ムの科学的な解明を進めていくことが必要である。

E．結論

化学物質による内分泌系への影響による高次の神経系や免疫系の発達や恒常性維持機能に対する有害影響が懸念されている。高次機能への影響については既存の試験法では、適切な評価が出来ないため、新たな評価手法の開発とその結果をもとにしたリスク評価の実施が求められている。化学物質による内分泌かく乱の検出法については、OECD を中心として、ガイドライン化に向けた検証が進められている。しかし、関与する化学物質が最終的に異なったエンドポイントに作用する場合、その最終的な評価を行うにあたり、必ずしも共通のアプローチは適用できない可能性もあり、複数の化学物質に関する曝露を評価する手法はもっと複雑である。内分泌かく乱は包含的機序であり、その潜在的な毒性影響には、多くの器官が関与するため実際の影響は多様である。また、化学物質だけでなく内因性ホルモンや食品等に含まれる天然化合物などが同時に内分泌系と相互作用することから、恒常性維持における生理学的変動や調整機能および感受性に関する年齢差や個体差に起因した影響についてもさらに研究を行う必要があるであろう。特に外的変動への適応性が低い子供や老人を含む高感受性集団における有害影響を適切にリスク評価するためには、高次恒常性維持機構に関する生物学的な基礎の解明と科学的根拠に基づいた評価系の構築を進める必要がある。

F．健康危険情報

特になし

G．研究発表

1．論文発表

2．学会発表

特になし

H．知的財産所有権の出願・登録状況

1．特許取得

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2．実用新案登録

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別添 5

III. 研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物 ・ 別刷り

雑　誌

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Original Article

Screening of toxicological properties of 4-methylbenzoic acid by oral administration to rats

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ABSTRACT — Oral toxicity of 4-methylbenzoic acid in male and female Sprague-Dawley rats was profiled through a twenty-eight-day repeated dose toxicity study (the 28-day study) and a screening test for reproductive/developmental toxicities (the reproduction/developmental study) conducted under Organisation for Economic Co-operation and Development (OECD) test guidelines. Daily administration of 4-methylbenzoic acid, at a dose level of 0, 100, 300 or 1,000 mg/kg, did not show any adverse effect on reproductive organs of animals in the 28-day study. In the reproductive/developmental study, however, 1,000 mg/kg/day of the compound reduced epididymal weights and increased incidence of cauda epididymal oligo/azoospermia. While the compound did not affect estrous cycle or mating performances, 1,000 mg/kg of the compound reduced fertility. Furthermore, 300 mg/kg or more of the compound increased pre-implantation loss, which resulted in a decrease in the number of offspring, and reduced body weight gain of the dams during the latter period of gestation. From these results, the no-observed-effect-level (NOEL) for reproductive/developmental toxicities is considered to be 100 mg/kg, whereas 1,000 mg/kg did not show any effect on neonates. In the 28-day study, NOEL is considered to be 300 mg/kg for male and female rats, since 1,000 mg/kg of the compound caused, in both sexes, a few minor changes, such as temporal salivation, a slight increase in food consumption and a moderate increase in blood aspartate aminotransferase (AST) activity. Thus, 4-methylbenzoic acid has the potential for reproductive toxicity, with diverse adverse effects on the epididymis, after repeated administration, observed in the two studies.

Key words: 4-methylbenzoic acid, Epididymis, OECD test guideline, Reproductive toxicant, Repeated dose toxicity, Rats

INTRODUCTION

Faced with an enormous number of existing chemicals that lacked hazard information, the Organisation for Economic Co-operation and Development (OECD) decided, in 1990, to undertake an investigation of such chemicals in cooperation with its member countries. They gave priority to high production volume (HPV) chemicals in collecting the data for an initial assessment of the hazard. A project was launched in 1990 to complete the dossiers of screening information data sets (SIDS) of toxicity for HPV chemicals through testing, and the work has been done cooperatively in Japan, as well as in other countries. Since 1991, we have participated in the testing under the auspices of the Ministry of Health (, Labour) and Welfare.

4-Methylbenzoic acid (*p*-toluic acid) was selected from the OECD List of HPVs (OECD, 2004). 4-Methylbenzoic acid is produced at levels greater than 1,000 tones per year and is used in the manufacture of dye stuffs, colorants and paints; agrochemicals; and anticorrosive additives, as well as being an intermediate in the manufacture of polyethylene terephthalate. Although oral LD_{50} values in rats and mice have been reported as 3,113 mg/kg and 2,115 mg/kg, respectively (Mineshita *et al.*, 1978), its toxicological properties are little known. The present study was performed to profile the oral toxicity of 4-methylbenzoic acid according to the standard protocols, "Repeated Dose 28-day Oral Toxicity Study" (the 28-day study) and "Reproduction/Developmental Toxicity Screening Test" (the reproduction/developmental study), in rodents (OECD, 1997a, 1997b).

