were conducted for comparisons between control and 3-methylphenol-treated groups. If not homogenous or in the case of quantitative urinalysis data, analysis was performed using the Kruskal-Wallis test. In consequence, if a significant difference was detected, Dunnett type, Scheffe type or Mann-Whitney's U tests (Mann and Whitney, 1947) were conducted. In the newborn rat study, categorical data for general appearance, reflex ontogeny, necropsy and histopathology were analyzed by Fisher's exact probability test. A probability less than 5% was considered statistically significant.

RESULTS

18-day study in newborn rats (including the dose-finding study)

In a dose-finding study at doses of 100, 300 and 1,000 mg/kg, all animals at 1,000 mg/kg died within two days after the first treatment (Table 1). These rats showed deep respiration, decrease in spontaneous activity and pale skin. At 300 mg/kg, deep respiration and tremors under contact stimulus were noted during the dosing period in all animals, but no deaths occurred. No clinical signs were observed at 100 mg/kg. Body weight gain was depressed in females at 300 mg/kg. Blood biochemical examination showed a slight increase in total bilirubin in both sexes receiving 300 mg/kg (males; 0.36 mg/dL, compared with 0.32 mg/dL in controls, females; 0.37 mg/dL, 0.32 mg/dL). Organ weights, shown in Table 2, demonstrated a significant increase in relative liver weight in males at 100 and 300 mg/kg, and in females at 300 mg/kg but not absolute liver weights. No dose-related changes in hematology or gross findings were observed. Based on these results, the clearly toxic dose of 300 mg/kg was selected as the top dose in the main study, and 30 and 100 mg/kg were derived by approximately one-third divisions.

In the main study, various toxic signs such as deep respiration, increase in motor activity and tremors under contact stimulus were noted during the dosing period in all animals receiving 300 mg/kg, but no deaths occurred (Table 1). With 100 mg/kg, although

Table 1. Clinical signs and mortality in 18-day studies of 3-methylphenol in newborn rats.

	Dose-finding study			Main study		
Dose (mg/kg)	100	300	1,000	30	100	300
Males						
No. of animals	5	5	5	12	12	12
No. of dead animals	–	–	5[a]	–	–	–
No. of animals with clinical signs						
Deep respiration	–	5	3	–	–	5
Increase in motor activity	–	–	–	–	–	12
Decrease in spontaneous activity	–	–	5	–	–	1
Hypersensitivity on handling	–	–	–	–	1[b]	7
Tremors under contact stimulus	–	5	–	–	3[c]	12
Pale skin	–	–	1	–	–	–
Females						
No. of animals	5	5	5	12	12	12
No. of dead animals	–	–	5[a]	–	–	–
No. of animals with clinical sign						
Deep respiration	–	5	2	–	–	3
Increase in motor activity	–	–	–	–	–	12
Decrease in spontaneous activity	–	–	5	–	–	1
Hypersensitivity on handling	–	–	–	–	–	10
Tremors under contact stimulus	–	5	–	–	–	12
Pale skin	–	–	2	–	–	–

–: No animals with clinical sign.

[a]: All animals died within 2 days after first treatment, [b]: Observed only at dosing day 18, [c]: Observed only at dosing day 4 in one rat and at dosing day 11 in another two.

no clinical signs were observed in the dose-finding study, three males showed tremors under contact stimulus only on single days, dosing days 4, 11 and 11, respectively, and another male showed hypersensitivity on handling on a single day, dosing day 18. No change in general behavior was observed at 30 mg/kg. Body weights of both sexes given 300 mg/kg were lowered during the dosing period, but at 100 and 30 mg/kg were comparable to control values (Fig.1). No definitive changes in developmental parameters, including sexual maturation, as well as reflex ontogeny were detected in any dose group. At the scheduled sacrifice, blood biochemical examination of the 300 mg/kg group showed increases in γ-GTP, total bilirubin and BUN in males (Table 3). Significant increase of relative liver weight but not absolute liver weight was noted in both sexes given this highest dose (Table 2). In addition, there was a decrease in absolute brain weight in both sexes, but this change was not noted in the dose-finding study. On histopathological examination, basophilic tubules in kidneys showed a tendency to increase in 300 mg/kg males (slight and moderate changes in 2/6 and 3/6, respectively, compared with only slight change in 5~6/6 animals in other groups). After the recovery-maintenance period, there were no dose-related changes in body weight, blood biochemistry and histopathology, but low absolute brain weights remained in males (1.90 g, compared with 2.08 g in controls). No dose-related changes in food consumption, urinalysis, hematology and gross finding were observed throughout this study, including the recovery-maintenance period.

Since the hypersensitivity on handling and tremors under contact stimulus observed in a small number of males of the 100 mg/kg group were considered as dose-related adverse effects, the NOAEL in the main study was concluded to be 30 mg/kg/day. However, these clinical signs at 100 mg/kg were observed only on single days during the dosing period in the main study and not in the dose-finding study. Therefore, as for the unequivocally toxic level, 300 mg/kg/day was concluded to be appropriate because significant toxic effects in the central nervous system were observed at this dose, along with decrease in body weight gain.

28-day study in young rats (including the dose-finding study)

In the dose-finding study for 14 days at doses of 125, 250, 500 and 1,000 mg/kg, no deaths occurred at any dose (Table 4). Salivation, tremors and prone/lateral position were observed during the dosing period in both sexes at 1,000 mg/kg. Body weights and food consumption were lowered in males receiving 1,000 mg/kg. At 500 mg/kg and less, no changes in clinical signs, body weight and food consumption were observed. Blood biochemical examination showed increase in total cholesterol in females at 1,000 mg/kg

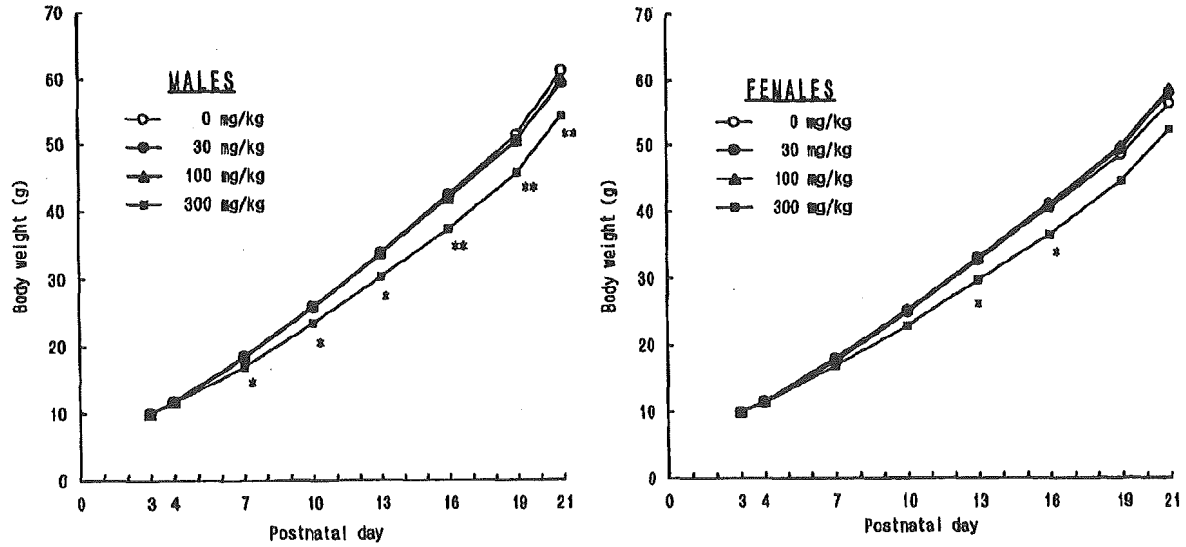

Fig. 1. Body weight change in 18-day study of 3-methylphenol in newborn rats (main study).
*: Significantly different from controls ($p < 0.05$), **: Significantly different from controls ($p < 0.01$).

