and quantitative RT-PCR

Total RNA from the testis was isolated using ISOGEN (NIPPON GENE, Tokyo, Japan), purified using an RNeasy kit (QIAGEN, Chatsworth, CA, USA) according to the manufacturer's protocol, and then stored at −80 °C until use. For RT-PCR, each RNA was treated with ribonuclease free deoxyribonuclease 1 (Invitrogen, Co., CA, USA). Then, 1 μg of total RNA was reverse transcribed using random primer and superscript II reverse transcriptase (Invitrogen) at 42 °C for 60 min. PCR was carried out in the ABI Prism 5700 Sequence Detection System (Applied Biosystems, CA, USA) using SYBER Green Master Mix (Applied Biosystems) in the presence of appropriate gene primers. Sequences of gene primer sets are given in Table 1. We examined the expression of ERα, ERβ, progesterone receptor (PR), androgen receptor (AR), cholesterol side-chain cleavage enzyme (P450scc), 17α-hydroxylase-17, 20-lyase (CYP17), 3β-hydroxysterod dehydrogenase (3β-HSD), 17β-hydroxysterod dehydrogenase (17β-HSD), and cytocrome P450 subfamily 19 (CYP19). Relative RNA equivalents for each sample were obtained by standardization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each PCR amplification was performed in triplicate, under the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by a total of 40 two-temperature cycles (15 s at 95 °C and 1 min at 60 °C in a volume of 15 μl).

2.6. Statistics

Data were statistically analyzed by multiple comparison tests as described below and compared for body weight, organ weight, parameters for observation of fetuses and preputial separation and for sperm analysis, and serum testosterone level between the control group and each of the groups treated with BPA. Group mean values and standard errors were calculated. Subsequently, the data on the BPA groups were tested by Bartlett's method for homogeneity of variance. When the variances were homogenous, Dunnett's method was used. When the variances were heterogeneous, a Dunnett-type method using a rank order was used. Data on the E2 group were tested by Student's *t*-test and compared with the control group. The copulatory rate and fertility rate obtained in the BPA groups and E2 group were tested by Fisher's exact probability test. The level of significance was set at 5%.

3. Results

3.1. General signs and body weight changes

The injection sites became white in all animals in the 1 mg BPA group a few days after the 1st injection. No such change was noted in any other groups. No animals died in any group until the day of necropsy. There were no significant differences in the body weight among the control, BPA, or E2 group (Table 2).

3.2. Preputial separation

Preputial separation was completed in all males in the BPA groups (Table 3). There were no differences in the day of completion for preputial separation between any of the BPA groups and the control group. On the other hand, preputial separation was incomplete in five of the eight males in the E2 group until PND 150. Preputial separation was noted 2 weeks later in the E2 group than in the control.

3.3. Copulatory rate, fertility rate, and observation of fetuses

Copulation was observed by the second paring in the BPA groups (Table 4). The copulatory rate was 100% in the BPA groups. The fertility rate was 100% in the BPA groups except for the 1 mg group showing 87.5%; the lower fertility rate in the 1 mg group was caused by one female in which no implantation scars were noted. However, one male which failed to fertilize this female was confirmed to have the capability to fertilize later when he was mated with another female. Accordingly, this male was judged to have normal fertilizability. Compared with the control, the copulatory rate was significantly lower in the E2 group. Only three of the eight pairs succeed in copulation; the preputial separation was completed in these pairs. Although they copulated with normal females, one of them failed to fertilize his partner. The male was paired and mated again with another female, but they failed to copulate. Therefore, it was not possible to confirm his fertilizability in the present study.

Table 1
Sequences of gene primer sets for Q-RT-PCR

Gene	GenBank Accession no.	Primer sequences (5′–3′)	
		Forwad	Reverse
ERα	NM_012689	CACACACGCTCTGCCTTGA	GACGGAAGGAAGGAATGTGC
ERβ	U57439	TGGAGATGCTGAATGCTCACAC	TGGCCATCACTGAGACTGTAGG
PR	NM_022847	TGAGAAAGTGCTGTCAGGCTG	TTAGGAAAGGCCACTGACTGG
AR	M23264	TACCCTCCCATGGCACATTT	AAACACAGGCAGGAGCACAAC
P450scc	J05156	CTGTGATTACCTGTCCACGTTAGC	GAAAGGGAGACAGGARGAAAGAGA
P450c17	M31681	ATCCATACCTCAACACCCACAGTA	GCACTGGGACTAGCACCTCTAAGA
3β-HSD	M38179	CAAGCAGAAAACCTCGGAGTG	GGAGGATCCAGTAAACACCCAG
17β-HSD	AF035156	TGGAGGGAGAGTTGAGGAGATC	CCGACGACTACCACCAAGGA
CYP19	M33986	CTGTCCATTCCAGCACCCTTAC	GCACGCAAAGCAGTAGTTTGG
GAPDH	BC059110	AGAGAGAGGCCCTCAGTTGCT	GTGAGGGAGATGCTCAGTGTTG

Table 2
Body weight changes in male rats treated neonatally with BPA or E2

	Control[a]	BPA[a]					E2[a]
	0 ng[b] 8[c]	24 ng[b] 8[c]	120 ng[b] 8[c]	600 ng[b] 8[c]	3 μg[b] 8[c]	1 mg[b] 8[c]	10 μg[b] 8[c]
PND 0	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	6.6 ± 0.1	7.0 ± 0.1
10	26.1 ± 0.7	26.1 ± 0.4	26.1 ± 0.5	26.2 ± 0.8	26.2 ± 0.7	25.3 ± 2.0	26.1 ± 2.0
21	64 ± 1	61 ± 2	62 ± 2	63 ± 2	62 ± 2	62 ± 5	62 ± 5
42	234 ± 6	233 ± 5	229 ± 7	234 ± 8	240 ± 8	243 ± 7	222 ± 7
63	409 ± 16	409 ± 13	397 ± 15	403 ± 8	420 ± 18	429 ± 16	411 ± 13
84	529 ± 27	528 ± 21	507 ± 24	506 ± 22	541 ± 24	549 ± 21	542 ± 15
105	594 ± 33	594 ± 24	554 ± 27	564 ± 25	605 ± 29	611 ± 22	616 ± 22
126	637 ± 40	631 ± 25	587 ± 28	595 ± 26	649 ± 32	652 ± 26	660 ± 23
140	674 ± 44	662 ± 25	612 ± 29	622 ± 28	682 ± 33	681 ± 30	680 ± 22
150	699 ± 46	688 ± 26	630 ± 30	644 ± 29	704 ± 33	708 ± 31	710 ± 22

Each value shows mean ± S.E.
[a] Group.
[b] Dose.
[c] Number of males.