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MATERIALS AND METHODS

Test substance

4-Methylbenzoic acid (CAS No. 99-94-5, purity 98.95%) was supplied by Toray (Tokyo, Japan), and was kept at room temperature until use. To prepare dosing samples, the compound was finely ground in a mortar, at first. Then, the ground compound for each dose was suspended in a 0.5% sodium carboxymethylcellulose solution, and each dose was adjusted to a constant volume of 5 ml/kg. The stability of the suspended compound in the vehicle and the content and uniformity of the compound of each dose were confirmed in Hatano Research Institute.

The doses used in the studies were determined based on a preliminary, 7-day, repeated dose, oral toxicity study of the compound, in which a dose level of 1,000 mg/kg did not show any toxic effects. This was considered sufficient for the highest dose, and the doses used in the present studies were set at 0, 100, 300 and 1,000 mg/kg.

Animals

Male and female rats of the Sprague-Dawley (Crj: CD(SD)) strain were purchased from Charles River Japan (Atsugi Breeding Center, Atsugi, Kanagawa, Japan). For the 28-day study, the animals were purchased at 4 weeks of age, and after an 8-day quarantine period, the animals were divided into 4 groups of each sex according to a stratified allocation based on body weight measured on the day before initial dosing. For the reproduction/developmental study, the animals were purchased at 7 weeks of age and quarantined for 7 days. After the quarantine period, the female rats were monitored for estrous cycle by observing daily vaginal smears for 2 weeks. At 10 weeks of age, the females revolving on a regular 4-day estrous cycle and the males were divided into each 4 groups by the same method as used in the 28-day study.

These animals were kept individually in metallic cages with metal-meshed floors, except copulated females in the reproduction and developmental study, which were kept in flat-bottomed plastic cages with bedding materials (Paper Clean, Nippon SLC, Hamamatsu, Japan) from 18 days after copulation. The animal rooms were maintained with a light-dark cycle of 12-hr (lights on 7 hr), and temperature and relative humidity were controlled to 21.0-25.0°C and to 40.0-75.0%, respectively, with air ventilation at 15 complete changes per hour. The animals were supplied with solid rodent chow (CE-2, CLEA Japan, Tokyo, Japan) and tap water, *ad libitum*.

Experimental design

All procedures described here were approved by the Committee on Animal Experiments of Hatano Research Institute, Food and Drug Safety Center.

The 28-day study

Groups given 0 and 1,000 mg/kg of 4-methylbenzoic acid comprised 10 animals of each sex, including 5 animals of each sex for the 2-week recovery study after the 28-day administration of the compound. The groups given 100 and 300 mg/kg comprised 5 animals of each sex.

Administration was begun at 5 weeks of age, and the initial day of the administration was designated as Day 1 of treatment. All the animals received daily administration of the compound by gavage for 28 days at a fixed time every day, and the initial day of recovery was designated as Day 1 of recovery.

Signs of toxicity were daily observed. Detailed clinical observations were made as specified in the OECD test guideline 407 (OECD, 1997a) in all animals prior to the initial administration, and once a week thereafter until the end of the recovery period. The observers were unaware of the treatment of each animal. Findings were recorded using a scoring system. In addition to the detailed observation of clinical signs, a 4-item neurobehavioral test battery assessing auditory and visual functions was administered at the last week of treatment.

Body weight was measured 3 times during the first week of the treatment, and twice a week thereafter. Food consumption was determined weekly.

Urinalysis was performed at the final week of treatment and at the final week of the recovery study. Urine of all animals was collected for 4 and 24 hr in a metabolic cage, and was examined for pH, occult blood, protein, ketone bodies, urobilinogen and bilirubin, semi-quantitatively using a urine test strip analyzer (Clinitek 200+, Bayer Medical, Tokyo, Japan), and for its color, turbidity and sediments. The volume and weight of the 24-hr urine was measured, and the specific gravity of the urine was calculated.

Necropsy of the animals was performed after 18-24 hr of fasting on the day following final treatment and on the day following the recovery period. Under anesthesia with pentobarbital sodium, blood samples were collected from the abdominal caval vein by syringe, with sodium citrate as an anticoagulant, for determination of coagulation times; with EDTA-2K potassium, for hematological examination; and with heparin, for blood chemical examination. Then, the animals were killed by exsanguination from the axillary artery. After gross observation, dissected organs, such as brain, thymus, heart, liver, kidneys, spleen, testes, adrenals and epididymides, were weighed.