(76.0 mg/dL, compared with 53.4 mg/dL at controls) and 500 mg/kg (72.2 mg/dL). Increase in relative liver weights in both sexes at 1,000 mg/kg (males: 3.86 g/100 g body weight, compared with 3.32 g/100 g body weight at controls, females: 3.70 g/100 g body weight, 3.34 g/100 g body weight) and 500 mg/kg (males: 3.60 g/100 g body weight, females: 3.68 g/100 g body weight), and in relative kidney weight in males at 1,000 mg/kg (0.47 g/100 g body weight, compared with 0.43 g/100 g body weight) was also observed. There were no dose-related changes evident on hematological and gross examination. Based on the results, the upper limit dose in the Test Guideline of 1,000 mg/kg was selected as the top dose for the main study, and 300 and 100 mg/kg were derived by division.

In the main study, deaths did not occur even at 1,000 mg/kg (Table 4). Salivation and tremors were observed throughout the dosing period at only 1,000 mg/kg in most males and females. At this dose, body weights were significantly lowered (finally 9% lower than controls for males and 11% for females) throughout the dosing period in males and from dosing day 14 in females, and food consumption was transiently lowered during the early dosing period in both sexes. At dosing week 4, increases in water consumption and urine volume were found in males and lowering of urinary pH in both sexes in the 1,000 mg/kg group. At 100 and 300 mg/kg, no changes in clinical signs, body weight, food consumption and urinalysis data were observed. Blood biochemical examination showed only slight increases in total cholesterol and BUN in males with a tendency for increase in total cholesterol in females receiving 1,000 mg/kg (Table 5). No dose-related changes in hematological findings were observed in any 3-methylphenol-treated group. There were significant increases in relative liver weights of

Table 2. Organ weights after 18-day repeat dosing of 3-methylphenol in newborn rats.

	Dose-finding study[a]			Main study			
Dose (mg/kg)	0	100	300	0	30	100	300
Males							
No. of animals	5	5	5	6	6	6	6
Body weight [b] (g)	62 ± 5	62 ± 6	59 ± 7	53.1 ± 3.3	52.7 ± 3.5	51.4 ± 3.5	46.7 ± 4.3*
Brain (g)	1.53 ± 0.05[c]	1.57 ± 0.06	1.52 ± 0.06	1.55 ± 0.04	1.58 ± 0.06	1.51 ± 0.06	1.47 ± 0.02*
	(2.47 ± 0.19[d])	(2.55 ± 0.18)	(2.61 ± 0.23)	(2.93 ± 0.18)	(3.00 ± 0.11)	(2.94 ± 0.13)	(3.16 ± 0.28)
Liver (g)	1.81 ± 0.13	1.91 ± 0.19	1.94 ± 0.24	1.74 ± 0.15	1.71 ± 0.13	1.75 ± 0.24	1.75 ± 0.20
	(2.90 ± 0.04)	(3.08 ± 0.19**)	(3.29 ± 0.05**)	(3.27 ± 0.12)	(3.24 ± 0.14)	(3.39 ± 0.25)	(3.74 ± 0.13**)
Kidney (g)	0.69 ± 0.06	0.72 ± 0.07	0.68 ± 0.05	0.64 ± 0.04	0.66 ± 0.06	0.62 ± 0.04	0.58 ± 0.02
	(1.11 ± 0.03)	(1.16 ± 0.03)	(1.16 ± 0.07)	(1.21 ± 0.05)	(1.26 ± 0.05)	(1.20 ± 0.06)	(1.25 ± 0.10)
Testis (mg)	310 ± 20	310 ± 30	320 ± 40	300 ± 28	293 ± 36	282 ± 14	270 ± 27
	(500 ± 20)	(500 ± 40)	(540 ± 20)	(566 ± 43)	(555 ± 52)	(549 ± 15)	(581 ± 51)
Females							
No. of animals	5	5	5	6	6	6	6
Body weight (g)	61 ± 4	59 ± 6	51 ± 6*	49.4 ± 3.8	50.5 ± 4.1	51.6 ± 3.3	45.5 ± 1.4
Brain (g)	1.50 ± 0.05	1.44 ± 0.03	1.43 ± 0.10	1.52 ± 0.05	1.48 ± 0.06	1.48 ± 0.05	1.42 ± 0.05*
	(2.46 ± 0.18)	(2.45 ± 0.24)	(2.81 ± 0.16*)	(3.09 ± 0.27)	(2.94 ± 0.27)	(2.88 ± 0.13)	(3.13 ± 0.10)
Liver (g)	1.77 ± 0.13	1.77 ± 0.12	1.67 ± 0.15	1.59 ± 0.18	1.59 ± 0.13	1.72 ± 0.08	1.61 ± 0.05
	(2.91 ± 0.08)	(3.01 ± 0.10)	(3.29 ± 0.15**)	(3.21 ± 0.13)	(3.16 ± 0.04)	(3.34 ± 0.11)	(3.54 ± 0.12**)
Kidney (g)	0.71 ± 0.05	0.70 ± 0.04	0.63 ± 0.06	0.63 ± 0.04	0.63 ± 0.04	0.65 ± 0.04	0.61 ± 0.04
	(1.19 ± 0.07)	(1.19 ± 0.10)	(1.24 ± 0.09)	(1.27 ± 0.03)	(1.25 ± 0.04)	(1.27 ± 0.09)	(1.34 ± 0.07)
Ovary (mg)	14.9 ± 2.8	14.4 ± 1.1	15.7 ± 2.4	15.6 ± 4.0	15.4 ± 3.0	13.8 ± 2.1	12.6 ± 2.3
	(24.4 ± 5.1)	(24.5 ± 1.5)	(30.9 ± 4.8)	(31.7 ± 8.9)	(30.5 ± 5.5)	(26.8 ± 4.7)	(27.8 ± 5.1)

Data are mean ± SD values.

[a]: In the 1,000 mg/kg group of the dose-finding study, since all animals died by dosing day 2, measurement of organ weights was not conducted, [b]: Body weight after overnight starvation following the last dosing, [c]: Absolute weight, [d]: Relative weight (g or mg/100 g body weight).

*: Significantly different from the control group ($p<0.05$), **: Significantly different from the control group ($p<0.01$).

both sexes at 1,000 mg/kg and of females at 300 mg/kg and in relative kidney weights of females at 1,000 mg/kg (Table 6). However, there was no change in absolute organ weights in any 3-methylphenol-treated group.

On histopathological examination, no dose-related changes were observed in any of the 3-methylphenol-treated groups. At the end of the recovery period, no significant changes in any parameters were observed.

Table 3. Blood chemical findings after dosing period in 18-day study of 3-methylphenol in newborn rats (main study).

Dose (mg/kg)	0	30	100	300
Males				
No. of animals	6	6	6	6
GOT (IU/L)	127 ± 13	121 ± 7	121 ± 11	132 ± 22
GPT (IU/L)	24 ± 4	21 ± 4	21 ± 3	21 ± 3
γ-GTP (IU/L)	0.84 ± 0.24	0.90 ± 0.15	1.07 ± 0.11	1.19 ± 0.15**
Total bilirubin (mg/dL)	0.40 ± 0.03	0.41 ± 0.04	0.41 ± 0.03	0.47 ± 0.02**
Total cholesterol (mg/dL)	74 ± 11	78 ± 9	81 ± 7	85 ± 9
Triglyceride (mg/dL)	29 ± 10	25 ± 6	32 ± 3	28 ± 7
BUN (mg/dL)	13.5 ± 1.8	11.8 ± 2.1	13.0 ± 2.1	17.9 ± 3.6*
Females				
No. of animals	6	6	6	6
GOT (IU/L)	122 ± 15	119 ± 12	131 ± 9	116 ± 10
GPT (IU/L)	16 ± 2	19 ± 4	19 ± 4	17 ± 2
γ-GTP (IU/L)	0.93 ± 0.21	0.85 ± 0.10	0.98 ± 0.26	1.20 ± 0.14
Total bilirubin (mg/dL)	0.41 ± 0.04	0.40 ± 0.03	0.40 ± 0.02	0.45 ± 0.03
Total cholesterol (mg/dL)	77 ± 11	77 ± 10	75 ± 8	78 ± 12
Triglyceride (mg/dL)	24 ± 5	26 ± 2	25 ± 3	23 ± 3
BUN (mg/dL)	13.5 ± 2.3	13.5 ± 2.5	13.2 ± 2.3	14.2 ± 2.8

Data are mean ± SD values.

*: Significantly different from control group ($p<0.05$), **: Significantly different from control group ($p<0.01$).

