Perinatal Phthalate/Adipate Esters Exposure in Rats: Effects on Maternal Body Weight Changes and Developmental Landmarks in Offspring Rats

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ABSTRACT

Di-n-butyl phthalate (DBP), diisononyl phthalate (DINP) and di-(2-ethylhexyl) adipate (DEHA) are ubiquitously distributed chemicals that are widely used as plasticizers and also found at low levels in foods. The aims of this study were to determine whether perinatal exposure to DBP, DINP and DEHA could alter normal patterns of neonatal development. Dams were provided with pulverized soy-free diet containing 20, 200, 2,000 and 10,000 ppm of DBP, 40, 400, 4,000 and 20,000 ppm of DINP, or 480, 2,400 and 12,000 ppm of DEHA from gestational day 15 to postnatal day 21. Exposure to the high doses of DBP, DINP and DEHA during gestational period significantly decreased food consumption and body weight gain of dams. These chemicals reduced neonatal body weight as well as that of the after maturation. Also, exposure to DINP of all the doses used and the higher doses (2,400 and 12,000 ppm) of DEHA decreased AGD at PND 1 in male neonates, though that to DBP did not affect AGD in males. In female neonates, an increase in AGD was observed in DBP- and DINP-exposed animals at the highest doses. Moreover, these chemicals affected survival rate of pups at PND 5, and delayed onset of eye opening in all chemical-exposed groups at PND 17. These results suggest that perinatal exposure to these chemicals may affect the normal development and / or growth of offspring.

(Key words : Di-n-butyl phthalate (DBP), Diisononyl phthalate (DINP), Di-(2-ethylhexyl) adipate (DEHA), Developmental Landmarks, Perinatal exposure)

INTRODUCTION

Phthalates are synthetic chemicals that are widely used as a plasticizer in the production of plastics (Thomas and Thomas, 1984), and are present in many consumer products, such as soaps, perfumes, cosmetics, shampoos, building products, shower curtains, aerosols, plastic toys, and plastic packaging (Agency for Toxic Substances and Disease Registry (ATSDR), 1995, 2001 and 2003). They have been also found at low levels in foods, particularly fatty foods such as dairy products because they are fat-soluble (Sharman *et al.*, 1994; Page and Lacroix, 1995).

Exposure of the general population to DBP has been estimated by at least four authoritative sources: the International Program on Chemistry Safety (IPCS) (1997), the UK Ministry of Agriculture, Fisheries, and Food (MAFF) (1998), Health Canada (Chan and Meek, 1994) and the US Agency of Toxic Substances and Disease Registry (ATSDR) (1990). MAFF (1998) estimated adult DBP exposure through dietary intake based on a 1993 survey of fatty foods in the UK. DBP was detected in carcass meat (0.09 mg/kg), poultry (0.2 mg/kg), eggs (0.1 mg/kg), and milk (0.003 mg/kg). In calculating dietary food exposures, MAFF assumed that these types of food likely account for 85% of dietary phthalate intake. Mean and high level DBP intakes were estimated at 13 μg DBP/person/day and 31 μg DBP/person/day, respectively. Using the IPCS-assumed (1997) adult body weight of 64 kg, the exposure values were converted to 0.20~0.48 μg/kg bw/day.

Humans may be exposed to DINP by the oral, dermal, and inhalation routes of exposure. Occupational exposure occurs primarily through inhalation and dermal contact, while consumer exposure occurs primarily through oral and dermal routes. Exposure of children to DINP through children's products is a public con-

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cern. Exposure of children to DINP from PVC toys was estimated by Steiner *et al.* (1998) in Austria. DINP levels were measured in the saliva of 10 adult volunteers who first sucked on and then sucked and chewed on 10~15 cm^2 pieces of teether (containing about 36% DINP) for 1 hr. In the experiment where the volunteers only sucked on the sample, the migration rates of DINP ranged from 297~1,452 $\mu g/dm^2/hr$ with a mean migration rate of 832±397 $\mu g/dm^2/hr$.

Consumer exposure to DEHA via food wrap has been reported for human populations in the US (Till *et al.*, 1982), Canada (Page and Lacroix, 1995), and elsewhere (Harrison, 1988; Kozyrod and Ziaziaris, 1989; Petersen *et al.*, 1995). DEHA can migrate from cling film wrappings to food. In general, higher levels of DEHA are found in food with high fat content (Harrison, 1988). DEHA has been used extensively as an additive in food contact plastics, mainly as a plasticizer in thin PVC cling-films. When such films are used in contact with fatty foodstuff such as cheese and meat, significant migration of DEHA to the foodstuff can occur in amounts higher than the specific migration limit of 18 mg/kg food (Petersen *et al.*, 1995; Petersen and Naamansen, 1998). The limit was proposed by the Scientific Committee on Food (SCF) and has been adopted by the Danish Veterinary and Food Administration. On the other hand, estimates of total DEHA consumption are low with the MAFF reporting an estimated total consumption of 8.2 mg/person/day (117 μg/kg/day for a 70 kg person). Studies in humans indicate that this value may be an upper limit with a median exposure of 2.7 mg/person/day determined from a limited sampling (Loftus *et al.*, 1994).

During recent years the phthalates have attracted much attention because several of these compounds are suspected of possessing endocrine disrupting effects. Also, although adipate, especially DEHA is currently being evaluated as potential substitutions for some phthalates, due to similarities in structure and metabolism of DEHP and DEHA, and of DEHA and DINP, it could be hypothesized that similarities in action may also exist. Recently, considerable concern has focused on the health effects of phthalate exposures to children from toys and other sources, and to pregnant woman from dialysis treatment or blood transfusions. Moreover, phthalates are able to cross the placenta and pass into breast milk (Parmar *et al.*, 1985; Dostal *et al.*, 1987; Srivastava *et al.*, 1987), and, therefore, exposure during gestational and lactational periods is of particular concern.