Table 3
Preputial separation in male rats treated neonatally with BPA or E2

	Control[a]	BPA[a]					E2[a]
	0 ng[b] 8[c]	24 ng[b] 8[c]	120 ng[b] 8[c]	600 ng[b] 8[c]	3 μg[b] 8[c]	1 mg[b] 8[c]	10 μg[b] 8[c]
Number of males in which preputial separation was completed	8	8	8	8	8	8	3
Number of males in which preputial separation was incompleted	0	0	0	0	0	0	5
Days when preputial separation was completed	45 ± 1.1	44 ± 0.7	44 ± 1.1	42 ± 0.6	44 ± 0.9	44 ± 0.3	590.9[d,**]

Each value shows mean ± S.E.
[a] Group.
[b] Dose.
[c] Number of males.
[d] Mean in three males.
** $P < 0.01$.

Table 4
Copulatory rate and fertilty rate in male rats treated neonatally with BPA or E2

	Control[a]	BPA[a]					E2[a]
	0 ng[b] 8[c]	24 ng[b] 8[c]	120 ng[b] 8[c]	600 ng[b] 8[c]	3 μg[b] 8[c]	1 mg[b] 8[c]	10 μg[b] 8[c]
Paring							
First paring	8	8	7	8	7	7	3
Second paring	–	–	1	–	1	1	0
Third paring	–	–	–	–	–	–	0
Fourth paring	–	–	–	–	–	–	0
Total	8	8	8	8	8	8	3
Copulatory rate (%)[d]	100.0	100.0	100.0	100.0	100.0	100.0	37.5*
Number of pregnant animals	8	8	8	8	8	7	2
Fertility rate (%)[e]	100.0	100.0	100.0	100.0	100.0	87.5	66.7

–: Not mated.
[a] Group.
[b] Dose.
[c] Number of males.
[d] (Number of females with successful copulation/number of females) × 100.
[e] (Number of pregnant females/number of females with successful copulation) × 100.
* $P < 0.05$.

Table 5
Observation of fetuses derived from male rats treated with neonatally BPA or E2

	Control[a]	BPA[a]					E2[a]
	0 ng[b] 8[c]	24 ng[b] 8[c]	120 ng[b] 8[c]	600 ng[b] 8[c]	3 μg[b] 8[c]	1 mg[b] 7[c]	10 μg[b] 2[c]
Number of copora lutea	16.1 ± 0.5	16.5 ± 0.6	17.0 ± 0.5	16.6 ± 0.6	16.5 ± 0.6	17.4 ± 1.6	16.0
Number of implantation sites	15.3 ± 0.6	14.3 ± 1.2	16.5 ± 0.4	15.8 ± 0.6	16.0 ± 0.5	16.0 ± 0.6	14.0
Implantation rate[d]	94.5 ± 2.2	84.7 ± 5.1	97.2 ± 1.1	94.8 ± 2.3	97.2 ± 2.0	91.9 ± 2.2	87.9
Post-implantation loss	0.8 ± 0.4	0.6 ± 0.2	2.5 ± 1.5	0.6 ± 0.2	0.9 ± 0.5	1.1 ± 0.6	0.0
Number of live fetuses	14.5 ± 0.4	13.6 ± 1.2	14.0 ± 1.5	15.1 ± 0.6	15.1 ± 0.7	14.9 ± 1.0	14.0

Each value shows mean ± S.E.
[a] Group.
[b] Dose.
[c] Number of dams.
[d] (Number of implantation sites/number of copora lutea) × 100.

Table 6
Sperm analysis in male rats treated neonatally with BPA or E2

	Control[a]	BPA[a]					E2[a]
	0 ng[b] 8[c]	24 ng[b] 8[c]	120 ng[b] 8[c]	600 ng[b] 8[c]	3 μg[b] 8[c]	1 mg[b] 8[c]	10 μg[b] 8[c]
Sperm motility							
Motile sperm rate (%)	79.7 ± 2.8	77.0 ± 1.1	79.4 ± 0.6	77.8 ± 2.2	79.9 ± 1.3	79.4 ± 1.5	80.0 ± 1.3
Progressive sperm rate (%)	41.3 ± 3.5	38.7 ± 1.9	40.1 ± 3.3	37.8 ± 3.3	40.9 ± 2.2	34.6 ± 1.9	36.8 ± 2.7
Sperm morphology							
Abnormal sperm rate (%)[d]	2.1 ± 0.5	0.7 ± 0.2*	1.0 ± 0.2	1.2 ± 0.3	0.9 ± 0.2*	2.2 ± 0.3	2.0 ± 0.6
Number of sperms in left cauda epididymis ($\times 10^6$)	380.2 ± 10.7	348.6 ± 22.2	342.9 ± 18.3	329.4 ± 23.8	357.5 ± 20.9	330.4 ± 15.1	232.2 ± 17**
Number of sperms/g of left cauda epididymis ($\times 10^6$)	1057 ± 49.5	981.4 ± 57.7	983.9 ± 47.8	945.8 ± 60.2	1017 ± 60.1	1012 ± 54.4	721.3 ± 42**

Each value shows mean ± S.E.
[a] Group.
[b] Dose.
[c] Number of males.
[d] (Number of abnormal sperms/number of sperms examined) × 100.
* $P < 0.05$.
** $P < 0.01$.

Results of the observation of fetuses in the BPA groups were not different from those in the control (Table 5). The fetal observation in the E2 group was similar to that of the control.

3.4. Sperm analysis

The motile sperm rate, progressive sperm rate, and number of sperm in left cauda epididymis in the BPA groups were not significantly different from the corresponding control values (Table 6). The significant decrease in the percent abnormal sperm in the 24 ng and 3 μg groups was considered spurious. The number of sperm per epididymis or per gram in the E2 group was significantly lower than in the control.

3.5. Organ weights

No changes in organ weights were noted in any of the BPA groups on PND 10, 35, or 150 (Table 7). In contrast, significant decreases in the organ weights were noted in the E2 group as follows: in the testis on PND 10, in the testis, seminal vesicle, ventral prostate, and penis on PND 35, and in the ventral prostate and penis on PND 150.

3.6. Serum testosterone levels

No changes in serum testosterone levels were noted in any of the BPA groups and the E2 group on PND 10, 35, or 150 (Table 8).

3.7. mRNA expression in the testis

No changes in mRNA expression in the testis were found in any of the BPA groups on PND 10 (Table 9), 35, or 150 (data not shown). In the E2 group, however, decreases in 3β-HSD and P450scc mRNA, and increases in ERβ, AR, and PR mRNA were evident on PND10 (Table 9), but not on PND 35 or 150 (data not shown).