Table 4. Clinical signs and mortality in repeated dose studies of 3-methylphenol in young rats.

	Dose-finding study (14-day)				Main study		
Dose (mg/kg)	125	250	500	1,000	100	300	1,000
Males							
No. of animals	5	5	5	5	7	7	14
No. of dead animals	–	–	–	–	–	–	–
No. of animals with clinical signs							
Salivation	–	–	–	3	–	–	11
Tremors	–	–	–	3	–	–	12
Prone/lateral position	–	–	–	1	–	–	1
Soiled perigenital fur	–	–	–	–	–	–	–
Females							
No. of animals	5	5	5	5	7	7	14
No. of dead animals	–	–	–	–	–	–	–
No. of animals with clinical signs							
Salivation	–	–	–	–	–	–	8
Tremors	–	–	–	4	–	–	13
Prone/lateral position	–	–	–	2	–	–	2
Soiled perigenital fur	–	–	–	–	–	–	2

–: No animals with clinical sign.

Table 5. Blood chemical findings after dosing period in repeated dose studies of 3-methylphenol in young rats (main study).

Dose (mg/kg)	0	100	300	1,000
Males				
No. of animals	7	7	7	7
GOT (IU/L)	68.6 ± 4.8	65.4 ± 5.4	62.7 ± 3.2	59.4 ± 5.4**
GPT (IU/L)	24.7 ± 2.9	25.4 ± 3.7	27.0 ± 2.9	28.0 ± 3.7
γ-GTP (IU/L)	0.17 ± 0.24	0.21 ± 0.13	0.60 ± 1.15	0.36 ± 0.23
Total bilirubin (mg/dL)	0.056 ± 0.005	0.049 ± 0.007	0.054 ± 0.010	0.050 ± 0.008
Total cholesterol (mg/dL)	52.7 ± 15.1	58.1 ± 11.8	58.3 ± 5.8	69.0 ± 9.4*
Triglyceride (mg/dL)	43.7 ± 19.8	54.7 ± 22.4	37.6 ± 3.1	50.0 ± 26.9
BUN (mg/dL)	13.89 ± 1.46	14.10 ± 0.85	14.56 ± 1.17	16.23 ± 2.14*
Females				
No. of animals	7	7	7	7
GOT (IU/L)	57.1 ± 4.3	65.9 ± 3.6	62.0 ± 5.7	59.1 ± 3.1
GPT (IU/L)	20.6 ± 2.2	21.4 ± 2.9	18.9 ± 3.1	20.1 ± 4.2
γ-GTP (IU/L)	0.83 ± 0.20	0.90 ± 0.16	1.00 ± 0.29	1.06 ± 0.10
Total bilirubin (mg/dL)	0.053 ± 0.011	0.056 ± 0.011	0.043 ± 0.008	0.054 ± 0.008
Total cholesterol (mg/dL)	63.4 ± 14.0	58.7 ± 10.6	61.4 ± 10.3	78.7 ± 13.7
Triglyceride (mg/dL)	15.4 ± 8.2	11.3 ± 4.8	9.9 ± 1.8	16.1 ± 5.2
BUN (mg/dL)	17.71 ± 1.96	16.63 ± 1.11	17.30 ± 2.14	18.03 ± 2.00

Data are mean ± SD values.

*: Significantly different from control group ($p<0.05$), **: Significantly different from control group ($p<0.01$).

Table 6. Organ weights after dosing period in repeated dose studies of 3-methylphenol in young rats (main study).

Dose (mg/kg)	0	100	300	1,000
Males				
No. of animals	7	7	7	7
Body weight[a] (g)	325.0 ± 23.5	345.6 ± 23.5	335.9 ± 16.7	298.3 ± 31.8
Brain (g)	2.04 ± 0.06[b]	2.11 ± 0.09	2.03 ± 0.08	2.05 ± 0.06
	(0.63 ± 0.05[c])	(0.61 ± 0.03)	(0.60 ± 0.02)	(0.69 ± 0.06*)
Liver (g)	10.55 ± 1.30	11.28 ± 1.08	11.29 ± 0.68	10.94 ± 2.01
	(3.24 ± 0.22)	(3.26 ± 0.19)	(3.36 ± 0.11)	(3.65 ± 0.34**)
Kidney (g)	2.65 ± 0.24	2.82 ± 0.24	2.78 ± 0.19	2.61 ± 0.23
	(0.82 ± 0.04)	(0.82 ± 0.03)	(0.83 ± 0.06)	(0.88 ± 0.05)
Testis (g)	2.96 ± 0.31	3.06 ± 0.21	2.95 ± 0.30	2.93 ± 0.22
	(0.91 ± 0.11)	(0.89 ± 0.08)	(0.88 ± 0.10)	(0.99 ± 0.14)
Females				
No. of animals	7	7	7	7
Body weight (g)	210.1 ± 15.4	207.6 ± 13.0	197.3 ± 19.3	186.4 ± 17.4*
Brain (g)	1.96 ± 0.06	1.90 ± 0.07	1.89 ± 0.07	1.88 ± 0.06
	(0.93 ± 0.06)	(0.92 ± 0.05)	(0.96 ± 0.08)	(1.01 ± 0.09)
Liver (g)	6.39 ± 0.68	6.59 ± 0.56	6.60 ± 0.67	6.51 ± 0.45
	(3.04 ± 0.17)	(3.17 ± 0.08)	(3.35 ± 0.13**)	(3.50 ± 0.20**)
Kidney (g)	1.66 ± 0.18	1.73 ± 0.11	1.65 ± 0.17	1.72 ± 0.14
	(0.79 ± 0.06)	(0.84 ± 0.05)	(0.84 ± 0.06)	(0.92 ± 0.03**)
Ovary (mg)	81.4 ± 13.7	82.0 ± 14.3	85.0 ± 14.0	78.9 ± 13.3
	(38.8 ± 6.3)	(39.5 ± 6.1)	(43.4 ± 8.3)	(42.4 ± 6.8)

Data are mean ± SD values.

[a]: Body weight after overnight starvation following last dosing, [b]: Absolute weight, [c]: Relative weight (g or mg/100 g body weight).

*: Significantly different from control group ($p<0.05$), **: Significantly different from control group ($p<0.01$).

Based on clinical signs of neurotoxicity with lowering of body weights, the unequivocally toxic level was concluded to be 1,000 mg/kg/day. Increase in relative liver weight without related changes at 300 mg/kg in the main study was not considered as an adverse effect. In the dose-finding study, effects on liver were noted at 500 mg/kg but no dose-related changes were evident at 250 mg/kg, which could not be taken into consideration of the estimation of the NOAEL because of the insufficient dosing period (14 days). Therefore, the NOAEL was concluded to be 300 mg/kg/day.

DISCUSSION

Concerning health of infants exposed to chemicals, our testing project has provided the following benefits. First, detailed examination of physical development and sexual maturation during the early postnatal period provides specific information on chemical toxicity towards newborn animals. Second, because the same experimental conditions, as much as possible, are set between newborn and young rat studies, this facilitates comparisons of toxicity. Furthermore, for toxicity levels, two additional analyses (estimation of unequivocally toxic levels in addition to NOAELs and careful incorporation of the dose-finding study) allow more precise / appropriate comparisons. So far, we have reported three comparative analyses of 4-nitrophenol, 2,4-dinitrophenol and 3-aminophenol (Koizumi *et al.*, 2001, 2002a, 2002b; Yamamoto *et al.*, 2001; Takano *et al.*, 2001; Nishimura *et al.*, 2002). As results, the toxicity profiles of these chemicals were similar in both ages, the susceptibility of newborn rats was 2 to 4 times higher than that of young rats, and no effects on physical development, sexual maturation and reflex ontogeny were observed.

