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Original Article

Toxicity in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine in rats

Shihori Tanabe[1], Masahito Ohara[2], Masaya Ito[2], Atsushi Noda[2], Katsumi Kobayashi[1], Mariko Matsumoto[1] and Akihiko Hirose[1]

[1]Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, 1-18-1, Kami-yoga, Setagaya-ku, Tokyo, 158-8501, Japan
[2]Research Institute for Animal Science in Biochemistry and Toxicology, 3-7-11, Hashimoto-dai, Midori-ku, Sagamihara, Kanagawa, 252-0132, Japan

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ABSTRACT — To assess the toxicity of *N*-phenyl-1-naphthylamine, Sprague Dawley rats were repeatedly administered with the chemical by oral gavage daily at doses of 0, 4, 20, 100, and 500 mg/kg/day for 28 days, followed by a 14-day recovery period. A significant decrease or decreasing trend of red blood cell counts, hemoglobin concentration, hematocrit, and mean corpuscular hemoglobin concentration and a significant increase in reticulocyte counts were observed at a dose of 500 mg/kg in both male and female rats. Increase in blood urea nitrogen and sodium levels was observed in male rats that received 500 mg/kg; increase in serum total protein, albumin, and calcium levels and in albumin/globulin ratio were observed in female rats that received 500 mg/kg. Increase in relative liver weight in female rats that received 100 mg/kg and increase in the absolute and relative liver weights in both male and female rats that received 500 mg/kg were observed; increases in the absolute and relative spleen weights and absolute kidney weight in female rats that received 500 mg/kg were observed. Hypertrophy of centrilobular hepatocyte and extramedullary hematopoiesis in the spleen were observed in both male and female rats at doses of 100 and 500 mg/kg. Renal tubular dilatation and papillary necrosis were observed in both male and female rats that received 500 mg/kg. These changes had the reversible trend in the recovery period. Based on these results, the no-observed-effect-level of *N*-phenyl-1-naphthylamine after a repeated daily oral administration for 28 days was determined to be 20 mg/kg/day for both sexes.

Key words: *N*-phenyl-1-naphthylamine, Liver toxicity, Hypertrophy of centrilobular hepatocyte, Spleen toxicity, Extramedullary hematopoiesis

INTRODUCTION

This toxicity study was conducted at the request of the Office of Chemical Safety, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare. We evaluated the toxicity of *N*-phenyl-1-naphthylamine after repeated daily oral administration for 28 days and examined the plasticity of the effects in the 14-day recovery period.

This study was designed to meet the test guidelines for toxicology studies issued by "Notification test methods of New Chemical Substances" of the Ministry of Health, Labour and Welfare (MHLW), Ministry of Economy, Trade and Industry (METI), Ministry of the Environment (MOE) (Yakuhatsu No. 1121002, 2003; Seikiyoku No. 2, 2003; Kanpoki No. 031121002; Yakuhatsu 1120001, 2006; Seikiyoku No. 2, 2006; and Kanpoki No. 061120001) and OECD Guidelines for the testing of chemicals for "repeated dose 28-day oral toxicity study in rodents" (TG 407). The experiments were conducted in compliance with the good laboratory practice standards of MHLW, METI, and MOE (Yakuhatsu No. 1121003, 2003; Seikiyoku No. 3, 2003; Kanpoki No. 031121004; Yakuhatsu No. 0704001, 2008; Seikiyoku No. 2, 2008; and Kanpoki No. 080704001) for criteria for test facilities for conducting tests on a new chemical substance in Japan.

Correspondence: Shihori Tanabe (E-mail: stanabe@nihs.go.jp)

MATERIALS AND METHODS

Test substance and reagent

The test substance *N*-phenyl-1-naphthylamine (lot no. 601006, purity 99.41%, purple yellow to light brown massive solid, MW: 219.29, CAS No. 90-30-2), which was used as lubrication and rubber antioxidants, was obtained from Ouchi-Shinko Chemical Industrial Co., Ltd. (Tokyo, Japan) and stored at approximately 2-6°C in a light-shielded storage room at the testing facility. Stability of the prepared sample was confirmed. Olive oil (lot no. BA26) for use as a vehicle was purchased from Miyazawa Pharmaceutical Co., Ltd. (Tokyo, Japan).

Animals and breeding condition

A total of 42 male and 42 female specific-pathogen-free Sprague Dawley (SD) rats [Crl:CD(SD)] (age: 4 weeks) were purchased from Atsugi Breeding Center of Charles River Japan Inc. (Kanagawa, Japan). After arrival at the testing facility, male and female rats were acclimatized to the testing environment for 7 and 8 days, respectively. Quarantine span was 4 and 5 days, respectively, from the day after the arrival. All animals were confirmed to be normal based on general condition and increase in body weight, and were divided into several groups. Administration of the test substance was initiated at 5 weeks of age for both sexes. The body weight range for male and female rats at treatment initiation was 145-166 g and 126-151 g, respectively. Group allocation was made to achieve comparable average body weight for each group. Assignment of an individual rat was based on a combination of random sampling method. Animals were individually housed in wire-mesh steel cage (W260 × D380 × H180 mm) of bracket type and kept in a room under controlled conditions: temperature, 21.2-22.9°C; humidity, 52-62%; ventilation, > 10 times per hr; and illumination, 12 hr per day (light on at 7:00 hr and off at 19:00 hr). The animals were fed with a pellet diet (Labo MR Stock, lot nos. 060873 and 060963, Nosan Corporation, Kanagawa, Japan) and provided *ad libitum* access to sterilized tap water [ultraviolet-irradiated after filtration with a cartridge filter (pore size: 1 μm)]. The animals were individually identified by ear punching, and each cage was labeled with experimental number, dose, animal ID and cage number. The experiments were performed with the approval of animal experimental committee at the test facility [Research Institute for Animal Science in Biochemistry and Toxicology (RIAS)].

Selection of dose levels

Dose levels of the test substance for the present study were selected based on the results of a 14-day dose range-finding study conducted on the same strain of rats (four males and four females for each group) at dose levels of 0, 4, 20, 100, and 500 mg/kg/day. In the dose range-finding study, a dose-dependent increase in the absolute liver weight was observed in rats of both sexes that received doses of 100 and 500 mg/kg/day. Moreover, salivation and decrease in hemoglobin, red blood cell (RBC) counts and hematocrit levels, and increase in reticulocyte counts, albumin, albumin/globulin (A/G) ratio, total bilirubin, absolute liver weight, and black-discoloration of spleen were observed in rats of both sexes that received 500 mg/kg/day dose. Decreasing trend of body weight gain, shortening of prothrombin time (PT), increases in inorganic phosphorus (IP) level and absolute spleen weight of male rats, and increases in total cholesterol level and relative kidney weight of female rats were observed in the 500 mg/kg/day group. Therefore, in the present 28-day oral administration toxicity study, 500 mg/kg/day was used as the highest dose (ensured toxicity on repeated administration) and 4 mg/kg/day was used as the lowest dose (no expected toxicity). The high and middle doses were set at 100 mg/kg/day and 20 mg/kg/day using a common ratio of 5.