Table 7
Organ weights in male rats treated neonatally with BPA or E2 PND10 Group

	Control[a]	BPA[a]					E2[a]
	0 ng[b] 8[c]	24 ng[b] 8[c]	120 ng[b] 8[c]	600 ng[b] 8[c]	3 μg[b] 8[c]	1 mg[b] 8[c]	10 μg[b] 8[c]
PND 10							
Testis (g/%)	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.3 ± 0.1**
PND 35							
Testis (g/%)	0.84 ± 0.02	0.86 ± 0.02	0.85 ± 0.02	0.82 ± 0.02	0.87 ± 0.01	0.81 ± 0.03	0.62 ± 0.04**
Epididymis (mg%)	98.0 ± 5.4	100.4 ± 3.7	102.0 ± 2.6	107.7 ± 7.6	99.5 ± 4.3	102.4 ± 2.7	91.8 ± 6.0
Seminal vesicle (mg%)	79.0 ± 4.3	94.2 ± 6.7	94.2 ± 3.0	88.9 ± 6.8	91.1 ± 5.1	81.6 ± 2.8	55.4 ± 4.1**
Ventral prostate (mg/%)	61.5 ± 3.7	63.9 ± 4.0	60.6 ± 3.9	58.7 ± 4.0	58.3 ± 2.8	53.9 ± 1.8	38.5 ± 3.8**
Penis (mg%)	40.3 ± 1.6	42.3 ± 1.6	42.4 ± 1.1	42.4 ± 1.0	39.7 ± 1.9	39.7 ± 0.6	25.9 ± 1.4**
PND 150							
Testis (g/%)	0.56 ± 0.01	0.55 ± 0.02	0.61 ± 0.04	0.59 ± 0.04	0.56 ± 0.02	0.52 ± 0.06	0.50 ± 0.03
Epididymis (mg%)	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.2 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
Seminal vesicle (g%)	0.33 ± 0.03	0.32 ± 0.02	0.35 ± 0.02	0.37 ± 0.01	0.34 ± 0.02	0.31 ± 0.01	0.26 ± 0.02
Ventral prostate (mg/%)	110 ± 10	95 ± 8	115 ± 13	101 ± 6	97 ± 9	87 ± 10	66 ± 6**
Penis (mg/%)	27 ± 2	25 ± 2	28 ± 3	27 ± 1	25 ± 1	24 ± 1	19 ± 2**

Each value shows mean ± S.E.
[a] Group.
[b] Dose.
[c] Number of males.
** $P < 0.01$.

Table 8
Serum testosterone levels in male rats treated neonatally with BPA or E2

	Control[a]	BPA[a]					E2[a]
	0 ng[b]	24 ng[b]	120 ng[b]	600 ng[b]	3 μg[b]	1 mg[b]	10 μg[b]
PND 10[c]	0.15 ± 0.08	0.18 ± 0.06	0.16 ± 0.05	0.22 ± 0.07	0.25 ± 0.14	0.13 ± 0.06	0.10 ± 0.04
PND 35[d]	0.20 ± 0.07	0.21 ± 0.08	0.12 ± 0.03	0.17 ± 0.05	0.27 ± 0.09	0.16 ± 0.06	0.38 ± 0.11
PND 150[d]	3.03 ± 0.72	1.97 ± 0.44	2.63 ± 0.44	2.37 ± 0.32	1.80 ± 0.29	2.07 ± 0.51	2.23 ± 0.31

Each value shows mean ± S.E.
[a] Group.
[b] Dose.
[c] Serum samples from two or three animals of the same group were put together and regarded as one test sample; a total of three test samples thus obtained from eight animals were subjected to measurement.
[d] Serum samples from eight animals per group, were individually subjected to measurement.

Table 9
mRNA relative expression[a] in the testis in male rats treated neonatally with BPA or E2 (PND 10)

	Control[b]	BPA[b]					E2[b]
	0 ng[c] 8[d]	24 ng[c] 8[d]	120 ng[c] 8[d]	600 ng[c] 8[d]	3 μg[c] 8[d]	1 mg[c] 8[d]	10 μg[c] 8[d]
3β-HSD	1.00 ± 0.09	1.01 ± 0.09	0.84 ± 0.09	0.85 ± 0.09	0.84 ± 0.07	0.84 ± 0.11	0.71 ± 0.08*
P450scc	1.00 ± 0.05	1.00 ± 0.08	0.92 ± 0.09	0.88 ± 0.13	1.09 ± 0.12	0.96 ± 0.08	0.31 ± 0.13**
ERβ	1.00 ± 0.09	1.03 ± 0.05	0.89 ± 0.11	0.92 ± 0.11	0.82 ± 0.14	0.77 ± 0.11	1.71 ± 0.27*
AR	1.00 ± 0.13	1.01 ± 0.15	0.79 ± 0.08	0.87 ± 0.09	0.74 ± 0.09	0.70 ± 0.13	1.70 ± 0.22*
PR	1.00 ± 0.15	1.32 ± 0.15	0.92 ± 0.13	1.12 ± 0.22	1.13 ± 0.25	1.34 ± 0.14	17.97 ± 5.83*

Each value shows mean ± S.E.
[a] Only for genes for which significant differences from the control group were seen on PND 10 in any group.
[b] Group.
[c] Dose.
[d] Number of males.
* $P < 0.05$.
** $P < 0.01$.