In the present study, we assessed the effects of perinatal exposure to DBP, DINP and DEHA on several developmental landmarks in rats. To this end, maternal rats were given a diet containing these chemicals from gestational day 15 to the day of weaning, and anogenital distance (AGD) at postnatal day (PND) 1, survival rate at PND 5 and onset of eye opening from PND 17 to PND were examined.

MATERIAL AND METHODS

Test Compounds

DBP (CAS No. 84-74-2, purity>98%, Cat No. P0292) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). DINP (CAS No. 28553-12-0, purity>98%, Cat No. 040-22805), and DEHA (CAS No. 103-23-1, purity>99%, Cat No. 027-13006) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Animals and Treatments

This study followed Guidelines for the Care and Use of Laboratory Animals of the Graduate School of Agricultural and Life Sciences, the University of Tokyo. Wistar-Imamichi rats (Imamichi Institute for animal Reproduction, Ibaraki, Japan) of approximately 8 weeks of age were obtained and maintained in a room with controlled illumination (lights on 0500~1900 o'clock) and temperature (23±1℃), and provided chow and water *ad libitum*. Animals were mated (mating confirmed by sperm presence in vaginal smears), and the day of mating was designated as gestational day (GD) 0. The pregnant females were allowed to deliver pups naturally (day of birth = PND 0), and the litter size was adjusted to eight on the PND 5. The dams were fed with pulverized soy-free diet (NIH-07-PLD (phytoestrogen-low diet); Oriental yeast Co. Ltd., Tokyo, Japan) to reduce the effect of phytoestrogen. From GD 15 to the day of weaning (PND 21), the dams were provided with pulverized soy-free diet that contained 20, 200, 2,000 and 10,000 ppm of DBP, 40, 400, 4,000 and 20,000 ppm of DINP, or 480, 2,400 and 12,000 ppm of DEHA. After weaning, all the animals were fed with a conventional pellet diet (CRF-1; Oriental yeast Co. Ltd., Tokyo, Japan).

Experimental Design

Food consumption and body weight gains of dams were measured during the exposure period, from GD 15 to PND 21. AGD was measured on PND 1, and was normalized by the cube root of body weight, it is likely that the cube root of body weight would be the appropriate standard for making adjustments to AGD when it is necessary to compensate for differences in weight among pups (Clark, 1999). Pups were held gently between thumb and forefinger while AGD measurements were taken using Vernier calipers which measure to an accuracy of 0.05 mm. AGD is commonly regarded as a hormonally sensitive developmental measure in rodents (Heinrichs, 1985). Body weight changes of offspring were monitored from PND 1 to postnatal week (PNW) 11. Survival rate of pups was examined at PND

5. Viability was calculated by averaging ratio of pups surviving from postnatal day 1 to postnatal day 5. Pups were also examined of their eye opening beginning from PND 17 to PND 21.

Statistical Analysis

Statistical analyses were conducted using StatView (version J5, Abacus Concepts, Inc.). A one-way analysis of variance and Dunnett's test were used to determine differences between treated and control groups. Differences were considered statistically significant at $p<0.05$.

RESULTS

Effects on Dams

Food Consumption and Body Weight Gain

As shown in Fig. 1~3, maternal food consumption (A) and body weight gain (B) were not statistically different among the groups during PND 10-14, which is before exposure to chemicals. Significant reductions of maternal food consumption were observed in 10,000 ppm DBP- (Fig. 1A), 4,000 and 20,000 ppm DINP- (Fig. 2A) and 2,400 and 12,000 ppm DEHA- (Fig. 3A) exposed dams on GD 15-17 and / or GD 15-19. A significant reduction of maternal food consumption during lactation period was only observed 10,000 ppm DBP-exposed rats on PND 9-17 (Fig. 1A). On the other hand, these chemicals reduced the maternal body weight gains during GD15-19. Significant reductions of maternal body weight gains were observed in 10,000 ppm DBP- (GD 15-19) (Fig. 1B), 400 and 20,000 ppm DINP- (GD 17-19 and GD 15-19, respectively) (Fig. 2B) and 12,000 ppm DEHA-exposed groups (Fig. 3B). However, after delivery, maternal body weight gains were not significantly different among all chemical exposed groups.

Effects on Offspring

Body Weight and Anogenital Distance

At PND 1, body weight of control males was significantly greater than that of control females (Fig. 4A). In DBP, DINP or DEHA-exposed neonatal rats, body weights were significantly decreased compared with those of control pups of corresponding sexes, to the exception of 20 ppm of DBP in both sexes and 200 ppm of DBP in females. As shown in Fig. 4B, AGD in male neonates was decreased in a dose-dependent manner by maternal exposure to all the chemicals used. AGD in 2,400 ppm and 12,000 ppm of DEHA-exposed female neonates was also decreased. However, when AGD was normalized by the cube root of body weight, exposure to DBP did not affect AGD in male neonates, though that of DINP of all the doses used and the higher doses (2,400 and 12,000 ppm) of DEHA still decreased AGD in males (Fig. 4C). In female neonates, an increase in AGD was observed in DBP- and DINP-exposed animals at the highest doses, while inhibitory effects of DEHA were no more discernible.

As shown in Table 1~6, pup's body weight was observed from PND 1 to PNW 11. Body weight of both sexes of offspring at weaning (PNW 3) was significantly decreased in DBP-, DINP, and DEHA-exposed groups except for 20 and 2,000 ppm of DBP-exposed male rats, and 480 ppm of DEHA-exposed male rats. These reductions of body weight were also persisted after maturation.