Experimental design

Main group comprised of five male and five female rats; recovery group comprised of five male and five female rats each in the highest dose and the control groups. Rats were administered with the test substance by oral gavage once daily at doses of 0 (equivalent volume of olive oil as vehicle control), 4, 20, 100, and 500 mg/kg/day for 28 days. The administered volume was calibrated to 5 mL/kg body weight. On the day after the last dose, five male and five female rats from each group were euthanized for the assessment of hematology, blood biochemistry, organ weights, and macroscopic and microscopic examination of tissues and organs. The respective remaining five male and five female rats that were administered doses of 0 and 500 mg/kg were kept without treatment during a 14-day recovery period and then fully examined.

Daily observation, detailed clinical examination, sensory functional observation, grip strength and spontaneous motor activity, body weight and food consumption

All animals were observed for clinical signs of toxicity at four time-points (before administration, after administration, 30 min to 1 hr after administration and 4 hr after administration) during the administration period and once

daily during the recovery period. Detailed clinical observations including observations in the home cage were recorded by scoring 1 day before the administration, at days 6, 13, 20, and 27 during the administration period and at days 6 and 13 during the recovery period. Sensory functional observation including eye sight, hearing, sense of touch, and pain reactions, pupillary reflexes and righting reflexes were scored at day 27 during the administration period and at day 13 during the recovery period. Grip strength was measured with MK-380R/FR (Muromachi Kikai Co., Ltd., Tokyo, Japan) and spontaneous motor activity was measured with SUPERMEX (Muromachi Kikai Co., Ltd., Tokyo, Japan). Observations were recorded at day 27 during the administration period and at day 13 during the recovery period.

Body weight was recorded at days 1 (just before administration), 7, 14, 21, and 28 during the administration period and at days 7 and 14 during the recovery period. Food consumption by male rats was recorded at days 5, 12, 19, and 26 during the administration period and at days 5 and 12 during the recovery period. Food consumption in female rats was recorded at days 4, 11, 18, and 25 during the administration period and at days 4 and 11 during the recovery period.

Urinalysis, hematology, and clinical biochemistry

Non-fasting urinalysis was performed at day 22 during the administration period and at day 8 during the recovery period using metabolic cages for rats. For these measurements, fresh urine was collected and analyzed by dipsticks (gross appearance, pH, occult blood, protein, glucose, and urobilinogen). Sediments obtained from 3-hr urine output were subjected to microscopic examination. Volume and specific gravity of 18-hr urine output was determined using measuring cylinder and refractometer, respectively.

At the end of the administration and recovery periods, blood samples were collected from the abdominal aorta under deep ether anesthesia after overnight fasting (for 16-20 hr). The collected blood samples were divided into three portions. One portion of the blood was treated with EDTA-2K and examined for hematologic parameters such as RBC count, hemoglobin level, hematocrit percent, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, platelet count, white blood cell (WBC) count, differential leukocyte counts (basophils, eosinophils, neutrophils, lymphocytes, and monocytes). The second portion of blood sample was treated with 3.8% sodium citrate, and blood coagulation parameters, such as PT and activated partial thromboplastin time (APTT) were determined by fully automated blood coagulation measuring device (KC-10A, Amelung Co., NJ, USA).

The third portion of blood was used for blood biochemistry. Serum levels of total protein (TP), albumin, A/G ratio, glucose, total cholesterol, triglyceride, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), calcium (Ca), and IP levels were determined using an automated biochemical analyzer (JCA-BM8, JEOL Ltd., Tokyo, Japan); Serum sodium (Na), potassium (K), and chlorine (Cl) were determined using an automated electrolyte analyzer (NAKL-131, DKK-TOA Corporation, Tokyo, Japan).

Gross necropsy and examination of organ weights

After blood collection, all animals were sacrificed by exsanguination, and all internal organs and tissues including mucous membrane at orifices were observed macroscopically. The brain, thymus, heart, liver, spleen, kidneys, adrenals, thyroids, pituitary, testes, epididymides, and ovaries were weighed by electric balance before fixation. In addition, relative organ weights were calculated (ratios of organ weights to body weight). Thyroids and pituitary weights were weighed after fixation in 10% neutral-buffered formalin.

The brain, eyeballs, pituitary, thyroids (including parathyroid), spinal marrow (cervical, thoracic and lumbar spine), heart, trachea and lungs (injected and immersed with the fixative), liver, kidneys, thymus, spleen, adrenals, stomach, intestine (including the duodenum, jejunum, ileum, cecum, colon, rectum, and Peyer's patch), testes or ovaries, epididymis, prostate, seminal vesicles or uterus, vagina, bladder, sciatic nerve (cervical lymph nodes, mesenteric lymph nodes), and bone marrow (femur) were fixed in 10% neutral-buffered formalin and stored. All the fixed tissues were routinely prepared and stained with hematoxylin-eosin; and histopathological examination was conducted on control and top dose (500 mg/kg) groups at the end of the administration period. Histopathological examination of liver, kidney and spleen for groups treated with 4, 20, and 100 mg/kg was conducted at the end of the administration period, and for control and top dose groups at the end of the recovery period, since the test substance affected the liver, kidney and spleen in both sexes at dose of 500 mg/kg in the administration period.

Data analysis

Data pertaining to parametric variables such as grip

strength, motor activity, body weight, weight gain, food consumption, urine volume, specific gravity, hematology, blood biochemistry, and absolute and relative organ weights are presented as mean ± standard deviation. For comparison between more than three groups, Bartlett's test for homogeneity of distribution was applied. When the distribution was homogenous, one-way analysis of variance was performed. When a significant difference was observed, Dunnett's multiple comparison test was used to assess differences between control and treatment groups. If the distribution was not homogenous, or in case of nonparametric variables, the Kruskal-Wallis ranking test was applied. If significant difference was observed, Dunnett's type mean rank sum test was used to assess the differences between the control and individual treatment groups. During the recovery test with two groups, parametric data were analyzed for homogeneity of distribution using the *F*-test. If the distribution was homogenous, the student's *t*-test was used to assess between-group differences; if the distribution was not homogenous, Aspin-Welch's *t*-test was used. Nonparametric data were analyzed using the Mann-Whitney's *U* test. Categorical data such as general clinical signs, detailed clinical parameters, sensory functional observation, and gross and histopathological findings were analyzed using Fisher's exact test. Significance level was set at 0.05 for all tests.

RESULTS

General clinical observation

Salivation and purple discoloration of urine were observed in male rats in the 100 mg/kg group and in both male and female rats in the 500 mg/kg group. In the 100 mg/kg group, salivation was observed in two female rats and chromaturia was observed in three female rats. Salivation disappeared within 30 min just after administration. However, salivation was observed reflexively by handling before administration. One male rat in the 500 mg/kg group died on the day before necropsy; soiling of fur over lower abdomen by feces and urine and chromodacryorrhea were observed in this male rat at day 28. During the recovery period, chromaturia was observed in both male and female rats in the 500 mg/kg group at day 1. No changes were observed on day 2 or thereafter (data not shown).

Detailed observation

No significant changes were observed in any groups during the administration and recovery periods (data not shown).

Responses in the sensory function

No significant changes were observed in any groups during the administration and recovery periods (data not shown).

Grip strength and spontaneous motor activity

Significant decrease in grip strength of forelimb was observed in male rats in the 20 mg/kg group; however, these changes during the administration period did not appear to be dose-dependent. There were no changes in grip strength during the recovery period. No significant change of spontaneous motor activity was observed in any of the groups during the administration and recovery periods (data not shown).