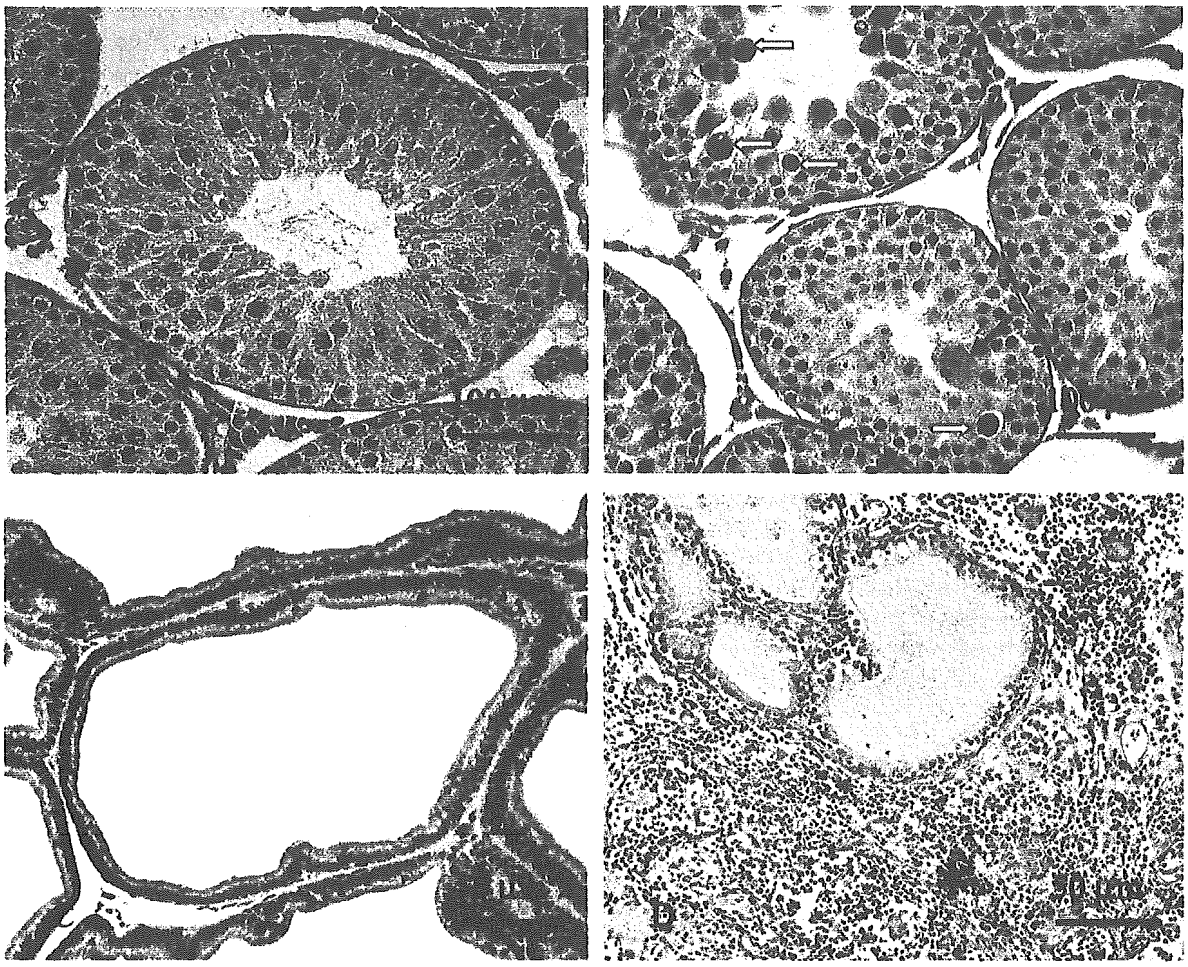

Fig. 1. Photomicrographs of the testicular tissue and on PND 35 from control group (A) and E2 group (B). The ventral prostate on PND 150 from control group (C) and E2 group (D). (A) Normal appearance in semiferous tubule; (B) degeneration of germinal epithelium with a multinucleated giant cell (black arrow) and apoptotic cells (white arrows) is found; (C) normal appearance in prostate; (D) chronic prostatitis with acute inflammation, which was mainly characterized by lymphocytic infiltration of the interstitium (asterisk). H.E. stain.

3.8. Histological changes in the testis and ventral prostate

Compared to the control group, no changes in the testis or ventral prostate were found in any of the BPA groups on PND 10, 35, or 150, or in the E2 group on PND 10. Degeneration of germinal epithelium with multinucleated giant cells was found in three males in the E2 group on PND 35 (Fig. 1B). Apoptotic cells were found in some seminiferous tubles in the same three males as above. Chronic prostatitis with acute inflammation (Fig. 1D), which was mainly characterized by lymphocytic infiltration of the interstitium, was found in one male of the E2 group on PND 150.

4. Discussion

The present data revealed that neonatal exspoure to BPA caused no adverse effects on males and estrogen-mediated response in the testis. In contrast, E2 given neonatally elicited incomplete preputial separation, lower copulatory rate, decrease in the reproductive organ weight and number of sperm, degeneration of germinal epithelium, and alteration of mRNA expression in the testis. In the males given E2, however, no changes were found in the body weight, observation of fetuses, sperm analysis, or serum testosterone levels.

The severe damage caused by E2 on reproductive functions has been reported in male rodents treated perinatally with DES [36,37]. Changes in the testis, that is, gene expression on PND 10, decrease in the testis weight on PNDs 10 and 35, and degeneration of germinal epithelium on PND 35, were temporary, and disappeared by PND 150. However, the effect on the number of sperm in the left cauda epididymis remained until PND 150. Also, the weights of the penis and prostate on PND 150 were affected by neonatal treatment with E2. Abnormalities in the prostate have also been reported in male rodents perinatally treated with DES [38]. Thus, potent estrogens, like E2, have temporary and permanent adverse effects on male reproductive functions and fetal development. Serum testosterone levels were normal at all ages examined in the E2 group despite the presence of effects of E2 on testosterone-dependent reproductive endpoints such as preputial separation, organ weights,

sperm counts, and expression of steroidogenic enzymes. This discrepancy may be ascribable to the decreased sensitivity of the seminiferous tubules and accessory organs to testosterone caused by estrogen given neonatally [39,40], since estrogen given neonatally is assumed to act directly on neonatal testes as well as on the hypothalamic mechanisms [30]. Occurrence of numerous multinucleated giant cells in the seminiferous tubules of the E2 group on PND 35 indicates the direct action of estrogen on the testis and/or on the hypothalamo-hypophyseal axis in neonates as has been suggested in neonatally estrogenized mice [39–41], although the effect of estrogen may be transient in rats as has been reported by others [42]. Reproductive abnormalities caused by neonatal estrogenization in male is known to depend on the dose and length of the treatment, and species of animals [30].

The doses of BPA per kg body weight were 2 μg (1–3.5 μg) in the 24 ng group, 11 μg (4.8–18 μg) in the 120 ng group, 56 μg (24–87 μg) in the 600 ng group, 277 μg (124–429 μg) in the 3 μg group, and 97 mg (43–152 mg/kg) in the 1 mg group. The dose of E2 per kg body weight was 0.9 mg (0.4–1.4 mg) in the 10 μg group. BPA even at the highest dose (97 mg/kg) has no effect on male reproductive functions or fetal development, being in agreement with Nagao's result [43] at a dose of 300 mg/kg. Atanassova et al. [44] showed that the testis weight was greater than the control values on PNDs 18 and 90–100 in Wistar rats treated neonatally with BPA at 0.5 mg daily from PNDs 1 to 11. In the present study, however, the testis weight of rats treated neonatally with BPA at 1 mg (97 mg/kg) daily from PNDs 0 to 9 was of the same order as that of the controls throughout the experimental period. In a 3-generation reproductive toxicity study of dietary BPA in rats [45], no adverse effect was observed at reproductive and postnatal stages with 750 ppm (50 mg/kg/day). Taking account of the results in the 1 mg group (97 mg/kg), the highest dose level in the present study, BPA may have no reproductive toxicity even at fairly high dose levels.

