mesenteric lymph node), bladder, seminal vesicle, prostate, vagina, parathyroid, spinal cord, sciatic nerve, eye ball, Hardarian gland, sternum, and femur were removed. The numbers of corpora lutea and implantation sites were counted in all the main group females. The testes and epididymides were fixed with Bouin's solution and 90% alcohol. The eye ball was fixed with glutaraldehyde formalin and 20% neutral buffered formalin. Other organs were stored in 20% neutral buffered formalin. Histopathological evaluations were performed on these organs. The caudal epididymis was used for determination of the sperm motility, sperm viability, sperm morphology, and sperm count.

The right epididymal cauda was minced and stored for 5 min in Medium 199 containing 0.5% bovine serum albumin (BSA) at 37°C. This original sperm suspension was used for tests of sperm motility, viability, and morphology. The original sperm suspension was diluted with Medium 199 containing 0.5% BSA and incubated for about 30 min in 5% CO_2 in air at 37°C. After the incubation, the sperm samples were loaded into a chamber (Microslides #HTR1099; VitroCom, Mountain Lakes, NJ), and sperm motility was analyzed using a TOX IVOS (Hamilton Thorne Research, Beverly, MA). The original sperm sample was stained with calcein acetoxy methyl ester (CAM) and ethidium homodimer-1 (EthD-1) and incubated for about 60 min in 5% CO_2 in air. The population of viable and dead spermatozoa was identified by using a fluorescence microscopy according to Kato et al. (2002), and sperm viability and survivability rates were calculated. The original sperm suspension was mounted on a slide glass, fixed with the 10% neutral-buffered formalin and stained with 1% eosin. Sperm morphology was observed by using a microscope. The left epididymal cauda was stored at −80°C and homogenized with 0.1% Triton X-100. Number of sperm was determined with the TOX IVOS (Hamilton Thorne Research).

Urine samples were tested for colour, pH, protein, glucose, ketone body, bilirubin, occult blood, and urobilinogen. Urinary sediment was stained and examined microscopically. Urine volume was calculated from the specific gravity and weight. The collected blood samples were measured for the red blood cell (RBC) count, hemoglobin, platelet count, and white blood cell count. In addition, mean cell volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration, reticulocyte rate, and differential leukocyte rates were calculated. Prothrombin time (PT), activated partial thromboplastin time, and fibrinogen were determined. Blood chemistry was tested for aspartate aminotransferase, alkaline phosphatase (ALP), γ-glutamyl transpeptidase, total protein, albumin, albumin/globulin (A/G) ratio, total bilirubin, blood urea nitrogen (BUN), creatinine, glucose, total cholesterol, triglycerides, Na, K, Cl, Ca, and inorganic phosphate.

Daily vaginal lavage samples of each female were evaluated for estrous cyclicity throughout the premating period. Each female rat was mated overnight with a single male rat of the same dosage group until copulation occurred or the 2-week mating period had elapsed. During the mating period, daily vaginal smears were examined for the presence of sperm. The presence of sperm in the vaginal smear and/or a vaginal plug was considered as evidence for successful mating. Once insemination was confirmed, the females were checked for signs of parturition before 10:00 from day 21 of pregnancy. The females were allowed to deliver spontaneously and nurse their pups until postnatal day (PND) 6. The day on which parturition was completed by 10:00 was designated as PND 0. Litter size and numbers of live and dead pups were recorded, and live pups were sexed, and individually weighed on PNDs 0 and 4. Pups were inspected for external malformations and malformations within the oral cavity on PND 0. On PND 4, the pups were euthanized by exsanguination under anesthesia, and gross internal examinations were performed.

Data Analysis

The statistical analysis of pups was carried out using the litter as the experimental unit. Mean and standard deviation in each dose group were calculated for the body weight gain, food consumption, grip strength, spontaneous motor activity, urine volume, urinary specific gravity, hematological test results, blood biochemical test results, absolute and relative organ weights, sperm test results, numbers of estrous cases and days till copulation after pairing, length of gestation, numbers of corpora lutea and implantations, implantation index, total number of pups born, number of live and dead pups, sex ratio, delivery index, birth index, live birth index, live pups and viability index on day 4 of lactation, and body weight of pups. These were analyzed with Bartlett's test (Bartlett, 1937) for homogeneity of variance. If it was homogeneous, the data were analyzed using Dunnett's multiple comparison test (Dunnett, 1955) to compare the mean of the control group with that of each dosage group, and if it was not homogeneous, the Dunnett's rank test (Miller, 1981) was applied. The copulation index, fertility index, and gestation index were analyzed with Fisher's exact test (Fisher, 1973). The 5% levels of probability were used as the criterion for significance.

RESULTS

General Findings

Table I shows the general findings in male rats given dinoseb. No deaths were observed in any of the groups. Transient salivation was observed immediately after administration in one male in the 0.78 mg/kg bw/day dose group on day 14, in 11 males on day 8 or later in the 2.33 mg/kg bw/day dose group and in all 12 males on day 2 or later in the

TABLE I. General findings in male rats given dinoseb

Dose (mg/kg bw/day)	0 (Control)	0.78	2.33	7.0
No. of male rats	12	12	12	12
No. of deaths	0	0	0	0
Initial body weight (g)[a]	362 ± 13	363 ± 11	361 ± 13	360 ± 16
Body weight gain (g)				
The administration period				
Days 0–7	18 ± 9	23 ± 6	17 ± 8	7 ± 7**
Days 7–14	18 ± 7	18 ± 4	16 ± 6	14 ± 6
Days 14–21	18 ± 7	17 ± 5	17 ± 8	9 ± 10*
Days 21–28	22 ± 8	18 ± 6	21 ± 6	16 ± 7
Days 28–35	20 ± 4	22 ± 5	19 ± 5	19 ± 5
Days 35–41	10 ± 8	11 ± 6	11 ± 6	9 ± 8
The recovery period				
Days 0–7	13 ± 6	18 ± 4	14 ± 10	26 ± 7**
Days 7–14	6 ± 9	11 ± 3	13 ± 7	18 ± 3*
Food consumption (g/day/rat)				
The administration period				
Days 0–1	20 ± 2	20 ± 2	17 ± 3*	17 ± 2*
Days 3–4	21 ± 3	21 ± 2	20 ± 2	23 ± 2
Days 7–8	21 ± 3	21 ± 2	21 ± 3	23 ± 4
Days 10–11	20 ± 3	21 ± 2	21 ± 2	24 ± 2**
Days 28–29	21 ± 2	20 ± 2	21 ± 2	23 ± 3
Days 31–32	21 ± 2	21 ± 3	21 ± 2	24 ± 3**
Days 35–36	20 ± 2	21 ± 3	19 ± 2	23 ± 2*
Days 38–39	22 ± 2	21 ± 2	22 ± 3	24 ± 2*
The recovery period				
Days 0–1	22 ± 2 (6)	22 ± 2 (6)	21 ± 2 (6)	25 ± 2* (6)
Days 3–4	24 ± 2 (6)	24 ± 2 (6)	21 ± 2 (6)	25 ± 2* (6)
Day 7–8	26 ± 3 (6)	24 ± 3 (6)	25 ± 4 (6)	27 ± 2 (6)
Days 10–11	22 ± 3 (6)	25 ± 3 (6)	27 ± 2** (6)	27 ± 1** (6)

Figures in parentheses indicate number of males.
[a] Values are given as the mean ± SD.
* Significantly different from the control group ($p < 0.05$).
** Significantly different from the control group ($p < 0.01$).

7.0 mg/kg bw/day dose group. Body weight gain was significantly decreased on days 0–7 and 14–21 at 7.0 mg/kg bw/day during the administration period, and it was significantly increased at 7.0 mg/kg bw/day during the recovery period. During the administration period, food consumption was significantly low on days 0–1 at 2.33 and 7.0 mg/kg bw/day, and it was significantly higher than controls on days 10–11, 31–32, 35–36, and 38–39 at 7.0 mg/kg bw/day. During the recovery period, no significant changes were observed in general condition and body weight gain at 0.78 and 2.33 mg/kg bw/day. Food consumption was significantly higher than controls on recovery days 10–11 at 2.33 and 7.0 mg/kg bw/day and on recovery days 0–1 and 3–4 at 7.0 mg/kg bw/day.

Table II presents the general findings in the main group female rats given dinoseb. In the 7.0 mg/kg bw/day main group, seven animals died on gestation day 19 and one on gestation day 21, and one animal was moribund on each of gestation days 19 and 20. Transient salivation was observed immediately after administration in five females on day 10 or later in the 2.33 mg/kg bw/day dose group and in 11 females on day 3 or later in the 7.0 mg/kg bw/day dose group. During the gestation period, transient salivation was observed immediately after administration in three animals at 2.33 mg/kg bw/day and in all females at 7.0 mg/kg bw/day. In addition, females in the highest dose group that died or were moribund showed a decrease in spontaneous motor activity, prone position, low body temperature, dyspnea, cyanosis, and bradypnea. In the 7.0 mg/kg bw/day main group, body weight gain was significantly lowered on administration days 0–7 compared with controls. During the gestation and lactation, no significant change was observed in body weight gain in any of the dinoseb-treated groups. There was significantly higher food consumption compared with controls on days 7–8 of the premating period and on gestation days 1–2, 8–9, and 15–16 at 7.0 mg/kg bw/day in the main groups.

In females of the recovery groups, no deaths were observed. Food consumption was significantly increased during the administration period at 7.0 mg/kg bw/day and

TABLE II. General findings in female rats given dinoseb

Dose (mg/kg bw/day)	0 (Control)	0.78	2.33	7.0
No. of female rats	12	12	12	12
No. of deaths during premating period	0	0	0	0
No. of deaths during pregnancy	0	0	0	10
Initial body weight (g)[a]	251 ± 9	250 ± 8	250 ± 9	251 ± 9
Body weight gain (g)				
Days 0–7	12 ± 9	8 ± 11	10 ± 6	2 ± 5**
Days 7–14	12 ± 7	9 ± 6	11 ± 4	8 ± 7
Days 0–7 of gestation	35 ± 9 (11)	35 ± 6 (11)	34 ± 6	37 ± 6
Days 7–14 of gestation	34 ± 4 (11)	34 ± 2 (11)	37 ± 4	38 ± 5
Days 14–21 of gestation	91 ± 12 (11)	79 ± 32 (11)	85 ± 13	65 (2)
Days 0–6 of lactation	6 ± 11 (11)	10 ± 9 (10)	14 ± 11	9 (1)
Food consumption (g/day/rat)				
Days 0–1	13 ± 2	15 ± 3	15 ± 1	14 ± 2
Days 3–4	15 ± 3	16 ± 4	15 ± 2	16 ± 3
Days 7–8	13 ± 2	14 ± 3	13 ± 3	17 ± 3**
Days 10–11	16 ± 4	16 ± 3	17 ± 3	18 ± 3
Days 1–2 of gestation	18 ± 2	18 ± 2	18 ± 1	22 ± 3**
Days 8–9 of gestation	19 ± 2	19 ± 2	19 ± 2	23 ± 2**
Days 15–16 of gestation	19 ± 2	20 ± 2	21 ± 2	23 ± 3*
Days 15–16 of gestation	19 ± 2	17 ± 5	20 ± 3	19 ± 5 (4)
Days 1–2 of lactation	23 ± 3	22 ± 4	21 ± 4	13 (1)

Figures in parentheses indicate number of dams.
[a] Values are given as the mean ± SD.
* Significantly different from the control group ($p < 0.05$).
** Significantly different from the control group ($p < 0.01$).

during the recovery period at 2.33 mg/kg bw/day and above (data not shown).

Functional Observation

No treatment-related effects on the detailed clinical observations, FOB, sensory reactivity such as pupillary reflex, approximation reflex, behavioural abnormality, tactile reflex, auditory reflex and pain reflex, grip strength, and spontaneous motor activity were observed in males and females in the test and recovery groups (data not shown).

Urinalysis

Urinalysis revealed no significant differences in any parameters between the control and dinoseb-treated groups in males and females in the test and recovery groups (data not shown).

Hematology

Table III shows hematological findings in male and female rats. In males of the main groups, RBC count was significantly higher at 0.78 mg/kg bw/day and hematocrit was significantly higher at 0.78 mg/kg bw/day and above. Hemoglobin and PT were significantly higher than controls at 2.33 and 7.0 mg/kg bw/day. In the 7.0 mg/kg bw/day dose group, MCV was significantly higher than controls. As for females in the main groups, RBC count was significantly higher, and MCV and MCH were significantly lower at 0.78 mg/kg bw/day. Fibrinogen was significantly lower at 2.33 mg/kg bw/day. RBC count was significantly lower, and MCV was significantly higher in the 7.0 mg/kg bw/day recovery dose group males.