Pup Surviving and Eye Opening

Fig. 5A displays the ratio of live pups at PND 5 for

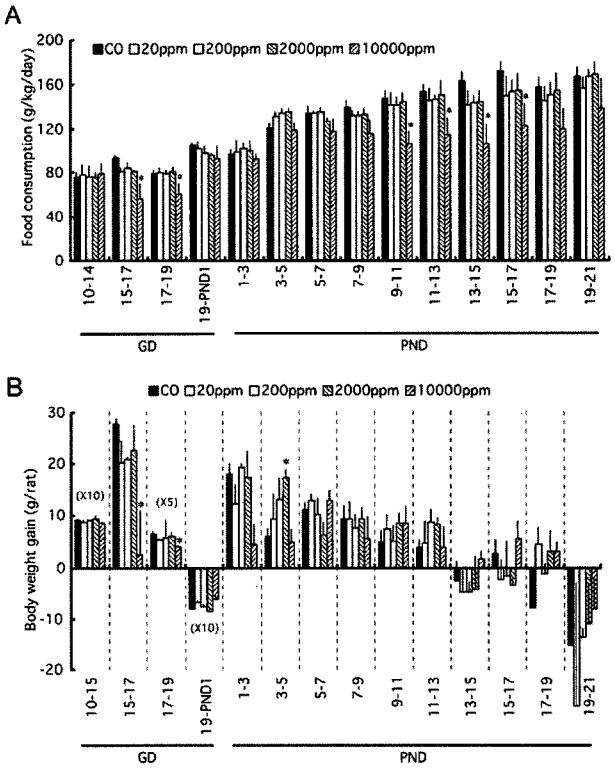

Fig. 1. Maternal food consumption (A) and body weight gains (B) during the gestation and lactation periods. Maternal food consumption at the 10,000 ppm DBP-exposed group showed significant decrease from control diet at GD 15-19 and PND 9-17. Maternal body weight gains at the 2,000 ppm and 10,000 ppm DBP-exposed group showed significant increase and decrease from control diet at PND 3-5 and GD 15-19, respectively. Data represent mean±SE., n=4 per group. Each column and vertical bar is multiplied by the value in parentheses. DBP, di-n-butyl phthalate; CO, control diet; GD, gestation day; PND, postnatal day. * $p<0.05$ vs. CO.

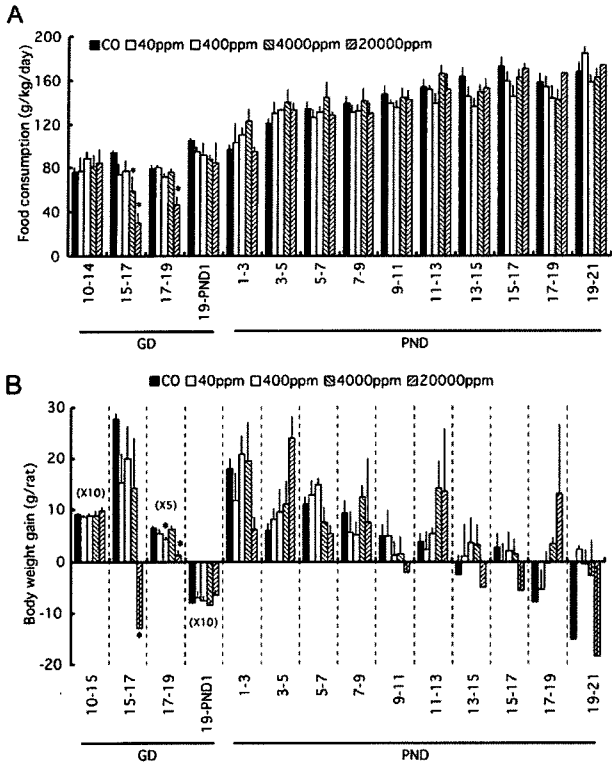

Fig. 2. Maternal food consumption (A) and body weight gains (B) during the gestation and lactation periods. Maternal food consumption at the 4,000 ppm and 20,000 ppm DINP-exposed group showed significant decrease from control diet at GD 15-17 and GD 15-19, respectively. Maternal body weight gains at the 400 ppm and 20,000 ppm DINP-exposed group showed significant decrease from control diet at GD 17-19 and GD 15-19, respectively. Data represent mean±SE., n=4 per group. Each column and vertical bar is multiplied by the value in parentheses. DINP, disononyl phthalate; CO, control diet; GD, gestation day; PND, postnatal day. * $p<$ 0.05 vs. CO.

number of live pups at PND 1. The percentage of the live pups was significantly lower at 10,000 ppm DBP-exposed male rats, and all of the 20,000 ppm DINP-exposed pups were died on PND 5. Ratio of eye opening is presented in Fig. 5B. The time of eye opening was significantly delayed in all chemical-exposed groups at PND 17. At PND 21, ratio of eye opening was 100 percent in all dose levels, to the exception of 12,000 ppm DEHA-exposed groups.

DISCUSSION

The lower maternal body weight gain during late gestation may relate to reduced maternal food consumption, because these chemicals have bitter taste, and fetal growth retardation by these chemicals causes of decreased birth body weight probably also contribute to the reduced