On the other hand, no effects of BPA at dose levels low enough to express in μg/kg, at which prostate weight increased [22] and daily sperm production reduced [21] in male mice treated prenatally, were noted in male rats treated neonatally with BPA at 2–277 μg/kg. Thus the results of the present study at low-dose levels were different from those obtained in previous studies [21,22]. It cannot be said, however, that the former was absolutely discrepant with the latter, since the experimental design was different in the dosing route, dose levels, dosing duration, and species. Pottenger et al. [46] reported that the relative bioavailability of BPA in rats was markedly lower following oral administration as compared to subcutaneous administration. Therefore, the subcutaneous dosages we employed have the possibility of causing higher bioavailability than the oral dosages vom Saal et al. [21] had employed. As described in this study, PR in the testis, an estrogen-responsible gene, was not up-regulated at any of the dosages used in the present study; it is safe to conclude that estrogen activity of BPA was not detected. The results of the present study are in agreement with those of Cagen et al. [26]. It seems highly probable, therefore, that the present findings verify a number of hypotheses on the low-dose effect of BPA on male rats.

The testis plays an important role in sex differentiation of the brain by testosterone secretion in the perinatal period. In the present study, some of the genes in steroidogenic enzymes were selected and determined as an indication of functions in the testis in addition to genes of sex steroid receptors, ERs, AR, and PR. Alterations of gene expression were noted in the E2 group only on PND 10. E2 caused high gene expression of PR about a 17-folds compared to the control. The up-regulation of PR mRNA by E2 demonstrates that ER is functional in the testis. Although it was shown in the rat hypothalamus that BPA increased PR immunoreactivity [47] and mRNA expression [48], BPA at any dose level evoked no up-regulation at least in the neonatal testis. P450scc, which metabolizes the cholesterol to the pregnenolone (3β-hydroxypregn-5-en-20-one), and 3β-HSD, which metabolizes the pregnenolone and/or its analog, both genes of steroidogenic enzymes, were down-regulated in the E2 group, but not in any of the BPA groups. In addition to the change in the steroidogenic enzyme, ERβ, and AR mRNA expression were affected by E2, but not by BPA. A transient increase in serum testosterone has been reported in F1 male rats from mothers treated prenatally with BPA at 25 and 250 μg/kg [49] or perinatally at 4 and 40 mg/kg [50], but in this study BPA caused no changes in serum testosterone levels at any dose level.

In a study of BPA using the same neonatal model as in the present study, some abnormalities have been detected in the reproductive organs of females treated neonatally with BPA at high dose levels (1 and/or 4 mg/female; almost the same doses as in the present study) [34]. However, since the neonatal treatment with BPA at any dose level had no effect on male reproductive functions, BPA produces hardly any injurious effect on reproduction in the males when exposed to BPA after birth.

In conclusion, neonatal BPA treatment causes no adverse effects both on male reproductive functions and on gene expression for the steroidogenic enzymes in the testis.

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CHARACTERIZATIONS OF THE FIRST FLUSH IN STORM WATER RUNOFF FROM AN URBAN ROADWAY

B.C. LEE[1]*, S. MATSUI[1], Y. SHIMIZU[2] AND T. MATSUDA[1]

[1]Department of Technology and Ecology, Kyoto University, Sakyo-Ku, Yoshida-Honmachi, Kyoto 606-8501, Japan
[2]Research Center for Environmental Quality Control, Kyoto University, 1-2 Yumihama, Otsu 520-0811, Japan

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ABSTRACT

Storm water runoff from urban roadways contains anthropogenic pollutants, which are mainly generated from traffic-related activities. The purpose of this study was to evaluate the characteristics of pollutants from the roadway runoff as well as first flush effects. Storm water runoff was sampled during five storm events from the experimental site in Otsu, Shiga, Japan. From the hydrographs and pollutographs for the roadway runoff, the concentration of pollutants increased with increasing runoff flow in the low flow rate event, but did not significantly increase in the high flow rate event. Moreover, according to the analysis of cumulative pollutant mass versus runoff volume curves from five storm events, the first 50% of the runoff volume transported 62% of TOC and Mo, 60% of SS, 59% of Fe, Mn and Cu, 58% of Ni, 57% of Cd and Pb, 56% of Al, 55% of Zn, and 54% of Cr, as the mean values. The first 30% and 80% of the runoff volume also transported 34-43% mass of the pollutants and 82-88% mass of the pollutants, respectively. This study for storm water runoff may also provide useful information to correctly design treatment facilities, such as detention tanks and ponds, filtration and adsorption systems.

Keywords: Roadway runoff, first flush, event mean concentration, metal elements, particle size distribution.

INTRODUCTION

Roadway storm water runoff may be responsible for serious environmental impacts. Storm water runoff from urban roadways contains significant loads of suspended solids, metal elements, organic and inorganic compounds [1-5]. These pollutants, especially during the first flush, are mainly discharged from the urban drainage system into the receiving water. The first flush generally relates to the initial period of storm water runoff during which the concentration of pollutants is significantly higher than those observed during the latter period of the storm water runoff [6]. Therefore, the first flush is influenced by various parameters, such as impervious area, rainfall intensity, rainfall duration, and the antecedent dry weather period [7-9].

Several studies have identified the first flush phenomenon for storm water runoff. To define the first flush phenomenon, many approaches have been based on the relationship between the normalized cumulative pollutant mass and the normalized cumulative runoff volume for a storm event [10-13]. This relationship depends on several parameters, such as the pollutant, the site, the storm event, and functioning of the sewer system [10]. First flush has been defined as when the normalized cumulative mass versus volume curve has an initial slope greater than 45° slope line, and the strength of the first flush is quantified by the point of maximum gap from the 45° slope line [14]. Thus, it has been suggested that there is a significant first flush if the maximum gap between the normalized cumulative curve and 45° slope line is greater than 0.2. Deletic [12] has also defined the first flush as the normalized cumulative pollutant mass transported by the first 20% of storm runoff volume. Wanielista and Yousef [8] proposed that first flush is when 50% of the total pollutant mass is transported in the first 25% of the runoff volume, presented as 25/50. Bertrand-Krajewski *et al.* [10] assumed that there is a significant first flush if at least 80% of the total pollutant mass is transported in the first 30% of the runoff volume during the storm event, presented as 30/80. However, the first flush by this definition is very rare and thus this definition is not sufficient to quantify precisely the first flush effect. Another approach defined the first flush in a combined sewer flow as a part of the storm up to the maximum divergence between the normalized cumulative pollutant mass and the normalized cumulative runoff volume plotted against the normalized cumulative time [7]. Sansalone and Buchberger [4] defined the first flush

as that which occurs when the normalized cumulative pollutant mass plotted against the normalized cumulative time exceeds the normalized cumulative runoff volume at all instants during the storm runoff.