Blood Biochemistry

Blood biochemical findings are shown in Table IV. Total cholesterol was significantly lower at 2.33 mg/kg bw/day, and creatinine was significantly higher at 7.0 mg/kg bw/day in the main group males. Albumin and A/G ratio were significantly higher in females of the main groups at 2.33 mg/kg bw/day. In the recovery group males, A/G ratio and Cl were significantly lower at 0.78 mg/kg bw/day. A/G ratio was significantly lower, and total cholesterol was significantly higher at 2.33 mg/kg bw/day. In the 7.0 mg/kg bw/day group, ALP and albumin were significantly lower and BUN was significantly higher.

Organ Weight

Table V shows organ weight of male and female rats given dinoseb. At 7.0 mg/kg bw/day, relative brain weight was significantly higher in the main group males. At the end of

TABLE III. Hematological findings in male and female rats given dinoseb

	Main Group				Recovery Group			
Dose (mg/kg bw/day)	0 (Control)	0.78	2.33	7.0	0 (Control)	0.78	2.33	7.0
No. of male rats	6	6	6	6	6	6	6	6
RBC ($10^4/\mu L$)[a]	801 ± 13	844 ± 30*	833 ± 31	834 ± 29	858 ± 21	831 ± 22	852 ± 38	817 ± 22*
Hemoglobin (g/dL)	14.9 ± 0.5	15.5 ± 0.7	15.7 ± 0.5*	16.2 ± 0.5**	15.9 ± 0.4	15.3 ± 0.4	15.6 ± 1.1	15.8 ± 0.3
Hematocrit (%)	43.9 ± 0.8	46.4 ± 1.9*	46.6 ± 1.4**	47.7 ± 1.2**	46.8 ± 0.9	45.2 ± 1.2	46.6 ± 3.4	46.0 ± 1.0
MCV (fL)	54.8 ± 1.3	55.0 ± 1.1	56.0 ± 1.6	57.2 ± 1.5*	54.5 ± 0.8	54.4 ± 1.5	54.6 ± 1.8	56.3 ± 1.0
MCH (pg)	18.6 ± 0.7	18.4 ± 0.6	18.8 ± 0.6	19.4 ± 0.7	18.6 ± 0.5	18.4 ± 0.6	18.3 ± 0.7	19.3 ± 0.4*
MCHC (g/dL)	33.8 ± 0.5	33.5 ± 0.7	33.6 ± 0.4	33.9 ± 0.7	34.0 ± 0.4	33.9 ± 0.6	33.6 ± 0.4	34.3 ± 0.4
Platelet count ($10^4/\mu L$)	91.2 ± 10.2	95.0 ± 15.4	88.1 ± 11.9	78.2 ± 7.5	91.2 ± 10.2	95.0 ± 15.4	88.1 ± 11.9	78.2 ± 7.5
Reticulocyte (‰)	28 ± 3	24 ± 3	25 ± 4	24 ± 3	24 ± 6	23 ± 4	24 ± 6	22 ± 3
PT (s)	13.2 ± 0.3	13.6 ± 0.5	13.8 ± 0.3*	14.1 ± 0.4**	19.1 ± 3.1	19.5 ± 3.5	19.9 ± 3.6	16.3 ± 1.5
APTT (s)	27.6 ± 1.1	29.8 ± 1.6	28.3 ± 3.2	28.2 ± 1.8	37.1 ± 4.0	37.0 ± 2.2	38.2 ± 1.8	34.2 ± 3.2
Fibrinogen (mg/dL)	220 ± 10	221 ± 23	224 ± 16	207 ± 10	220 ± 10	221 ± 23	224 ± 16	207 ± 10
WBC ($10^2/\mu L$)	78 ± 22	61 ± 14	66 ± 23	55 ± 16	61 ± 16	54 ± 22	76 ± 20	49 ± 10
Differential leukocyte (%)								
Lymphocyte	94.7 ± 2.3	91.2 ± 2.9	91.7 ± 2.1	90.8 ± 5.9	90.2 ± 2.8	90.8 ± 4.2	92.2 ± 4.2	88.7 ± 7.2
Neutrophil	4.5 ± 1.8	7.8 ± 2.1	7.7 ± 1.5	8.5 ± 5.5	9.0 ± 3.0	8.2 ± 4.7	6.8 ± 3.9	10.0 ± 6.8
Eosinophil	0.5 ± 0.5	0.5 ± 0.8	0.3 ± 0.5	0.5 ± 0.5	0.3 ± 0.5	0.5 ± 0.8	0.5 ± 0.5	0.7 ± 0.5
Basophil	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte	0.3 ± 0.5	0.5 ± 0.5	0.3 ± 0.5	0.2 ± 0.4	0.5 ± 0.5	0.5 ± 0.5	0.5 ± 0.5	0.7 ± 0.5
No. of female rats	6	6	6	1	6	6	6	6
RBC ($10^4/\mu L$)	702 ± 13	749 ± 25*	735 ± 41	725	739 ± 21	751 ± 45	758 ± 30	753 ± 26
Hemoglobin (g/dL)	14.4 ± 0.4	14.7 ± 0.5	14.8 ± 0.7	15.2	14.6 ± 0.4	14.8 ± 0.8	14.8 ± 0.5	14.9 ± 0.4
Hematocrit (%)	41.8 ± 1.1	42.8 ± 1.1	42.8 ± 1.9	44.6	41.4 ± 1.1	42.1 ± 2.1	42.3 ± 1.6	42.4 ± 1.5
MCV (fL)	59.5 ± 2.1	57.1 ± 1.1*	58.2 ± 1.2	61.5	56.0 ± 0.9	56.1 ± 1.4	55.8 ± 1.6	56.3 ± 0.6
MCH (pg)	20.6 ± 0.7	19.7 ± 0.2*	20.1 ± 0.6	21.0	19.7 ± 0.5	19.7 ± 0.6	19.4 ± 0.7	19.8 ± 0.4
MCHC (g/dL)	34.6 ± 0.6	34.4 ± 0.4	34.5 ± 0.5	34.1	35.2 ± 0.6	35.2 ± 0.3	34.8 ± 0.3	35.1 ± 0.5
Platelet count ($10^4/\mu L$)	105.2 ± 8.4	115.9 ± 9.9	112.6 ± 11.9	109.5	96.1 ± 13.0	94.9 ± 5.4	94.2 ± 11.4	90.3 ± 2.7
Reticulocyte (‰)	77 ± 16	64± 15	71 ± 9	52	27 ± 3	25 ± 5	24 ± 5	23 ± 3
PT (s)	13.5 ± 0.4	13.7 ± 0.5	13.3 ± 0.3	13.3	13.5 ± 0.6	13.1 ± 0.2	13.4 ± 0.4	13.2 ± 0.3
APTT (s)	25.1 ± 2.0	23.9 ± 2.1	23.6 ± 2.6	25.8	23.9 ± 1.6	22.9 ± 1.4	23.2 ± 1.2	24.4 ± 1.4
Fibrinogen (mg/dL)	257 ± 21	255 ± 28	217 ± 19*	210	177 ± 20	179 ± 13	180 ± 7	188 ± 29
WBC ($10^2/\mu L$)	49 ± 14	57 ± 10	54 ± 16	74	32 ± 15	39 ± 11	38 ± 10	37 ± 14
Differential leukocyte (%)								
Lymphocyte	86.7 ± 8.0	87.2 ± 5.6	89.0 ± 5.2	75.0	90.3 ± 3.7	89.8 ± 4.3	87.5 ± 5.5	88.7 ± 4.4
Neutrophil	12.0 ± 7.6	11.8 ± 5.5	9.5 ± 4.4	23.0	9.0 ± 3.8	9.2 ± 3.9	11.2 ± 5.3	10.3 ± 4.7
Eosinophil	0.5 ± 0.5	0.2 ± 0.4	0.5 ± 0.8	0.0	0.3 ± 0.5	0.7 ± 0.8	0.7 ± 0.8	0.7 ± 0.5
Basophil	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte	0.8 ± 0.8	0.8 ± 0.8	1.0 ± 0.6	2.0	0.3 ± 0.5	0.3 ± 0.5	0.7 ± 0.8	0.3 ± 0.5

[a] Values are given as the mean ± SD.
* Significantly different from the control group ($p < 0.05$).
** Significantly different from the control group ($p < 0.01$).

the recovery period, only dose independent changes were found as follows. Relative brain weight was significantly lower and absolute liver weight was significantly higher at 0.78 mg/kg bw/day in males. In females, relative heart weight was significantly lower at 0.78 mg/kg bw/day. In addition, relative brain weight was significantly lower than controls at 2.33 mg/kg bw/day, and absolute heart weight was significantly higher than controls at 7.0 mg/kg bw/day. Absolute and relative weights of the testes, epididymides, ovaries, and uterus showed no significant difference from controls in any dinoseb-treated groups.

Necropsy and Histopathological Findings

Extramedullary hematopoiesis in the spleen was observed in six animals in the control female group with severity ranging from slight to moderate. Reduction in extramedullary hematopoiesis in the spleen was significant at 2.33 mg/kg bw/day in the main group females with severity ranging from slight to mild. At 7.0 mg/kg bw/day, one surviving female showed slight extramedullary hematopoiesis in the spleen, but the other surviving female did not show splenic extramedullary hematopoiesis. No histopathological

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TABLE IV. Blood biochemical findings in male and female rats given dinoseb