In addition to these organs, spinal cord, lungs, bronchi, stomach, ileum, colon, seminal vesicles, ovaries, uterus, vagina, urinary bladder, thyroid gland, femoral marrow, mesenteric lymph nodes, mandibular lymph nodes and ischiadic nerves were dissected out and fixed in buffered formalin for histopathological examination. The testes were fixed in Bouin's solution, with post fixation in buffered formalin. These organs/tissues were then processed for paraffin embedded block, and sections cut from the blocks were stained with hematoxylin-eosin (HE).

Hematological examination was carried out using automatic blood analysis apparatus (CELL-DYN3500SL, Abbot Diagnostics, IL, USA) for cell counts (erythrocytes (RBCs), leucocytes (WBCs) and platelets), hemoglobin concentration and differential WBC counts. Hematocrit, mean concentration of hemoglobin in the RBC (MCHC) and mean content of hemoglobin in the RBC were calculated. The prothrombin time (PT) and activated partial thromboplastin time were measured using a fully automatic analyzer for blood coagulation (CA-1000, Toa Medical Electronics, Saitama, Japan).

In the blood chemical examination, plasma concentrations of total protein, albumin, total cholesterol, glucose, blood urea nitrogen, creatinine, triglyceride, total bilirubin, inorganic phosphorus and calcium and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GTP) were determined using a centrifugal automatic blood chemical analyzer (COBAS-FARA, Roche Diagnostics, Basel, Switzerland). Ratios of albumin to globulin were calculated, and plasma concentrations of sodium and potassium were measured using an automated electrolyte analyzer (EA05, A & T, Yokohama, Japan).

The reproduction/developmental study

The study consisted of 4 dosing groups, and each group comprised 13 males and 13 females. Administration was begun at 10 weeks of age, 2 weeks prior to mating in both, males and females, and was performed by gavage at a fixed time every day. The initial day of the administration was designated as Day 1 of treatment. Mating was performed by co-housing one male and one female from the same treatment group for a maximum of 2 weeks and was confirmed by observation of a copulatory plug or by the presence of sperm in a vaginal specimen. After confirmation of mating, each female was housed individually, and the day on which mating was confirmed was designated as Day 0 of gestation. Administration was continued during the mating period and gestation period, until 3 days after delivery for females. For males, the administration was continued through and after the mating period, for a total of 42 days. For females that had copulated but did not deliver or for females that had not copulated, the administration was continued until 25 days after copulation or to Day 52 of treatment, respectively.

Clinical conditions were observed daily, and body weights and food consumptions were measured weekly. After confirming copulation, the females were weighed on Days 0, 7, 14 and 20 of gestation, and their food consumptions were determined on Days 0-1, 7-8, 14-15 and 20-21 of gestation. When the females delivered live fetuses, they were weighed on Days 0 (the day of delivery) and 4 of lactation, and their food consumptions were determined on Day 3-4 of lactation.

The estrous cycle was monitored daily until copulation. The females that had copulated were allowed to deliver spontaneously and to nurse their own pups until Day 4 of lactation. During the lactation period, the number and sex of dead and live pups were recorded for each dam, and the external morphology and general condition of the live pups were examined daily. The dead pups were examined for external and visceral abnormalities, when possible.

All the males were killed for necropsy on the day after Day 42 of treatment by exsanguination under sodium pentobarbital anesthesia. Maternal animals were similarly killed on Day 4 of lactation, while their offspring were killed for necropsy on the same day by ether inhalation. The females that had not copulated during the mating period and the females that had copulated but did not deliver fetuses were also similarly killed.

At necropsy, males were examined grossly for abnormalities, and the testes and epididymides were dissected, weighed and fixed in Bouin's solution, with post fixation with a buffered formalin solution for processing for histopathological examination. The other reproductive organs, such as ventral prostate and seminal vesicles with coagulating glands, were also dissected and fixed in a buffered formalin solution for histopathological examination. Females were examined grossly for abnormalities. Organs with lesions were dissected for histopathological examination. Ovaries, uterus and vagina from dams were dissected to determine the number of corpora lutea and implantation sites. Then, these organs were processed for histopathological examination. The absence of implantation sites in the uterus of females that had not copulated and females that had copulated but did not deliver fetuses was confirmed under a stereomicroscope. Live pups were euthanized by ether inhalation and were examined for of external and visceral abnormalities.

Statistical analysis

Fisher's direct probability test was applied to analysis

of the incidence of animals in which the estrous cycle was altered, the copulation rate, the fertility index, the incidence of offspring with morphological abnormalities, and the incidence of histopathological findings. Graded findings in the histopathological examination were analyzed using the Mann-Whitney U-test. Data from the urinalysis urine quality test were analyzed using a chi-square test.