In the present study, 3-methylphenol was selected as a fourth chemical. Clinical signs, indications of neurotoxicity to the central nervous system, were observed in both ages but not at the same dose level. Decrease in body weight gain also occurred in both ages but at a 3 times lower dose in newborn animals. In the newborn study, significant decrease in absolute brain weight was also evident at the highest dose, but no abnormalities on histopathology in the brain or in terms of functional development (reflex ontogeny) were observed. Brain weight changes were observed only in the groups showing 10% and more lowering of body weight and were not noted in the dose-finding study. Brain weight might be affected by decrease in body weight gain. As unequivocally toxic levels were clearly judged to be 300 mg/kg/day and 1,000 mg/kg/day for newborn and young studies, respectively, based on neurotoxic effects and decrease in body weight gain, newborn rats were considered to be approx. 3 times more susceptible to this chemical than young rats. NOAELs were concluded to be 30 mg/kg/day and 300 mg/kg/day for newborn and young rats, respectively, indicating a 10 times higher susceptibility in the newborn. However, tremors under contact stimulus were observed in only three males on single days and hypersensitivity on handling was noted only in one male on a single day in the 100 mg/kg newborns. Furthermore, no such toxic clinical signs were noted at 100 mg/kg in the dose-finding study under the same experimental conditions. It appears that the realistic no adverse effect dose for the newborn is slightly lower than 100 mg/kg/day rather than around 30 mg/kg/day. Based on this speculation and equal toxicity at the unequivocally toxic levels, the difference in the sensitivity to 3-methylphenol between newborn and young rats could be considered to be 3- to 4-fold.

As for the toxicity of 3-methylphenol, much information is available including unpublished data reported in reviews on this chemical or cresols (ATSDR, 1991; EHC, 1995; IRIS, 1997). In a 28-day feeding study (NTP, 1992), F344 rats were given diet containing 3-methylphenol at 0, 300, 1,000, 3,000, 10,000, 30,000 mg/kg diet. Depression of body weight gain, increase in relative liver and kidney weight and uterus atrophy were observed at 30,000 mg/kg diet (about 2,390 mg/kg/day). Increase in relative liver weight was also noted at 10,000 mg/kg diet (866 mg/kg/day). These results are consistent with our present results for young rats. However, clinical signs observed in our young rat study (daily administration by gavage) were not found at any doses in this NTP study, which might be due to the lower blood concentration with dietary application than in our gavage study. In a 90-day study (MBA, 1988), SD rats were administered 3-methylphenol by gavage at 50, 150, 450 mg/kg. In addition to depression of body weight gain at 150 mg/kg and more, a pronounced increase in the incidence of salivation, tremors and urination was observed at 450 mg/kg. In another 90-day study under the same test conditions with more detailed neurotoxic analysis, hypoactivity, rapid labored respiration and excessive salivation were observed sporadically in all treated groups, although few significant changes were found in performance on neurobehavioral test batteries, and no brain weight changes and no gross or histopathological changes in the brain or other nervous tissues (TRL,

1986). These clinical signs observed at lower doses than our young rat study might be due to the longer dosing period, but no information was provided on the incidence or dose-relationship. As for developmental toxicity, no effects on fetuses were observed in rats treated with 3-methylphenol by gavage at 450 mg/kg or less on days 6-15 of gestation (BRRC, 1988a). However, in a 2-generation reproductive toxicity study on rats by gavage (BRRC, 1989), some effects on pup body weights and survival (no details on the incidence and the degree) were evident with 450 mg/kg, which caused severe maternal toxicity including death and various clinical signs. There were occasional body weight changes in lower dose groups, but it is not clear whether these changes were treatment-related.

Some causes of differences in susceptibility of newborn and young rats to 3-methylphenol can be considered, such as specific physiological characteristics and immaturity of the brain-blood barrier and metabolism in the newborn. It is reported that 3-methylphenol is mainly eliminated as glucuronides in urine (Bray *et al.*, 1950). UDP-glucuronyltransferase activity in rat liver is known to be substrate-specific and generally low in neonates, and the activity against phenolic substances, p-nitrophenol and 1-naphthol, at birth has been shown to be comparable to adults but nearly 50% lower during the suckling period (exposure period in our newborn study) (Watkins and Klaassen, 1985; Rachmel and Hazelton, 1986). Therefore, the low capacity of glucuronidation might be one of the major causes for higher susceptibility of newborn rats to 3-methylphenol. In the case of humans, hepatic glucuronidation at birth is known to be relatively immature (Gow *et al.*, 2001), and it has been shown that *in vitro* bilirubin glucuronidation activity at birth is much lower than that of mature-phase values (Kawade and Onishi, 1981). These data suggest that human infants may be more susceptible to chemicals that are detoxified by this pathway.

The effects on the central nervous system, leading to death, are a major toxicological outcome characteristic of some phenolic compounds (Koizumi *et al.*, 2001, 2002b); however, the mechanism(s) responsible for eliciting neurotoxicity is unknown. As for hepatotoxicity, several studies on the mechanism and the structure activity relationship, using hepatocytes or liver slices, have been reported for three isomers of methylphenols and some para-alkylphenols (Bolton *et al.*, 1992; Thompson *et al.*, 1994, 1995, 1996; Kitagawa, 2001). In these studies, it has been shown that the quinone intermediates are most likely to be the causative agents for hepatotoxicity, possibly via mitochondrial toxicity, and the hepatotoxicity of alkylphenols depends on the position and the kind of alkyl groups with 4-methylphenol exerting the greatest degree of hepatotoxicity. In the case of 3-methylphenol, the neurotoxicity seems to be the most sensitive endpoint in both newborn and young animals, since only minor increases in relative liver weight have been observed without any histopathological changes.

In conclusion, 3-methylphenol showed the same toxicity profile—that is neurological symptoms and growth inhibition—in both newborn and young rats. However, the susceptibility of the newborn rats was 3 to 4 times higher than that of young rats, consistent with our previous results for three chemicals, 4-nitrophenol, 2,4-dinitrophenol and 3-aminophenol, which showed 2 to 4 times differences in susceptibility between newborn and young rats.

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Unexpected nephrotoxicity induced by tetrabromobisphenol A in newborn rats

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Abstract

The repeated dose toxicity of tetrabromobisphenol A (TBBPA), a flame retardant, was examined in male and female newborn rats given TBBPA orally at 0, 40, 200, or 600 mg/kg per day for 18 days from 4 days of age until weaning at 21 days of age. Half the rats in each dose group were sacrificed for a full gross necropsy and a histopathology on the organs and the tissues at 22 days of age and the remaining rats were reared without any treatment from post-weaning until 84 days of age to examine the recovery and the delayed occurrence of toxic effects. Treatment with 200 or 600 mg/kg TBBPA-induced nephrotoxicity characterized by the formation of polycystic lesions, and some deaths occurred in the 600 mg/kg group. There was no gender difference of nephrotoxicity and there were no other critical toxicities. At 85 days of age, nephrotoxic lesions were still present in the 200 and 600 mg/kg groups, but no abnormalities indicating delayed occurrence of toxic effects were found in the treated groups. In order to investigate the specificity of the nephrotoxicity induced by TBBPA in newborn rats, TBBPA was given to male and female young rats (5 weeks old) by oral administration at 0, 2000, or 6000 mg/kg per day for 18 days. The kidneys showed no histopathological changes even at the high dose. These results clearly indicate that the nephrotoxicity of TBBPA is specific for newborn rats although the toxic dose level was relatively high. To gain insight into the possible effects on human infants, the mechanism of this unexpected nephrotoxicity of TBBPA in newborn rats should be examined.

Keywords: Tetrabromobisphenol A; 4,4′-Isopropylidene bis(2,6-dibromophenol); Unexpected nephrotoxicity; Polycystic kidney; Newborn rats

1. Introduction

Recently, there is growing concern about the effects of environmental chemicals on children,

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particularly infants, who may be more sensitive on a body weight basis than adults to a given toxicant exposure (Scheuplein et al., 2002). To address this issue, we have conducted repeated toxicity studies of 18 chemicals in newborn rats as a Japanese National Project. So far, comparative evaluation of the toxicity in newborn and young rats has been conducted for four chemicals, 4-nitrophenol, 2,4-dinitrophenol, 3-aminophenol, and 3-methylphenol (Koizumi et al., 2001, 2002, 2003). The results showed that the susceptibility of newborn rats to these chemicals was approximately two to four times higher than that of young rats, although the toxicological profiles were almost the same at both ages.