	Main Group				Recovery Group			
Dose (mg/kg bw/day)	0 (control)	0.78	2.33	7.0	0 (control)	0.78	2.33	7.0
No. of male rats	6	6	6	6	6	6	6	6
AST (IU/L)[a]	85.3 ± 19.0	85.9 ± 17.7	93.5 ± 20.1	91.3 ± 7.3	120.7 ± 44.1	115.1 ± 29.7	103.2 ± 24.4	102.7 ± 16.8
ALT (IU/L)	37.6 ± 6.7	34.3 ± 3.3	37.8 ± 5.5	40.1 ± 4.6	39.0 ± 13.8	35.9 ± 9.8	34.9 ± 12.1	29.9 ± 4.9
ALP (IU/L)	584.0 ± 91.4	641.4 ± 106.4	651.0 ± 166.2	950.3 ± 338.4	373.8 ± 56.4	310.4 ± 53.7	304.9 ± 77.7	256.2 ± 56.9**
γ-GTP (IU/L)	0.34 ± 0.15	0.46 ± 0.19	0.53 ± 0.20	0.53 ± 0.11	0.41 ± 0.22	0.29 ± 0.21	0.27 ± 0.12	0.39 ± 0.08
Total protein (g/dL)	5.8 ± 0.2	5.9 ± 0.2	5.8 ± 0.2	5.6 ± 0.2	5.8 ± 0.2	5.8 ± 0.2	5.8 ± 0.4	5.4 ± 0.1
Albumin (g/dL)	2.95 ± 0.16	2.92 ± 0.12	2.91 ± 0.07	2.98 ± 0.15	2.93 ± 0.19	2.75 ± 0.12	2.84 ± 0.16	2.71 ± 0.07*
A/G ratio	1.04 ± 0.09	0.99 ± 0.03	1.00 ± 0.05	1.14 ± 0.10	1.04 ± 0.07	0.91 ± 0.05**	0.96 ± 0.05*	1.00 ± 0.04
Total bilirubin (mg/dL)	0.07 ± 0.02	0.06 ± 0.03	0.07 ± 0.04	0.05 ± 0.03	0.13 ± 0.03	0.12 ± 0.04	0.14 ± 0.02	0.11 ± 0.02
BUN (mg/dL)	17.7 ± 3.7	16.6 ± 1.3	16.4 ± 1.8	20.2 ± 1.3	15.3 ± 1.6	16.9 ± 2.2	16.7 ± 2.3	18.5 ± 2.1*
Creatinine (mg/dL)	0.27 ± 0.04	0.28 ± 0.02	0.28 ± 0.04	0.34 ± 0.04*	0.26 ± 0.03	0.30 ± 0.06	0.29 ± 0.04	0.29 ± 0.01
Glucose (mg/dL)	118.4 ± 7.3	119.6 ± 10.3	120.1 ± 9.2	112.7 ± 9.1	115.6 ± 15.2	121.4 ± 17.2	114.0 ± 22.5	102.7 ± 7.1
Total cholesterol (mg/dL)	68.0 ± 5.4	61.0 ± 4.4	56.7 ± 9.0*	60.6 ± 6.4	45.9 ± 10.0	60.6 ± 8.8	61.3 ± 10.6*	58.8 ± 11.2
Triglyceride (mg/dL)	63.4 ± 9.7	55.9 ± 21.3	62.8 ± 13.3	56.8 ± 15.4	37.5 ± 25.6	35.7 ± 17.7	36.9 ± 13.5	29.2 ± 10.7
Na (mEq/L)	144.5 ± 1.1	144.0 ± 1.3	144.3 ± 1.2	145.0 ± 0.7	144.8 ± 0.7	144.4 ± 0.7	145.2 ± 1.3	145.5 ± 0.9
K (mEq/L)	4.34 ± 0.27	4.35 ± 0.33	4.46 ± 0.29	4.12 ± 0.18	4.02 ± 0.34	4.23 ± 0.25	4.28 ± 0.22	4.16 ± 0.19
Cl (mEq/L)	105.8 ± 1.6	105.4 ± 1.0	105.1 ± 1.6	026.1 ± 1.1	106.8 ± 0.7	105.1 ± 1.4*	105.8 ± 0.9	106.9 ± 1.1
Ca (mg/dL)	9.9 ± 0.3	9.7 ± 0.3	9.6 ± 0.3	9.5 ± 0.3	9.4 ± 0.3	9.4 ± 0.3	9.5 ± 0.1	9.3 ± 0.2
Inorganic phosphate (mg/dL)	6.2 ± 1.3	6.2 ± 0.9	7.0 ± 0.6	5.7 ± 0.9	7.0 ± 0.5	7.3 ± 0.9	7.3 ± 0.7	7.4 ± 0.6
No. of female rats	6	6	6	1	6	6	6	6
AST (IU/L)	90.0 ± 16.4	98.7 ± 29.3	94.4 ± 21.4	78.4	86.0 ± 17.0	91.7 ± 11.7	85.8 ± 11.1	92.5 ± 11.2
ALT (IU/L)	23.8 ± 2.2	25.3 ± 3.9	26.6 ± 5.1	32.4	23.2 ± 3.9	26.5 ± 6.0	22.8 ± 1.6	26.2 ± 5.9
ALP (IU/L)	192.6 ± 46.8	178.8 ± 48.0	202.2 ± 23.1	194.7	129.3 ± 39.4	122.4 ± 17.5	135.5 ± 27.6	169.8 ± 49.1
γ-GTP (IU/L)	0.45 ± 0.12	0.65 ± 0.23	0.49 ± 0.17	0.55	0.46 ± 0.25	0.58 ± 0.25	0.59 ± 0.21	0.66 ± 0.26
Total protein (g/dL)	6.5 ± 0.2	6.4 ± 0.3	6.7 ± 0.5	6.1	6.5 ± 0.5	6.5 ± 0.3	6.2 ± 0.2	6.2 ± 0.4
Albumin (g/dL)	3.19 ± 0.15	3.16 ± 0.20	3.48 ± 0.25*	3.29	3.61 ± 0.36	3.59 ± 0.26	3.40 ± 0.16	3.32 ± 0.30
A/G ratio	0.98 ± 0.05	0.98 ± 0.07	1.10 ± 0.02*	1.17	1.24 ± 0.08	1.25 ± 0.11	1.23 ± 0.08	1.18 ± 0.09
Total bilirubin (mg/dL)	0.11 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.05	0.10 ± 0.03	0.11 ± 0.03	0.10 ± 0.03	0.12 ± 0.03
BUN (mg/dL)	20.5 ± 2.5	18.9 ± 4.4	23.5 ± 1.9	18.4	18.2 ± 1.4	16.1 ± 1.7	18.1 ± 2.5	18.0 ± 1.6
Creatinine (mg/dL)	0.39 ± 0.01	0.38 ± 0.05	0.40 ± 0.01	0.44	0.37 ± 0.02	0.34 ± 0.04	0.39 ± 0.02	0.36 ± 0.05
Glucose (mg/dL)	127.2 ± 13.2	129.0 ± 14.3	123.3 ± 13.7	130.7	120.7 ± 20.5	123.2 ± 9.8	126.3 ± 11.4	119.2 ± 15.1
Total cholesterol (mg/dL)	72.2 ± 5.5	81.9 ± 20.7	70.3 ± 9.3	86.4	81.5 ± 8.4	87.0 ± 10.8	75.7 ± 11.2	84.4 ± 17.4
Triglyceride (mg/dL)	41.8 ± 13.7	56.9 ± 24.5	54.3 ± 16.7	82.4	25.7 ± 5.4	38.9 ± 22.7	42.7 ± 29.2	22.7 ± 6.4
Na (mEq/L)	141.1 ± 1.0	140.7 ± 0.8	140.5 ± 0.9	142.2	143.6 ± 0.9	142.9 ± 1.4	142.7 ± 1.3	144.2 ± 0.5
K (mEq/L)	4.23 ± 0.32	4.14 ± 0.34	4.08 ± 0.31	3.38	4.04 ± 0.27	4.16 ± 0.17	3.98 ± 0.25	3.83 ± 0.26
Cl (mEq/L)	104.6 ± 1.8	104.3 ± 0.9	104.3 ± 2.1	104.9	108.0 ± 1.3	107.1 ± 1.5	107.0 ± 1.0	107.2 ± 1.7
Ca (mg/dL)	10.9 ± 0.3	10.8 ± 0.3	10.7 ± 0.5	10.4	9.6 ± 0.3	9.7 ± 0.2	9.5 ± 0.2	9.5 ± 0.3
Inorganic phosphate (mg/dL)	7.8 ± 1.2	7.6 ± 0.8	6.8 ± 1.1	5.6	4.3 ± 0.7	4.4 ± 0.7	4.3 ± 0.6	4.7 ± 0.4

[a] Values are given as the mean ± SD.

* Significantly different from the control group ($p < 0.05$).

** Significantly different from the control group ($p < 0.01$).

TABLE V. Organ weight of male and female rats given dinoseb

Dose (mg/kg bw/day)		Main Group				Recovery Group			
		0 (Control)	0.78	2.33	7.0	0 (Control)	0.78	2.33	7.0
No. of male rats		6	6	6	6	6	6	6	6
Body weight	(g)[a]	451 ± 19	449 ± 27	438 ± 42	409 ± 29	458 ± 22	474 ± 19	461 ± 29	461 ± 20
Brain	(g)[b]	2.04 ± 0.05	2.02 ± 0.04	2.07 ± 0.04	2.04 ± 0.06	2.12± 0.08	2.02 ± 0.10	2.09 ± 0.09	2.03 ± 0.13
	(g%)[c]	0.45 ± 0.02	0.45 ± 0.02	0.48 ± 0.05	0.50 ± 0.03*	0.46 ± 0.02	0.43 ± 0.02*	0.46 ± 0.03	0.44 ± 0.02
Pituitary	(mg)	12.7 ± 1.1	13.3 ± 1.9	13.2 ± 1.0	11.7 ± 1.1	14.3 ± 1.2	15.1 ± 1.5	13.6 ± 0.8	15.4 ± 1.0
	(mg%)	2.8 ± 0.2	3.0 ± 0.5	3.0 ± 0.3	2.9 ± 0.4	3.1 ± 0.2	3.2 ± 0.2	3.0 ± 0.2	3.4 ± 0.2
Thyroids	(mg)	20.7 ± 5.6	19.1 ± 6.4	23.0 ± 3.1	20.3 ± 3.5	18.5 ± 2.8	22.1 ± 4.2	20.6 ± 2.0	21.1 ± 4.5
	(mg%)	4.6 ± 1.3	4.3 ± 1.6	5.3 ± 0.6	5.0 ± 1.0	4.0 ± 0.6	4.7 ± 0.7	4.5 ± 0.4	4.6 ± 1.0
Thymus	(mg)	367 ± 126	260 ± 76	296 ± 120	245 ± 54	268 ± 41	316 ± 90	265 ± 86	296 ± 64
	(mg%)	82 ± 30	58 ± 16	67 ± 22	60 ± 14	59 ± 10	66 ± 17	58 ± 18	65 ± 15
Heart	(g)	1.41 ± 0.14	1.40 ± 0.07	1.37 ± 0.14	1.35 ± 0.11	1.52 ± 0.19	1.56 ± 0.09	1.62 ± 0.28	1.55 ± 0.25
	(g%)	0.31 ± 0.02	0.31 ± 0.01	0.31 ± 0.02	0.33 ± 0.02	0.33 ± 0.05	0.33 ± 0.03	0.35 ± 0.05	0.33 ± 0.04
Liver	(g)	15.98 ± 1.00	15.65 ± 1.04	15.49 ± 1.93	15.57 ± 1.02	10.44 ± 1.36	11.87 ± 0.72*	11.48 ± 1.09	11.17 ± 0.49
	(g%)	3.55 ± 0.21	3.49 ± 0.18	3.54 ± 0.28	3.81 ± 0.18	2.88 ± 0.24	2.51 ± 0.17	2.50 ± 0.27	2.42 ± 0.05
Spleen	(mg)	763 ± 110	791 ± 77	704 ± 61	682 ± 111	774 ± 70	696 ± 93	785 ± 208	780 ± 80
	(mg%)	170 ± 30	177 ± 17	162 ± 20	166 ± 16	170 ± 21	147 ± 20	173 ± 58	169 ± 13
Kidneys	(g)	2.91 ± 0.19	2.97 ± 0.15	2.96 ± 0.19	2.82 ± 0.19	3.10 ± 0.51	2.86± 0.07	2.87 ± 0.24	2.92 ± 0.15
	(g%)	0.65 ± 0.05	0.66 ± 0.05	0.68 ± 0.07	0.69 ± 0.05	0.68 ± 0.11	0.60 ± 0.03	0.62 ± 0.04	0.64 ± 0.02
Adrenals	(mg)	50.7 ± 8.3	57.4 ± 8.6	54.8 ± 6.3	58.8 ± 11.2	55.6 ± 7.8	52.4 ± 6.8	53.2 ± 9.9	61.4 ± 7.3
	(mg%)	11.3 ± 2.1	12.8 ± 2.1	12.5 ± 1.2	14.4 ± 2.8	12.2 ± 1.8	11.0 ± 1.2	11.5 ± 1.8	13.3 ± 1.5
Testes	(g)	3.11 ± 0.35	3.18 ± 0.42	3.36 ± 0.18	3.33 ± 0.30	3.29 ± 0.34	3.30 ± 0.40	3.25 ± 0.28	3.36 ± 0.27
	(g%)	0.69 ± 0.10	0.71 ± 0.11	0.78 ± 0.10	0.82 ± 0.06	0.72 ± 0.08	0.70 ± 0.09	0.71 ± 0.05	0.73 ± 0.06
Epididymides	(mg)	1120 ± 71	1170 ± 100	1158 ± 74	1150 ± 88	1250 ± 135	1255 ± 101	1222 ± 80	1224 ± 101
	(mg%)	249 ± 23	262 ± 28	267 ± 29	281 ± 14	274 ± 31	265 ± 25	265 ± 8	265 ± 17
No. of female rats		6	6	6	2	6	6	6	6
Body weight	(g)	301 ± 12	298 ± 14	301 ± 11	295	297 ± 26	306 ± 8	327 ± 13**	310 ± 9
Brain	(g)	1.97 ± 0.08	1.95 ± 0.05	2.01 ± 0.08	1.98	1.99 ± 0.09	1.91 ± 0.09	1.95 ± 0.08	1.99 ± 0.02
	(g%)	0.66 ± 0.03	0.66 ± 0.03	0.67 ± 0.03	0.68	0.67 ± 0.08	0.63 ± 0.03	0.60 ± 0.03*	0.64 ± 0.02
Pituitary	(mg)	16.7 ± 2.8	16.2 ± 2.1	16.7 ± 2.3	15.8	18.9 ± 3.18	20.1 ± 3.0	18.9 ± 3.7	18.3 ± 2.6
	(mg%)	5.5 ± 0.8	5.5 ± 0.7	5.6 ± 0.9	5.3	6.5 ± 1.5	6.6 ± 1.1	5.8 ± 1.3	5.9 ± 0.9
Thyroids	(mg)	16.8 ± 2.4	16.5 ± 3.6	17.7 ± 4.0	17.4	20.0 ± 3.9	20.2 ± 1.9	18.0 ± 3.0	22.7 ± 4.4
	(mg%)	5.6 ± 0.8	5.6 ± 1.2	5.9 ± 1.3	5.9	6.8 ± 1.6	6.6 ± 0.7	5.5 ± 1.1	7.4 ± 1.5
Thymus	(mg)	234 ± 48	266 ± 73	292 ± 77	225	284 ± 59	273 ± 29	282 ± 75	303 ± 65
	(mg%)	78 ± 17	90 ± 25	97 ± 26	76	96 ± 19	89 ± 8	86 ± 22	98 ± 21
Heart	(g)	1.03 ± 0.08	0.99 ± 0.08	1.00 ± 0.05	1.03	0.95 ± 0.06	0.90 ± 0.03	1.00 ± 0.05	1.04 ± 0.07**
	(g%)	0.34 ± 0.03	0.33 ± 0.02	0.33 ± 0.02	0.35	0.32 ± 0.03	0.29 ± 0.01*	0.31 ± 0.01	0.34 ± 0.02
Liver	(g)	9.92 ± 0.90	9.66 ± 0.92	9.84 ± 0.55	10.70	7.33 ± 0.67	7.62 ± 0.59	8.03 ± 0.66	7.95 ± 0.59
	(g%)	3.29 ± 0.26	3.24 ± 0.21	3.27 ± 0.15	3.64	2.47 ± 0.15	2.49 ± 0.14	2.46 ± 0.24	2.57 ± 0.13
Spleen	(mg)	690 ± 79	679 ± 145	719 ± 100	606	537 ± 86	522 ± 93	560 ± 69	593 ± 28
	(mg%)	229 ± 26	227 ± 42	239 ± 31	203	182 ± 31	170 ± 28	172 ± 28	192 ± 8
Kidneys	(g)	2.14 ± 0.15	2.05 ± 0.20	2.04 ± 0.11	1.99	1.94 ± 0.09	1.98 ± 0.07	1.95 ± 0.16	2.01 ± 0.19
	(g%)	0.71 ± 0.06	0.69 ± 0.05	0.68 ± 0.04	0.67	0.66 ± 0.06	0.65 ± 0.02	0.60 ± 0.05	0.65 ± 0.05
Adrenals	(mg)	74.6 ± 7.6	70.0 ± 10.4	69.9 ± 7.2	83.5	72.9 ± 6.1	62.1 ± 12.2	71.1 ± 6.2	70.5 ± 11.1
	(mg%)	24.9 ± 3.1	23.5 ± 2.9	23.2 ± 2.1	28.6	24.7 ± 3.1	20.3 ± 3.9	21.8 ± 2.5	22.7 ± 3.1
Ovaries	(mg)	110.0 ± 15.3	100.9 ± 8.3	102.2 ± 8.9	120.1	82.9 ± 20.3	79.9 ± 10.0	91.9 ± 14.9	96.3 ± 4.2
	(mg%)	36.6 ± 4.8	34.0 ± 3.2	34.0 ± 2.9	40.9	27.7 ± 4.8	26.1 ± 3.0	28.2 ± 4.7	31.1 ± 0.9
Uterus	(mg)	605 ± 165	570 ± 123	631 ± 135	2133	672 ± 139	622 ± 174	706 ± 168	576 ± 161
	(mg%)	202 ± 58	193 ± 46	210 ± 47	742	229 ± 59	203 ± 55	216 ± 49	186 ± 48