The first flush effect in storm water runoff has been identified as one of the major causes of the deterioration of the quality of receiving water environments. Therefore, the characterization of storm water runoff, especially the first flush phenomenon, is necessary not only to manage urban drainage systems but also to meet the water quality objectives for the receiving water bodies. The main objective of this study was to evaluate the characteristics of pollutants from the roadway runoff as well as first flush effect. In addition, the fractionation of metal elements between the dissolved and particulate-bound form in the roadway runoff was also investigated.

MATERIALS AND METHODS

Experimental Site and Sampling

The experimental site is located along Route 161 in Otsu, Shiga, Japan, which is located nearby to Southern Lake Biwa, and is shown in Figure 1. The area of Otsu is 302.34 km^2 and the resident population is 289,601 [15]. The average traffic density between 7 am and 7 pm at the experiment site is 38,086 vehicles per 12 hrs. The experimental site consists of four lanes that have two lanes on each side separated by a central reservation. The pavement is a traditional asphalt surface that has a transverse cross slope of 0.015 to 0.02. Characteristics of five storm events from the experimental site during the study period are summarized in Table 1. The 30 March 2004 event had high flow rate with mean runoff flow

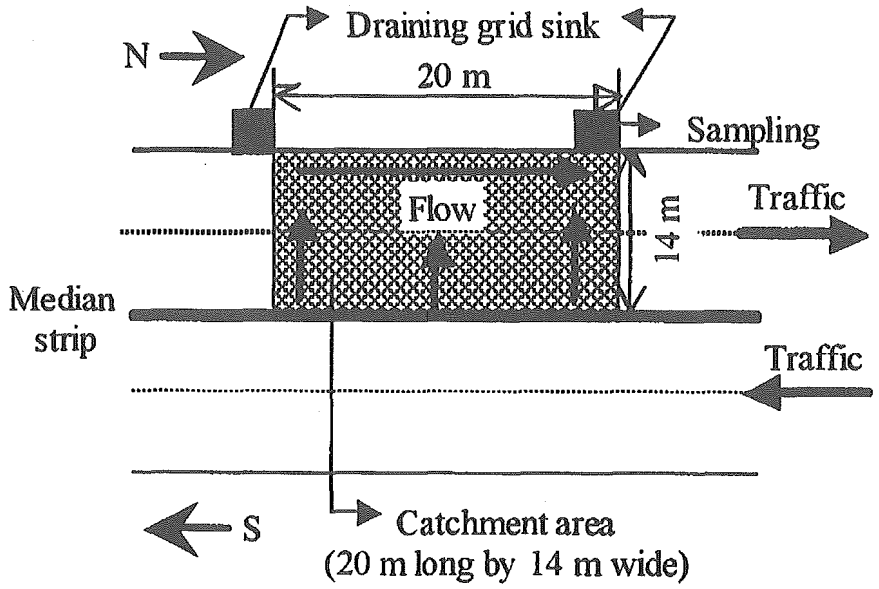

Figure 1. Experimental site on Route 161 in Otsu, Japan.

Table 1. Characteristics of five storm events and runoff from the experimental site.

Parameter	Event				
	30 Mar. 04	2 Apr. 04	4 Apr. 04	7 Apr. 04	14 Apr. 04
Antecedent dry period (day)	4	2	1	2	6
Rain duration (hr)	9	2	13	4	8
Rain maximum intensity (mm hr^{-1})	9	2	3	3	2
Rain depth (mm)	32	3	21	8	11
Runoff flow peak (l min^{-1})	27	9.8	13.4	11.4	10.4
Runoff mean flow (l min^{-1})	10.3	3.7	3.4	4.8	3.3
Runoff volume (l)	4480	446	2396	1376	1466
Runoff duration (min)	435	120	705	285	450

rate greater than 10 l min^{-1} and had high runoff volume. The other four events had low flow rate with mean runoff flow rate less than 10 l min^{-1} and had low runoff volume. The 4 April 2004 event had two distinct periods separated by a period of no flow in the runoff hydrograph and pollutograph. The storm water runoff was sampled from the roadway catchment area (i.e., 20 m long by 14 m wide) as a function of time, immediately after the runoff left the pavement surface. The samples were collected using polypropylene bottles (1000 ml) and transported to the laboratory for analysis.

Analytical Methods

Electrical conductivity (EC) and pH were determined using an EC meter (CM-5S, TOA Electronics, Japan) and pH meter (PHM210, Radiometer Analytical, France), respectively. Total organic carbon (TOC) was measured by the combustion oxidizing method using a TOC meter (TOC-5000A, Shimadzu, Japan). Suspended solids (SS) were determined by using glass fiber filters (GF/B, Whatman), drying at 105°C for 2 hr and weighing. Particle size distribution (PSD) was measured with a laser diffraction particle size analyzer (SALD-2100, Shimadzu, Japan). Particle sizes of range from 0.03 to 1000 μm were measured as follows; range of absorbance and refraction index were from 0.01 to 0.2 and from 1.70 to 0.20, respectively. Metal element fractionation entailed the physical separation of the total metal mass into dissolved and particulate-bound fractions using filtration. Dissolved fraction was measured as follows; nitric acid (2% HNO_3) was added into the sample (50 ml) filtered through glass fiber filters (GF/B, Whatman). Total fraction was measured as follows; nitric acid (2% HNO_3) was added into the sample (50 ml) which was not filtered and shaken rigorously to suspend all particulate matter. And then the solution was filtered after 24 hr through glass fiber filters (GF/B, Whatman). Therefore, the particulate fraction was calculated as the difference between total fraction and dissolved fraction. The metal elements (i.e., Al, Fe, Cr, Mn, Ni, Cu, Zn, Mo, Cd and Pb) were determined by using inductively coupled plasma with mass spectroscopy (HP4500/ICP-MS, Yokogawa Analytical Systems, Japan).