Body weight

During the administration period, body weight of male rats in the 500 mg/kg group was slightly lower than that of their counterparts in the control group. Body weight gain was significantly small, although the body weight at measurement points was not significantly different compared with those of the control group. No change in body weights was observed in female groups compared with control. At day 7 of the recovery period, the body weight of male rats in the 500 mg/kg group was significantly lower than the body weight of their counterparts in the control group. No difference was observed between male rats in the 500 mg/kg and control groups with respect to weight gain. Both body weight and body weight gain of female rats were comparable to the control group at each measurement points (data not shown).

Food consumption

A trend of decreasing food consumption was observed during the administration period in male rats in the 500 mg/kg group; however, the difference was not statistically significant. At week 1, significant increase in food consumption was observed among female rats in the 20, 100, and 500 mg/kg groups; however, these changes did not appear to be dose-dependent and were deemed to be incidental changes. During the recovery period, a trend of decreasing food consumption was observed among male rats in the 500 mg/kg group at week 1; however, the decrease was not statistically significant. No changes in food consumption were observed among female rats (data not shown).

Urinalysis

A significant increase in urine volume was observed in both male and female rats in the 500 mg/kg group. A significant decrease and decreasing trend in specif-

ic gravity were observed in male and female rats in the 500 mg/kg group, respectively. During the administration period, chromaturia was observed in female rats in the 100 mg/kg group, and in both male and female rats in the 500 mg/kg group. No significant changes were observed during the recovery period in this respect (data not shown).

Hematology

Hematological results are summarized in Table 1. Significant decrease in hemoglobin, hematocrit level, and MCHC, and a significant increase in reticulocyte counts in both sexes, significant decrease of RBC count in females and decreasing trend of RBC count in males were observed in 500 mg/kg group at the end of the administration period. At the end of the recovery period, the changes in sacrificed animals showed a tendency for recovery. However, significant differences were observed in the 500 mg/kg group with respect to RBC and hemoglobin level in both sexes, MCHC and reticulocyte rate in male rats, and hematocrit level in female rats. Further, increase in MCV in both sexes, and increase in MCH in male rats was observed in the 500 mg/kg group. Moreover, with respect to differential leucocyte count, a significant decrease in eosinophil rate was observed in male rats; however, the number of eosinophil (67 ± 25/μL) were comparable to that in the control (123 ± 53/μL) ($P > 0.05$).

Table 1. Hematological findings in male and female rats in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine and the 14-day recovery period.

Dose (mg/kg/day):	At the end of administration period					At the end of recovery period	
	0	4	20	100	500	0	500
Males							
Number of animals:	5	5	5	5	4	5	5
RBC, $\times 10^4$/μL	753 ± 14	777 ± 14	784 ± 10	732 ± 23	681 ± 54	849 ± 39	738 ± 28**
Hemoglobin, g/dL	15.1 ± 0.4	15.0 ± 0.6	15.3 ± 0.4	14.5 ± 0.3	12.8 ± 0.5**	15.5 ± 0.8	14.4 ± 0.5*
Hematocrit, %	45.9 ± 1.0	45.6 ± 2.0	47.2 ± 1.0	44.6 ± 1.0	40.5 ± 0.6**	46.5 ± 2.1	44.7 ± 1.6
MCV, fL	61 ± 2	59 ± 2	60 ± 1	61 ± 2	60 ± 4	55 ± 0	61 ± 2**
MCH, pg	20.0 ± 0.6	19.3 ± 0.6	19.5 ± 0.3	19.8 ± 0.6	18.8 ± 0.9	18.3 ± 0.2	19.6 ± 0.6**
MCHC, %	32.8 ± 0.4	32.9 ± 0.3	32.4 ± 0.2	32.5 ± 0.2	31.5 ± 0.8*	33.4 ± 0.3	32.3 ± 0.3**
Reticulocyte, ‰	31.7 ± 1.9	34.9 ± 9.4	34.0 ± 6.9	44.7 ± 4.7	73.7 ± 13.8**	22.0 ± 3.6	43.8 ± 9.9**
PT, sec.	13.4 ± 0.7	13.5 ± 1.2	13.1 ± 0.2	13.0 ± 0.3	13.0 ± 0.3	12.8 ± 0.6	12.7 ± 0.2
APTT, sec.	19.8 ± 1.4	22.0 ± 2.3	19.5 ± 1.0	19.7 ± 1.7	22.5 ± 2.1	21.7 ± 0.4	21.7 ± 1.8
Platelet, $\times 10^4$/μL	131 ± 9	141 ± 10	133 ± 14	142 ± 23	158 ± 17	146 ± 6	136 ± 18
WBC, $\times 10^2$/μL	74 ± 25	60 ± 11	75 ± 18	90 ± 44	102 ± 26	88 ± 19	91 ± 13
Differential leukocyte count (%)							
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
Eosinophil	0.7 ± 0.2	0.8 ± 0.1	0.6 ± 0.3	0.6 ± 0.2	0.4 ± 0.2	1.4 ± 0.4	0.7 ± 0.3*
Neutrophil	14.6 ± 2.2	15.8 ± 2.4	16.5 ± 4.5	17.1 ± 0.7	15.4 ± 5.3	15.6 ± 3.1	13.9 ± 4.2
Lymphocyte	81.3 ± 3.0	80.2 ± 2.5	79.2 ± 4.2	79.0 ± 0.7	81.3 ± 6.3	80.3 ± 3.5	82.1 ± 3.7
Monocyte	3.4 ± 0.9	3.2 ± 0.7	3.6 ± 0.6	3.4 ± 0.8	3.0 ± 1.0	2.7 ± 0.6	3.2 ± 0.8
Females							
Number of animals:	5	5	5	5	5	5	5
RBC, $\times 10^4$/μL	753 ± 58	734 ± 16	746 ± 25	744 ± 29	638 ± 32**	779 ± 17	694 ± 44**
Hemoglobin, g/dL	14.5 ± 0.7	14.3 ± 0.5	14.6 ± 0.7	14.3 ± 0.5	12.3 ± 0.8**	14.9 ± 0.3	13.7 ± 0.5**
Hematocrit, %	42.8 ± 1.6	42.8 ± 1.4	43.3 ± 1.8	43.2 ± 1.4	38.7 ± 3.1*	43.6 ± 0.6	40.9 ± 1.8*
MCV, fL	57 ± 3	58 ± 2	58 ± 1	58 ± 1	61 ± 3	56 ± 1	59 ± 1**
MCH, pg	19.2 ± 0.6	19.4 ± 0.5	19.6 ± 0.4	19.2 ± 0.5	19.3 ± 0.8	19.1 ± 0.6	19.8 ± 0.7
MCHC, g/dL	33.7 ± 0.4	33.4 ± 0.4	33.7 ± 0.3	33.1 ± 0.5	31.8 ± 0.5**	34.2 ± 0.8	33.6 ± 0.4
Reticulocyte, ‰	27.9 ± 6.4	34.9 ± 6.8	25.1 ± 6.3	28.8 ± 3.3	102.5 ± 16.0*	20.4 ± 1.6	29.3 ± 10.3
PT, sec.	13.3 ± 0.5	13.2 ± 0.3	13.4 ± 0.4	13.6 ± 0.5	13.2 ± 0.3	13.4 ± 0.6	13.5 ± 0.4
APTT, sec.	17.4 ± 1.4	17.8 ± 1.3	18.7 ± 1.1	18.7 ± 0.6	19.6 ± 2.0	18.1 ± 0.9	18.1 ± 1.3
Platelet, $\times 10^4$/μL	146 ± 13	146 ± 15	132 ± 5	138 ± 8	130 ± 58	127 ± 6	114 ± 54
WBC, $\times 10^2$/μL	39 ± 11	46 ± 14	50 ± 11	57 ± 15	51 ± 17	43 ± 12	54 ± 19
Differential leukocyte count (%)							
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
Eosinophil	1.4 ± 0.5	1.6 ± 0.6	1.2 ± 0.5	0.9 ± 0.6	1.0 ± 0.6	1.3 ± 0.3	1.0 ± 0.5
Neutrophil	16.0 ± 4.7	19.9 ± 4.9	17.8 ± 2.4	18.7 ± 3.7	17.1 ± 3.7	14.1 ± 3.5	14.4 ± 2.2
Lymphocyte	80.3 ± 5.4	76.1 ± 5.8	79.4 ± 3.4	78.3 ± 4.6	80.2 ± 4.8	81.2 ± 3.6	81.3 ± 2.1
Monocyte	2.3 ± 0.8	2.4 ± 0.5	1.6 ± 0.7	2.0 ± 1.1	1.6 ± 0.6	3.4 ± 0.5	3.2 ± 0.6