The other data were analyzed with multiple comparisons when comparing the data from more than 2 groups with those from the control. When comparing the data from a single group with those from the control, Student's t-test or Aspin-Welch's t-test was applied, following an F-test. In the multiple comparisons, an analysis of variance (ANOVA) test or Kruskal-Wallis's rank test was applied, following examination of the uniformity of variations by Bartlett's method. Significant differences from the control were determined by Dunnett's test. A p value of less than 0.5% was judged a significant difference.

RESULTS

Effects of repeated dosing in the 28-day study

Neither, death nor moribund condition, was observed in any animal, but a few male and female animals in the 1,000 mg/kg group showed temporary salivation after dosing. Except that, there were no clinical signs related to the treatment, and the scores of the detailed observation of clinical signs in the compound treated groups were comparable to those in the control (data not shown). In addition, no abnormalities were observed in the neurobehavioral test at the last week of treatment (data not shown).

Changes in body weight and food consumption are illustrated in Figs. 1 and 2, respectively. While body weights of males and females in the compound treated groups changed similarly to those in the control group at any period, food consumption of females in the 1,000 mg/kg treated group was slightly greater than in the control group, from Day 7 to 8 of treatment. Whereas the food consumption of females in this group tended to be greater thereafter, until the end of the treatment, no differences in food consumption were observed in males among any groups throughout the study.

As summarized in Table 1, urine volume measured on the Day 23 of treatment was larger in the males given 300 mg/kg or more of the compound and in females given 1,000 mg/kg when compared with those in the control. In addition, urine specific gravities were decreased in the males of these groups. These changes accompanied by an increase in water consumption, which was confirmed by an observation of water feeding bottles placed in the metabolic cages. Urinalysis showed some minor changes and was not suggestive of any toxic effects (data not shown).

The hematology data are presented in Table 2. At the end of treatment period, platelet counts were slightly lower in the 1,000 mg/kg treated females, but were statistically insignificant when compared with those in the control. No changes were observed in any of the hematology parameters of males at the end of treatment period. At the end of recovery period, there were statistically significant differences between the males of compound treated and

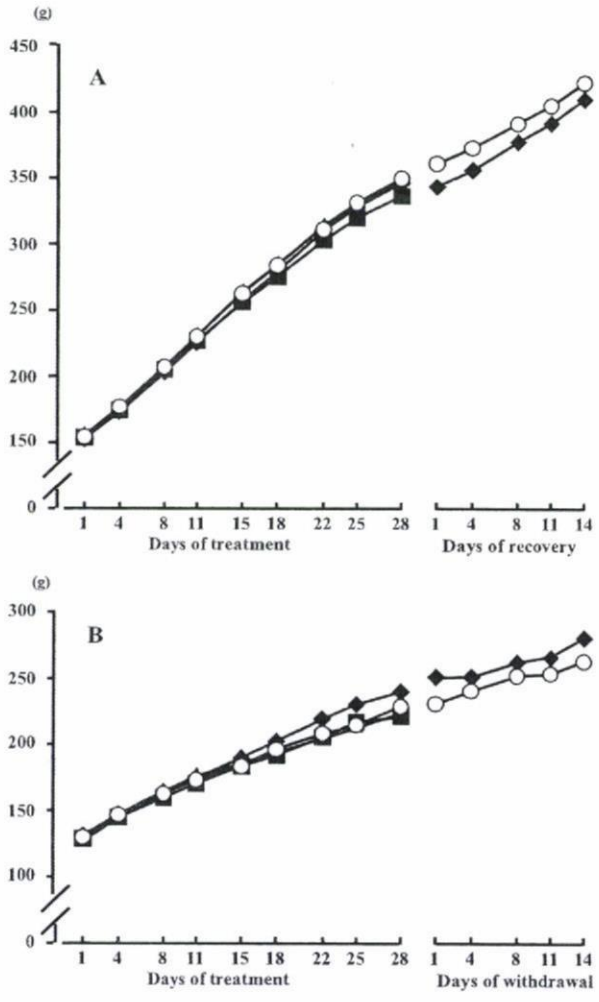

Fig. 1. Body weight changes of male (A) and female (B) rats treated orally with 4-methylbenzoic acid for 28-days at dose levels of 0 (○), 100 (■), 300 (▲) and 1,000 mg/kg/day (♦) in the repeated dose 28-day oral toxicity study. Values during the treatment period represent the average for 5 animals in the 100 or 300 mg/kg-treated group and for 10 animals in the 0 or 1,000 mg/kg-treated group. Those during the recovery period represent the average for 5 animals.

control groups, and were not suggestive any toxic effects.

The blood chemistry data are presented in Table 3. At the end of treatment period, a moderate increase in AST activity and a slight decrease in total protein concentration were observed in the 1,000 mg/kg-treated females, compared with those of control. In those data from males and those at the end of the recovery period from animals of both sexes, no significant differences were observed between the compound treated and control groups.