RESULTS AND DISCUSSION

Characteristics of Water Quality of Roadway Runoff

The use of an event mean concentration (EMC) is appropriate for evaluating the effects of storm water runoff, because pollutant concentrations transported in storm water runoff may vary in order of magnitude during a storm event. The EMC has often been used as an important parameter to characterize pollutant concentrations [4], which are represented as the total pollutant mass divided by the total runoff volume for the entire event duration, shown as follows:

$$EMC = M/V = \frac{\int_0^{t_r} Q(t)C(t)dt}{\int_0^{t_r} Q(t)dt} \quad \text{(i)}$$

where, M is the total mass of pollutant over an entire duration (mg); V is the total volume of flow over an entire duration (l); $Q(t)$ is the time variable flow (l min^{-1}); $C(t)$ is the time variable concentration (mg l^{-1}); t_r is the event duration (min); and t is time (min).

The event mean concentration (EMC) of pollutants from the roadway runoff for each event is shown in Table 2. The average EMC of SS, TOC, Al, Fe, Cr, Mn, Ni, Cu, Zn, Mo, Cd and Pb was 54.4 mg l^{-1}, 17.3 mg l^{-1}, 3.68 μg l^{-1}, 1.57 mg l^{-1}, 3.43 μg l^{-1}, 51.6 μg l^{-1}, 4.07 μg l^{-1}, 47.2 μg l^{-1}, 501.9 μg l^{-1}, 2.94 μg l^{-1}, 0.14 μg l^{-1} and 14.1 μg l^{-1}, respectively, for five storm events. A relatively high level of Fe and Zn was observed in the

Table 2. Event mean concentration (EMC) of pollutants from the runoff.

Parameter	Event					Mean	Ref*
	30 Mar. 04	2 Apr. 04	4 Apr. 04	7 Apr. 04	14 Apr. 04		
SS (mg l^{-1})	45.6	75.6	47.9	30.4	72.6	54.4	154.7
TOC (mg l^{-1})	13.1	18.9	10.9	11.6	32.2	17.3	-
Al (μg l^{-1})	3.02	4.06	3.65	2.43	5.24	3.68	2294
Fe (μg l^{-1})	1423	2038	1239	928	2241	1574	4136
Cr (μg l^{-1})	3.23	3.37	2.80	3.15	4.60	3.43	21.2
Mn (μg l^{-1})	43.6	67.7	45.4	38.5	63.0	51.6	323.6
Ni (μg l^{-1})	3.17	4.24	3.99	3.26	5.69	4.07	43.4
Cu (μg l^{-1})	41.8	46.1	36.4	32.1	79.8	47.2	135.0
Zn (μg l^{-1})	438	496	499	510	567	502	4274
Mo (μg l^{-1})	1.89	3.22	2.26	2.35	4.98	2.94	-
Cd (μg l^{-1})	0.09	0.13	0.12	0.15	0.23	0.14	7
Pb (μg l^{-1})	11.7	12.0	13.5	12.9	20.5	14.1	64.4

*Sansalone and Buchberger [4]; Sansalone *et al.* [13]

roadway runoff, while similar results were reported by other studies [4, 5]. Generally, Fe and Zn are mainly derived from vehicular component wear such as tires, brakes, frame and body [4, 16]. The levels of EMC of pollutants for the 14 April 2004 event were higher than the average EMC of pollutants for the five storm events. This event had a longer antecedent dry weather period than the other events. Thus, this result indicated that the EMC of pollutants at the same roadway condition was mainly affected by the antecedent dry weather period. Gupta and Saul [7] reported that the first flush load was related to the peak rainfall intensity, the storm duration and the antecedent dry period. Especially, the antecedent dry period was found to be the most important parameter for determining the build-up of pollutants.

Compared to the average EMC of roadway in Cincinnati [4, 13], the average EMC in this study was lower for SS and metals (Table 2). The average EMC was about 3 times lower for SS, Fe and Cu, about 6 times lower for Pb, Cr and Mn, about 10 times lower for Zn and Ni, about 50 times lower for Cd, and about 620 times lower for Al. The average daily traffic density (150,000 vehicles) of the roadway in Cincinnati was 4 times higher than that of the roadway in this study. Generally, the EMCs of pollutants in the site with high traffic density are higher than those in the site with low traffic density.

Pollutant Runoff Pattern and Particle Size Distribution (PSD) from Roadway Surface

The runoff hydrographs and pollutographs at the experimental site (about 20 m long x 14 m wide) for each event are shown in Figure 2. Here, the runoff time was initialized to zero at the beginning of runoff after rainfall. The concentrations of pollutants were typically higher at the beginning or early period of runoff and decreased afterwards for storm events. The peak pollutant concentrations also occurred before the flow peak of the roadway runoff. Similar results were reported by another study [9]. The concentrations of pollutants significantly increased with increasing runoff flow in the low flow rate event, but did not increase in the high flow rate event. This result indicated that the appearance of the peak of pollutant concentrations followed by the flow peak in the low flow rate event was more evident than that in the high flow rate event due to the fact that most of the