[a] Values are given as the mean ± SD.
[b] Absolute organ weight.
[c] Relative organ weight.
* Significantly different from the control group ($p < 0.05$).
** Significantly different from the control group ($p < 0.01$).

TABLE VI. Sperm analysis of male rats at completion of the administration period

Dose (mg/kg bw/day)	0 (Control)	0.78	2.33	7.0
No. of animals	6	66	6	
Sperm motility[a]				
Motile sperm rate (%)	71.3 ± 2.8	72.8 ± 5.3	75.0 ± 7.4	59.2 ± 6.0**
Progressive sperm rate (%)	34.4 ± 3.7	37.3 ± 7.5	38.2 ± 8.4	22.9 ± 4.5*
Path velocity (μm/s)	150.7 ± 4.8	152.3 ± 6.1	153.6 ± 6.9	143.8 ± 6.0
Straight line velocity (μm/s)	110.4 ± 4.2	112.6 ± 5.5	111.7 ± 6.4	97.7 ± 8.2**
Curvilinear velocity (μm/s)	339.4 ± 11.4	335.9 ± 17.0	344.9 ± 12.8	319.4 ± 15.9
Amplitude of lateral head displacement (μm)	19.9 ± 0.3	19.5 ± 0.7	19.9 ± 1.0	20.9 ± 0.4*
Beat cross frequency (Hz)	29.5 ± 0.8	28.3 ± 1.6	29.6 ± 1.4	28.5 ± 1.7
Viability rate (%)[b]	99.8 ± 0.2	99.4 ± 0.5	99.4 ± 0.9	97.9 ± 2.2*
Survivability rate (%)[c]	76.7 ± 8.2	83.4 ± 6.4	80.9 ± 6.8	66.9 ± 10.1
Sperm morphology[d]				
Abnormal sperm rate (%)	2.5 ± 1.8	1.9 ± 1.0	2.0 ± 1.0	6.9 ± 4.6*
Abnormal head rate (%)	2.5 ± 1.8	1.9 ± 0.9	1.8 ± 0.8	5.6 ± 4.3
Abnormal tail rate (%)	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.3	1.3 ± 1.0**
No. of sperm in left cauda epididymis ($\times 10^6$)	268.8 ± 25.1	342.9 ± 50.1	334.8 ± 49.2	308.4 ± 114.2
No. of sperm/g weight of left cauda epididymis ($\times 10^6$)	1070.0 ± 94.9	281.1 ± 109.5	1238.2 ± 114.3	1227.4 ± 279.5

[a] Values are given as the mean ± SD.
[b] (Number of live sperm + number of sperm that died during incubation/number of sperm examined) × 100.
[c] (Number of live sperm/number of sperm examined) × 100.
[d] (Number of abnormal sperm/number of sperm examined) × 100.
* Significantly different from the control group ($p < 0.05$).
** Significantly different from the control group ($p < 0.01$).

changes were found in the epididymides, ovaries, and uterus in the dinoseb-treated groups (data not shown).

Sperm Analysis

Table VI shows results of sperm analysis at the end of the administration period in male rats given dinoseb. Sperm analysis showed that there were no significant differences between the 0.78 and 2.33 mg/kg bw/day groups and controls in any of the sperm tests. The motile sperm rate, progressive sperm rate, straight line velocity, and viability rate in the 7.0 mg/kg bw/day dose group were significantly lower than controls, and the amplitude of lateral head displacement, abnormal sperm rate, and abnormal tail rate were significantly higher than controls.

Table VII shows results of sperm analysis at the end of the recovery period in male rats given dinoseb. At completion of the recovery period, sperm analysis revealed no significant changes at 0.78 and 2.33 mg/kg bw/day. At 7.0 mg/kg bw/day, a significantly lower viability rate and survivability rate, and higher abnormal sperm rate and abnormal head rate were found.

Reproductive and Developmental Findings

Table VIII shows the reproductive and developmental findings in rats given dinoseb. No change attributable to the chemical was noted in the number of estrous cases, copulation index, number of days till copulation after pairing, number of pregnant females, fertility index, gestation length, number of corpora lutea, number of implantation sites, implantation index or delivery, and nursing conditions. A number of dams delivered their pups and of dams with live pups at delivery was significantly lowered in the 7.0 mg/kg bw/day group. Slight focal atrophy of the seminiferous tubule was observed in one male at 7.0 mg/kg bw/day. No changes attributable to the chemical were noted in the total number of births, number of stillbirths, number of live pups born, sex ratio, delivery index, birth index, live birth index, general condition, number of live pups on day 4 of lactation, viability index, body weight of offspring, appearance, and necropsy findings in the 0.78 and 2.33 mg/kg bw/day dose groups. Acaudate or short tail was observed in each one pup at 0.78 mg/kg bw/day.

DISCUSSION

The present study was conducted to obtain initial information on the possible repeated dose toxicity and reproductive and developmental toxicity of dinoseb in rats. The data show that dinoseb exerts general toxicity and reproductive and developmental toxicity, but is unlikely to possess teratogenic potential under this test condition.

The dosage used in the present study was sufficiently high such that it should be expected to induce general toxic effects such as body weight and food consumption changes. A significant decrease in body weight gain was found at

TABLE VII. Sperm analysis of male rats at completion of the recovery period

Dose (mg/kg bw/day)	0 (Control)	0.78	2.33	7.0
Number of animals	6	6	6	6
Sperm motility[a]				
Motile sperm rate (%)	78.5 ± 6.5	76.6 ± 10.3	72.7 ± 3.3	71.5 ± 4.4
Progressive sperm rate (%)	29.3 ± 9.5	30.4 ± 12.2	33.3 ± 11.2	26.5 ± 6.7
Path velocity (μm/s)	144.2 ± 8.0	147.9 ± 8.0	147.8 ± 8.6	141.3 ± 5.5
Straight line velocity (μm/s)	101.9 ± 6.2	104.1 ± 6.8	107.0 ± 10.3	97.7 ± 6.4
Curvilinear velocity (μm/s)	327.4 ± 22.2	335.4 ± 26.4	324.6 ± 22.6	324.2 ± 13.9
Amplitude of lateral head displacement (μm)	19.9 ± 0.3	19.5 ± 0.7	19.9 ± 1.0	20.9 ± 0.4*
Beat cross frequency (Hz)	20.2 ± 0.9	20.4 ± 0.8	19.8 ± 1.0	20.3 ± 0.8
Viability rate (%)[b]	99.9 ± 0.2	99.8 ± 0.3	99.7 ± 0.3	98.8 ± 1.1*
Survivability rate (%)[c]	84.1 ± 4.1	83.1 ± 3.3	81.0 ± 4.6	78.2 ± 3.1*
Sperm morphology[d]				
Abnormal sperm rate (%)	1.5 ± 1.0	2.4 ± 2.2	2.3 ± 1.4	4.6 ± 2.8*
Abnormal head rate (%)	1.5 ± 1.0	2.3 ± 2.2	2.2 ± 1.4	4.2 ± 2.5*
Abnormal tail rate (%)	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.4
No. of sperm in left cauda epididymis ($\times 10^6$)	358.4 ± 17.4	385.5 ± 70.5	347.8 ± 48.8	332.1 ± 28.8
No. of sperm/g weight of left cauda epididymis ($\times 10^6$)	1134.5 ± 72.6	1201.3 ± 1191.9	1118.9 ± 97.2	1087.3 ± 90.0

[a] Values are given as the mean ± SD.
[b] (Number of live sperm + number of sperm that died during incubation/number of sperm examined) × 100.
[c] (Number of live sperm/number of sperm examined) × 100.
[d] (Number of abnormal sperm/number of sperm examined) × 100.
* Significantly different from the control group ($p < 0.05$).

7.0 mg/kg bw/day in both sexes as expected. On the other hand, a significant increase in food consumption was observed from the mid stage of the administration period at 7.0 mg/kg bw/day in both sexes, and this effect was continued during the recovery period. In the 1930s, 2,4-dinitrophenol, a compound very similar to dinoseb, was prescribed as a weight-reducing agent. It is estimated that about 100 000 people in the United States used this drug while it was available. Because the energy is released as heat by uncoupling of electron transport from ATP synthesis, weight loss was remarkable (ATSDR, 1995; DOH, 2005). The decreased body weight gain with increased food consumption observed in this study is consistent with dinoseb's action as a metabolic activator. Because body weight gain was significantly increased during the recovery period at 7.0 mg/kg bw/day in males, the metabolic activation due to dinoseb seems temporary. At the end of the administration period, relative brain weight was significantly higher than controls with no significant difference in absolute weight in males at 7.0 mg/kg bw/day, suggesting that the difference in body weight in comparison with controls was responsible.

Transient salivation was observed immediately after administration at 0.78 mg/kg bw/day and higher, but it was considered as a result of stimulation by dinoseb because neither morphological changes in the salivary gland nor other neurological changes were observed. Females that died or were moribund showed a decrease in spontaneous motor activity, prone position, low body temperature, dyspnea, cyanosis, and bradypnea. In the acute toxicity, dinoseb can cause death because of respiratory and circulatory disorders (Rotterdam Convention, 2003). In this study, females that died or were moribund also showed adverse effects in the respiratory and circulatory systems with congestion in the lungs and livers.