No significant differences from the control were observed in absolute organ weights or in organ weights relative to body weight in any of the compound treated groups of males (Table 4) or females (data not shown).

No abnormalities suggestive of any toxic effects were observed in any organs or tissues on gross examination at necropsy or histopathological examination (data not shown).

Effects of repeated dosing in the reproduction/ developmental study

As found in the 28-day study, temporary salivation was observed in a few animals given 1,000 mg/kg (data not shown). Except that, there were no clinical signs relating to the treatment.

Changes in body weight and food consumption of males are shown in Figs. 3 and 4, respectively. The compound did not affect body weight increase or food consumption, at any dose level.

Changes in body weight and food consumption of females are shown in Figs. 5 and 6, respectively. The compound did not affect body weights until Day 14 of gestation, at any dose level, while food consumption of the 1,000 mg/kg treated group was slightly higher than that of the control at the beginning of the dosing. During Days 14-20 of gestation, however, maternal body weight gain was reduced in the 300 mg/kg or more treated groups, while the food consumption that was determined during this period (Day 14-15 of gestation) and body weight on

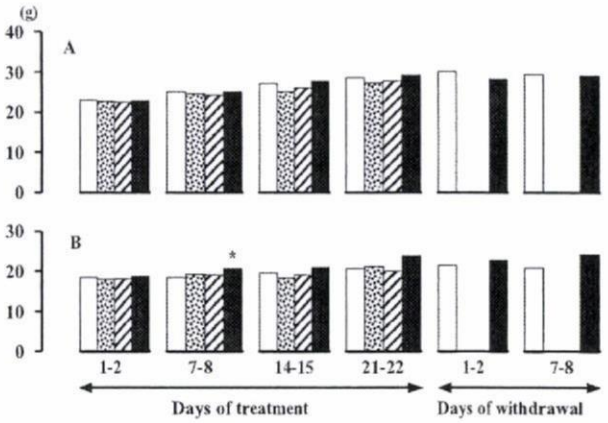

Fig. 2. Changes in food consumption of male (A) and female (B) rats treated orally with 4-methylbenzoic acid for 28-days at dose levels of 0 (open column), 100 (dashed column), 300 (hatched column) or 1,000 mg/kg/day (closed column) in the repeated dose 28-day oral toxicity study. Each column represents average for 5 animals in the 100 or 300 mg/kg-treated group and for 10 animals in the 0 or 1,000 mg/kg-treated group. During the recovery period, it represents the average for 5 animals. * indicates significant difference from control at $p < 0.05$.

Table 1. Urinalysis of rats treated orally with 4-methylbenzoic acid in repeated dose 28-day oral toxicity study

	On Day 23 of treatment				On Day 9 of recovery	
Dose (mg/kg)	0	100	300	1,000	0	1,000
Number of animals (males/females)						
	10/10	5/5	5/5	10/10	5/5	5/5
Urinary volume (ml/24 hr)						
Males	15.6 ± 2.2	15.6 ± 2.1	20.9 ± 4.7*	23.8 ± 4.7**	18.3 ± 4.7	24.3 ± 4.6
Females	11.7 ± 3.5	11.9 ± 3.4	13.0 ± 4.3	22.1 ± 7.5**	13.3 ± 2.8	18.3 ± 5.6
Specific gravity						
Males	1.058 ± 0.008	1.051 ± 0.007	1.043 ± 0.011**	1.045 ± 0.006**	1.056 ± 0.009	1.038 ± 0.006**
Females	1.045 ± 0.012	1.046 ± 0.008	1.043 ± 0.007	1.038 ± 0.009	1.041 ± 0.010	1.044 ± 0.011

Values represent average ± S.D.

* and **, significant difference from control at $p < 0.05$ and 0.01, respectively.

Table 2. Hematology data of rats treated orally with 4-methylbenzoic acid in the repeated dose 28-day oral toxicity study