Tetrabromobisphenol A (TBBPA), the fifth chemical subjected to the comparative analysis, has been widely used as a flame retardant. Its toxicity was previously investigated using young or young adult animals as follows: in 28- and 90-day feeding studies using rats, no toxic effects were observed up to 50 and 100 mg/kg per day, respectively (Goldenthal and Geil, 1972; Quast et al., 1975). In mice given TBBPA in their food for 90 days, all animals at 7100 mg/kg per day died while suffering from malnutrition and anemia (Tobe et al., 1986). Inhibition of body weight gain and anemia, but not death, were observed at 2200 mg/kg per day, and the non-toxic level was 700 mg/kg per day. There were no signs of maternal or developmental toxicity when rats were given this chemical during pregnancy up to 3000 mg/kg per day (Goldenthal et al., 1978; Noda et al., 1985). Recently, a 28-day repeated dose toxicity study of this chemical was conducted in rats using the Japanese test guidelines (equivalent to OECD guideline for testing of chemicals for repeated dose 28-day toxicity study in rodents (407)) under the Principles of Good Laboratory Practice, and showed no chemical-related effects up to 1000 mg/kg per day (MHLW, 2001).

In the present study, we performed a 18-day repeated dose oral toxicity study using newborn rats from 4 days of age under the same experimental conditions reported previously (Koizumi et al., 2001), and unexpectedly found severe nephrotoxicity. Therefore, a young rat study was also conducted at a dose up to 6000 mg/kg per day to confirm the specificity of the nephrotoxicity in newborn rats.

2. Materials and methods

2.1. Materials

Tetrabromobisphenol A: TBBPA (4,4′-isopropylidene bis(2,6-dibromophenol), molecular weight 543.88, CAS No.79-94-7, 99.5% purity) was obtained from Toso Co. Ltd. (Yamaguchi, Japan) and suspended in 0.5% (w/v) carboxymethylcellulose-Na (Kanto Chemicals Co. Ltd., Tokyo, Japan) solution with 0.1% (w/v) Tween 80 (Difco Laboratories, Detroit, Michigan, USA). The suspension was prepared at least once a week and stored hermetically in a cool and dark place (4 °C) until dosing. The stability of TBBPA under these conditions was confirmed to be at least 8 days by an analysis of dosing suspensions.

2.2. Animals

Sprague-Dawley SPF rats (Crj:CD(SD)IGS) were purchased from Charles River Japan Inc. (Atsugi, Japan) and maintained in an environmentally controlled room at 22 ± 3 °C with a relative humidity of 55 ± 10%, an air exchange rate of more than 10 times per hour, and a 12:12 h light/dark cycle. All animals were allowed free access to commercial solid diet (Labo MR Stock, Nihon Nosan Kogyo Co. Ltd., Yokohama, Japan) and tap water. The animals used in the present study were reared, treated, and sacrificed in accordance with "The Provisions for Animal Welfare" of the Research Institute for Animal Science in Biochemistry and Toxicology, which follow the guidelines for animal experimentation issued by Japanese Association for Laboratory Animal Science.

2.3. Newborn rat study

For the study of newborn rats, 20 pregnant rats (gestation day 15) were purchased and were allowed to deliver spontaneously. Among all newborns separated from each dam at the age of 3 days, 48 males and 48 females were randomly selected and assigned to four dose groups, including controls. Twelve foster mothers suckled four males and four females assigned to each group up to weaning on day 21 after birth. After weaning, the animals of the recovery-maintenance group were individually maintained for 9 weeks.

In the dose finding study, newborn rats (five/sex/group) were administered TBBPA by gastric intubation at 0, 40, 200 or 1000 mg/kg per day from days 4–21 after birth. They were examined daily for general behavior and measured twice a week for body weight, and sacrificed at postnatal day 22, after overnight starvation, for assessment of hematology, blood biochemistry, macroscopic findings and weight of organs.

In the main study, newborn rats were administered TBBPA at 0 (vehicle as a control), 40, 200 or 600 mg/kg per day, based on the results of the dose finding study, by gastric intubation daily from 4 to 21 days after birth, and sacrificed under ether anesthesia after overnight starvation following the last treatment (scheduled-sacrifice group). Recovery-maintenance groups at the same dosages were maintained for 9 weeks without chemical treatment and sacrificed at 12 weeks of age. The number of animals at each sex/dose was six for both the scheduled-sacrifice and recovery-maintenance groups.

General behavior was observed daily. Body weights were measured twice a week during the dosing period and once a week during the recovery-maintenance period. Food consumption during 24 h was measured once a week during the recovery-maintenance period. At day 20 after birth for males and day 21 for females, gait condition, pupillary reflex, auricular reflex, corneal reflex, visual placing reflex, surface and mid-air righting reflexes, and ipsilateral flexor reflex were examined (Moser et al., 1991). Furthermore, fur appearance, incisor eruption and eye opening were examined in all animals from postnatal days 7, 9 and 11, respectively, and testes descent or vaginal opening was observed only in the recovery-maintenance group from postnatal day 17 or 29, respectively. During the period from days 78–82 after birth (only in the recovery-maintenance group), urine samples were obtained for the determination of pH, protein, glucose, ketone bodies, bilirubin, urobilinogen and occult blood using Multistix (Biel-Sankyo, Tokyo, Japan). Color, sediment, specific gravity and volume of the urine were also examined. For hematology and blood biochemistry, blood was collected from the abdominal aorta under ether anesthesia at sacrifice after overnight starvation for both the scheduled-sacrifice and recovery-maintenance groups. One part of the blood was examined for hematological parameters such as the red blood cell count, hemoglobin, hematocrit, white blood cell count, platelet count using a automatic blood cell analyzer (Sysmex E-4000,Toa Medical Electronics Co. Ltd., Kobe, Japan). The reticulocyte count and the differential leukocyte count were obtained by examining brilliant-cresyl-blue-stained and May-Giemsa-stained blood smears, respectively. In addition, blood clotting parameters such as prothrombin time (PT) and activated thromboplastin time (APTT) were measured using a coagulometer (Amelung-Coagulometer KC-10, Baxter Co. Ltd., Tokyo, Japan). Plasma obtained from the other portion of the blood was analyzed for blood biochemical parameters such as total protein, albumin, albumin–globulin ratio, glucose, total cholesterol, triglycerides, phospholipid, total bilirubin urea nitrogen (BUN), creatinine, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), γ-glutamyl transpeptidase, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), choline esterase, calcium, inorganic phosphorus using a clinical biochemistry analyzer (JCA-BM8, JEOL. Ltd., Tokyo, Japan). In addition, serum levels of sodium, potassium and chloride were determined using an auto electrolyte analyzer (NAKL 132, TOA Electronics Ltd., Tokyo, Japan). After recording the macro findings for all organs of animals sacrificed under ether anesthesia, the brain, pituitary gland, heart, thymus, liver, kidneys, spleen, adrenal glands, thyroids, lungs, testes, epididymides, prostate, ovaries, and uterus were removed and weighed. Histopathological examination was conducted for the control and the highest dose groups. The trachea, stomach, intestine, pancreas, lymph node, urinary bladder, spinal cord, sciatic nerve, seminal vesicles, bone, and bone marrow as well as the above organs were fixed with 10% buffered formalin-phosphate (following Bouin's fixation for testes and epididymides), and paraffin sections were prepared using routine methods and stained with hematoxylin-eosin for microscopic examination. For other groups, the organs in which dose-related effects were evident on microscopic examination for the highest dose group were examined.

2.4. Young rat study

In the study of young rats, 4-week-old male and female rats were obtained and used when they were 5-week-old, after 1 week acclimation. Five male and

female SD young rats for each group were administered TBBPA at 0, 2000 or 6000 mg/kg per day by gavage for 18 days. General behavior was observed daily and body weight was measured twice a week. At the termination of the treatment, animals were sacrificed under ether anesthesia and macroscopic findings of the major organs were recorded. The kidneys were removed and weighed, and histopathological examination was performed.

2.5. Statistical analysis

Continuous data were analyzed by Bartlett's test (Bartlett, 1937) for homogeneity of distribution. When homogeneity was recognized, Dunnett's test (Dunnett, 1964) ($P < 0.01$ or 0.05) was conducted for comparison between control and individual treatment groups after one-way layout analysis of variance (Yoshimura, 1997). If the data were not homogenous, they were analyzed using the Kruskal–Wallis test (Kruskal and Wallis, 1952) following a mean rank test of the Dunnett type (Hollander and Wolfe, 1973) ($P < 0.01$ or 0.05). Quantitative data were analyzed by Fisher's exact test (Fisher, 1973) ($P < 0.01$ or 0.05).