Significant increases in RBC count, hematocrit, haemoglobin, or MCV found in the treated group animals indicate that dinoseb has a potential to induce polycythemia in rats. These changes were considered to be a result of regulation to low oxygen levels of cells. Dinoseb increases the rate of oxygen consumption in cells (Palmeira et al., 1994), and RBC production can be regulated by erythropoietin for need of oxygen (MedicineNet, 2001). Splenic extramedullary hematopoiesis, which was substantial in control pregnant rats, was significantly decreased in the 2.33 mg/kg bw/day females. This effect was also observed in one surviving female at 7.0 mg/kg bw/day. Reduction in splenic extramedullary hematopoiesis was considered to be a physiologic response to the decreased need for hematopoiesis because of polycythemia condition. In the blood chemistry, creatinine was significantly higher at 7.0 mg/kg bw/day in the main group males. However, no histopathological changes were found in the kidney, and other related parameters were not affected, suggesting that this change seems unlikely to be due to kidney failure but rather due to changes in muscle metabolism by dinoseb as an uncoupling agent. PT was significantly higher at 2.33 mg/kg bw/day (13.8 ± 0.3 s) and 7.0 mg/kg bw/day (14.1 ± 0.4 s), but

TABLE VIII. Reproductive and developmental findings in rats given dinoseb

Dose (mg/kg bw/day)	0 (control)	0.78	2.33	7.0
No. of females	12	12	12	12
No. of estrous cases before pairing (14 days)[a]	3.5 ± 0.5	3.3 ± 0.5	3.4 ± 0.5	3.3 ± 0.5
No. of pairs with successful copulation	12	12	12	12
Copulation index (%)[b]	100	100	100	100
No. of days till copulation after pairing[a]	2.3 ± 1.1	2.5 ± 1.2	2.4 ± 1.2	2.5 ± 1.3
No. of pregnant females	11	11	12	12
Fertility index (%)[c]	91.7	91.7	100.0	100.0
Length of gestation (days)[a]	22.2 ± 0.4	22.4 ± 0.9	22.2 ± 0.6	22.0
No. of corpora lutea[a]	16.6 ± 2.2	16.3 ± 2.1	16.1 ± 1.4	15.3 ± 1.2
No. of implantations[a]	14.6 ± 2.4	14.8 ± 1.8	14.6 ± 1.6	13.9 ± 1.1
Implantation index (%)[a,d]	87.6 ± 7.4	91.3 ± 5.5	90.6 ± 4.5	91.4 ± 6.6
No. of dead or moribund pregnant females during pregnancy	0	0	0	10
No. of dams delivered pups	11	11	12	2**
No. of dams without live pups	0	0	0	1
No. of dams with live pups	11	10	12	1**
No. of pups born[a]	13.9 ± 1.9	13.2 ± 3.4	13.2 ± 2.6	11.0
No. of stillbirths[a]	0.2 ± 0.4	1.2 ± 1.6	0.2 ± 0.4	6.0
No. of live pups born[a]	13.7 ± 1.8	12.0 ± 4.3	13.0 ± 2.5	5.0
Sex ratio at birth (male/female)[a,e]	1.09 ± 0.65 (74/77)	1.12 ± 0.44 (67/65)	1.28 ± 0.94 (80/76)	1.00 (5/5)
Delivery index (%)[a,f]	95.5 ± 5.5	88.2 ± 20.3	89.9 ± 14.1	84.5
Birth index (%)[a,g]	94.4 ± 5.7	79.4 ± 28.4	88.8 ± 13.4	41.5
Live birth index (%)[a,h]	98.8 ± 2.6	85.4 ± 29.7	98.8 ± 2.7	50
No. of live pups on day 4 of lactation[a]	13.6 ± 1.7	12.5 ± 1.6	12.8 ± 2.6	8.0
Viability index (%)[a,i]	99.4 ± 2.1	95.4 ± 10.3	98.1 ± 4.7	80
Body weight of pups[a]				
Male				
Day 0	6.8 ± 0.5	6.5 ± 0.4	6.7 ± 0.5	6.8
Day 4	11.1 ± 1.4	10.8 ± 1.1	10.6 ± 1.2	8.2
Female				
Day 0	6.4 ± 0.6	6.2 ± 0.5	6.3 ± 0.6	6.8
Day 4	10.2 ± 1.5	10.5 ± 1.3	9.9 ± 1.5	8.4
No. of fetuses (litters) with external malformation	0	2 (2)	0	0
Acaudate	0	1 (1)	0	0
Short tail	0	1 (1)	0	0

[a] Values are given as the mean ± SD.
[b] (Number of pairs with successful copulation/number of pairs) × 100.
[c] (Number of pregnant females/number of pairs with successful copulation) × 100.
[d] (Number of implantation scars/number of corpora lutea) × 100.
[e] Number of male pups/number of female pups.
[f] (Number of pups born/number of implantation scars) × 100.
[g] (Number of live pups born/number of implantation scars) × 100.
[h] (Number of live pups born/number of pups born) × 100.
[i] (Number of live pups on day 4/number of live pups born) × 100.
** Significantly different from the control group ($p < 0.01$).

these were within the range of historical background data for the laboratory that performed this study (16.2 ± 1.8 s). In the 7.0 mg/kg bw/day group, ALP and albumin were significantly lower and BUN was significantly higher. All of these differences were not observed at completion of the administration period and suggested not to be attributable to administration of the test substance. Other changes in hematology or blood chemistry were dose independent.

It seems unlikely that dinoseb exerts reproductive toxicity to female rats when administered during the premating and early pregnancy period because no adverse effects on the estrous cyclicity, copulation, or fertility were caused by the administration of dinoseb in females. However, dinoseb is suggested to be reproductively toxic during mid and late pregnancy because death was found during late pregnancy in the main group females but not in the recovery group

females, more specifically in nonpregnant females, at 7.0 mg/kg bw/day.

In the 7.0 mg/kg bw/day dose group males, motile sperm rate, progressive sperm rate, straight line velocity, and viability rate were significantly lower than controls, and the amplitude of lateral head displacement, abnormal sperm rate, and abnormal tail rate were significantly higher than controls at the end of the administration period. The viability rate and survivability rate were also significantly lower than controls and the abnormal sperm rate and abnormal head rate were significantly higher than controls at the end of the recovery period. These effects are thought to be caused by the uncoupling effects of dinoseb rather than due to a body weight loss and a body temperature increase (Linder et al., 1982). On the histopathological findings of this study, focal atrophy of the seminiferous tubule in the testis was observed in one male at 7.0 mg/kg bw/day. However, this change was considered to be spontaneous because the degree of histopathological change was slight, and the incidence at this dose was low and not significantly different from the control group. There were no dose-related effects on spermatogenesis at stages of spermatogonia and spermatocyte in this study. However, in a feeding study of dinoseb (0–22.2 mg/kg bw/day) to Sherman rats, histopathological changes to spermatogonia, spermatocyte, spermatid, or sperm in the testes were observed after 20 or 30 days of administration at 22.2 mg/kg bw/day, and a critical effect to the spermatogonia was observed after 50 days of treatment at 22.2 mg/kg bw/day. After 11-week administration, sperm counts were significantly decreased at 9.1 mg/kg bw/day and above. In addition, spermatozoa were not found in sections of the epididymides at 22.2 mg/kg bw/day (Linder et al., 1982). Another spermatotoxicity study, in which males rats were treated with dinoseb for 5 days, showed no effect of treatment on sperm parameters in the cauda epididymis 3 days after the final dose but after 14 days, reduced sperm motility and increased incidence of tailless sperm were noted (Takahashi et al., 2004). These findings suggest that dinoseb affects sperm indirectly by disturbing spermatogenesis or maturation process of sperm in the epididymis and that prolonged exposure to dinoseb in feed affects the early stage of spermatogenesis.

As for the developmental parameters, no changes attributable to the chemical were noted in the 0.78 and 2.33 mg/kg bw/day dose groups. Developmental toxicity of dinoseb was not completely estimated in the present study, because only one dam with live pups was obtained at the highest dose. At 0.78 mg/kg bw/day, one pup was observed to be acaudate and another to have a short tail. However, incidences of fetuses with an external malformation at this dose were very low and not significantly different from those in the control group. The external malformations observed in the present study are of the types that occur spontaneously among control rat fetuses (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Barnett et al., 2000). Prenatal developmental toxicology studies by feeding administration of dinoseb showed teratogenic effects such as hypoplastic tails or microphthalmia in rats (Spencer and Sing, 1982; Giavini et al., 1986), but teratogenic effects were not induced by gavage dose (Giavini et al., 1986) or intraperitoneal administration (McCormack et al., 1980; Daston et al., 1988) in rats. Giavini et al. (1986) reported that gavage dose of dinoseb (0, 2.5, 5, 10, or 15 mg/kg bw/day) induced both maternal toxicity and developmental toxicity without teratogenic effects; however, administration of dinoseb in a diet induced microphthalmia at 200 ppm (~15 mg/kg bw/day in feed) with reduction in maternal body weight gain. Based on the results of the present study, dinoseb is considered to be unlikely to exert teratogenic effects by gavage dose, which is in agreement with the literature. There are no studies showing differences in toxicokinetics of dinoseb by gavage dose and feed administration, but there are some examples that gavage and feed administration show differences in toxicokinetics of chemicals (Yuan et al., 1994, 1995). Differences in absorption, distribution, metabolism, and/or elimination may explain the different teratogenic responses of dinoseb observed in these studies. Further studies are needed to clarify the differences in the toxicokinetics of dinoseb by gavage dose and feeding.

The present study was performed in compliance with the OECD guideline 422 (OECD, 1996), and this screening test does not provide complete information on all aspects of reproduction and development because of the relatively small numbers of animals in the dose groups. In the present study, only two females delivered their pups at the highest dose group; therefore, the developmental toxicity to pups was not fully assessed. The LOAEL for males and NOAEL for females were 0.78 mg/kg bw/day based on increases in hematocrit in males at 0.78 mg/kg bw/day and higher and decreased extramedullary hematopoiesis of the spleen in females at 2.33 mg/kg bw/day. The NOAEL for reproductive/developmental toxicity was considered to be 2.33 mg/kg bw/day based on sperm motility and morphology in males and decreased number of dams with live pups in females.

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Potential adverse effects of phthalic acid esters on human health: A review of recent studies on reproduction

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Abstract

Various phthalic acid esters (PAEs) have been used for a wide range of products. PAEs and their metabolites produce reproductive and developmental toxicities in laboratory animals. These findings have raised concern about the possibility of PAEs as contributors to reproductive and developmental adverse effects in humans. This paper focuses on PAE exposure and health effects in human populations and summarizes recent studies. The exposure data in human populations indicate that the current methodology of estimation of PAE exposure is inconsistent. It is therefore important to obtain improved data on human PAE exposure and better understanding of the toxicokinetics of PAEs in each subpopulation. Studies on health effects of PAEs in humans have remained controversial due to limitations of the study designs. Some of findings in human populations are consistent with animal data suggesting that PAEs and their metabolites produce toxic effects in the reproductive system. However, it is not yet possible to conclude whether phthalate exposure is harmful for human reproduction. Studies in human populations reviewed in this paper are useful for showing the strength of the association. It is sometimes claimed that the use of animal data for estimating human risk does not provide strong scientific support. However, because it is difficult to find alternative methods to examine the direct toxic effects of chemicals, animal studies remain necessary for risk assessment of chemicals including PAEs.

Keywords: Phthalic acid ester; Human health; Reproduction; Development

1. Introduction

Various phthalic acid esters (PAEs) have been used for a wide range of products, and the largest use of these esters is in plasticizers for polyvinyl chloride (PVC) products (Autian, 1973). When used as plasticizers, PAEs are not irreversibly bound to the polymer matrix; therefore, they can migrate from the plastic to the external environment under certain conditions. PAEs are ubiquitous environmental pollutants because of their widespread manufacture, use, and disposal as well as their high concentration in and ability to migrate from plastics (Marx, 1972; Mayer et al., 1972). Humans are exposed to PAEs from food contaminated during growth, processing, and packaging or from storage and indoor air. Di-(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl phthalate (DBP), and butyl benzyl phthalate (BBP) were particularly found in fatty foods including dairy products (Kavlock et al., 2002a,b,c). Women have been exposed to DEHP, DBP, and diethyl phthalate (DEP) in cosmetics on a daily basis (Koo and Lee, 2004).