Values are expressed as the mean ± standard deviation. *: Significant compared to the control group at $P < 0.05$. **: Significant compared to the control group at $P < 0.01$.

Clinical biochemistry

Clinical biochemistry results are summarized in Table 2. At the end of the administration period, significant increase in total bilirubin in both sexes, and significant increase in albumin level and A/G ratio in male rats was observed in the 100 and 500 mg/kg groups. Moreover, significant increases in BUN, sodium in males and TP, albumin levels, A/G ratio, and calcium level in females were observed in 500 mg/kg groups. Significant decrease in triglyceride level was observed in females that received 4, 20, and 100 mg/kg; however, these changes were not observed in a dose-dependent manner. At the end of the recovery period, significant decrease in total bilirubin level was observed in males that received 500 mg/kg; however, grades of changes were reduced. No other changes from that observed at the end of the administration period were observed. Moreover, significant increase in potassium level in males and significant decreases in ALT activity and increase in IP levels were observed in 500 mg/kg; these changes were not observed at the end of the administration period.

Gross necropsy

Large sized liver was observed in all animals (both

Table 2. Biochemical findings in male and female rats in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine and the 14-day recovery period.

Dose (mg/kg/day):	At the end of administration period					At the end of recovery period	
	0	4	20	100	500	0	500
Males							
Number of animals:	5	5	5	5	4	5	5
LDH, IU/L	303 ± 43	523 ± 306	245 ± 92	397 ± 257	423 ± 226	253 ± 85	208 ± 85
AST, IU/L	64 ± 5	70 ± 18	61 ± 4	69 ± 7	68 ± 4	65 ± 7	69 ± 9
ALT, IU/L	32 ± 5	35 ± 10	29 ± 4	31 ± 2	34 ± 5	35 ± 6	39 ± 4
ALP, IU/L	824 ± 111	783 ± 242	680 ± 77	695 ± 121	910 ± 104	547 ± 95	548 ± 38
γ-GTP, IU/L	0.50 ± 0.22	0.29 ± 0.14	0.40 ± 0.21	0.44 ± 0.28	0.71 ± 0.31	0.61 ± 0.20	0.58 ± 0.03
TP, g/dL	5.66 ± 0.15	5.81 ± 0.09	5.75 ± 0.16	5.87 ± 0.21	6.04 ± 0.24	6.04 ± 0.13	5.88 ± 0.12
Albumin, g/dL	2.68 ± 0.08	2.88 ± 0.10	2.88 ± 0.25	3.05 ± 0.14**	3.48 ± 0.16**	2.91 ± 0.12	2.81 ± 0.12
A/G ratio	0.90 ± 0.07	0.98 ± 0.08	1.01 ± 0.12	1.08 ± 0.05*	1.36 ± 0.11**	0.93 ± 0.05	0.92 ± 0.07
Total cholesterol, mg/dL	68 ± 15	73 ± 15	73 ± 7	65 ± 6	57 ± 13	73 ± 15	83 ± 16
Triglyceride, mg/dL	73 ± 41	67 ± 7	78 ± 13	75 ± 21	35 ± 5	90 ± 13	74 ± 22
Glucose, g/dL	151 ± 16	141 ± 13	145 ± 30	142 ± 19	142 ± 21	167 ± 17	149 ± 16
BUN, mg/dL	10.8 ± 1.0	10.3 ± 1.5	11.9 ± 1.2	12.0 ± 0.9	13.1 ± 1.2*	14.4 ± 2.0	13.1 ± 1.3
Creatinine, mg/dL	0.38 ± 0.05	0.40 ± 0.03	0.40 ± 0.04	0.41 ± 0.03	0.44 ± 0.02	0.46 ± 0.03	0.42 ± 0.03
Total bilirubin, mg/dL	0.34 ± 0.03	0.41 ± 0.07	0.42 ± 0.06	0.52 ± 0.07*	1.03 ± 0.29**	0.34 ± 0.02	0.41 ± 0.05*
Ca, mg/dL	9.6 ± 0.3	9.7 ± 0.3	9.9 ± 0.2	9.5 ± 0.3	9.6 ± 0.6	9.7 ± 0.2	9.4 ± 0.3
IP, mg/dL	8.2 ± 0.7	8.3 ± 0.6	9.2 ± 1.2	8.1 ± 0.3	9.1 ± 1.0	7.5 ± 0.5	7.9 ± 0.7
Na, mEq/L	146 ± 1	147 ± 1	147 ± 1	147 ± 1	148 ± 2*	147 ± 1	148 ± 1
K, mEq/L	5.04 ± 0.32	5.17 ± 0.45	5.53 ± 0.80	5.30 ± 0.42	5.89 ± 0.55	4.79 ± 0.14	5.05 ± 0.10**
Cl, mEq/L	105 ± 1	105 ± 1	103 ± 3	105 ± 2	106 ± 2	105 ± 1	105 ± 2
Females							
Number of animals:	5	5	5	5	5	5	5
LDH, IU/L	482 ± 79	492 ± 182	443 ± 118	581 ± 466	411 ± 180	325 ± 87	356 ± 182
AST, IU/L	76 ± 2	70 ± 5	88 ± 32	117 ± 60	66 ± 5	72 ± 12	68 ± 12
ALT, IU/L	25 ± 3	24 ± 4	28 ± 5	35 ± 12	36 ± 10	36 ± 3	27 ± 4**
ALP, IU/L	589 ± 101	578 ± 22	489 ± 134	386 ± 56	521 ± 151	337 ± 113	333 ± 63
γ-GTP, IU/L	0.54 ± 0.32	0.73 ± 0.32	0.96 ± 0.37	0.71 ± 0.49	0.80 ± 0.45	1.27 ± 0.55	0.79 ± 0.20
TP, g/dL	5.87 ± 0.18	5.92 ± 0.29	5.81 ± 0.22	5.90 ± 0.39	6.58 ± 0.14**	6.34 ± 0.24	6.28 ± 0.21
Albumin, g/dL	2.96 ± 0.11	2.96 ± 0.21	3.05 ± 0.24	3.08 ± 0.22	3.87 ± 0.17**	3.27 ± 0.29	3.20 ± 0.15
A/G ratio	1.02 ± 0.05	1.00 ± 0.11	1.11 ± 0.10	1.09 ± 0.09	1.43 ± 0.13**	1.07 ± 0.11	1.04 ± 0.09
Total cholesterol, mg/dL	71 ± 7	59 ± 7	61 ± 11	67 ± 10	80 ± 16	86 ± 11	85 ± 25
Triglyceride, mg/dL	31 ± 10	18 ± 6**	16 ± 5**	16 ± 3**	29 ± 4	25 ± 12	41 ± 18
Glucose, g/dL	136 ± 10	109 ± 11*	130 ± 11	123 ± 14	124 ± 16	145 ± 23	132 ± 16
BUN, mg/dL	13.3 ± 1.7	13.2 ± 1.7	14.6 ± 2.8	14.1 ± 2.5	15.4 ± 4.7	18.0 ± 3.1	16.1 ± 3.3
Creatinine, mg/dL	0.47 ± 0.03	0.43 ± 0.03	0.43 ± 0.03	0.46 ± 0.05	0.46 ± 0.05	0.49 ± 0.01	0.45 ± 0.05
Total bilirubin, mg/dL	0.26 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.43 ± 0.05*	1.20 ± 0.30**	0.29 ± 0.04	0.25 ± 0.03
Ca, mg/dL	9.4 ± 0.2	9.3 ± 0.2	9.2 ± 0.2	9.6 ± 0.2	10.0 ± 0.2**	9.5 ± 0.2	9.6 ± 0.3
IP, mg/dL	6.8 ± 0.6	7.4 ± 0.7	7.1 ± 0.9	7.8 ± 1.1	7.6 ± 0.8	6.3 ± 0.4	7.2 ± 0.7*
Na, mEq/L	146 ± 1	146 ± 2	146 ± 1	146 ± 2	148 ± 2	146 ± 2	147 ± 1
K, mEq/L	4.83 ± 0.17	5.13 ± 0.16	4.64 ± 0.21	4.81 ± 0.30	5.16 ± 0.38	5.10 ± 0.26	4.91 ± 0.24
Cl, mEq/L	107 ± 1	108 ± 1	108 ± 2	106 ± 2	107 ± 1	107 ± 1	107 ± 1