3. Results

3.1. Newborn rat study

In the dose finding study, various abnormalities were observed at 1000 mg/kg as follows: diarrhea, lowering of body weight, decreases in prothrombin time, activated thromboplastin time and hemoglobin, increase in platelet count, LDH, GOT, BUN, total bilirubin and creatinine, remarkable enlargement of kidneys, slight dilation of the cecum, and increases in the absolute and relative weights of the liver and kidneys (Table 1). Unexpectedly, the relative weights of the kidneys for both sexes reached approximately six times higher than those in controls. No histopathological information on the kidneys was obtained because of the lack of an examination schedule in the protocol. In the 200 mg/kg group, there were no significant changes except for a decrease in prothrombin time in females. Based on these results, 600 mg/kg, at which toxic effects should be clearly observed, was selected as the high dose, 40 mg/kg as the low (non-toxic) dose, and 200 mg/kg as the medium dose in the main study.

Table 1
Relative weights of the major organs at the termination of treatment in dose finding and main newborn studies

	mg/kg per day	Number of rats	Body weight (g)	Brain	Liver	Kidney	Testis	Ovary
Dose finding								
Males	0	5	56 ± 4	2.71 ± 0.08	2.94 ± 0.10	1.15 ± 0.04	0.53 ± 0.02	
	40	5	57 ± 3	2.74 ± 0.11	2.86 ± 0.04	1.16 ± 0.06	0.53 ± 0.03	
	200	5	55 ± 5	2.79 ± 0.24	2.92 ± 0.14	1.17 ± 0.06	0.54 ± 0.04	
	1000	5	53 ± 2	2.79 ± 0.10	3.42 ± 0.13**	6.96 ± 2.21	0.51 ± 0.04	
Females	0	5	56 ± 4	2.84 ± 0.12	2.92 ± 0.06	1.24 ± 0.05		0.036 ± 0.015
	40	5	57 ± 3	2.83 ± 0.16	2.96 ± 0.09	1.26 ± 0.08		0.031 ± 0.006
	200	5	55 ± 5	2.78 ± 0.17	3.01 ± 0.12	1.15 ± 0.08		0.032 ± 0.009
	1000	5	53 ± 2	2.84 ± 0.11	3.47 ± 0.23**	7.61 ± 3.05		0.024 ± 0.010
Main								
Males	0	6	51 ± 3	2.93 ± 0.23	3.25 ± 0.14	1.26 ± 0.04	0.57 ± 0.07	
	40	6	52 ± 3	2.97 ± 0.14	3.27 ± 0.11	1.28 ± 0.04	0.60 ± 0.04	
	200	6	52 ± 3	3.01 ± 0.14	3.37 ± 0.09	1.22 ± 0.03	0.60 ± 0.04	
	600	6	51 ± 2	3.02 ± 0.14	3.60 ± 0.17**	3.57 ± 0.77*	0.58 ± 0.04	
Females	0	6	48 ± 4	3.18 ± 0.21	3.21 ± 0.21	1.33 ⊥ 0.07		0.028 = 0.004
	40	6	48 ± 2	3.04 ± 0.10	3.24 ± 0.05	1.33 ± 0.06		0.033 = 0.008
	200	6	48 ± 2	3.06 ± 0.17	3.32 ± 0.11	1.37 ± 0.10		0.031 ± 0.008
	600	6	48 ± 4	3.01 ± 0.22	3.44 + 0.26	4.86 ± 4.47**		0.029 ± 0.007

Values are given as mean ± S.D.
Significantly different from control (*$P < 0.05$; **$P < 0.01$).

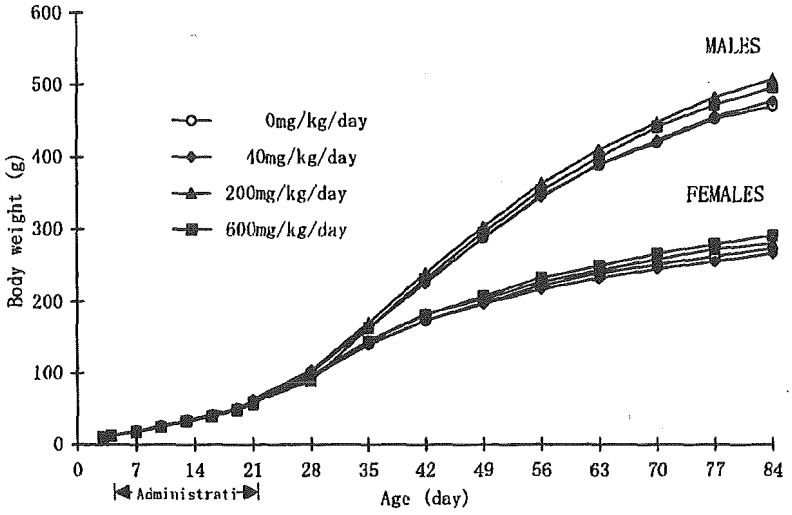

Fig. 1. Body weight changes of rats treated orally with TBBPA for 18 days from 4 days of age until weaning.

In the main study, diarrhea occurred sporadically during the treatment period in some males and females in the 200 and 600 mg/kg groups. There were no differences in body weight gain between the control and TBBPA-treated groups (Fig. 1). No definitive changes in physical development or reflex ontogeny were detected in any dose group. At the scheduled-sacrifice, the hematological and blood biochemical examinations showed decreases in hemoglobin in females and activated thromboplastin time in males, and increase of 600 mg/kg in total bilirubin in both the sexes (Table 2). The absolute and relative kidney weights dramatically increased in both sexes and the relative liver weight increased slightly in males (Table 1). The relative kidney weights were 2.8 times higher in males and 3.7 times higher in females than those in the control groups. The macroscopic appearance of the kidneys is shown in Fig. 2.

Histopathological findings of the kidneys are shown in Table 3. In the kidneys of two of six males in the 200 mg/kg group and all six males and six females in the 600 mg/kg group, polycystic lesions associated with the dilation of the tubules were noticed bilaterally from the cortico-medullary junction to the inner cortex (Fig. 3A). The changes of the lesions in the 600 mg/kg group were so severe that the tissue specimen looked like a sponge in gross examinations. In addition, hyperplasia of the renal tubular epithelium was observed from the cortico-medullary junction to the inner cortex (Fig. 3B), and the outer cortex was contracted due to the pressure produced by the cysts. Some rats also had marked hyaline casts within tubules and/or regenerating basophilic tubules or suppurative inflammatory reactions. Regarding other histopathological changes,

Fig. 2. Gross appearance of kidney (lower right) and its cross-section (upper right) in a 22-day-old rat treated with TBBPA (600 mg/kg body weight daily, orally) for 18 days. The kidney is markedly larger than that of a non-treated rat (left).

Table 2
Hematological and blood biochemical findings at 22 days of age of rats treated orally with TBBPA for 18 days from 4 days of age until weaning