Some PAEs and their metabolites produce reproductive and developmental toxicities in laboratory animals. The major toxicities are known to be testicular effects (Zhang et al., 2004), embryolethality (Ema et al., 1994, 1997a; Tyl et al., 1988), malformations such as cleft palate and fusion of the sternebrae, and adverse effects on sexual differentiation (Ema et al., 1997b, 1998; Gray et al., 2000). There are considerable homologies among different

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mammalian species for androgen activities during sex differentiation (Gray et al., 1994). Chemicals that adversely affect human sex differentiation (Schardein, 2000) also produce predictable alterations of this process in rodents (Gray et al., 1994). The anti-androgenic effects of some PAEs were observed in a Hershberger assay in castrated male rats (Stroheker et al., 2005; Lee and Koo, 2007) or in an AR reporter gene assay (Satoh et al., 2004). These findings have raised concern about the possibility of PAEs as contributors to reproductive and developmental adverse effects in humans. Available data on primates are currently limited but show significant differences from rodents regarding the reproductive effects of PAEs (Kurata et al., 1998; Pugh et al., 2000; Tomonari et al., 2006), indicating the possibility of species-related differences.

The lower sensitivity of primates is thought to arise from differences between rodents and primates in the absorption, distribution, metabolism, and excretion (ADME) of PAEs. Monoester metabolites of PAEs such as mono-2-ethylhexyl phthalate (MEHP) and mono-butyl phthalate (MBP) have been reported to be the active metabolites responsible for adverse effects (Elcombe and Mitchell, 1986; Ema and Miyawaki, 2001; Tomita et al., 1986). DEHP is hydrolyzed to MEHP by the catalytic action of lipase (Ito et al., 2005). Lipase activities in the liver, small intestine, and kidneys are higher in rodents than in primates (Ito et al., 2005). The maximum concentrations of MEHP in the blood of marmosets were up to 7.5 times lower than in rats (Kessler et al., 2004). In rats, MEHP is oxidized to other secondary metabolites, and both MEHP and secondary metabolites are found in the blood and amniotic fluid primarily in their free form (Kurata et al., 2005; Calafat et al., 2006). Urinary MEHP was mostly found as a glucuronide conjugate in rats (Calafat et al., 2006). On the other hand, in humans and primates, MEHP is present in blood and urine primarily as glucuronide conjugates, which enhance urinary excretion and reduce the biological activity of the active metabolites (Ito et al., 2005; Kurata et al., 2005; Silva et al., 2003), but DEHP metabolites with a carboxylated ester side-chain were found as both conjugates and free forms in human urine (Silva et al., 2006a). Plasma radioactivity measurements of DEHP in rats and marmosets revealed that radioactivity in rat testis was about 20-fold higher than that in marmosets. About 60% of the dose was excreted in urine in rats primarily as unconjugated MEHP-metabolites. For marmosets, the majority of the dose was excreted in the feces (Kurata et al., 1998).

The potential of PAEs to produce adverse effects in humans has been the subject of considerable discussion. Many toxicity studies have been conducted in laboratory animals, especially in rats, and review papers are available based on these animal data (Corton and Lapinskas, 2005; Ema, 2002; Foster, 2006); however, studies in human populations have not been adequate to assess the toxic potential on human health. Lately, several review papers were published regarding PAE exposure in human populations (Koch et al., 2006; Latini, 2005; Schettler, 2006). These review studies are worthwhile for knowing exposure levels and routes of PAE exposure in human populations; however, review works regarding the relationships between PAE exposure and human health are not adequate. In the late 20th century, only a few papers have reported a relationship between environmental PAE exposures and human health (Aldyreva et al., 1975; Fredricsson et al., 1993; Murature et al., 1987). Studies in human populations have been receiving much attention for the last 2 or 3 years, and the number of studies in human populations has increased. Some recent studies have suggested possible associations between environmental exposure to PAEs and adverse effects on human reproductive health. It will be useful to review them to determine whether there is concordance between animal models and human populations in order to develop hypotheses for future studies. This paper focuses on the PAE exposure and health effects in human populations and summarizes recent human studies published up to 2006.

2. Exposure to PAEs

Many studies have suggested that PAEs and their metabolites produce reproductive and developmental toxicities in laboratory animals. Although the most of these animals were exposed to PAEs at relatively high level to exam toxicological effects, some studies showed that relatively low doses of PAEs caused toxic effects (Arcadi et al., 1998; Lee et al., 2004; Poon et al., 1997). Thus, there is a question of whether humans are exposed to PAEs at a severe enough level to generate human health effects. Several studies have been conducted to estimate the exposure level of PAEs in humans.

2.1. Estimate of PAE exposure in human populations

Levels of human exposure to PAEs were estimated from the urinary metabolite of PAEs. Table 1 shows the urinary PAE metabolite in US populations. A pilot study was conducted for measurement of levels of seven urinary phthalate metabolites, MEHP, MBP, mono-benzyl phthalate

Table 1
Total urinary phthalate monoester concentrations (in μg/g of creatinine)

Metabolites	Diester	Measurement in 289 individuals (Blount et al., 2000)		Measurement in 2541 individuals (Silva et al., 2004a)	
		Geometric mean	95th percentile	Geometric mean	95th percentile
MEP	DEP	345	2610	163	1950
MBP	DBP/BBP	36.9	162	22.4	97.5
MBzP	BBP	20.2	91.9	14.0	77.4
McHP	DcHP	0.3	1.0	<LOD	3.00
MEHP	DEHP	3.0	15.2	3.12	18.5
MOP	DOP	0.5	2.1	<LOD	3.51
MINP	DINP	1.3	6.8	<LOD	4.29

LOD, limit of detection.

(MBzP), mono-cyclohexyl phthalate (McHP), mono-ethyl phthalate (MEP), mono-isononyl phthalate (MINP) and mono-*n*-octyl phthalate (MOP), in 289 US adults (Blount et al., 2000). A subsequent study involving a group of 2541 individuals from participants of the National Health and Nutrition Examination Survey (NHANES) aged ⩾ 6 years in US provided similar findings to the previous study although urinary levels for MEP, MBP and MBzP were lower than the previously reported values (Silva et al., 2004a).

These urinary metabolite levels were used to calculate the ambient exposure levels for five PAEs, BBP, DBP, DEHP, di-*n*-octyl phthalate (DOP) and di-isononyl phthalate (DINP), in human populations (David et al., 2001; Kohn et al., 2000). The estimation of daily intake of phthalates was calculated by applying the following equation according to David et al. (2001):

$$\text{Intake } (\mu g/kg/day) = \frac{\text{UE } (\mu g/g) \times \text{CE } (mg/kg/day)}{f \times 1000\ (mg/g)} \times \frac{MW_d}{MW_m}$$

where UE is the urinary concentration of monoester per gram creatinine, CE is the creatinine excretion rate normalized by body weight, f is the ratio of urinary excretion to total elimination, and MW_d and MW_m are the molecular weights of the diesters and monoesters, respectively.

Table 2 shows the estimated ambient exposure to PAEs. As shown in Table 2, all estimated PAE intakes in the US population were lower than the tolerable daily intake (TDI) values settled by the EU Scientific Committee for Toxicity, Ecotoxicity and the Environment (BBP: 200 μg/kg/day, DBP: 100 μg/kg/day, DEHP: 37 μg/kg/day, DOP: 370 μg/kg/day, and DINP:150 μg/kg/day) (CSTEE, 1998), the reference dose (RfD) of the US EPA (BBP: 200 μg/kg/day, DBP: 100 μg/kg/day, and DEHP: 20 μg/kg/day) (US EPA, 2006) and the TDI values established by the Japanese Government (DEHP: 40–140 μg/kg/day and DINP: 150 μg/kg/day) (MHLW, 2002). Among these PAEs, DEHP is most commonly used plasticizer for flexible PVC formulations and is a widespread environmental contaminant (Kavlock et al., 2002c); however, the estimated daily intake level of DEHP was not high as expected.

Koch et al. (2004a, 2003) and Barr et al. (2003) cast doubt on the sensitivity of the biomarker MEHP for assessing DEHP exposure, and they explored mono- (2-ethyl-5oxo-hexyl) phthalate (5oxo-MEHP) and mono- (2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP) as additional biomarkers for DEHP. After a single oral dose of DEHP in a male volunteer, peak concentrations of MEHP, 5OH-MEHP, and 5oxo-MEHP were found in the serum after 2 h, and in urine after 2 h (MEHP) and 4 h (5OH-MEHP and 5oxo-MEHP). The major metabolite was MEHP in serum and 5OH-MEHP in urine (Koch et al., 2004a). Barr et al. (2003) analyzed 62 urine samples for metabolites of DEHP, and the mean urinary levels of 5oxo-MEHP and 5OH-MEHP were 4-fold higher than MEHP.

Koch et al. (2003) determined a median DEHP intake of 13.8 μg/kg/day based on urinary oxidative metabolites of DEHP, 5OH-MEHP and 5oxo-MEHP, in male and female Germans ($n = 85$; aged 18–40). Twelve percent of the subjects exceeded the TDI of the EU-CSTEE (37 μg/kg/day) and 31% of the subjects exceeded the RfD of the US EPA (20 μg/kg/day). For DBP, BBP, DEP, and DOP, the 95th percentile intake values were estimated to be 16.2, 2.5, 22.1, and 0.42 μg/kg/day, respectively. Subsequently, urine samples from 254 German children aged 3–14 were also analyzed for concentrations of these three metabolites of DEHP. The geometric means for MEHP, 5OH-MEHP and 5oxo-MEHP in urine were 7.9, 52.1, and 39.9 μg/L, respectively (Becker et al., 2004). The median daily intake of DEHP in children was estimated to be 7.7 μg/kg. Four children exceeded the TDI of the EU-CSTEE (37 μg/kg/day) and 26 children also exceeded the RfD of the US EPA (20 μg/kg/day) (Koch et al., 2006).

Although these findings showed that German populations could be exposed to DEHP at a higher level than previously estimated values (David et al., 2001; Kohn et al., 2000), these results should be interpreted carefully. In the above-mentioned equation, Kohn et al. (2000) and David et al. (2001) applied the fractional urinary excretion value ($f = 0.106$: MEHP) determined by Peck and Albro (1982). On the other hand, Koch et al. (2003) applied the fractional urinary excretion values ($f = 0.074$: 5OH-

Table 2
Comparison of calculated intakes of phthalates based on the geometric mean values for urinary metabolites and the tolerable daily intake levels as well as the reference dose of phthalates (in μg/kg/day)

PAEs	Estimated by David et al. (2001) for 289 US individuals (Blount et al., 2000)		Estimated by Kohn et al. (2000) for 2541 US individuals (Silva et al., 2004a)		TDI (EU) (CSTEE, 1998)	RfD (US) (US EPA, 2006)	TDI (Japan) (MHLW, 2002)
	Geometric mean	95th percentile	Geometric mean	95th percentile			
BBP	0.73	3.34	0.88	4.0	200	200	Not established
DBP	1.56	6.87	1.5	7.2	100	100	Not established
DEHP	0.60	3.05	0.71	3.6	37	20	40–140
DOP	<LOD	—	0.0096	0.96	370	Not established	Not established
DINP	0.21	1.08	<LOD	1.7	150	Not established	150

LOD, limit of detection.

MEHP, 0.055: 5oxo-MEHP and 0.024: MEHP) determined by Schmid and Schlatter (1985). Using different fractional urinary excretion values can yield several fold differences in estimated values even if the levels of the urinary metabolites are the same.

Table 3 shows a comparison of the estimated median exposure levels of DEHP. Koo and Lee (2005) and Fujimaki et al. (2006) applied the same fractional urinary excretion values of Koch et al. (2003) for calculating daily DEHP intake. Koo and Lee (2005) estimated daily intake of DEHP in Korean children aged 11–12 years old ($n = 150$) and in Korean women aged 20–73 years old ($n = 150$) with a fractional urinary excretion value of 0.024 for MEHP. Median intake levels of DEHP were estimated to be 6.0 μg/kg/day in children and 21.4 μg/kg/day in adult women. TDI of the EU (37 μg/kg/day) was reached at the 56th percentile for women and the 95th percentile for children. Fujimaki et al. (2006) estimated the daily intake of DEHP in forty pregnant Japanese women. The median concentrations of MEHP, 5OH-MEHP and 5oxo-MEHP in the urine were 9.83, 10.4, and 10.9 μg/L, respectively. The median DEHP intake based on MEHP, 5OH-MEHP, and 5oxo-MEHP were estimated to be 10.4 (3.45–41.6), 4.55 (0.66–17.9), and 3.51 (1.47–8.57) μg/kg/day, respectively. These two studies showed higher exposure levels than the previously estimated values in the US population (David et al., 2001; Kohn et al., 2000). Koo and Lee (2005) also showed that a different estimation model can yield 10-fold lower values when estimating DEHP intake, indicating that methods for estimation of daily intake values of PAEs remain inconsistent.