	End of treatment								The end of recovery			
	Males				Females				Males		Females	
Dose (mg/kg)	0	100	300	1,000	0	100	300	1,000	0	1,000	0	1,000
RBC ($\times 10^4/\mu l$)	755 ± 53	780 ± 15	770 ± 27	755 ± 18	742 ± 19	771 ± 26	750 ± 25	751 ± 30	797 ± 13	766 ± 22**	749 ± 25	766 ± 24
Hemoglobin (g/dl)	14.9 ± 0.6	15.2 ± 0.2	15.2 ± 0.3	15.0 ± 0.2	14.8 ± 0.3	15.1 ± 0.5	14.9 ± 0.3	14.7 ± 0.5	15.2 ± 0.1	14.6 ± 0.3 **	14.5 ± 0.4	14.4 ± 0.7
Hematocrit (%)	44.8 ± 2.9	46.0 ± 0.7	45.5 ± 1.1	45.2 ± 0.4	44.2 ± 1.0	44.9 ± 1.3	44.4 ± 0.7	43.6 ± 1.9	45.3 ± 0.8	43.5 ± 1.0**	42.6 ± 1.3	42.7 ± 1.9
MCV (fl)	59.4 ± 2.2	58.9 ± 0.8	59.0 ± 1.3	60.0 ± 1.5	59.5 ± 1.1	58.3 ± 1.2	59.2 ± 1.4	58.0 ± 1.1	56.8 ± 1.4	56.8 ± 0.8	56.9 ± 1.2	55.8 ± 2.0
MCH (pg)	19.8 ± 0.8	19.5 ± 0.6	19.8 ± 0.6	19.9 ± 0.5	20.0 ± 0.6	19.6 ± 0.3	19.8 ± 0.6	19.6 ± 0.3	19.1 ± 0.4	19.0 ± 0.3	19.4 ± 0.4	18.8 ± 0.7
MCHC (g/dl)	33.3 ± 0.1	33.0 ± 0.7	33.6 ± 0.3	33.2 ± 0.2	33.6 ± 0.4	33.7 ± 0.3	33.5 ± 0.2	33.8 ± 0.6	33.5 ± 0.6	33.5 ± 0.4	34.0 ± 0.3	33.7 ± 0.3
Platelet ($\times 10^4/\mu l$)	102.9 ± 13.9	104.5 ± 16.3	106.6 ± 8.1	101.6 ± 10.4	96.3 ± 7.0	100.5 ± 9.4	93.3 ± 9.3	83.9 ± 3.1	96.1 ± 19.6	99.3 ± 8.4	101.8 ± 12.7	102.7 ± 10.2
PT (sec)	18.4 ± 3.0	18.9 ± 3.1	17.2. ± 4.0	14.9 ± 1.7	12.6 ± 0.7	12.7 ± 0.5	12.9 ± 0.6	13.2 ± 0.6	16.4 ± 2.7	15.2 ± 1.4	11.9 ± 0.7	11.5 ± 0.7
APTT (sec)	22.1 ± 0.8	22.9 ± 1.7	20.5 ± 2.4	20.1 ± 1.0	18.5 ± 1.0	18.2 ± 1.4	17.8 ± 1.2	16.7 ± 1.4	21.6 ± 1.5	21.0 ± 0.4	17.1 ± 0.8	16.9 ± 0.8
WBC ($\times 100/\mu l$)	90.8 ± 16.7	61.5 ± 20.5	67.7 ± 17.6	74.4 ± 21.7	61.0 ± 18.1	54.9 ± 14.8	46.7 ± 12.4	59.8 ± 7.8	69.4 ± 13.4	65.4 ± 14.2	43.9 ± 2.9	58.8 ± 14.5
Differential leukocyte counts (%)												
Neutrophil	10 ± 5	11 ± 2	10 ± 2	11 ± 3	7 ± 2	6 ± 3	8 ± 4	8 ± 4	13 ± 5	15 ± 3	10 ± 4	10 ± 3
Eosinophil	1 ± 0	1 ± 0	1 ± 0	0 ± 0*	1 ± 0	1 ± 1	1 ± 0	1 ± 0	1 ± 0	1 ± 1	2 ± 1	1 ± 1
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
Monocyte	3 ± 1	4 ± 1	5 ± 1	4 ± 1	3 ± 1	4 ± 1	3 ± 1	3 ± 1	4 ± 1	4 ± 2	4 ± 1	4 ± 2
Lymphocyte	85 ± 5	85 ± 3	85 ± 3	85 ± 3	89 ± 3	88 ± 3	88 ± 4	88 ± 4	81 ± 4	79 ± 4	85 ± 5	85 ± 5

Values represent average for five animals ± S.D.

* and **, significant difference from control at $p < 0.05$ and 0.01, respectively.

Table 3. Blood chemistry data of rats treated orally with 4-methylbenzoic acid in repeated dose 28-day oral toxicity study