Item	Dose (mg/kg per day)			
	0	40	200	600
Males				
Number of animals	6	6	6	6
Erythrocyte ($10^4/\mu l$)	480 ± 9	479 ± 14	483 ± 24	483 ± 19
Hemoglobin (g/dl)	9.7 ± 0.4	9.6 ± 0.5	9.3 ± 0.9	8.9 ± 0.7
Hematocrit (%)	30.9 ± 1.2	30.7 ± 1.7	30.4 ± 2.3	29.1 ± 1.6
Leukocyte ($10^2/\mu l$)	16 ± 5	18 ± 5	16 ± 7	18 ± 7
Platelet ($10^4/\mu l$)	145 ± 14	139 ± 11	141 ± 9	153 ± 15
PT (s)	13.6 ± 0.3	13.7 ± 0.3	13.7 ± 0.3	13.3 ± 0.4
APTT (s)	15.2 ± 0.4	14.4 ± 0.7	14.4 ± 0.8	14.2 ± 0.3*
LDH (IU/l)	521 ± 120	462 ± 198	557 ± 143	536 ± 143
GOT (IU/l)	127 ± 12	129 ± 11	132 ± 10	139 ± 17
GPT (IU/l)	25 ± 1	28 ± 5	28 ± 4	31 ± 5
ALP (IU/l)	995 ± 184	1079 ± 138	1075 ± 96	1224 ± 146
Total bilirubin (mg/dl)	0.41 ± 0.02	0.40 ± 0.03	0.43 ± 0.03	0.50 ± 0.05**
Total protein (g/dl)	4.93 ± 0.12	4.71 ± 0.24	4.69 ± 0.23	4.70 ± 0.16
Albumin (g/dl)	3.13 ± 0.07	2.98 ± 0.21	2.95 ± 0.18	2.99 ± 0.14
Total cholesterol (mg/dl)	80 ± 15	82 ± 7	74 ± 13	80 ± 12
BUN (mg/dl)	15.2 ± 3.4	15.4 ± 3.0	16.0 ± 4.0	14.8 ± 4.2
Creatinine (mg/dl)	0.45 ± 0.03	0.43 ± 0.05	0.45 ± 0.02	0.45 ± 0.02
Na (meq./l)	143 ± 1	142 ± 1	142 ± 1	142 ± 1
K (meq./l)	6.93 ± 0.65	7.07 ± 0.31	7.19 ± 0.60	6.80 ± 0.54
Cl (mcq./l)	107 ± 2	108 ± 1	107 ± 1	106 ± 2
Females				
Number of animals	6	6	6	6
Erythrocyte ($10^4/\mu l$)	507 ± 26	512 ± 12	507 ± 23	503 ± 12
Hemoglobin (g/dl)	10.0 ± 0.8	10.2 ± 0.4	9.9 ± 0.5	9.0 ± 0.4**
Hematocrit (%)	31.5 ± 2.0	32.7 ± 1.3	32.0 ± 1.8	29.5 ± 1.0
Leukocyte ($10^2/\mu l$)	23 ± 7	23 ± 6	26 ± 14	25 ± 4
Platelet ($10^4/\mu l$)	142 ± 17	155 ± 15	152 ± 22	160 ± 23
PT (s)	13.9 ± 0.2	14.0 ± 0.6	13.6 ± 0.4	13.6 ± 0.4
APTT (s)	14.4 ± 0.7	15.6 ± 0.9*	14.2 ± 0.6	13.5 ± 0.9
LDH (IU/l)	598 ± 249	613 ± 48	479 ± 88	615 ± 158
GOT (IU/l)	135 ± 18	137 ± 16	119 ± 11	148 ± 23
GPT (IU/l)	19 ± 2	21 ± 4	20 ± 4	23 ± 4
ALP (IU/l)	925 ± 189	1007 ± 99	983 ± 150	1109 ± 94
Total bilirubin (mg/dl)	0.38 ± 0.03	0.39 ± 0.03	0.41 ± 0.02	0.50 ± 0.13**
Total Protein (g/dl)	5.01 ± 0.25	4.94 ± 0.07	4.77 ± 0.17	4.82 ± 0.39
Albumin (g/dl)	3.25 ± 0.20	3.15 ± 0.11	3.03 + 0.18	3.03 ± 0.08
Total cholesterol (mg/dl)	69 ± 13	74 ± 23	72 ± 11	93 ± 31
BUN (mg/dl)	18.5 ± 4.1	16.6 ± 2.6	14.6 ± 2.0	21.6 ± 13.4
Creatinine (mg/dl)	0.46 ± 0.04	0.47 ± 0.03	0.43 ± 0.03	0.48 ± 0.11
Na (meq./l)	142 ± 1	142 ± 1	142 ± 1	142 ± 1
K (meq./l)	7.18 ± 0.69	7.29 ± 0.43	4.24 ± 0.20	7.01 ± 0.53
Cl (meq./l)	108 ± 2	108 ± 1	107 ± 1	106 ± 3

Each value is expressed as mean ± S.D.
Significantly different from control (*$P < 0.05$; **$P < 0.01$).

Table 3
Incidence of renal histopathological findings of rats treated orally with TBBPA for 18 days from 4 days of age until weaning

Findings	Grade	22 Days of age								85 Days of age								FD/KE	
		0[a]		40[a]		200[a]		600[a]		0[a]		40[a]		200[a]		600[a]		600[a]	
		M 6[b]	F 6[b]	M 6[b]	F 6[b]	M 6[b]	F 6[b]	M 6[b]	F 6[b]	M 6[b]	F 6[b]	M 6[b]	F 6[b]	M 6[b]	F 6[b]	M 4[b]	F 5[b]	M 2[b]	F 1[b]
Cyst, multiple	+	0	0	0	0	2	0	0	0	0	0	0	0	1	1	0	1	0	0
	++	0	0	0	0	0	0	0	6	0	0	0	0	0	0	3	4	0	0
	+++	0	0	0	0	0	0	6	0	0	0	0	0	0	0	1	0	2	1
Cast, hyaline	+	0	0	0	0	0	0	2	3	0	0	0	0	0	0	1	2	0	0
	++/+++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	1
Cast, granular	+/++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1
Necrosis, tublar epithelium	+/++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1
Basophilic tubules	+	4	6	5	5	5	5	4	4	2	0	3	2	3	1	1	3	0	0
	++/+++	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2	1	2	0
Cellular infiltration, lymphocytes	+	0	0	1	0	0	0	0	0	0	1	1	0	1	0	2	1	0	0
	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0
Inflammation, suppurative	+++	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Hyperplasia, tublar epithelium	+	0	0	0	0	2	0	6	3	0	0	0	0	0	0	2	2	1	0
	++	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1
Atrophy, cortical	+	0	0	0	0	0	0	5	5	0	0	0	0	0	0	1	0	0	0
	++/+++	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	1
Fibrosis, interstitial	+	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	++/+++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0

M: male; F: female; (+): slight; (++): moderate; (+++): severe; FD/KE: found dead or killed in extremis.
[a] Doses in milligram per kilogram per day.
[b] Number of animals.

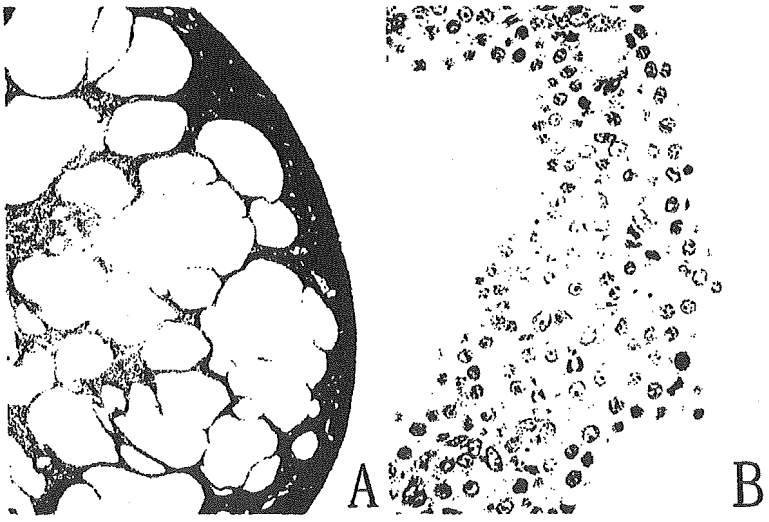

Fig. 3. Polyeystic renal lesion observed in a 22-day-old rat treated with TBBPA (600 mg/kg body weight daily, orally) for 18 days. H-E stain. (A) Dilatation of the tubules from the cortico-mudullary junction to the inner cortex, 40×; (B) hyperplasia of the tubular epithelium, 125×.

only a slight change of the liver (centrilobular hypertrophy of the hepatocytes in 3/6 males) of 600 mg/kg group was observed.

During the recovery-maintenance period, clinical signs such as emaciation, decrease in spontaneous activity and pale skin were observed only in two males and one female of the 600 mg/kg group from 4 days after the termination of the treatment. On day seven after termination of the treatment, one male and one female were found dead and one male was killed in moribund condition in this group. The kidneys of these three rats had necrosis of the tubular epithelium and formation of granular casts in addition to multiple cystic lesions. No dose-related changes in body weight, food consumption, parameters of sexual maturation or urinalysis were detected.