Recently, other secondary oxidized metabolites of DEHP have been recognized (Koch et al., 2005b). Although 5OH-MEHP and 5oxo-MEHP in the urine reflect short-term exposure levels of DEHP, other secondary oxidized metabolites of DEHP such as mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) and mono-[2-(carboxymethyl)hexyl] phthalate (2cx-MMHP) are considered excellent parameters for measurement of the time-weighted body burden of DEHP due to their long half-times of elimination. Biological monitoring in a German population ($n = 19$) indicated that 5cx-MEPP is the major urinary metabolite of DEHP. Median concentrations of the metabolites of DEHP were 85.5 μg/L (5cx-MEPP), 47.5 μg/L (5OH-MEHP), 39.7 μg/L (5oxo-MEHP), 9.8 μg/L (MEHP) and 36.6 μg/L (2cx-MMHP) (Preuss et al., 2005). Furthermore, oxidized metabolites of DINP have been recently introduced as new biomarkers for measurement of DINP exposure (Koch and Angerer, 2007; Silva et al., 2006b). These new findings imply that more accurate methods for estimation of PAE exposure can be developed.

2.2. Exposure in fetuses and infants

PAE exposure to the fetus in utero is a great concern because some PAEs are considered to be developmental toxicants. Adibi et al. (2003) measured of urinary phthalate metabolites in pregnant women ($n = 26$) in New York. The median creatinine-adjusted concentrations of MEP, MBP, MBzP, and MEHP were 236, 42.6, 12.1, and 4.06 μg/g, respectively. Metabolites levels in pregnant women were comparable with those in US general population (Blount et al., 2000; Silva et al., 2004a). Another study in 24 mother–infant pairs confirmed DEHP and/or MEHP exposure during human pregnancies (Latini et al., 2003a). The mean DEHP concentrations in maternal plasma and cord plasma were 1.15 and 2.05 μg/mL, respectively, and the mean MEHP concentration was 0.68 μg/mL in both maternal plasma and cord plasma. The levels of phthalate metabolites in the amniotic fluid may reflect fetal exposure to PAEs. Only three metabolites, MEP, MBP, and/or MEHP, were detected in the amniotic fluid samples ($n = 54$). The levels of mono-methylphthalate (MMP), MBzP, McHP, MINP, MOP, 5OH-MEHP, and 5oxo-MEHP were under the limits of detection. Levels of MEP, MBP, and MEHP ranged from under the limits of detection to 9.0 ng/mL ($n = 13$), 263.9 ng/mL ($n = 50$), and 2.8 ng/mL ($n = 21$), respectively (Silva et al., 2004b). These studies suggest that human exposure to PAEs can begin in utero.

Breast milk and infant formula can be routes of PAE exposure for infants. Table 4 shows phthalate monoesters levels in human milk, infant formula, and consumer milk. Levels of phthalate monoesters in pooled breast milk

Table 3
Comparison of estimated mean daily intake of DEHP (μg/kg/day)

Metabolites	German[a] adults ($n = 85$) (Koch et al., 2003)	Korean[a] (Koo and Lee, 2005)		Japanese[a] pregnant women ($n = 40$) (Fujimaki et al., 2006)	US[b] adults ($n = 289$) (David et al., 2001)	US[b] aged ⩾6 years ($n = 2541$) (Kohn et al., 2000)
		Adults (women) ($n = 150$)	Children ($n = 150$)			
MEHP	10.3 (38.3)	21.4 (158.4)	6.0 (37.2)	10.4	0.60 (3.05)	0.71 (3.6)
5OH-MEHP	13.5 (51.4)	No data	No data	4.55	No data	No data
5oxo-MEHP	14.2 (52.8)	No data	No data	3.51	No data	No data
Oxidative DEHP metabolites[c]	13.8 (52.1)	No data	No data	No data	No data	No data

Figures in parentheses show the 95th percentile.
[a] Applying the equation of David et al. (2001) and the fractional urinary excretion value determined by Schmid and Schlatter (1985).
[b] Applying the equation of David et al. (2001) and the fractional urinary excretion value determined by Peck and Albro (1982).
[c] Average of estimated intakes of DEHP based on 5OH-MEHP and 5oxo-MEHP.

Table 4
Phthalate monoester levels (μg/L) in human milk, infant formula and consumer milk

Monoester	Diester	Three pooled breast milk samples (Calafat et al., 2004b)	Thirty-six samples of Danish mother's milk (Mortensen et al., 2005)	Ten samples of infant formula (Mortensen et al., 2005)	Seven samples of consumer milk (Mortensen et al., 2005)
MMP	DMP	<LOD	0.17 ± 0.26[a]	<LOD	<LOD
MEP	DEP	<LOD	1.78 ± 2.74	<LOD	<LOD
MBP	DBP/BBP	1.3 ± 1.5[a]	359 ± 1830	0.6–3.9[b]	1.4–2.8[b]
MBzP	BBP	<LOD	1.2 ± 1.6	<LOD	<LOD
MEHP	DEHP	7.8 ± 6.8	13 ± 11	5.6–9.1	7.1–9.9
MINP	DINP	15.9 ± 7.7	114 ± 69	<LOD	<LOD

LOD, limit of detection.
[a] Values are given as mean ± standard deviation.
[b] Values are given as range.

($n = 3$) were reported by Calafat et al. (2004b). A subsequent study for 36 individual human milk samples provided higher values for all metabolites; in particular, levels of MBP were two magnitudes higher (Mortensen et al., 2005) than that in the previous study by Calafat et al. (2004b). Phthalate metabolites in breast milk were detected in their free forms unlike the metabolites found in urine and blood. Therefore, infants may receive active PAE metabolites from breast milk on a daily basis. Only MBP and MEHP were detected in consumer milk and infant formula (Mortensen et al., 2005).

The levels of PAEs were determined for 27 infant formulae sold in several countries, and DEHP and DBP were found (Yano et al., 2005). The amounts of DEHP (34–281 ng/g) were much higher than DBP (15–77 ng/g). DEHP, DBP, and DEP were also found in a total of 86 human milk samples collected from 21 Canadian mothers over a 6-month postpartum period. DEHP was the major ester with a mean value of 222 ng/g (8–2920 ng/g), followed by DBP with a mean of 0.87 ng/g (undetectable to 11.39 ng/g). DEP with a mean of 0.31 ng/g (undetectable to 8.1 ng/g) was detected in only a small number of samples. Dimethyl phthalate (DMP), BBP, and DOP were not detected in any samples (Zhu et al., 2006). Table 5 presents estimated maximum daily intakes of PAEs in infants, which was calculated by assuming that the body weight of infants is 7 kg and the daily intake of milk is 700 mL. Although the total estimated maximum daily intake of DEHP in infants was generally less than in general adults (Koch et al., 2003), the estimated maximum daily intake per body weight was higher than adults due to the low body weight of the infants. Assuming that milk was the only exposure route for PAEs in the infants, it is likely that infants had less exposure to DBP and DEP than the general adult population (Koch et al., 2003). These studies suggest that some infants may also be exposed to DEHP at higher levels than the established safe standard levels.

2.3. Possible variation of PAE exposure

Some humans may be exposed to PAEs at higher level than the established safe standard levels. Measurements of urinary metabolites of PAEs have revealed notable differences in concentrations of specific metabolites based on age, gender and race (Blount et al., 2000; Silva et al., 2004a). Concentrations of MBP, MBzP, and MEHP were higher in the youngest age group (6–11 years) and decreased with age. Non-Hispanic blacks tended to have higher levels of phthalate metabolites than non-Hispanic whites or Mexican Americans. Females tended to have a higher level of phthalate metabolites than did males (Silva et al., 2004a). Blount et al. (2000) also indicated that women of reproductive age (20–40 years) had significantly higher levels of MBP than other age/gender groups. Measurement of the three urinary metabolites MEHP, 5OH-MEHP and 5oxo-MEHP in male and female children ($n = 254$) aged 3 to 14 showed that boys had higher concentrations of these three metabolites of DEHP than girls (Becker et al., 2004). The higher levels of PAE metabolites in the young age group may be due to a different food category, dairy products, or the use of PVC toys (CSTEE, 1998), and the higher levels of MBP in females may be

Table 5
Estimated maximum daily intake (μg/kg/day) of PAEs in infants and general German population

Compounds	Human milk ($n = 21$) (Zhu et al., 2006)	Infant formula ($n = 27$) (Yano et al., 2005)	General population ($n = 85$) (Koch et al., 2003)
DEHP	301 (41.1)	6.9	166 (52.1)
DBP	1.21 (0.12)	1.07	22.6 (16.2)
DEP	0.87	Not measured	69.3 (22.1)

Daily PAE intake levels were calculated by assuming that the average daily milk consumption is 700 mL (722 g: specific gravity of human milk = 1.031) and average body weight is 7 kg.
Figures in parentheses show 95th percentile.

due to use of cosmetic products that contain high levels of DBP (Koo and Lee, 2004).

Koo et al. (2002) approached this issue from a different point of view. Their statistical examination concluded that higher levels of MBP in urine were associated with a lower level of education (only a high school education) and/or lower family income (less than $1500) in the month before sampling. Slightly higher levels of MEP were found in urban populations, low income groups, and males. PAE exposure occurred from food, water, and indoor air, although dietary intake of PAEs from contaminated food was likely to be the largest source (Schettler, 2006). Education level and family income may therefore influence the dietary pattern.

It is still unknown whether the variations in these metabolites represent differences in the actual exposure levels. Metabolism of PAEs may vary by age, race, or sex; for example, the ratios of 5OH-MEHP/5oxo-MEHP and 5oxo-MEHP/MEHP decrease with increasing age (Becker et al., 2004). The mean relative ratios of urinary MEHP to 5OH-MEHP to 5oxo-MEHP were 1 to 7.1 to 4.9 in German male and female children and 1 to 3.4 to 2.1 in German male and female adults. This might indicate enhanced oxidative metabolism in children (Koch et al., 2004b). The ratios for urinary MEHP, 5OH-MEHP and 5oxo-MEHP in Japanese pregnant women were reported to be approximately 1 to 1 to 1 (Fujimaki et al., 2006). The variation seen in these three populations may be due to differences in the analytical methods; however, these variations in human populations are still not negligible for accurate risk assessment. Because the current estimates of PAE intake in humans can be imprecise and ADMEs of PAEs in each subpopulation are not clear, the significance of exposure to PAEs with regard to health effects is yet unknown.

2.4. Exposure from medical devices

DEHP has been used for a wide variety of PVC medical devices such as i.v. storage bags, blood storage bags, tubing sets, and neonatal intensive care units (NICUs), and known treatments that involve high DEHP exposures include blood exchange transfusions, extracorporeal membrane oxygenation and cardiovascular surgery.

Serum concentrations of DEHP were significantly increased in platelet donors and receptors (Buchta et al., 2005, 2003; Koch et al., 2005c). A median increase of 232% of serum DEHP was detected after plateletpheresis in healthy platelet donors (Buchta et al., 2003). Mean DEHP doses for discontinuous-flow platelet donors and continuous-flow platelet donors were 18.1 and 32.3 μg/kg/day on the day of apheresis, which were close to or exceeded health standard levels such as the TDI or RfD (Koch et al., 2005c).

Premature infants who experience medical procedures may have a higher risk of exposure to DEHP than the general population. Because the same size of each medical device is used for all ages, infants may receive a larger dose of PAEs on a mg/kg basis than adults due to their smaller size. Calafat et al. (2004a) provided the first quantitative evidence confirming that infants who undergo intensive therapeutic medical interventions are exposed to higher concentrations of DEHP than the general population. They assessed exposure levels of DEHP in 6 premature newborns (23–26 weeks old) by measuring levels of urinary MEHP, 5OH-MEHP and 5oxo-MEHP. The geometric mean concentrations of MEHP (100 μg/L), 5oxo-MEHP (1617 μg/L), and 5OH-MEHP (2003 μg/L) were found to be one or two orders of magnitude higher than German children aged 3–5 (MEHP: 6.96 μg/L, 5OH-MEHP: 56.7 μg/L and 5oxo-MEHP:42.8 μg/L). Koch et al. (2005a) estimated DEHP exposure due to medical devices by using five major DEHP metabolites. Forty-five premature neonates (2–31 days old) with a gestational age of 25–40 weeks at birth were exposed to DEHP up to 100 times over the RfD value set by the US EPA depending on the intensity of medical care (median: 42 μg/kg/day; 95th percentile: 1780 μg/kg/day).