Dose (mg/kg)	The end of treatment								The end of recovery			
	Males				Females				Males		Females	
	0	100	300	1,000	0	100	300	1,000	0	1,000	0	1,000
TP (g/dl)	5.0 ± 0.2	5.2 ± 0.2	5.1 ± 0.1	5.0 ± 0.0	5.4 ± 0.3	5.3 ± 0.3	5.2 ± 0.3	4.8 ± 0.3**	5.6 ± 0.4	5.4 ± 0.1	5.5 ± 0.1	5.7 ± 0.3
Albumin (g/dl)	3.0 ± 0.2	3.0 ± 0.3	3.0 ± 0.1	3.0 ± 0.1	3.2 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	2.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.1	3.2 ± 0.2	3.3 ± 0.4
A/G	1.48 ± 0.15	1.46 ± 0.27	1.38 ± 0.16	1.44 ± 0.08	1.51 ± 0.17	1.48 ± 0.17	1.64 ± 0.11	1.56 ± 0.09	1.14 ± 0.08	1.28 ± 0.14	1.41 ± 0.20	1.37 ± 0.24
BUN (mg/dl)	16 ± 2	17 ± 2	16 ± 3	16 ± 1	23 ± 3	21 ± 2	23 ± 1	20 ± 2	19 ± 3	15 ± 3	21 ± 1	24 ± 3
Creatinine (mg/dl)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.8 ± 0.1
Glucose (mg/dl)	130 ± 13	137 ± 32	1 43 ± 13	155 ± 24	109 ± 13	113 ± 21	118 ± 7	108 ± 10	137 ± 10	129 ± 16	125 ± 12	128 ± 10
T. Cholest.(mg/dl)	34 ± 8	33 ± 4	37 ± 5	40 ± 5	41 ± 8	48 ± 9	45 ± 10	31 ± 8	41 ± 15	38 ± 10	48 ± 9	54 ± 4
Triglyceride (mg/dl)	19 ± 5	22 ± 7	28 ± 14	35 ± 15	11 ± 5	9 ± 3	10 ± 3	9 ± 2	24 ± 13	29 ± 12	13 ± 2	22 ± 12
T. Bil.(mg/dl)	0.07 ± 0.04	0.08 ± 0.03	0.09 ± 0.05	0.07 ± 0.03	0.09 ± 0.03	0.07 ± 0.03	0.09 ± 0.04	0.09 ± 0.03	0.08 ± 0.02	0.09 ± 0.03	1.10 ± 0.04	0.08 ± 0.02
Inorg. P. (mg/dl)	9.0 ± 0.7	8.7 ± 0.8	8.4 ± 0.3	8.6 ± 0.4	7.9 ± 0.8	8.1 ± 0.7	8.2 ± 1.1	8.4 ± 0.5	7.2 ± 0.9	6.5 ± 0.6	7.4 ± 0.9	7.5 ± 0.5
Ca (mg/dl)	8.9 ± 0.1	8.9 ± 0.3	9.0 ± 0.1	9.2 ± 0.2	9.1 ± 0.1	9.1 ± 0.2	9.1 ± 0.2	8.8 ± 0.4	8.9 ± 0.2	8.8 ± 0.1	9.1 ± 0.2	9.2 ± 0.2
Na (mEq/l)	145.8 ± 0.6	145.1 ± 0.9	145.8 ± 1.3	145.5 ± 0.9	145.5 ± 0.8	144.7 ± 0.7	145.6 ± 0.8	146.0 ± 1.4	146.2 ± 0.4	145.9 ± 0.6	143.1 ± 0.8	143.4 ± 0.2
K (mEq/l)	4.48 ± 0.53	4.33 ± 0.36	4.08 ± 0.26	4.29 ± 0.24	4.18 ± 0.39	4.31 ± 0.20	4.20 ± 0.28	4.05 ± 0.38	4.03 ± 0.26	3.96 ± 0.12	4.11 ± 0.28	3.98 ± 0.32
Cl (mEq/l)	109.0 ± 1.7	108.3 ± 1.7	108.1 ± 1.2	107.8 ± 1.4	109.1 ± 1.2	107.5 ± 1.0	109.9 ± 1.1	109.3 ± 2.3	108.2 ± 1.1	108.7 ± 0.6	107.2 ± 0.6	107.8 ± 0.6
ALP (U/l)	441 ± 143	451 ± 58	511 ± 190	459 ± 56	319 ± 65	261 ± 62	299 ± 75	266 ± 46	366 ± 80	374 ± 29	219 ± 36	231 ± 39
ALT (U/l)	31 ± 6	27 ± 4	29 ± 3	37 ± 15	25 ± 2	22 ± 2	22 ± 5	29 ± 5	35 ± 5	32 ± 4	24 ± 3	28 ± 5
AST (U/l)	72 ± 10	66 ± 11	67 ± 7	88 ± 24	69 ± 3	66 ± 5	69 ± 8	94 ± 16*	83 ± 14	66 ± 2	62 ± 3	66 ± 5
γ-GTP (U/l)	0 ± 1	0 ± 1	1 ± 0	1 ± 1	1 ± 0	1 ± 0	1 ± 1	1 ± 0	0 ± 1	1 ± 1	1 ± 1	1 ± 1

Values represent average for five animals ± S.D.

* and **, significant difference from control at $p < 0.05$ and 0.01, respectively.