Values are expressed as the mean ± standard deviation. *: Significant compared to the control group at $P < 0.05$. **: Significant compared to the control group at $P < 0.01$.

sexes) in the 500 mg/kg group. At the end of the administration period, red area in left kidney was observed in one female rat in the 100 mg/kg group, and large sized kidney was observed in one dead male that received 500 mg/kg. At the end of the recovery period, phyma in the left kidney was observed in one female rat in the 500 mg/kg group.

Organ weights

The results of organ weight analyses are summarized in Tables 3 and 4. Findings at the end of the administration period are as follows: significant increase in relative liver weight was observed in females that received 100 mg/kg. Significant increases in absolute and relative liver weights in both sexes, absolute and relative spleen weights in female rats, relative brain and thyroid weights in male rats, absolute kidney weight in female rats, and significant decrease in absolute thymus weight in male rats were observed in 500 mg/kg groups. Significant increases in absolute weights of kidney and ovary were observed in females that received 20 mg/kg. Findings at the end of the recovery period included significant increase in relative liver weight in females in the 500 mg/kg group; however, the increasing trend in liver weight in both sexes tended to recover. Moreover, significant decrease in body weight as assessed just before necropsy, and significant increase in relative weights of brain and heart, and absolute and relative weights of spleen were observed in males that received 500 mg/kg.

Histopathology

The results of histopathological findings are summarized in Tables 5 and 6. Findings at the end of the administration period are as follows: Influence of the test substance on rats was observed in liver, kidney, and spleen in both males and females. Among the surviving animals, hypertrophy of centrilobular hepatocytes was observed in one male and one female that received 100 mg/kg, and in all animals (both sexes) that received 500 mg/kg. In these lesions, only mild foci of hypertrophy of centrilobular hepatocyte were observed in 100 mg/kg groups; however, 500 mg/kg groups showed widespread moderate hypertrophy of centrilobular hepatocytes. In the kidney, slight dilatation of distal and collecting tubules was observed in three males and three females that received 500 mg/kg; slight to moderate signs of papillary necrosis were observed in one male and two females that received 500 mg/kg. Moreover, basophilic renal tubule was observed

Table 3. Organ weights in male rats in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine and the 14-day recovery period.

Organ	Absolute and relative weights	At the end of administration period					At the end of the recovery period	
		Dose (mg/kg/day)					Dose (mg/kg/day)	
		0	4	20	100	500	0	500
Number of animals:		5	5	5	5	4	5	5
Body weight	g	347 ± 18	332 ± 32	334 ± 44	339 ± 31	286 ± 35	432 ± 33	388 ± 27**
Brain	g	1.91 ± 0.07	1.89 ± 0.06	1.92 ± 0.12	1.91 ± 0.07	1.86 ± 0.08	1.97 ± 0.04	1.97 ± 0.02
	%	0.55 ± 0.05	0.57 ± 0.04	0.58 ± 0.05	0.57 ± 0.05	0.66 ± 0.06*	0.46 ± 0.03	0.51 ± 0.04*
Thymus	g	0.58 ± 0.09	0.49 ± 0.08	0.47 ± 0.09	0.54 ± 0.13	0.36 ± 0.12*	0.52 ± 0.14	0.53 ± 0.16
	%	0.17 ± 0.03	0.15 ± 0.02	0.14 ± 0.03	0.16 ± 0.03	0.13 ± 0.04	0.12 ± 0.03	0.14 ± 0.04
Heart	g	1.20 ± 0.12	1.15 ± 0.07	1.14 ± 0.11	1.16 ± 0.12	1.02 ± 0.11	1.30 ± 0.07	1.28 ± 0.12
	%	0.34 ± 0.02	0.34 ± 0.02	0.34 ± 0.03	0.34 ± 0.04	0.36 ± 0.02	0.30 ± 0.01	0.33 ± 0.01**
Liver	g	10.90 ± 0.78	10.53 ± 1.41	10.53 ± 1.37	13.26 ± 1.92	15.31 ± 2.42**	12.91 ± 0.98	12.37 ± 1.66
	%	3.14 ± 0.12	3.17 ± 0.22	3.16 ± 0.03	3.90 ± 0.35	5.34 ± 0.23**	2.99 ± 0.04	3.18 ± 0.28
Kidney	g	2.65 ± 0.25	2.57 ± 0.33	2.63 ± 0.34	2.64 ± 0.32	2.56 ± 0.47	2.95 ± 0.14	2.87 ± 0.16
	%	0.77 ± 0.08	0.78 ± 0.11	0.79 ± 0.06	0.78 ± 0.05	0.90 ± 0.13	0.68 ± 0.03	0.74 ± 0.06
Adrenal	mg	58.6 ± 4.3	55.8 ± 7.4	54.5 ± 13.3	58.3 ± 4.6	53.4 ± 7.7	67.8 ± 9.1	66.1 ± 4.4
	10^{-3}%	16.9 ± 1.4	16.8 ± 1.4	16.2 ± 2.3	17.2 ± 0.9	19.0 ± 3.9	15.7 ± 1.7	17.0 ± 1.3
Spleen	g	0.70 ± 0.11	0.61 ± 0.07	0.67 ± 0.11	0.78 ± 0.22	0.71 ± 0.27	0.73 ± 0.08	0.94 ± 0.10**
	%	0.20 ± 0.03	0.18 ± 0.02	0.20 ± 0.01	0.22 ± 0.05	0.25 ± 0.07	0.17 ± 0.01	0.25 ± 0.04**
Testis	g	3.22 ± 0.17	2.93 ± 0.26	3.26 ± 0.23	3.01 ± 0.15	2.95 ± 0.23	3.29 ± 0.12	3.26 ± 0.25
	%	0.93 ± 0.10	0.89 ± 0.10	0.99 ± 0.09	0.89 ± 0.11	1.04 ± 0.11	0.76 ± 0.07	0.84 ± 0.09
Epididymis	g	0.79 ± 0.05	0.76 ± 0.05	0.79 ± 0.08	0.76 ± 0.08	0.66 ± 0.10	1.06 ± 0.07	1.08 ± 0.05
	%	0.23 ± 0.03	0.23 ± 0.02	0.24 ± 0.02	0.22 ± 0.02	0.23 ± 0.04	0.25 ± 0.02	0.28 ± 0.03
Pituitary gland	mg	12.2 ± 0.6	11.0 ± 0.8	11.9 ± 1.9	11.4 ± 1.6	10.2 ± 1.7	13.0 ± 1.6	12.3 ± 0.6
	10^{-3}%	3.5 ± 0.3	3.3 ± 0.3	3.6 ± 0.3	3.4 ± 0.3	3.5 ± 0.2	3.0 ± 0.2	3.2 ± 0.1
Thyroid	mg	25.6 ± 2.4	25.1 ± 3.7	26.4 ± 2.7	29.5 ± 4.6	28.5 ± 1.7	31.2 ± 3.3	28.1 ± 2.5
	10^{-3}%	7.3 ± 0.4	7.6 ± 1.2	7.9 ± 0.4	8.7 ± 0.8	10.1 ± 1.2**	7.2 ± 0.4	7.3 ± 0.9