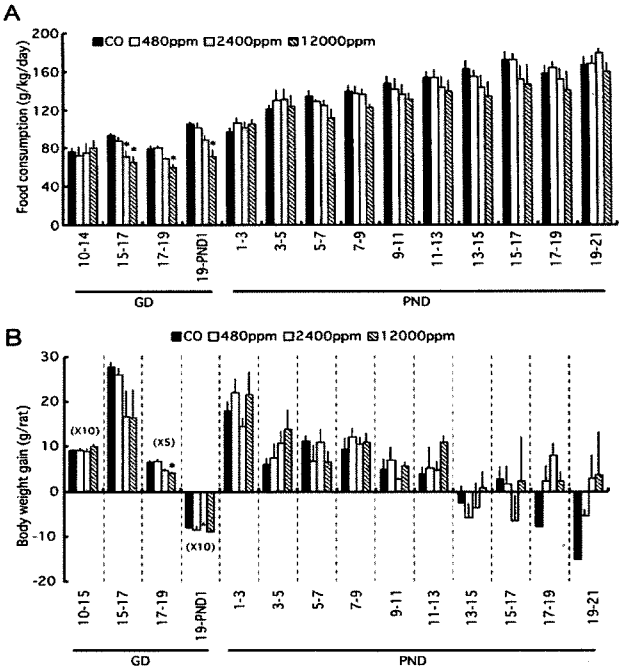

Fig. 3. Maternal food consumption (A) and body weight gains (B) during the gestation and lactation periods. Maternal food consumption at the 2,400 ppm and 12,000 ppm DEHA-exposed group showed significant decrease from control diet at GD 15-17 and GD 15-19, respectively. Maternal body weight gains at the 12,000 ppm DEHA-exposed group showed significant decrease from control diet at GD 17-19. Data represent mean±SE., n=4 per group. Each column and vertical bar is multiplied by the value in parentheses. DEHA, di-2-ethylhexyl adipate; CO, control diet; GD, gestation day; PND, postnatal day. * $p<0.05$ vs. CO.

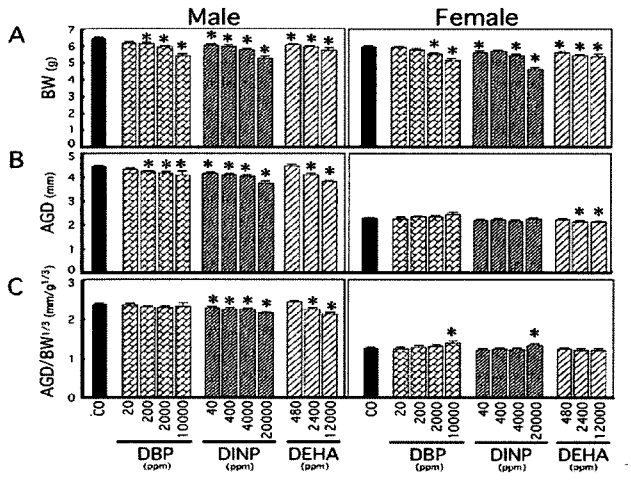

Fig. 4. Body weight (BW) (A), anogenital distance (AGD) (B) and AGD per cube root of BW ratio (C) at postnatal day 1 in male and female rats exposed to DBP, DINP and DEHA from gestational day 15 to postnatal day 1. Data represent mean±SE., n= 16-47 pups from five to six litters per group. CO, control diet; DBP, di-n-butyl phthalate; DINP, diisononyl phthalate; DEHA, di-2-ethylhexyl phthalate. * $p<0.05$ vs. CO.

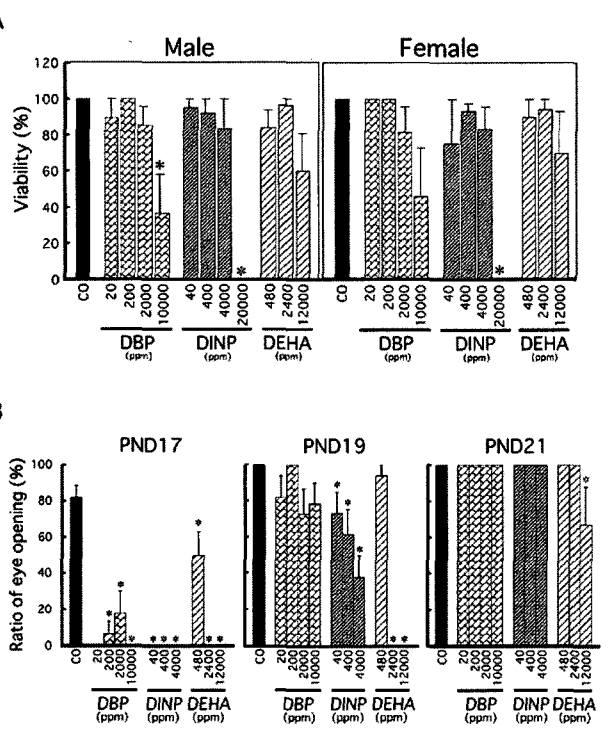

Fig. 5. Pups survival rate (A) from gestational day 15 to postnatal day 5 and ratio of eye opening (B) on PND 17, 19 and 21 after perinatal exposure to DBP, DINP and DEHA. Data represent mean ± SE. CO, control diet; DBP, di-n-butyl phthalate; DINP, diisononyl phthalate; DEHA, di-2-ethylhexyl phthalate. * $p<0.05$ vs. CO.

maternal body weight gain, in partially. However, the number of pups per litter at birth was similar, but ratio of pups surviving at PND 5 was significantly lower than control groups in 10,000 ppm of DBP and 20,000 ppm of DINP (males and both sexes, respectively). Although phthalates were rapidly metabolized and eliminated in animals and humans (Foster *et al.*, 1983; Group, 1986), it is generally accepted that fetuses or younger animals have greater susceptibilities to DBP-induced toxicity than adults (Dostal *et al.*, 1988; Foster *et al.*, 1988; Wellejus *et al.*, 2002). Moreover, phthalates have been shown in animal studies to cross the placenta and pass into breast milk (Parmar *et al.*, 1985; Dostal *et al.*, 1987; Srivastava *et al.*, 1987), so prenatal exposure and exposure from breastfeeding may occur in humans. Infants and young children consume more calories per kilogram of body weight, consume relatively more dairy and other fatty foods, and have higher minute ventilation than adults do, so dietary exposures and exposure from indoor air would be expected to be higher in infants and young children (Etzel and Balk, 1999).