TP, total protein; A/G, albumin ratio to globulin (TP – albumin); BUN, blood urea nitrogen; T. Cholest., total cholesterol; T. Bil., total bilirubin; Inorg. P., Inorganic phosphorus; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ -GTP, γ-glutamyl transpeptidase

Table 4. Organ weights of male rats treated orally with 4-methylbenzoic acid in repeated dose 28-day oral toxicity study

	The end of treatment				End of recovery	
Dose (mg/kg)	0	100	300	1,000	0	1,000
Number of animals	5	5	5	5	5	5
Body weight (g)	307.9 ± 24.7	301.4 ± 32.0	311.2 ± 4.2	311.8 ± 30.7	380.8 ± 43.4	370.2 ± 15.1
Absolute weight						
Brain (g)	1.92 ± 0.07	1.87 ± 0.06	1.89 ± 0.07	1.84 ± 0.07	1.92 ± 0.11	1.91 ± 0.07
Thymus (mg)	587 ± 86	493 ± 61	537 ± 95	535 ± 98	423 ± 42	481 ± 132
Heart (g)	1.10 ± 0.09	1.01 ± 0.06	1.00 ± 0.05	1.06 ± 0.11	1.21 ± 0.15	1.18 ± 0.12
Liver (g)	9.62 ± 0.99	9.97 ± 1.87	9.73 ± 0.46	10.26 ± 1.15	10.95 ± 2.00	10.88 ± 0.46
Kidneys (g)	2.39 ± 0.20	2.42 ± 0.24	2.43 ± 0.17	2.42 ± 0.28	2.72 ± 0.30	2.69 ± 0.27
Spleen (mg)	707 ± 192	611 ± 74	643 ± 85	591 ± 106	743 ± 114	694 ± 93
Testes (g)	2.69 ± 0.01	2.84 ± 0.14	3.00 ± 0.28	2.87 ± 0.35	2.94 ± 0.16	2.95 ± 0.15
Epididymides (g)	0.67 ± 0.04	0.71 ± 0.07	0.70 ± 0.02	0.69 ± 0.10	0.91 ± 0.06	0.90 ± 0.04
Adrenal glands (mg)	50.9 ± 3.6	45.6 ± 5.6	54.6 ± 4.6	47.1 ± 6.3	52.7 ± 6.0	55.8 ± 6.8
Relative weight (g/100 g)						
Brain	0.63 ± 0.04	0.62 ± 0.05	0.61 ± 0.03	0.60 ± 0.06	0.51 ± 0.03	0.52 ± 0.01
Thymus	0.19 ± 0.02	0.16 ± 0.02	0.17 ± 0.03	0.17 ± 0.02	0.11 ± 0.02	0.13 ± 0.04
Heart	0.36 ± 0.04	0.34 ± 0.03	0.32 ± 0.01	0.34 ± 0.02	0.32 ± 0.05	0.32 ± 0.03
Liver	3.12 ± 0.16	3.29 ± 0.36	3.13 ± 0.17	3.29 ± 0.14	2.87 ± 0.32	2.94 ± 0.08
Kidneys	0.78 ± 0.05	0.80 ± 0.05	0.78 ± 0.05	0.78 ± 0.06	0.72 ± 0.09	0.73 ± 0.07
Spleen	0.23 ± 0.04	0.20 ± 0.02	0.21 ± 0.03	0.19 ± 0.03	0.20 ± 0.02	0.19 ± 0.02
Testes	0.88 ± 0.13	0.95 ± 0.11	0.97 ± 0.09	0.92 ± 0.05	0.78 ± 0.07	0.80 ± 0.06
Epididymides	0.22 ± 0.03	0.24 ± 0.02	0.23 ± 0.00	0.22 ± 0.02	0.24 ± 0.02	0.24 ± 0.02
Adrenal glands	0.017 ± 0.002	0.015 ± 0.001	0.018 ± 0.002	0.015 ± 0.001	0.014 ± 0.001	0.015 ± 0.002

Values represent average ± S.D.

the day of delivery (Day 0 of lactation) were higher in the 1,000 mg/kg treated group and in the 300 mg/kg or more treated groups, respectively, compared with those in the control. During the lactation period, maternal body weight gain and food consumption in the 1,000 mg/kg treated group were smaller than those in the control.

Terminal body weight and the weights of testes and epididymides of the male rats treated for 42 days are shown in Table 5. The compound did not affect the terminal body weight, at any dose level. While no differences were observed in the absolute or relative testicular weights between the control and the compound treated groups, those of the epididymides were significantly lower in the 1,000 mg/kg treated group than in the control group. Histopathological examination revealed that there were lumens containing no or few spermatozoa, i.e., oligo/azoospermia, in the cauda epididymis in all the males of this group (Fig. 7), while none of their caput epididymis showed the same abnormality. In addition to this, the number of animals with cell debris in the cauda epididymal lumen was increased in this group, although it was statistically insignificant when compared with that in the