Values are expressed as the mean ± standard deviation. *: Significant compared to the control group at $P < 0.05$. **: Significant compared to the control group at $P < 0.01$.

Table 4. Organ weights in female rats in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine and the 14-day recovery period.

Organ	Absolute and relative weights	At the end of administration period Dose (mg/kg/day) 0	4	20	100	500	At the end of the recovery period Dose (mg/kg/day) 0	500
Number of animals:		5	5	5	5	5	5	5
Body weight	g	203 ± 20	206 ± 8	219 ± 7	209 ± 16	198 ± 7	235 ± 20	224 ± 28
Brain	g	1.80 ± 0.09	1.83 ± 0.06	1.83 ± 0.07	1.75 ± 0.10	1.79 ± 0.10	1.80 ± 0.07	1.86 ± 0.11
	%	0.89 ± 0.07	0.89 ± 0.02	0.84 ± 0.04	0.83 ± 0.05	0.90 ± 0.05	0.77 ± 0.04	0.84 ± 0.09
Thymus	g	0.42 ± 0.10	0.44 ± 0.11	0.47 ± 0.08	0.49 ± 0.07	0.37 ± 0.08	0.45 ± 0.12	0.37 ± 0.09
	%	0.21 ± 0.07	0.21 ± 0.05	0.21 ± 0.04	0.24 ± 0.03	0.19 ± 0.04	0.19 ± 0.05	0.16 ± 0.03
Heart	g	0.72 ± 0.06	0.77 ± 0.02	0.79 ± 0.06	0.78 ± 0.09	0.78 ± 0.06	0.81 ± 0.09	0.82 ± 0.09
	%	0.36 ± 0.01	0.37 ± 0.02	0.36 ± 0.02	0.37 ± 0.03	0.39 ± 0.03	0.35 ± 0.02	0.37 ± 0.02
Liver	g	5.88 ± 0.76	5.90 ± 0.50	6.85 ± 0.49	7.04 ± 0.68	10.06 ± 0.97**	6.53 ± 0.80	6.88 ± 0.54
	%	2.90 ± 0.13	2.86 ± 0.20	3.13 ± 0.22	3.37 ± 0.11*	5.08 ± 0.36**	2.78 ± 0.15	3.09 ± 0.18*
Kidney	g	1.50 ± 0.09	1.62 ± 0.18	1.76 ± 0.12*	1.70 ± 0.10	1.72 ± 0.13*	1.69 ± 0.13	1.76 ± 0.33
	%	0.75 ± 0.06	0.78 ± 0.08	0.80 ± 0.07	0.82 ± 0.07	0.87 ± 0.07	0.72 ± 0.10	0.78 ± 0.07
Adrenal	mg	62.6 ± 5.5	72.6 ± 11.3	63.8 ± 16.8	62.9 ± 5.8	63.4 ± 7.6	77.8 ± 11.1	65.5 ± 5.1
	10^{-3}%	31.1 ± 3.8	35.2 ± 5.1	29.2 ± 8.0	30.0 ± 1.3	32.1 ± 4.8	33.3 ± 4.7	29.5 ± 3.4
Spleen	g	0.40 ± 0.03	0.47 ± 0.09	0.48 ± 0.07	0.48 ± 0.02	0.56 ± 0.05**	0.47 ± 0.05	0.50 ± 0.10
	%	0.20 ± 0.02	0.23 ± 0.04	0.22 ± 0.03	0.23 ± 0.01	0.29 ± 0.02**	0.20 ± 0.02	0.22 ± 0.03
Ovary	mg	74.6 ± 5.5	76.8 ± 7.6	96.0 ± 10.7*	84.2 ± 9.2	79.8 ± 14.9	86.5 ± 18.1	94.7 ± 18.6
	10^{-3}%	37.1 ± 4.3	37.2 ± 3.2	43.8 ± 4.7	40.5 ± 6.2	40.5 ± 8.6	37.3 ± 10.2	42.0 ± 5.0
Pituitary gland	mg	12.7 ± 1.8	13.3 ± 2.5	14.9 ± 1.0	13.9 ± 1.1	11.7 ± 1.1	15.1 ± 1.2	13.0 ± 1.9
	10^{-3}%	6.3 ± 0.6	6.5 ± 1.2	6.8 ± 0.6	6.7 ± 0.7	5.9 ± 0.7	6.5 ± 0.8	5.8 ± 0.9
Thyroid	mg	18.0 ± 2.7	20.0 ± 2.0	20.7 ± 3.7	21.8 ± 3.3	23.9 ± 3.3	20.5 ± 1.8	21.2 ± 3.8
	10^{-3}%	9.0 ± 1.9	9.7 ± 0.8	9.5 ± 1.7	10.5 ± 2.1	12.1 ± 1.8	8.8 ± 1.2	9.4 ± 0.9

Values are expressed as the mean ± standard deviation. *: Significant compared to the control group at $P < 0.05$. **: Significant compared to the control group at $P < 0.01$.

in four males that received 500 mg/kg. This finding was a noticeable change as compared to the few cases with mild lesions in the control group. In the spleen, extramedullary hematopoiesis was observed in three male and one female rats in the 100 mg/kg group and in three male and three female rats in the 500 mg/kg group. Increase in deposits of brown pigment was observed in five female rats in the 500 mg/kg group.

Hypertrophy of centrilobular hepatocytes, dilatation of renal tubules, basophilic tubule, and renal papillary necrosis were observed in one dead male rat that received 500 mg/kg. However, these lesions were more severe as compared to those observed in survivors and hyaline droplet was observed in this rat. Moreover, congestion in spleen and atrophy and hemorrhage in thymus were also observed.