Eye opening and AGD is developmental landmark for the physical development and the differentiation of the external genitalia. The eye opening may be controlled by thyroid hormones. The mechanisms by which thyroid hormones accelerate or delay normal development are complex. But, the effects of thyroid hormone depletion and enhancement were also evident in substantial differences observed for eye opening. For example, hypothyroidism typically results in a 1~2 day delay in eye opening (Bakke *et al.*, 1970; Davenport *et al.*, 1976),

Table 1. Male pup's body weight changes after gestational and lactational exposure to DBP

Day	CO	DBP (ppm)			
		20	200	2,000	10,000
PND 1	6.4±0.0 (70)	6.1±0.1 (21)	6.1 ±0.0 (28)*	5.8±0.0 (26) *	5.4±0.1 (13)*
PND 3	7.7±0.1 (39)	7.2±0.2 (20)	7.1 ±0.1 (28)	7.0±0.1 (25)*	6.9±0.1 (10)*
PND 5	10.2±0.3 (39)	9.0±0.4 (19)	9.4 ±0.3 (23)	9.3±0.2 (23)	8.4±0.1 (8)*
PND 7	14.1±0.6 (24)	12.3±0.7 (14)	11.62±0.6 (16)*	12.0±0.6 (15)	10.4±0.4 (8)*
PND 9	17.6±0.7 (24)	15.3±1.0 (14)	13.8 ±0.8 (16)*	15.4±0.8 (15)	12.5±0.5 (8)*
PND 11	22.3±0.9 (24)	18.7±1.4 (14)	16.6 ±1.2 (16)*	19.7±0.9 (14)	14.3±0.5 (8)*
PND 13	26.7±1.0 (24)	23.3±1.6 (13)	19.4 ±1.6 (16)*	23.2±1.1 (14)	16.2±0.7 (8)*
PND 15	30.9±1.0 (24)	26.7±1.9 (13)	22.8 ±1.9 (15)*	26.5±1.3 (14)	18.2±0.6 (8)*
PND 17	34.9±1.1 (24)	29.7±2.1 (13)	25.7 ±2.0 (15)*	29.4±1.5 (14)	20.3±0.8 (8)*
PND 19	38.7±1.0 (24)	32.1±2.3 (13)	28.3 ±2.1 (15)*	31.9±1.7 (14)	22.4±0.9 (8)*
PNW 3	43.1±1.1 (24)	37.3±2.8 (13)	32.0 ±2.4 (15)*	36.1±2.0 (14)	25.5±1.0 (8)*
PNW 4	76.0±2.3 (24)	63.6±4.0 (13)	56.5 ±4.2 (15)*	66.4±2.8 (14)	50.4±2.6 (8)*
PNW 5	131.0±3.5 (24)	113.8±7.0 (13)	102.6 ±6.7 (15)*	117.7±4.6 (14)	91.2±3.8 (8)*

Table 1. Continued

Day	CO	DBP (ppm)			
		20	200	2,000	10,000
PNW 6	187.8±4.8 (24)	165.6± 9.2 (13)	150.8± 8.4 (15)*	171.6± 6.0 (14)	139.3± 4.8 (8)*
PNW 7	250.2±5.7 (24)	227.8±12.2 (13)	209.7±11.6 (15)*	233.9± 8.2 (14)	189.5± 6.4 (8)*
PNW 8	303.3±6.2 (18)	265.1±13.6 (11)	258.3±12.8 (12)*	274.8± 9.2 (12)	234.1±10.0 (6)*
PNW 9	351.7±6.6 (18)	317.8±14.2 (11)	304.9±14.7 (12)*	326.8±10.3 (12)	293.2±11.1 (6)*
PNW 10	387.7±7.0 (18)	354.4±13.7 (11)	345.9±15.5 (12)	353.9±12.1 (12)	325.3±11.8 (6)*
PNW 11	423.3±7.8 (18)	392.1±11.5 (11)	378.8±14.7 (12)*	398.2±10.5 (12)	356.9±17.1 (6)*

The data represent the mean ± SE. Values in parentheses are the number of rats. DBP, di-n-butyl phthalate; PND, postnatal; PNW, postnatal weeks; The litter size was adjusted to eight for each group on PND 5.
* Significantly different from the control group ($p<0.05$).

Table 2. Female pup's body weight changes after gestational and lactational exposure to DBP