3. Health effects of PAEs in human populations

In the late 20th century, a few studies reported a relationship between environmental exposure of PAEs and human health. For example, Murature et al. (1987) reported that there was a negative correlation between DBP concentration in the cellular fraction of ejaculates and sperm production. Fredricsson et al. (1993) reported that human sperm motility was affected by DEHP and DBP. In females, decreased rates of pregnancy and higher levels of miscarriage in factory workers were associated with occupational exposure of DBP (Aldyreva et al., 1975). More recent studies in human males, females and infants are summarized below.

3.1. Studies of the male reproductive system

Table 6 shows a summary of studies of the male reproductive system in human populations. Two studies are available for 168 male subjects who were members of subfertile couples (Duty et al., 2003a,b). Eight urinary PAE metabolites, MEP, mono-methyl phthalate (MMP), MEHP, MBP, MBzP, MOP, MINP and McHP, were measured with a single spot urine sample. Urinary MEHP, MOP, MINP, or McHP showed no relevance to sperm parameters or DNA damage (Duty et al., 2003a,b). Urinary MBP was associated with lower sperm concentration and lower motility, and urinary MBzP was associated with lower sperm concentration. There was limited evidence suggesting an association of increased MMP with poor sperm morphology (Duty et al., 2003a). A neutral comet assay revealed that urinary MEP levels were associated with increased DNA damage in sperm (Duty et al., 2003b). This result was confirmed by a recent study in 379 men from an infertility clinic in which sperm DNA damage was associated with MEP (Hauser et al., 2007).

Table 6
Male reproductive effects in human populations

Compounds	Number of subjects	Related effects	Reference
Total PAEs[a]	$n = 21$	↓Sperm normal morphology, ↑Percent of single-stranded DNA in sperm	Rozati et al. (2002)
Phthalic acid	$n = 234$	↑Large testis[c], ↑Sperm motility[c]	Jonsson et al. (2005)
DEHP	$n = 37$	↓Semen volume, ↑Rate of sperm malformation	Zhang et al. (2006)
MEHP	$n = 187$	↓Straight-line velocity and curvilinear velocity of sperm[d]	Duty et al. (2004)
	$n = 74$	↓Plasma free testosterone	Pan et al. (2006)
%MEHP[b]	$n = 379$	↑Sperm DNA damage	Hauser et al. (2007)
MEP	$n = 168$	↑DNA damage in sperm	Duty et al. (2003b)
	$n = 234$	↑Large testis[c], ↓Sperm motility, ↓Luteinizing hormone	Jonsson et al. (2005)
	$n = 379$	↑DNA damage in sperm	Hauser et al. (2007)
	$n = 187$	↓Sperm linearity[d], ↑Straight-line velocity and curvilinear velocity of sperm[c,d]	Duty et al. (2004)
DBP	$n = 37$	↓Semen volume	Zhang et al. (2006)
MBP	$n = 168$	↓Sperm concentration, ↓Sperm motility	Duty et al. (2003a)
	$n = 463$	↓Sperm concentration, ↓Sperm motility	Hauser et al. (2006)
	$n = 187$	↓Straight-line velocity and curvilinear velocity of sperm[d]	Duty et al. (2004)
	$n = 74$	↓Plasma free testosterone	Pan et al. (2006)
	$n = 295$	↑Inhibin B level[c,d]	Duty et al. (2005)
MBzP	$n = 168$	↓Sperm concentration	Duty et al. (2003a)
	$n = 463$	↓Sperm concentration[d]	Hauser et al. (2006)
	$n = 187$	↓Straight-line velocity and curvilinear velocity of sperm[d]	Duty et al. (2004)
	$n = 295$	↓Follicle-stimulating hormone[c]	Duty et al. (2005)
MMP	$n = 168$	↑Poor sperm morphology[d]	Duty et al. (2003a)

[a] Total level of DMP, DEP, DBP, DEHP and DOP.
[b] The urinary concentrations of MEHP divided by sum of MEHP, 5OH-MEHP and 5oxo-MEHP concentrations and multiplied by 100.
[c] Data do not support the association of PAEs with reproductive adverse effects in male human populations.
[d] Only suggestive association was observed (statistically not significant).

In another study, semen volume, sperm concentration, motility, sperm chromatin integrity and biochemical markers of epididymal and prostatic function were analyzed together with MEP, MEHP, MBzP, MBP, and phthalic acid levels in urine in 234 young Swedish men (Jonsson et al., 2005). Urinary MEP level was associated with fewer motile sperm, more immotile sperm, and lower serum luteinizing hormone (LH) values. However, higher phthalic acid levels were associated with more motile sperm and fewer immotile sperm; therefore, the results for phthalic acid were opposite what had been expected.

A similar study was conducted in 463 male partners of subfertile couples (Hauser et al., 2006). Phthalate metabolites were measured in a single spot urine sample. There were dose–response relationships of MBP with low sperm concentration and motility. There was suggestive evidence of an association between the highest MBzP quartile and low sperm concentration. There were no relationships between MEP, MMP, MEHP or oxidative DEHP metabolites with any of the semen parameters.

Although there were associations between some metabolites of PAEs and sperm count, motility, or morphology, no statistically significant associations between MEP, MBzP, MBP, MEHP, or MMP and sperm progression, sperm vigor, or swimming pattern were observed in 187 subjects. There were only suggestive associations as follows: negative associations between MBzP with straight-line velocity (VSL) or curvilinear velocity (VCL), between MBP with VSL and VCL and between MEHP with VSL and VCL. MEP was positively associated with VSL and VCL but negatively associated with linearity (Duty et al., 2004).

Duty et al. (2005) explored the relationship between urinary phthalate monoester concentrations and serum levels of reproductive hormones in 295 men. In their previous studies (Duty et al., 2003a,b), MBP and MBzP were associated with sperm parameters, and the investigators had hypothesized that inhibin B, a sensitive marker of impaired spermatogenesis (Uhler et al., 2003), would be inversely associated with MBP and MBzP. However, MBP exposure was associated with increased inhibin B, although this was of borderline significance. Additionally, MBzP exposure was significantly associated with a decrease in serum follicle-stimulating hormone (FSH) level. The serum FSH level has been used as a marker of spermatogenesis for infertile males in clinical evaluation (Subhan et al., 1995), and it is increased in comparison to normal males (Sina et al., 1975). Therefore, the hormone concentrations did not change in the expected patterns.

DEHP is known to cause adverse effects on the male reproductive system in rodents (Gray et al., 2000), and DNA damage in human lymphocytes is also induced by DEHP and MEHP (Anderson et al., 1999). A Hershberger assay with DEHP or MEHP showed anti-androgenic effects in castrated rats (Stroheker et al., 2005; Lee and Koo, 2007). However, only a few studies have suggested that DEHP could be a reproductive toxicant in humans. Urine and blood samples from 74 male workers at a factory producing unfoamed polyvinyl chloride flooring exposed to DBP and DEHP were compared with samples from 63 unexposed male workers. The exposed workers had significantly elevated concentrations of MBP (644.3 vs. 129.6 μg/g creatinine) and MEHP (565.7 vs. 5.7 μg/g

creatinine) in their urine. The plasma free testosterone level was significantly lower (8.4 vs. 9.7 μg/g creatinine) in the exposed workers than in the unexposed workers. Free testosterone was negatively correlated to MBP and MEHP in the exposed worker group (Pan et al., 2006). Another recent study showed that although the urinary MEHP concentration was not associated with sperm DNA damage, the percentage of DEHP metabolites excreted as MEHP (MEHP%) was associated with increased sperm DNA damage. It is of interest that the oxidative metabolites had inverse relationships with sperm DNA damage (Hauser et al., 2007).

Unlike other studies, the following two studies used diester concentrations for measurement of PAEs. Rozati et al. (2002) reported that the concentration of total PAEs (DMP, DEP, DBP, BBP, DEHP, and DOP) in the seminal plasma was significantly higher in infertile men ($n = 21$) compared to controls ($n = 32$). Correlations were observed between seminal PAEs and sperm normal morphology ($r = -0.769$, $p < .001$), in addition to the % of single-stranded DNA in the sperm ($r = 0.855$, $p < .001$). This study examined only total PAEs, and relationships between individual PAEs and sperm parameters were not identified. Another study in a human male population was carried out by measurement of semen parameters and DEHP, DBP, and DEP in human semen ($n = 37$) (Zhang et al., 2006). The three PAEs were detected in most of the samples, and mean levels of DEHP, DBP, and DEP were 0.28, 0.16, and 0.47 μg/L, respectively. There was a negative correlation between semen volume and concentration of DBP or DEHP. There was also a positive association between the rate of sperm malformation and DEHP concentrations. These diester concentrations may directly reflect PAE exposure levels.

Animal data have suggested that mature exposure to DBP and DEHP affects sperm parameters (Agarwal et al., 1986; Higuchi et al., 2003). Dietary exposure of mature male F344 rats (15–16 weeks old) to DEHP (0–20,000 ppm) for 60 consecutive days resulted in a dose dependent reduction in testis, epididymis and prostate weights at 5000 and 20,000 ppm (284.1 and 1156.4 mg/kg/day). Epididymal sperm density and motility were also reduced and there was an increased occurrence of abnormal sperm at 20,000 ppm (Agarwal et al., 1986). Exposure of BBP from adolescence to adulthood showed changes in reproductive hormones in CD(SD)IGS rats at 100 and 500 mg/kg/day (Nagao et al., 2000). In Dutch-Belted rabbits, exposure of DBP during adolescence and in adulthood decreased the amount of normal sperm whereas in utero exposure of DBP decreased the amount of normal sperm, sperm counts, ejaculated volume, and accessory gland weight (Higuchi et al., 2003). Preadolescent male rats appear to have a greater sensitivity to the adverse testicular effects of DEHP than older rats. Akingbemi et al. (2001) demonstrated that preadolescent male rats (21 days old) were more sensitive than young adult animals (62 days old) to 14- or 28-day DEHP exposures that induced decreases in Leydig cell production of testosterone. PAE effects on male reproductive organs could be influenced by the stage of development, but the data also support the possibility that mature animals are susceptible to PAE exposure. The studies in human populations were in accord with these animal data.

Some studies in human populations have suggested associations between MEP, a metabolite of DEP, and changes in sperm; however, these results regarding to MEP are not supported by animal studies. According to Foster et al. (1980), oral dosing of DEP (1600 mg/kg/day) for 4 days did not damage the testes in young SD rats. In another study, male and female CD-1 mice were given diets with DEP (0–2.5%) for 7 days prior to and during a 98-day cohabitation period. There were no apparent effects on reproductive function in animals exposed to DEP (Lamb et al., 1987).

Furthermore, studies in rodents may have little relevance to humans for the reason that DEHP and DINP do not cause reproductive effects in non-human primates. Pugh et al. (2000) showed no evidence of testicular lesions in young adult cynomolgus monkeys (~2 years old) gavage dosed with 500 mg/kg bw/day DEHP and DINP for 14 days. A study with matured marmosets (12–15 months old) showed that repeated dosing of DEHP at up to 2500 mg/kg bw/day for 13 weeks resulted in no differences in testicular weight, prostate weight, blood testosterone levels, blood estradiol levels or any other aspect of the reproductive system (Kurata et al., 1998). DEHP treatment up to 2500 mg/kg bw/day in marmosets from weaning (3 months old) to sexual maturation (18 months old) produced no evidence of testicular damage. Sperm head counts, zinc levels, glutathione levels and testicular enzyme activities were also not affected (Tomonari et al., 2006). In contrast to data from rabbits and rodents, no testicular effects of DEHP or DINP were found in non-human primates at any ages. The current understanding of how PAEs affects semen parameters, sperm DNA damage, and hormones in human populations is limited and further investigation is required.

3.2. Studies of the female reproductive system

Studies of adult female humans are less numerous than those of adult males. Cobellis et al. (2003) compared plasma concentrations of DEHP and MEHP in endometriotic women ($n = 55$) with control women ($n = 24$), and higher plasma DEHP concentrations were observed in endometriotic women. Similar results were observed in a recent study reported by Reddy et al. (2006). The investigators collected blood samples from 49 infertile women with endometriosis (the study group), 38 infertile women without endometriosis (control group I) and 21 women with proven fertility (control group II). Women with endometriosis showed significantly higher concentrations of DBP, BBP, DOP, and DEHP when compared to both control groups. Upon analysis of cord blood samples of 84 newborns, Latini et al. (2003b) revealed that MEHP-positive