Findings at the end of the recovery period were as follows: hypertrophy of centrilobular hepatocytes in one male and one female; slight renal papillary necrosis in three females, post-necrotic mineralization of papilla in one male, extramedullary hematopoiesis in two males and deposit of brown pigment in two male and five female rats. Other changes were recovered.

Unilateral hemorrhagic necrosis in kidney cortex (localized lesion) was observed at the end of the administration period in one female that received 100 mg/kg, in which the red area in kidney was observed at necropsy performed at the end of the administration period. Unilateral nephroblastoma was observed in one female that received 500 mg/kg, in which phyma was observed in left kidney at necropsy performed at the end of the recovery period. In addition, focal necrosis in liver, microgranuloma and peripheral lobular fatty degeneration in hepatocytes, hyaline droplet in proximal renal tubular epithelium, hyaline casts, solitary and multiple cyst, and focal renal cortical fibrosis, and hemorrhage in thymus were observed; there were no significant differences between the treated groups.

DISCUSSION

This report examined the toxicity of *N*-phenyl-1-naphthylamine in male and female rats after daily administration for 28 days, and the plasticity of the effects in the 14-day recovery period. In previous toxicity studies, LD_{50} value of oral *N*-phenyl-1-naphthylamine in rats was reported to be 1625 mg/kg (NIOSH, 2011). *N*-phenyl-1-naphthylamine was shown to be capable of skin sensitization (Boman *et al.*, 1980), but did not induce genotoxicity (IPCS, 1998).

Table 5. Histopathological findings in male rats in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine and the 14-day recovery period.

Organ	Findings	Grade	At the end of administration period							At the end of recovery period	
			Dose (mg/kg/day)	0	4	20	100		500	0	500
			Fate	KA	KA	KA	KA	FD	KA	Total	KR	KR
			No of animal	5	5	5	5	1	4	5	5	5
Lung	Mineralization, artery	-		4	#	#	#	1	4	5	#	#
		+		1	#	#	#	0	0	0	#	#
Heart	Myocardial degeneration/fibrosis	-		4	#	#	#	1	4	5	#	#
		+		1	#	#	#	0	0	0	#	#
Liver	Hypertrophy, hepatocyte, centrilobular	-		5	5	5	4	0	0	0	5	4
		+		0	0	0	1	0	0	0+ 5=5**	0	1
		++		0	0	0	0	1	4		0	0
	Necrosis, focal	-		5	4	3	4	1	3	4	4	3
		+		0	1	2	0	0	1	1	1	1
		++		0	0	0	1	0	0	0	0	1
	Microgranuloma	-		4	5	5	5	1	4	5	4	5
		+		1	0	0	0	0	0	0	1	0
Kidney	Dilatation, distal/collecting, tubules	-		5	5	5	5	0	1	1	5	5
		+		0	0	0	0	0	3	3+	0	0
		++		0	0	0	0	1	0	1=4*	0	0
	Necrosis, papilla	-		5	5	5	5	0	3	3	5	5
		++		0	0	0	0	1	1	2	0	0
	Mineralization, papilla	-		5	5	5	5	1	4	5	5	4
		+		0	0	0	0	0	0	0	0	1
	Hyaline droplet, proximal tubular epithelium	-		0+ 5=5	0+ 4=4	1+ 4=5	1+ 4=5	1	1	2+ 3=5	0+ 5=5	1+ 4=5
		++		0	1	0	0	0	0	0	0	0
		+++										
	Basophilic tubule	-		3+ 2=5	4+ 1=5	4+ 1=5	5+ 0=5	0	0	0+ 0=0	4+ 1=5	0+ 3=3
		+		0+ 0=0	0+ 0=0	0+ 0=0	0+ 0=0	0	4	4+ 1=5**	0+ 0=0	2+ 0=2
		++						1	0			
		+++										
	Eosinophilic body, proximal tubular epithelium	-		5	5	5	5	1	4	5	4	5
		+		0	0	0	0	0	0	0	1	0
	Cast, hyaline	-		4	5	5	5	0	3	3	5	5
		+		1+ 0=1	0+ 0=0	0+ 0=0	0+ 0=0	0	1	1+ 1=2	0	0
		++						1	0		0	0
	Cyst, solitary	-		5	4	5	4	1	4	5	4	3
		++		0	1	0	1	0	0	0	1	2
	Cyst, multiple	-		5	5	5	5	1	4	5	5	4
		++		0	0	0	0	0	0	0	0	1
	Fibrosis, cortex, focal	-		4	5	5	5	1	4	5	5	5
		+		1	0	0	0	0	0	0	0	0
Thymus	Atrophy	-		5	#	#	#	0	4	4	#	#
		++		0	#	#	#	1	0	1	#	#
	Hemorrhage	-		5	#	#	#	0	4	4	#	#
		+		0	#	#	#	1	0	1	#	#
Spleen	Hematopoiesis, extramedullary	-		0+ 5=5	0+ 5=5	0+ 5=5	0+ 2=2	0	0	0+ 2=2	0+ 5=5	0+ 3=3
		+		0	0	0	3	1	1	3	0	2
		++						0	3			
	Deposit, pigment, brown	-		0+ 5=5	0+ 5=5	0+ 5=5	0+ 5=5	0	0	0+ 5=5	0+ 5=5	0+ 3=3
		+						1	4			
		++		0	0	0	0	0	0	0	0	2
	Congestion	-		5	5	5	5	0	4	4	5	5
		++		0	0	0	0	1	0	1	0	0
Prostate	Cellular infiltration, lymphocyte, interstitium	-		4	#	#	#	1	4	5	#	#
		+		1	#	#	#	0	0	0	#	#

KA: Killed by design at the end of the administration period. KR: Killed by design at the end of the recovery period. FD: Found dead. #: Not examined. Numbers underlined indicate the sum of the lesion for statistical analysis. Grade, -: Negative; +: Slight; ++: Moderate; +++: Severe. No abnormalities were found in brain, pituitary, thyroid, parathyroid, trachea, stomach, small intestine, large intestine, adrenal, urinary bladder, testis, seminal vesicle, spinal cord, sciatic nerve, bone marrow, lymph nodes, and eye ball from animals of control and 500 mg/kg group. *: Significant compared to the control group at $P < 0.05$. **: Significant compared to the control group at $P < 0.01$.

Table 6. Histopathological findings in female rats in repeated 28-day oral administration of *N*-phenyl-1-naphthylamine and the 14-day recovery period.