Day	CO	DBP (ppm)			
		20	200	2000	10000
PND 1	5.8±0.0 (76)	5.8±0.1 (25)	5.7±0.1 (21)	5.3±0.0 (26)*	5.2±0.1 (13)*
PND 3	7.2±0.1 (26)	6.9±0.2 (23)	6.7±0.1 (21)	6.4±0.1 (23)*	5.8±0.2 (12)*
PND 5	9.4±0.2 (26)	9.2±0.4 (21)	8.6±0.3 (18)	8.3±0.3 (23)	6.8±0.4 (9)*
PND 7	13.0±0.4 (16)	11.4±0.9 (15)	10.9±0.5 (16)	10.5±0.8 (14)*	8.5±0.5 (8)*
PND 9	17.0±0.5 (16)	14.7±1.1 (14)	13.4±0.7 (16)*	13.4±1.1 (14)*	10.1±0.7 (8)*
PND 11	21.7±0.6 (16)	18.1±1.5 (14)	16.5±0.9 (16)*	17.3±1.3 (14)*	12.0±0.9 (7)*
PND 13	26.3±0.7 (16)	21.5±1.9 (14)	19.4±1.1 (16)*	21.2±1.6 (13)	14.0±0.8 (6)*
PND 15	30.6±0.7 (16)	24.4±2.1 (14)*	22.7±1.3 (16)*	24.4±1.8 (13)*	15.4±1.1 (6)*
PND 17	34.6±0.9 (16)	27.1±2.3 (14)*	25.5±1.6 (16)*	27.0±2.0 (13)*	17.2±1.2 (6)*
PND 19	37.9±1.0 (16)	29.8±2.5 (14)*	27.7±1.7 (16)*	29.7±2.2 (13)*	18.9±1.3 (6)*
PNW 3	42.2±1.0 (16)	33.8±2.9 (14)*	31.0±1.9 (16)*	33.7±2.5 (13)*	21.5±1.4 (6)*
PNW 4	73.2±2.0 (16)	58.4±4.5 (14)*	55.6±3.1 (16)*	58.2±4.5 (13)*	41.8±2.7 (6)*
PNW 5	118.1±3.3 (16)	98.3±7.1 (14)	94.8±4.9 (16)*	97.9±8.0 (13)	76.3±5.0 (6)*
PNW 6	158.8±4.3 (16)	136.3±7.5 (14)	133.1±6.1 (16)	145.9±6.0 (12)	116.7±5.4 (6)*
PNW 7	188.2±4.6 (16)	171.1±7.5 (14)	164.6±6.8 (16)	179.2±5.8 (12)	151.1±5.6 (6)*
PNW 8	208.3±5.3 (12)	190.6±8.2 (12)	183.9±8.3 (12)	198.2±6.7 (10)	172.3±4.8 (6)*
PNW 9	232.6±6.2 (12)	211.8±7.1 (12)	208.3±8.2 (12)	219.0±6.8 (10)	199.9±5.4 (6)
PNW 10	249.0±6.6 (12)	233.7±7.3 (12)	225.8±8.1 (12)	237.8±6.8 (10)	220.4±5.0 (6)
PNW 11	265.0±7.8 (12)	248.0±6.3 (12)	245.7±8.3 (12)	254.7±8.3 (10)	238.9±4.8 (6)

The data represent the mean ± SE. Values in parentheses are the number of rats. DBP, di-n-butyl phthalate; PND, postnatal; PNW, postnatal weeks; The litter size was adjusted to eight for each group on PND 5.
* Significantly different from the control group ($p<0.05$).

Table 3. Male pup's body weight changes after gestational and lactational exposure to DINP

Day	CO	DINP (ppm)			
		40	400	4,000	20,000
PND 1	6.4±0.0 (70)	6.1± 0.1 (27)	5.8± 0.1 (30)*	5.4± 0.1 (26)*	5.4±0.1 (12)*
PND 3	7.7±0.1 (39)	7.0± 0.1 (26)*	6.6± 0.2 (30)*	6.5± 0.1 (25)*	5.8±0.1 (10)*
PND 5	10.2±0.3 (39)	8.5± 0.2 (26)*	9.0± 0.4 (22)*	8.9± 0.3 (15)	x
PND 7	14.1±0.6 (24)	11.4± 0.5 (16)*	10.6± 0.7 (14)*	11.0± 0.4 (15)*	x
PND 9	17.6±0.7 (24)	13.8± 0.6 (16)*	13.2± 0.9 (14)*	13.1± 0.5 (15)*	x
PND 11	22.3±0.9 (24)	16.3± 0.8 (16)*	16.1± 1.3 (14)*	16.0± 0.8 (15)*	x
PND 13	26.7±1.0 (24)	19.4± 1.1 (16)*	18.8± 1.8 (14)*	18.2± 1.2 (15)*	x
PND 15	30.9±1.0 (24)	22.6± 1.4 (16)*	21.5± 2.0 (14)*	20.6± 1.5 (15)*	x
PND 17	34.9±1.1 (24)	24.9± 1.6 (16)*	23.8± 2.3 (14)*	22.9± 1.8 (15)*	x
PND 19	38.7±1.0 (24)	27.3± 1.8 (16)*	26.4± 2.6 (14)*	24.8± 2.2 (15)*	x
PNW 3	43.1±1.1 (24)	31.7± 1.9 (16)*	30.5± 2.7 (14)*	29.7± 2.7 (15)*	x
PNW 4	76.0±2.3 (24)	57.6± 3.0 (16)*	54.4± 4.6 (14)*	52.7± 3.6 (15)*	x
PNW 5	131.0±3.5 (24)	100.1± 4.7 (16)*	95.9± 7.1 (14)*	95.4± 5.8 (15)*	x
PNW 6	187.8±4.8 (24)	151.4± 6.0 (16)*	144.2± 8.5 (14)*	144.9± 7.3 (15)*	x
PNW 7	250.2±5.7 (24)	208.5± 7.8 (16)*	199.1±11.2 (14)*	201.5± 9.4 (15)*	x
PNW 8	303.3±6.2 (18)	253.4±10.1 (12)*	239.8±12.4 (12)*	245.0±10.0 (12)*	x
PNW 9	351.7±6.6 (18)	311.5±10.5 (12)	294.2±12.0 (12)*	299.2± 9.2 (12)*	x
PNW 10	387.7±7.0 (18)	344.8±11.4 (12)	324.4±12.2 (12)*	326.8±10.0 (12)*	x
PNW 11	423.3±7.8 (18)	382.2±11.9 (12)	359.1±12.2 (12)*	356.9±10.3 (12)*	x

The data represent the mean ± SE. Values in parentheses are the number of rats. DINP, diisononyl phthalate; PND, postnatal; PNW, postnatal weeks; The litter size was adjusted to eight for each group on PND 5.
* Significantly different from the control group ($p<0.05$).