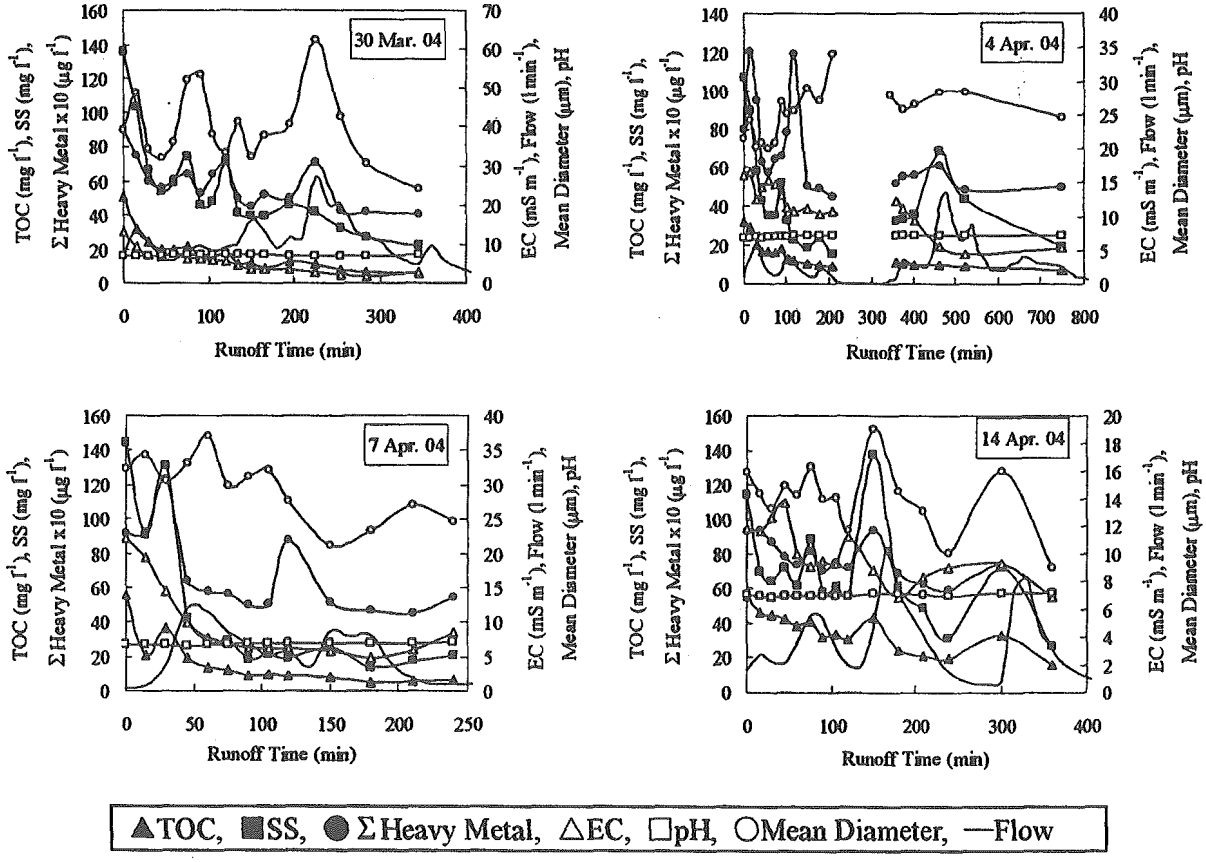

Figure 2. Runoff hydrographs and pollutographs for storm events (∑Heavy Metal = Cr + Mn + Ni + Cu + Zn + Mo + Cd + Pb).

pollutants were rapidly flushed at the beginning or early period of runoff in the high flow rate event. In the case of the 4 April 2004 event which had two distinct runoff hydrographs, the initial hydrograph showed a stronger first flush than the latter hydrograph for most pollutants.

Figure 2 also shows the delivery of particle sizes in response to the flow rate of roadway runoff. The mean particle diameter is the mean diameter determined from the distribution of particle sizes measured by particle size analyzer. The peak of mean particle diameter occurred at the flow rate peak in the roadway runoff. Therefore, the mean particle diameter was influenced by flow rate for both high and low flow rate runoff. Sansalone *et al.* [13] reported that the pattern of particle delivery was driven by runoff intensity and duration in a roadway runoff. Temporal variations of particle size distribution for both high and low flow rate runoff are also shown in Figure 3. For both events, the relative larger particles were observed at the beginning of runoff. This is due to the fact that the large particles nearby the sampling point were washed. For the high flow rate runoff (Figure 3a), the peak of PSD was shifted from about 80 μm (at 0 min) to about 30 μm (at 60 min), and thus the relatively smaller particles were rapidly washed from the roadway surface during initial runoff. However, significant variation of PSD in the low flow rate runoff (Figure 3b) was not observed during initial runoff. This means that the relatively smaller particles were slowly washed during low flow rate runoff. Therefore, PSD variation in the roadway runoff occurred in the high flow rate runoff.

Fractionation of Metal Elements in Roadway Runoff

The dissolved fraction (f_d) of metal elements has implications for metal transport and immobilization [4]. Thus, the dissolved fraction (f_d) for metal elements directly indicates whether a metal element mass is predominately in dissolved

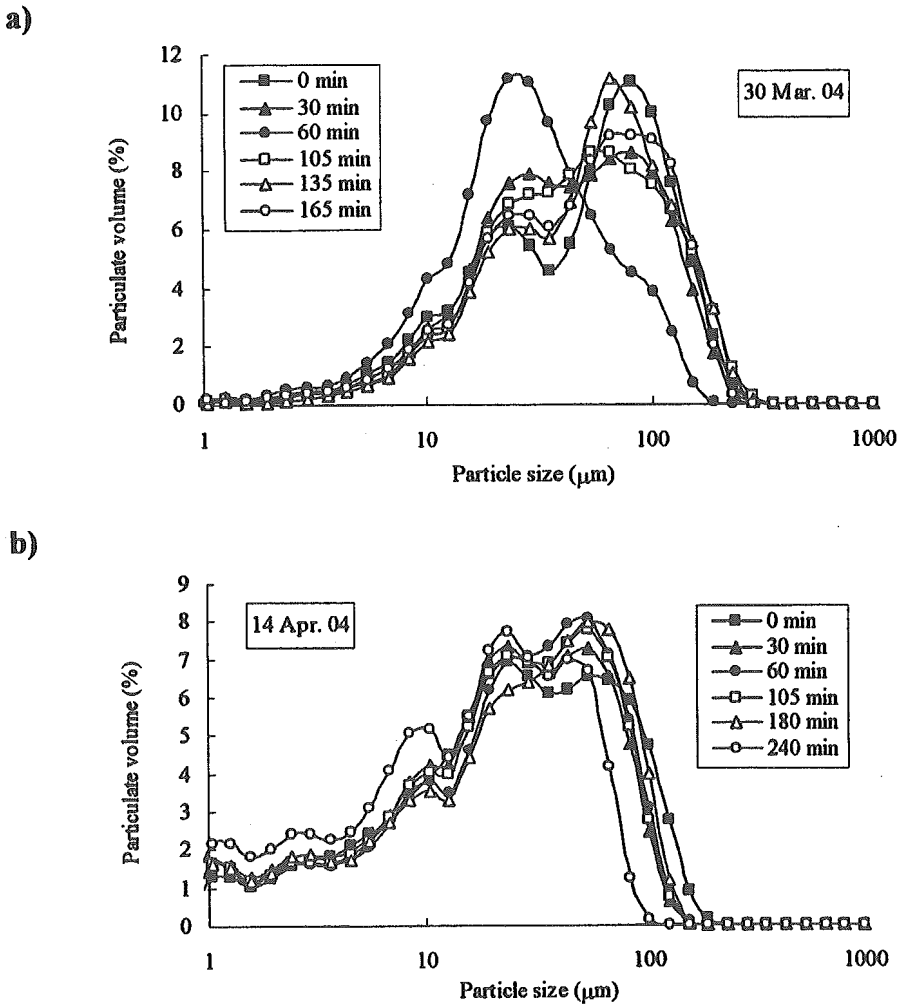

Figure 3. Temporal variations of particle size distribution (PSD) for both high (a) and low (b) flow rate runoff.