Organ	Findings	Grade	At the end of administration period					At the end of recovery period	
			Dose (mg/kg/day)	0	4	20	100	500	0	500
			Fate	KA	KA	KA	KA	KA	KR	KR
			No of animal	5	5	5	5	5	5	5
Lung	Mineralization, artery	-		4	#	#	#	5	#	#
		+		1	#	#	#	0	#	#
Liver	Hypertrophy, hepatocyte, centrilobular	-		5	5	5	4	0	5	4
		+		0+	0+	0+	1+	0+	0+	1+
		++		0=0	0=0	0=0	0=1	5=5**	0=0	0=1
	Necrosis, focal	-		5	5	5	5	4	4	5
		+		0	0	0	0	1	1	0
	Microgranuloma	-		4	4	4	4	4	4	5
		+		1	1	1	1	1	1	0
	Degeneration, fatty, hepatocyte, peripheral	-		4	4	5	5	5	5	5
		+		1	1	0	0	0	0	0
	Fibrosis, focal	-		5	5	5	5	5	5	4
		+		0	0	0	0	0	0	1
Kidney	Dilatation, distal/collecting, tubules	-		5	5	5	5	2	5	5
		+		0	0	0	0	3	0	0
	Necrosis, papilla	-		5	5	5	5	3	5	2
		+		0+	0+	0+	0+	1+	0+	3+
		+++		0=0	0=0	0=0	0=0	1=2	0=0	0=3
	Basophilic tubule	-		3	4	4	5	1	3	5
		+		2	1	1	0	4	2	0
	Cyst, solitary	-		5	5	5	5	5	5	4
		+		0	0	0	0	0	0	1
	Fibrosis, cortex	-		5	5	4	5	5	5	5
		+		0	0	1	0	0	0	0
	Mineralization, cortico-medullary junction	-		4	5	5	5	5	4	5
		+		1	0	0	0	0	1	0
	Hemorrhagic necrosis, cortex, unilateral	-		5	5	5	4	5	5	5
		++		0	0	0	1	0	0	0
	Nephroblastoma, unilateral (malignant)			0	0	0	0	0	0	1
Thymus	Hemorrhage	-		4	#	#	#	4	#	#
		+		1	#	#	#	1	#	#
Spleen	Hematopoiesis, extramedullary	-		0+	0+	0+	0+	0+	0+	0+
		++		5=5	5=5	5=5	4=4	2=2	5=5	5=5
		+++		0	0	0	1	3	0	0
	Deposit, pigment, brown	-		0+	0+	0+	0+	0+	0+	0+
		++		5=5	5=5	5=5	5=5	0=0	5=5	0=0
		+++		0	0	0	0	5**	0	5**

**: Significant compared to the control group at $P < 0.01$. KA: Killed by design at the end of the administration period. KR: Killed by design at the end of the recovery period. #: Not examined. Numbers underlined indicate the sum of the lesion for statistical analysis. Grade, -: Negative; +: Slight; ++: Moderate; +++: Severe. No abnormalities were found in brain, pituitary, thyroid, parathyroid, trachea, heart, stomach, small intestine, large intestine, adrenal, urinary bladder, ovary, uterus, spinal cord, sciatic nerve, bone marrow, lymph nodes, and eye ball from animals of control and 500 mg/kg group.

We evaluated the toxicity of *N*-phenyl-1-naphthylamine after oral administration at dose levels of 0, 4, 20, 100, and 500 mg/kg/day for 28 days and examined the plasticity of the effects during the recovery period of 2 weeks. Main toxic effects of the test substance were observed in liver, kidneys, and blood.

We observed increase in relative liver weight in males that received 100 mg/kg and increase in absolute and relative liver weights in both male and female rats that received 500 mg/kg. Hypertrophy of centrilobular hepatocyte was observed in both male and female rats that received 100 and 500 mg/kg. On macroscopic examination, increase in liver size was observed after administration of 500 mg/kg dose in both male and female rats.

Increases in albumin level and A/G ratio were observed in male rats that received 100 mg/kg and in both male and female rats that received 500 mg/kg. These changes represented the effects of the test substance on the liver.

Moreover, increase in total bilirubin level was observed in both male and female rats that received 100 and 500 mg/kg doses; these changes likely represent the effects of the test substance on the liver including hemolytic anemia (described later). The increase in calcium level in female rats that received 500 mg/kg dose appeared to be secondary to the increase in albumin levels, since no possible changes in calcium metabolism were observed.

With respect to the influence of the test substance on kidney, tubular dilatation and papillary necrosis were observed in both male and female rats that received 500 mg/kg; moreover, basophilic tubules that appear to reflect the regeneration of renal tubules were observed in male rats that received 500 mg/kg. Increase in absolute kidney weight was observed in female rats that received 500 mg/kg. Moreover, tubular dilatation, basophilic tubules, papillary necrosis, severe lesions with hyaline droplets in kidneys, and hypertrophy of hepatocytes were observed in one dead male rat that received 500 mg/kg. This animal was determined to have died due to the effect of the test substance. Results of urinalyses indicated the renal effects of the test substance. The changes included increase in urine volume and the tendency for decrease in specific gravity of urine in both male and female rats that received 500 mg/kg, and increase in BUN and sodium levels in males that received 500 mg/kg.

With respect to the influence of the test substance on hematology, decrease or tendency for decrease in RBC counts, hemoglobin, hematocrit levels and MCHC were observed in both male and female rats that received 500 mg/kg. The effect of *N*-phenyl-1-naphthylamine is known to be similar to that of aromatic amines, i.e., production of methemoglobin and hemolytic effect (Winder and Balouet, 2002). In the present study, increase in reticulocyte counts and increase in extramedullary hematopoiesis in spleen suggested enhancement of hematopoietic function. Similarly, increase in total bilirubin level indicated increased destruction of RBCs as reflected in brown colored deposits of pigments with hemosiderin derived from RBCs. These findings confirmed that hemolytic anemia was a direct effect of the test substance. Increase in absolute spleen weight in females that received 500 mg/kg seems to be related to the histopathological changes observed in the spleen. Moreover, decrease in body weight gain and tendency for lower food consumption during the administration period are considered to be related to the toxicity of the test substance.

Appearance of salivation in both male and female rats that received 100 and 500 mg/kg was not attributed to the test substance. It reflected the repellent response to the solution administered, since it appeared just after the administration; moreover, the reflex response was observed with the handling of the animals in a few cases.

On detailed clinical observation, the sensory function, grip strength, and spontaneous motor activity were found to be unaffected. Neurobehavioral toxicity was not observed in any of the treatment groups and no histopathological changes were observed in nervous system organs. Purple colored urine is believed to be derived from the test substance or its metabolites. Increase in relative weights of brain and thyroid and decrease in absolute weight of thymus were observed in male rats that received 500 mg/kg. However, no histopathological changes were observed in these organs or tissues compared with controls. Therefore, these changes likely represent the secondary effects associated with low value trends in body weight and toxicity to these organs was not observed. Significant increase in absolute weights of kidney and ovary were observed in female rats that received 20 mg/kg; however, this change was not observed in a dose-dependent manner. Therefore, it is likely to be an incidental change. Histological changes in liver and kidneys of survivors at the end of the administration period were clearly recovered by the end of the recovery period. With respect to the hematological effects, significant differences and associated pathological changes were observed at the end of the recovery period; however, the symptoms of anemia tended to reduce, which indicates that these changes were reversible.

The increase in potassium level in male rats, increase in IP level in female rats, and decrease in ALT activity in female rats were in the range of background data (K: 4.23-5.65 mEq/L, IP: 7.0-9.5 mg/dL, and ALT: 18-38 IU/L) of the test facility (RIAS), and the related changes were not observed at the end of the recovery period in both male and female rats that received 500 mg/kg. Therefore, these changes are believed to be incidental. At the end of the recovery period, nephroblastoma was observed in one female rat that received 500 mg/kg. This tumor was an embryonal tumor of epithelium derived from metanephric anlage. It has been reported to naturally occur in rats (JSTP, 2000; Hard and Noble, 1981). Since the symptoms were observed only in a rat, the tumor was likely unrelated to the administration of the test substance.

Based on these results, the toxicity of *N*-phenyl-1-naphthylamine in liver, kidney and blood were observed in both male and female rats that received 100 mg/kg. Therefore, the no-observed-effect-level of *N*-phenyl-1-naphthylamine was determined to be 20 mg/kg/day for both male and female rats.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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