Table 4. Female pup's body weight changes after gestational and lactational exposure to DINP

Day	CO	DINP (ppm)			
		40	400	4,000	20,000
PND 1	5.8±0.0 (76)	5.6±0.1 (18)	5.6±0.1 (22)	5.2±0.1 (30)*	4.5±0.0 (5)*
PND 3	7.2±0.1 (26)	6.5±0.1 (18)	6.3±0.2 (22)*	5.9±0.1 (29)*	5.0±0.1 (2)*
PND 5	9.4±0.2 (26)	8.1±0.2 (17)*	7.7±0.3 (18)*	7.9±0.3 (21)*	x
PND 7	13.0±0.4 (16)	9.4±0.5 (12)*	9.8±0.6 (14)*	9.3±0.3 (17)*	x
PND 9	17.0±0.5 (16)	11.5±0.6 (12)*	12.1±0.7 (14)*	11.1±0.4 (17)*	x
PND 11	21.7±0.6 (16)	13.8±0.9 (11)*	14.5±1.2 (13)*	13.3±0.6 (17)*	x
PND 13	26.3±0.7 (16)	16.6±1.1 (11)*	16.8±1.6 (13)*	15.2±0.9 (17)*	x
PND 15	30.6±0.7 (16)	19.6±1.6 (11)*	20.1±1.9 (12)*	17.8±1.2 (17)*	x
PND 17	34.6±0.9 (16)	21.8±2.1 (11)*	22.4±2.2 (12)*	20.1±1.5 (17)*	x
PND 19	37.9±1.0 (16)	23.8±2.2 (11)*	25.2±2.5 (12)*	22.2±1.8 (17)*	x
PNW 3	42.2±1.0 (16)	27.7±2.3 (11)*	29.3±2.6 (12)*	25.8±2.1 (17)*	x

Table 4. Continued

Day	CO	DINP (ppm)			
		40	400	4,000	20,000
PNW 4	73.2±2.0 (16)	49.4±3.7 (11)*	51.1±3.7 (12)*	44.4±2.8 (17)*	x
PNW 5	118.1±3.3 (16)	85.6±5.6 (11)*	88.7±5.5 (12)*	78.3±4.2 (17)*	x
PNW 6	158.8±4.3 (16)	124.6±5.6 (11)*	126.2±5.5 (12)*	116.8±5.1 (17)*	x
PNW 7	188.2±4.6 (16)	158.3±6.1 (11)*	158.7±6.4 (12)*	148.5±6.1 (17)*	x
PNW 8	208.3±5.3 (12)	182.3±6.5 (9)	177.6±7.5 (12)*	170.7±7.6 (12)*	x
PNW 9	232.6±6.2 (12)	208.7±6.3 (9)	201.3±7.1 (12)*	196.2±7.8 (12)*	x
PNW 10	249.0±6.6 (12)	226.6±5.0 (9)	217.6±6.9 (12)*	210.9±6.9 (12)*	x
PNW 11	265.0±7.8 (12)	247.0±4.4 (9)	236.0±6.4 (12)*	228.3±6.6 (12)*	x

The data represent the mean ± SE. Values in parentheses are the number of rats. DINP, diisononyl phthalate; PND, postnatal; PNW, postnatal weeks; The litter size was adjusted to eight for each group on PND 5.
* Significantly different from the control group ($p<0.05$).

Table 5. Male pup's body weight changes after gestational and lactational exposure to DEHA

Day	CO	DEHA (ppm)		
		480	1,200	2,400
PND 1	6.4±0.0 (70)	6.1± 0.4 (27)	5.8± 0.1 (21)*	5.4± 0.1 (20)*
PND 3	7.7±0.1 (39)	7.0± 0.1 (25)*	7.2± 0.2 (20)	6.3± 0.1 (18)*
PND 5	10.2±0.3 (39)	8.8± 0.2 (23)*	9.2± 0.4 (20)	7.5± 0.2 (18)*
PND 7	14.1±0.6 (24)	11.4± 0.5 (16)*	11.0± 0.7 (15)*	8.8± 0.5 (11)*
PND 9	17.6±0.7 (24)	13.9± 0.8 (16)*	13.4± 1.0 (15)*	10.9± 0.7 (10)*
PND 11	22.3±0.9 (24)	17.6± 1.2 (15)*	16.5± 1.5 (15)*	13.0± 0.8 (10)*
PND 13	26.7±1.0 (24)	21.8± 1.4 (15)	19.4± 1.9 (15)*	15.5± 1.0 (10)*
PND 15	30.9±1.0 (24)	25.2± 1.5 (15)*	22.0± 2.2 (15)*	18.6± 1.4 (10)*
PND 17	34.9±1.1 (24)	28.3± 1.5 (15)*	24.3± 2.5 (15)*	21.7± 1.8 (10)*
PND 19	38.7±1.0 (24)	31.4± 1.4 (15)*	26.5± 2.8 (15)*	24.9± 2.2 (10)*
PNW 3	43.1±1.1 (24)	35.7± 1.6 (15)	30.7± 3.1 (15)*	28.0± 2.5 (10)*
PNW 4	76.0±2.3 (24)	63.3± 3.6 (15)*	54.1± 5.3 (15)*	44.3± 3.5 (10)*
PNW 5	131.0±3.5 (24)	107.8± 5.8 (15)*	95.0± 8.6 (15)*	79.3± 6.7 (10)*
PNW 6	187.8±4.8 (24)	158.6± 7.4 (15)*	142.3±10.7 (15)*	124.2± 9.2 (10)*
PNW 7	250.2±5.7 (24)	216.4± 9.9 (15)	200.1±14.6 (15)*	176.4±11.5 (10)*
PNW 8	303.3±6.2 (18)	259.4±12.6 (12)*	235.4±17.8 (12)*	207.1±14.7 (8)*
PNW 9	351.7±6.6 (18)	304.3±12.6 (12)*	293.0±17.2 (12)*	256.3±14.0 (8)*
PNW 10	387.7±7.0 (18)	343.3±13.1 (12)	325.1±17.9 (12)*	284.5±12.9 (8)*
PNW 11	423.3±7.8 (18)	378.8±14.1 (12)*	361.0±17.3 (12)*	319.0±11.4 (8)*

The data represent the mean ± SE. Values in parentheses are the number of rats. DEHA, di-2-ethylhexyl adipate; PND, postnatal; PNW, postnatal weeks; The litter size was adjusted to eight for each group on PND 5.
* Significantly different from the control group ($p<0.05$).