At the end of the recovery-maintenance period, the absolute kidney weights of males and females in the 600 mg/kg group were still 1.3 times higher than those in the control group. Histopathological examinations revealed multiple cysts of the kidneys in one male and one female of the 200 mg/kg group and in all males and females of the 600 mg/kg group (Table 3). However, these kidneys contained reparative changes with interstitial fibrosis, in contrast to the kidneys at the scheduled-sacrifice.

3.2. *Young rat study*

In order to compare the nephrotoxic effects of TBBPA in newborn rats with those in young rats, young rats were administered TBBPA by gavage at 2000 or 6000 mg/kg per day for 18 days. There were no TBBPA-induced changes in general behavior, body weight or kidney weight. The histopathological examination of the kidneys showed no abnormalities in either sex in any group.

4. Discussion

It has been generally accepted that TBBPA has no critical toxicity for major organs, including the kidneys, in young and adult rats or mice (IPCS/WHO, 1995). The marked nephrotoxicity characterized by the formation of polycystic lesions (polycystic kidney) observed at 200 and 600 mg/kg in our newborn rat study was completely unexpected based on the general repeated dose toxicity studies and teratogenicity studies in young and adult animals. This nephrotoxicity is likely to be reproducible because the dose finding study in newborn rats showed a six-fold increase of the relative kidney weight at 1000 mg/kg. Since it was not observed in our young rat study after 18 days of TBBPA treatment even at the extremely high dose of 6000 mg/kg, the nephrotoxicity of TBBPA was considered to be specific for newborn rats versus young rats.

Lau and Kavlock (1994) have reviewed publications on the breadth of critical periods for renal toxicity of therapeutic agents, hormonal manipulations and environmental agents. Chlorambucil is highly effective in inducing renal hypoplasia and altered function when exposure occurs at the time of induction of the metanephric blstema (Kavlock et al., 1987). 2,3,7,8-Tetrachloro-1,4-dibenzodioxine (TCDD) and some other chemicals induce hydronephrosis specifically in fetal/newborn animals after maternal exposure during pregnancy and/or the lactating period (Couture-Haws et al., 1991). Enalapril, an angiotensin-converting enzyme inhibitor (Minsker et al., 1990) and glucocorticoids (Slotkin et al., 1991, 1992) are renal developmental toxicants when exposure occurs during late gestation, and difluoromethylornithine induces persistent effects on the kidney when exposure occurs in the early postnatal period (Gray and Kavlock, 1991). On the other hand, it is well-known that mercuric chloride is a potent nephrotoxicant in adult rats, but has little effect on newborns (Daston et al., 1983, 1984). Clinically, it is known that antibacterial agent-induced kidney damage (especially that caused by amino glycosides or glycopeptides) is less frequent and severe in newborns than in adults (Fanos and Cataldi, 1999).

Some investigations on the mechanism of the context of morphologic events occurring during those periods have been reported. Angiotensin-converting enzyme inhibitors cause excessive disturbances in normal physiology in a system with immature feedback loops in late fetal development (Brent and Beckman, 1991; Hanssens et al., 1991). Mercuric chloride is thought to interact initially with the brush border of the proximal tubules (Daston et al., 1983), whereas dichlorovinylcysteine requires activation by renal β-lyase before achieving toxicity (Darnerud et al., 1991), thus suggesting a biochemical immaturity

of the neonatal kidney that may offer a degree of protection from the effects of some nephrotoxicants. On the other hand, chlorambucil is thought to cause renal hypoplasia by a direct action on rapidly proliferating cell populations during induction of the renal anlagen (Kavlock et al., 1987). The mechanism of the hydronephrosis caused by methylsalicylate was suggested to be differences in the growth rate between the papillae and the parenchyma in the developing kidney (Woo and Hoar, 1972).

These reports suggest that there does not appear to be a good concordance between agents that induce renal toxicities in the fetus, newborn or adult.

Polycystic kidneys, in which the renal parenchyma is occupied by innumerable cysts of various sizes, have been reported to be induced by diphenylamine (Gardner et al., 1976), nordihydroguaiaretic acid (Evan and Gardner, 1979), diphenylthiazole (Gardner and Evan, 1983), alloxan (Kovacs et al., 1998), ferric-nitrilotriacetate (Kovacs et al., 1998), streptozocin (Kovacs et al., 1998), and 2-amino-4,5-diphenylthiazole (Tsumatani et al., 1997) in young and adult animals. Polycystic kidney is also known as an inherited disease in humans and some other species.

As a pathogenesis of the cyst formation in human cases, it is considered that epithelial hyperplasia results in tubular enlargement and obstruction (Bernstein, 1992). Pathogenesis of chemical-induced polycystic kidneys is also considered that chemicals cause some changes in metabolism of the epithelium or basement membrane of the tubules, resulting in abnormal extracellular matrices and hyperplasia of the epithelium, leading to the occlusion of the tubules (Carone et al., 1992; Avner, 1988). Then, an increase in the pressure of the lumen of occluded tubules is considered to cause formation of multiple renal cysts. In the present study, hyperplasia of the renal tubular epithelium was observed. Although no initial changes of hyperplasia of the tubular epithelium were detected, it is assumed that TBBPA may also have a damaging effect on the tubular epithelium and cause reactive hyperplasia of the damaged epithelium, leading to occlusion of the tubules.

As the same nephrotoxicity as that induced by TBBPA, characterized by polycystic kidney, para-nonylphenol was reported in rat neonates exposed via the maternal placenta and breast milk, but was not so obvious in adults (Latendresse et al., 2001). Since this effect on the kidneys was affected by phytoestrogens in the diet, the authors discussed the possible role of the estrogenic activity in this nephrotoxicity. In the case of TBBPA, the possibility of an estrogenic mechanism appears to be unlikely because there was no evidence of estrogenic activity in our previous and present studies.

It was known that nephrons in the kidneys of rats are formed in the period from the advanced stage of pregnancy until 2 weeks after birth (Chevalier, 1998), and only 10% of nephrons are present at birth (Merlet-Benichou et al., 1994). This period is analogous to that of the midtrimester human fetus, during which the major features of obstructive nephropathy including cystic changes evolve (Daikha-Dahmane et al., 1997).

Compared to the adult, the rapidly growing neonatal rat kidney appears to be particularly susceptible to interference with cellular proliferation and stimulation of apoptosis (programmed cell death) as a result of chronic unilateral ureteral obstruction (Chevalier et al., 1998). The mechanisms underlying these effects are complex, involving the interaction of multiple growth factors and cytokines (Chevalier, 1996).

These observations suggest that developing renal tubules in neonatal rats may be easy to cause hyperplasia of the tubular epithelium by a cellular damage due to a toxic effect of the agents.

On the other hand, a recent study using bile duct-cannulated rats showed that approximately 70% of ^{14}C-TBBPA orally administered at 2.0 mg/kg was excreted to the bile (Hakk et al., 2000). As bile synthesis, conjugation, transport and secretion are known to be immature at birth and the maturation usually occurs after weaning in animals (Scheuplein et al., 2002; Chuang and Haber, 1998), it is possible that the kidneys of the newborn were exposed to higher levels of TBBPA than the young adults. However, the mechanism of vulnerability specific to TBBPA in newborn but not young rats remains to be elucidated.

The relative weight of the liver increased slightly in the males of the 600 mg/kg group in the newborn rat study. Although some animals showed a slight centrilobular hepatocellular hypertrophy, the results of the biochemistry examinations did not indicate any abnormality in the liver function. Although no hepatotoxicity was found in adult animals (IPCS/WHO,

1995), a recent study suggested the possibility that TBBPA may disturb the heme metabolism in the rat liver (Szymanska et al., 2000).

TBBPA is a commercial product used as a polymer in resins such as acrylonitrile, butadiene, styrene, epoxy, polycarbonates and polystyrene. In general, the intake of TBBPA at home is estimated not to be harmful or to pose any risk, because most of the general population is only indirectly exposed to TBBPA through products made from these polymers (IPCS/WHO, 1995). Additionally, as the nephrotoxicity occurred only at relatively high TBBPA doses in the newborn rats, the results of the present study do not indicate a warning of any risk of TBBPA to human infants. However, the reason why TBBPA-induced polycystic kidney is specific to newborn rats should be determined.

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