Table 6. Female pup's body weight changes after gestational and lactational exposure to DEHA

Day	CO	DEHA (ppm)		
		480	1,200	2,400
PND 1	5.8±0.0 (76)	5.7±0.0 (29)	5.2±0.0 (30)*	5.0±0.0 (22)*
PND 3	7.2±0.1 (26)	6.6±0.1 (27)	6.3±0.1 (28)*	5.7±0.1 (24)*
PND 5	9.4±0.2 (26)	8.2±0.2 (27)*	7.9±0.2 (25)*	7.0±0.1 (19)*
PND 7	13.0±0.4 (16)	10.7±0.4 (16)*	10.0±0.6 (16)*	8.1±0.5 (11)*
PND 9	17.0±0.5 (16)	13.3±0.6 (16)*	12.2±0.8 (16)*	9.6±0.7 (11)*
PND 11	21.7±0.6 (16)	16.8±0.9 (16)*	14.9±1.3 (16)*	11.6±0.9 (11)*
PND 13	26.3±0.7 (16)	20.4±1.1 (16)*	17.5±1.7 (16)*	14.0± 1.2 (11)*
PND 15	30.6±0.7 (16)	23.9±1.2 (16)*	20.0±2.0 (16)*	16.5±1.6 (11)*
PND 17	34.6±0.9 (16)	27.4±1.2 (16)*	22.1±2.3 (16)*	19.5±2.1 (11)*
PND 19	37.9±1.0 (16)	30.3±1.2 (16)*	24.0±2.5 (16)*	22.2±2.5 (11)*
PNW 3	42.2±1.0 (16)	34.1±1.3 (16)*	27.9±2.9 (16)*	24.6±2.7 (11)*
PNW 4	73.2±2.0 (16)	57.7±2.4 (16)*	47.5±4.6 (16)*	38.4±3.5 (11)*
PNW 5	118.1±3.3 (16)	96.9±3.6 (16)*	82.0±7.1 (16)*	69.1±6.9 (11)*
PNW 6	158.8±4.3 (16)	135.8±3.5 (16)	119.6±8.2 (16)*	102.9±8.6 (11)*
PNW 7	188.2±4.6 (16)	166.0±3.4 (16)	153.3±9.1 (16)*	139.1±9.0 (11)*
PNW 8	208.3±5.3 (12)	184.0±4.3 (12)	170.6±8.8 (12)*	157.6±8.9 (10)*
PNW 9	232.6±6.2 (12)	209.9±4.6 (12)	198.4±8.1 (12)*	182.6±8.8 (10)*
PNW 10	249.0±6.6 (12)	228.7±4.3 (12)	215.5±7.7 (12)*	199.5±8.1 (10)*
PNW 11	265.0±7.8 (12)	243.9±4.7 (12)	235.2±7.3 (12)*	216.7±7.5 (10)*

The data represent the mean ± SE. Values in parentheses are the number of rats. DEHA, di-2-ethylhexyl adipate; PND, postnatal; PNW, postnatal weeks; The litter size was adjusted to eight for each group on PND 5.
* Significantly different from the control group ($p<0.05$).

whereas, the administration of exogenous thyroid hormones accelerate the day of eye opening by 1~4 day (Schapiro, 1968; Davenport *et al.*, 1976). Although thyroid hormones is related to eye opening, the present study suggests that low body weight by perinatal exposure to these chemicals until weaning may also affect the eye opening.

On the other hand, AGD is commonly used as an androgen-sensitive parameter of sexual differentiation in rodents. Previous studies have shown that in utero exposure to some phthalates produces a malformation of the male reproductive tract including a decrease in AGD probably by reducing fetal testicular testosterone production (Mylchreest *et al.*, 1998; Shultz *et al.*, 2001; Barlow and Foster, 2003). Numerous studies have reported that the effects of phthalates are caused by an antiandrogenic mechanism resulting in leydig cell hyperplasia, gonocyte degeneration, malformations of the epididymis, seminal vesicles, and reduction of the AGD (Mylchreest *et al.*, 2002; Akingbemi *et al.*, 2004; Zhang *et al.*, 2004; Mahood *et al.*, 2005). In this study, although the we did not undertake a histological examination, the we not only observed that the AGDs on PND 1 were significantly decreased in the male pups but also the body weights significantly decreased in exposed groups compared to the control group. However, these significant reduction disappeared when AGD were normalized by the cube root of body weight. It has been proposed that the relationship between AGD and body weight should be more properly evaluated using the cube root of body weight (Wise *et al.*, 1991; Clark, 1999). In rats, it is known that pups body weight may vary widely as a function of litter size, neonatal toxicity. Wise *et al.* (1991) and Clark (1999) proposed that the relationship between AGD and body weight should be more properly evaluated using the cube root of body weight. This suggestion was based on the fact that, as an animal grows rapidly, growth occurs in three dimensions so that body weight can be viewed as a cubic measure.

In summary, perinatal exposure to DINP and DEHA indeed decreased AGD in a dose-dependent manner even after normalization by the cube root of body weight in male neonates, but the effect of DBP was no more discernible. Since AGD is commonly regarded as an androgen-sensitive developmental measure in rodents (Heinrichs, 1985), it is suggested the anti-androgenic effect of DINP and DEHA appears to be more potent than that of DBP at least under the present experimental conditions. Moreover, the present study suggests that delayed in eye opening may be due to the reducing the neonatal growth resulting in low body weight, as well as interfering the thyroid hormone receptor following perinatal exposure to these chemicals.

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